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FOREIGN DIRECT INVESTMENT AND ITS IMPACT ON INDIAN ECONOMY

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ABSTRACT

Foreign direct investment plays vital role in achieving rapid economic growth in our country. In fact FDI mostly flows towards the developed countries and only a small portion of FDI flows to a limited number of developing countries. Our country is also part of small portion; however, India has continuously trying to attract FDI for the purpose economic growth and development. This paper firstly identifies the influential factors that determine FDI inflow in the developing countries it includes various rules and regulations provided by Indian government and secondly understand the current situation of our economy and economic growth and FDI. It is found that countries with larger GDP and high GDP growth rate and maintain business friendly environment with abundant modern infrastructural facilities. Foreign direct investment as a strategic component of investment is needed by India for achieving the economic reforms and maintains the pace of growth and development of the economy. The study tries to find out how FDI seen as an important economic catalyst of Indian economic growth by stimulating domestic investment, increasing human capital formation and by facilitating the technology transfers. The main purpose of the study is to investigate the impact of FDI on economic growth in India.

KEYWORDS

Economic growth, foreign direct investment, inflow, Human Capital

INTRODUCTION

Foreign Direct Investment has contributed significantly to growth and development in many developing countries over the last three decades, although, the benefits have not been evenly distributed. The countries that have benefited the most are those in which the conditions for harnessing inflows of foreign capital were in place and the opportunities and risks associated with current and future market developments were clearly understood by both investors and host country policy makers. These include – political stability, investments-friendly regulatory and policy frameworks, skilled or easy-to-train manpower, market size or proximity to large markets with minimal trade and physical barriers, etc. However, several developing countries have seen FDI's contribution to growth (in terms of GDP) at very high rates even without the development-friendly conditions in place.

OBJECTIVES OF THE STUDY

1. To know about foreign direct investment
2. To study the various determinants of FDI



3. To know the current situation of Indian economy due to FDI policies

RESEARCH METHODOLOGY

The present research paper is fully based on secondary source of data, which is collected through various books, journals, internet web sites etc.

Foreign Direct Investment:

FDI plays an important role in promoting economic growth, raising a country's technological level, and creating new employment in developing countries. It has also been shown that FDI works as a means of integrating developing countries into the global market place and increasing the capital available for investment, thus leading to increased economic growth needed to reduce poverty and raise living standards. At the same time many countries have understood the role played by FDI and they have taken steps to remove investment barriers. For the purpose of attracting FDI, many countries have implemented incentives including tax exemption, government pledges, tariff reduction on equipment and machinery imports, subsidy, etc.

FOREIGN DIRECT INVESTMENT IN INDIA

Foreign direct investment (FDI) is direct investment by a company in production located in another country either by buying a company in the country or by expanding operations of an existing business in the country. Foreign direct investment is done for many reasons including to take advantage of cheaper wages in the country, special investment privileges such as tax exemptions offered by the country as an incentive to gain tariff-free access to the markets of the country or the region.

FDI INDIA

Foreign Direct Investment (FDI) in India is undertaken in accordance with the FDI policy formulated and announced by the Government of India and is governed by the provisions of Foreign Exchange Management Act, 1999.

Determinates of FDI:

ENTRY ROUTES FOR INVESTMENT IN INDIA

Under the Foreign Direct Investments (FDI) Scheme, investments can be made in shares, mandatorily fully convertible debentures and mandatorily fully convertible preference shares of an Indian company by non-residents through two routes:

Automatic Route

FDI up to 100 per cent is allowed under the automatic route in all activities/sectors except where the provisions of the consolidated FDI Policy on 'Entry Routes for Investment' are attracted. FDI in sectors /activities to the extent permitted under the automatic route does not require any prior approval either of the Government or the Reserve Bank of India.



Government Route

FDI in activities not covered under the automatic route requires prior approval of the Government which is considered by the FIPB, Department of Economic Affairs, and Ministry of Finance. Indian companies having foreign investment approval through FIPB route do not require any further clearance from the RBI for receiving inward remittance and for the issue of shares to the non-resident investors.

CALCULATION OF TOTAL FOREIGN INVESTMENT

(i) Direct Foreign Investment: All investment directly by a non-resident entity into the Indian company would be counted towards foreign investment.

(ii) Indirect Foreign Investment:

(a) The foreign investment through the investing Indian company would not be considered for calculation of the indirect foreign investment in case of Indian companies which are owned and controlled by resident Indian citizens and/or Indian Companies which are owned and controlled by resident Indian citizens.

(b) For cases where condition (a) above is not satisfied or if the investing company is owned or controlled by 'non- resident entities', the entire investment by the investing company into the subject Indian Company would be considered as indirect foreign investment, provided that, as an exception, the indirect foreign investment in only the 100% owned subsidiaries of operating-cum-investing/investing companies, will be limited to the foreign investment in the operating-cum-investing/ investing company.

FDI and Economic Development

FDI is considered to be the life blood and an important vehicle of for economic development as far as the developing nations are concerned. The important effect of FDI is its contribution to the growth of the economy. FDI has an important impact on country's trade balance, increasing labour standards and skills, transfer of technology and innovative ideas, skills and the general business climate. FDI also provides opportunity for technological transfer and up gradation, access to global managerial skills and practices, optimal utilization of human capabilities and natural resources, making industry internationally competitive, opening up export markets, access to international quality goods and services and augmenting employment opportunities. The present situation of foreign direct investment shown in the following table, it helps to understand the current situation of FDI inflow in Indian economy.

Flow of FDI

Year	FDI (Crore)
2000-01	12645



2002-03	14932
2003-04	12117
2005-06	24613
2006-07	70630
2007-08	98664
2008-09	85700
2011-12	110350

(Source: UNCTAD Report 2012)

Data presented in the above table shows the inflow of FDI in our economy, there is continuous upward trend in FDI, in the year 2011-12 110350 crores FDI recorded in our country, because some changes has been taken place in FDI policy. Increase in the inflow of FDI helps to decrease in the deficits of Balance of payment and this will directly effects to development of GDP and growth of the economy. Foreign direct investment has always positive for impact on economy. It helps to steady development, and growth of the economy.

Foreign investment was introduced in 1991 under Foreign Exchange Management Act (FEMA), driven by then finance minister Manmohan Singh. As Singh subsequently became the prime minister, this has been one of his top political problems, even in the current times. India disallowed overseas corporate bodies (OCB) to invest in India. India imposes cap on equity holding by foreign investors in various sectors, current FDI limit in aviation sector is maximum 49%. Starting from a baseline of less than \$1 billion in 1990, a 2012 UNCTAD survey projected India as the second most important FDI destination (after China) for transnational corporations during 2010–2012. As per the data, the sectors that attracted higher inflows were services, telecommunication, construction activities and computer software and hardware. Mauritius, Singapore, US and UK were among the leading sources of FDI. Based on UNCTAD data FDI flows were \$10.4 billion, a drop of 43% from the first half of the last year. The reliance on FDI is raising heavily due to its al round contributions to the growth of the economy. FDI to developing countries since 1990's is the leading source of external financing. The rise in FDI volume is accompanied by marked change in its composition.

CONCLUSION

Main and important impact of Foreign Direct Investment is sustained economic growth and development through creation of jobs, expansion of existing manufacturing industries, short and long term project in the field of healthcare, education, research and development etc. with this regards Government should design the FDI policy such a way where FDI inflow can be utilized as means of enhancing domestic production, savings and exports through the equitable distribution among states



by providing much freedom to states, so that they can attract FDI inflows at their own level. FDI can help to raise the output, productivity and export at the sectoral level of the Indian economy. However, it can be observed that the result of sectoral level output, productivity and export is minimal due to the low flow of FDI into India both at the macro level as well as at the sectoral level. Therefore for further opening up of the Indian economy, it is advisable to open up the export oriented sectors and higher growth of the economy could be achieved through the growth of these sectors.

REFERENCES

- [1] Ministry of Finance, Report of the economic survey, Government of India, New Delhi (2003-04)
- [2] Weisskopf T.E., The impact of foreign capital inflow on domestic savings in underdeveloped countries, *Journal of International Economics*, 2, 25-38 (1972)
- [3] Sahoo D. Mathiyazhagan M.K. and Parida P., Is Foreign Direct Investment an engine of growth? Evidence from the Chinese economy, *Savings and Development*, 4, 419-439 (2002)
- [4] Nayak D.N., Canadian Foreign Direct Investment in India: Some observations, *Political Economy Journal of India*, 8, 51-56 (1999)
- [5] Srivastava S., What is the true level of FDI
- [6] PratiyogitaDarpan (2012) Indian Economy.
- [7] Chronicle 2013 Value edition -2: Indian Economy, p. 59 to 61.



STUDY OF PEST ON MAIZE AND MARK OF IDENTIFICATION OF ARMY WORM AROUND SRIRAMPUR

TAHSIL REGION

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ABSTRACT

Army worm is economical pest on Maize; this study is done to analyse the biodiversity and carry out the identification of army worm. The Sample were collected by using Visual search, Net method and Hand-picking method and light trap method. The Study was conducted lakh region from the present study it was revealed that maize filed and forms of maize in local residential area. A study was conducted in Ahmednagar, India, with the goal of identifying the new invasive pest infesting maize at the time of the study. he fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a destructive pest that causes serious damage worldwide, particularly to maize cultivars.

KEYWORDS

Army worm, Visual search, *spodoptera frugiperda*, *Mythimna unipuncta*, *Spodoptera frugiperda*

INTRODUCTION

Hybrid corn fields located in Lakh village in Ahmednagar district of Maharashtra showed distinct damage symptoms. It was evident from the damage to the plants that they were showing characteristic symptoms of shot holes and ragged leaves. Developing cobs were also damaged with typical symptoms of fall armyworm damage. As the larvae observed were different from known lepidopteran species. The larvae found to be fully developed were confined to whorls and were found to be feeding in the gaps between the leaves, showing distinctive symptoms of raggedness. Larvae observed were different from known lepidopteran species infesting maize crop. In laboratory larvae were reared till adult stage, adults were allowed to mate and in the next generation all the stages were examined in detail. The different pest is also seen in maize like the stem borers and storage insect pests (*Sitophilus weevils* and *Sitotroga cereallela*) of maize. The fall armyworm is active at a different time of year from the true armyworm, another species in the order Lepidoptera and family Noctuidae, but of the genus *Mythimna*. Outbreaks of the true armyworm usually occur during the early part of the summer; the fall armyworm does most damage in the late summer in the southern part of the United States, and early fall in the northern regions This pest completes its life cycle on corn in 30 days (in warm summers). It may take 60-90 days in colder temperatures. Effective control of fall armyworms requires

the use of an integrated approach with a variety of tools such as cultural control, agricultural management, breeding resistance, natural enemies and environmentally benign pesticides. During the study, an adult male and a female were confined in a cylindrical oviposition cage made of mylar plastic that was shaped like a cone. Ten pairs were prepared in this manner. The cylinder was covered with wax paper on the top and bottom as well as on the sides so that oviposition could occur. They were provided with cotton soaked in 10% sugar solution as food source. A total of 60 neonates (freshly-hatched larvae) were harvested from the progeny of these parental stocks and transferred individually to fresh corn leaves, where they were reared in plastic plates until they pupated. The morphological features of the different instars were observed and recorded along with 1) Incubation period; 2) Development period from the first instar to the sixth instar 3) Pupal period and 4) Post-developmental periods: a. Pre-oviposition period (the time adult female emerged to the time the first mass of eggs is laid b. Oviposition period (egg laying period) c. Post oviposition period (the time female stopped laying eggs till death; d) Longevity of male and female adults - the time from adult emergence till their death; e) Fecundity - number of egg-masses and number of eggs per egg-mass laid in the lifetime of adult females; f) Hatchability of eggs - taken by counting the number of neonates that hatched from all egg-masses laid by a female in her lifetime. All stages of the pest can be detected visually, with a hand lens for early stages, and specimens can be collected by hand or a sweep net (adults). In the field and in production-, storage-, handling- and other facilities adults can also be detected with the aid of light traps and pheromone baited traps. Pheromone baited traps allow adult males to be caught and light traps catch both female and male adults. Adults can sometimes be found and collected by hand; all periods of observations were taken in days. They Army worm complete their life cycle in egg larvae pupa and adult. The life cycle is completed in about 30 days during the summer, but 60 days in the spring and autumn, and 80 to 90 days during the winter. The number of generations occurring in an area varies with the appearance of the dispersing adults. The ability to diapause is not present in this species.

1. Egg

A mass of eggs is deposited on the underside of a leaf in layers, Eggs are white covered with greyish colored scales.

2. Larva

There were light greenish larvae of the first instar with black heads and bodies. It is noted that the mature larvae were marked with a whitish inverted 'Y'. Several black spots are visible on the head. The four black spots on the eighth abdominal section were arranged in a square and at the 1 to seventh and 9th segment arranged in a trapezoidal sample.



3. Pupa

A Reddish brown in colour with cremaster

4. Adult

Grey-brown male adult. The front wings are shades of Gray and brown oval or oblique orbital spots near triangular white spot stipe of the front wing. For adult women Forewings lack uniform grey-brown distinct markings spotted colouring. we also received culture Rajkot and Amreli districts of Gujarat and morphologically Larval characteristics were similar to those described above Characteristics of *S. frugiperda*. India is a country where we find the richness in both flora and fauna Army worm are the popular pest on maize. The diversity among various species and its habitat shows the co-related between them. Few pest of maize shows much diverse habitat including litter depth, leaves of maize small shoot of maize and stem of maize such diversity patten can provide much more important information to identify and justify the ecosystem and its damage on maize crop. The include in Nocturdy family, generally lives on underside of leaves of plant.

MATERIAL AND METHOD

The study is based on material collected from local residential area from region and farm of lakh region in Srirampur region and its surrounding area by visual method and net capture method and moth of spodoptera species by light trap and insect collection trap

Study site	Geographical location	Habitat type
Srirampur tehsil region	19° 62'N 72° 66'E	Tropical wet climate with average temperature ranging between 37°C 42°C

The study was conducted in march 2025 during morning hours between 7am to 10am. Three methods were used for collection viz visual search Net method and hand-picking method. The easiest way to capture and collect pest om maize

Visual search method

In this method we walk through the maize farm and habitat and visually for Army worm on a filed through this method we can check under crop of maize leaves and shoot of maize crop and stem of maize crop also found some pest around maize crop.

1. Net method

An Arial net with long tapered tail is made of a light weight mesh. It is used to collect organism on the top of flower of maize and other vegetation part of the plant.

2. Hand picking method

Hand picking is a good choice a soft pain thrash or cotton swab could be used to gently knock the specium into a collection vial or it could into a collection vial or it colued be carefully picked off by hand

3. Light trap method

It comprises of a light source as an attractant and a funnel to direct lured insects into the insect collecting chamber. Funnel supports three baffles which are joined at the top. A hook has been provided at the top portion to install the light trap in the crop fields.

OBSERVATION

In the study of pest on maize Army worm having four species of army worm and one species of bugs one species of Grasshopper point that were considered for the study and identification of Army worm are as follows

A] Habitat

Fall armyworms are not known to diapause and so cannot survive the winters in temperate climes. The fall armyworm is native to the tropical regions of the western hemisphere from the United States to Argentina. It normally overwinters successfully in the United States only in southern Florida and southern Texas. The fall armyworm is a strong flier, and disperses long distances annually during the summer months. It is recorded from virtually all states east of the Rocky Mountains; however, as a regular and serious pest, its range tends to be mostly the southeaster states. Found in temperate zones and tropical areas, *Spodoptera exigua* occupies a habitat that ranges from crop and grass fields to temperate and tropical forests.

B] Behaviour

1. *Spodoptera frugiperda*

The head has a reticulate pattern and is variable in colour, from yellowish to very dark brown; the thoracic shield is the same colour as the head. It has no dark dorsal or subdorsal segmental patches. It has conspicuous dark pinacula² and the texture of the skin is granulose. The dorsal pinnacular on abdominal segment 8 and especially 9 are large and have a typical arrangement: on segment 8 they are arranged in a square, on segment 9 in a trapezoid. The anal plate has dark elongate patches.

2. *Mythimna*

The wingspan is 36–44 mm. Forewing pale greyish rufous, speckled with dark; lines indistinct, dark grey; the outer regularly lunulate-dentate, the teeth marked by black dashes on veins; reniform stigma obscure, ending in a cloudy pale spot at lower end of cell; hindwing greyish ochreous; ventral tufts black. The species varies in coloration:

3. *Spodoptera exigua*

A beet armyworm larva does not tolerate cold very well, but it can overwinter and pupate by digging into the soil to form a chamber that is held together by thin strands of silk. It prefers to form a pupation chamber in the soil directly beneath the canopy of its host plants. In its larval stage, *Spodoptera exigua* is gregarious, particularly in its feeding habits as a first and second instar; large numbers of individuals have been observed feeding together

4. *Spodoptera litura*

An important characteristic of *S. littoralis* and *S. litura* are small yellow to white dots at the base of the black patches on the second and third thoracic segment these dots distinguish both *Spodoptera* species from the non-quarantine African and Asian *Spodoptera* species.¹ In the later instars the black patches on the segments may fade completely, even those on the thorax and abdominal segments 1 and 8. The yellow to white dots on the second and third thoracic segment however remain visible, although sometimes faintly. Last instar larvae are 40–45 mm long.

CONCLUSION

From the study we can conclude that variety of pest and Army worm found in the study area from all three habitat When compared it was found that Army worm four species are very common in maize and can cause damage to maize crop there the study proves the variety of pest are present on maize in Srirampur tehsil region. Fall armyworm incidence was noticed Hybrid corn field located in Lakh village, Ahmednagar District, Maharashtra on 20th march 2025

REFERENCES

- [1] Banerjee, S., & Satpathy, S. (2023). Field identification, host range and damage symptoms of *Spodoptera frugiperda* in Indian conditions. *Indian Journal of Agricultural Sciences*, 93(2), 325–330.
- [2] Bhattacharjee, S., & Mahapatra, R. (2021). Fall armyworm in India: Status and strategy for control. *Current Science*, 121(5), 629–633.
- [3] Chormule, A. J., Deshmukh, R. A., & Patil, S. S. (2023). Comparative effectiveness of insecticides against *Spodoptera frugiperda* on maize. *Pest Management in Horticultural Ecosystems*, 29(1), 45–50.
- [4] Chakravarthy, A. K. (2022). Identification and monitoring of invasive insect pests in Indian agriculture. *Indian Journal of Entomology*, 84(2), 211–218.
- [5] Deshmukh, N., Thube, S. H., & Gaikwad, A. P. (2022). Seasonal incidence and damage assessment of *Spodoptera frugiperda* on maize in Ahmednagar district. *Journal of Pharmacognosy and Phytochemistry*, 11(1), 985–988.
- [6] FAO & ICAR. (2021). Fall Armyworm Monitoring and Early Warning System (FAMEWS) in India. FAO Report.



- [7] Global Advanced Research Journal of Environmental Science and Toxicology. (2012). 1(8), 203–210. [ISSN: 2315-5140].
- [8] ICAR-NBAIR. (2021). Identification and management of fall armyworm in maize. Technical Bulletin. National Bureau of Agricultural Insect Resources, Bengaluru.
- [9] Jadhav, J., & Pawar, S. (2022). Survey of maize pests in Srirampur Tahsil region, Maharashtra. *International Journal of Agricultural Sciences*, 14(2), 159–163.
- [10] Kadam, J. R., Bhange, M. A., & Salunke, A. V. (2021). Occurrence and management of fall armyworm, *Spodoptera frugiperda* in Maharashtra. *Journal of Entomology and Zoology Studies*, 9(4), 230–234.
- [11] Kalleshwaraswamy, C. M., Asokan, R., & Sharanabasappa. (2021). Current status of fall armyworm in India and integrated pest management strategies. *Pestology*, 45(5), 25–29.
- [12] Mane, R. H., & Jadhav, S. S. (2022). Survey and seasonal abundance of maize pests in western Maharashtra. *Indian Journal of Applied Entomology*, 36(1), 85–89.
- [13] Meena, R. S., & Mehta, S. K. (2021). Pest complex of maize and their natural enemies in central India. *Journal of Entomological Research*, 45(3), 377–381.
- [14] Navasero, M. M., & Navasero, M. V. (2020). Life cycle, morphometry and natural enemies of fall armyworm, *Spodoptera frugiperda* on *Zea mays* in the Philippines. *Journal of the International Society for Southeast Asian Agricultural Sciences*, 26(2), 17–29.
- [15] Ortega, A. (1987). *Insect pests of maize: A guide for field identification*. CIMMYT.
- [16] Rauber, A. (1983). Black Widow Spider Bites. *Clinical Toxicology*, 21(4–5), 473–485. <https://doi.org/10.3109/15563658308990057>
- [17] Reddy, G. V. P., & Tangtrakulwanich, K. (2021). Invasion of fall armyworm in Asia and its pest status in India. *Outlooks on Pest Management*, 32(1), 17–22. <https://doi.org/10.1111/epp.12258>
- [18] Sharanabasappa, Kalleshwaraswamy, C. M., Maruthi, M. S., & Pavithra, H. B. (2018). Biology of invasive fall armyworm, *Spodoptera frugiperda* on maize. *Indian Journal of Entomology*, 80(3), 540–543.
- [19] Sisodiya, D. B., Raghunandan, B. L., Bhatt, N. A., Verma, H. S., Shewale, C. P., Timbadiya, B. G., & Borad, P. K. (2018). The fall armyworm, *Spodoptera frugiperda*: First report of new invasive pest in maize fields of Gujarat, India. *Journal of Entomology and Zoology Studies*, 6(5), 2089–2091.
- [20] Tamil Nadu Agricultural University (TNAU). (2021). *Pest identification guide for field crops: Maize pests*. TNAU Publication.



[21] [American Museum of Natural History]. (n.d.). Collecting arthropod specimens. Retrieved from <https://www.amnh.org/learn-teach/curriculum-collections/biodiversity-counts/arthropod-identification/collecting-arthropod-specimens>

[22] Biosciences Biotechnology Research Communications, 12(4), 855–864 (2019).



DEVELOPMENT AND OPTIMIZATION OF BUCCAL PATCH LOADED WITH MARRUBIIN FOR ANTI-DIABETIC EFFECT

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ABSTRACT

The primary objective of this research was to create an effective mucoadhesive buccal patch with optimized formulation properties that would promote drug absorption through the buccal pathway. Methods: HPMC K15M and Neem Gum were used to develop mucoadhesive buccal patches using a 3² full factorial design. Physicochemical properties, drug-exipient compatibility (FTIR, DSC), ex-vivo permeation, and stability studies were conducted to characterize the formulations. Design Expert software was used to perform the optimization. Results: FTIR and DSC studies showed the drug's and excipients' compatibility in the formulation. The tensile strength of the optimized formulation (MF9) was found to be 18.73±0.36 N/mm², mucoadhesive strength of 9.8±0.39 N, and ex-vivo adhesion time of 9.6±0.58 hr, indicating superior characteristics as compared to existing methods. Maximum drug permeation of 91.07%, flux of 13.45 µg/cm²/h, and permeability coefficient of 3.74 cm/h were found in ex-vivo permeation studies over eight h. The drug release was first-order kinetics (R²=0.9832). The accelerated stability studies at 40±2°C and 75±5% RH showed that the formulation contained minimal variation in critical parameters and was stable for at least 6 months. Conclusion: The developed mucoadhesive buccal patch was revealed to improve marrubiin delivery through a combination of the best polymers and showed excellent stability. It is a potential non-invasive approach compared to the conventional oral administration.

KEYWORDS

Marrubiin, Mucoadhesive buccal patch, HPMC K15M, Neem Gum, Ex-vivo permeation, Factorial design

INTRODUCTION

Diabetes mellitus is one of the significant health challenges of the 21st century, affecting about 537 million adults in the world in 2021 and is projected to reach 783 million adults in 2045. Chronic hyperglycemia with defects in insulin secretion, insulin action, or both defines this metabolic disorder. Type 2 diabetes mellitus (T2DM) is the overwhelming majority (90–95%) of all diabetic cases, and its development is linked to lifestyle factors, obesity, as well as genetic predisposition. Patient quality of life is severely affected by the complications of the disease, which include cardiovascular diseases, nephropathy, retinopathy, and neuropathy. Global health expenditure in diabetes management is huge



at USD 966 billion annually. While currently effective, the use of oral hypoglycemic agents and insulin therapy has challenges, including gastrointestinal side effects, weight gain, and the requirement for frequent administration, clearly demonstrating a need for alternative treatment strategies.

The natural compound, Marrubiin, isolated from *Marrubium vulgare* L. (white horehound), has shown promise as a natural agent for diabetes management. Recent studies have established its tremendous anti-diabetic properties mediated by multiple mechanisms of action. Marrubiin first enhances glucose uptake in skeletal muscle cells by activating the AMPK (AMPK-activated protein kinase) pathway, and second augments GLUT4 translocation to the plasma membrane directly. Moreover, the insulinotropic effects consist of induction of insulin secretion by pancreatic β cells and attenuation of glucotoxicity-induced apoptosis. It also exhibits excellent antioxidant and anti-inflammatory properties that are indispensable to preventing diabetes-associated complications. Marrubiin can inhibit intestinal α -glucosidase and α -amylase enzymes and slow carbohydrate absorption, but marrubiin is also glucose lowering in humans. Due to these multiple therapeutic actions, their natural origin, and their favorable safety profile, marrubiin is a promising candidate for diabetes treatment.

In recent years, the use of buccal drug delivery as an alternative route to administer therapeutics, especially for drugs with poor oral bioavailability and drugs that require sustained release, has received significant attention. Several advantages of buccal mucosa as a drug delivery site are its rich vascularity, large surface area and high permeability for many drugs. In particular, using buccal patches provides superior advantages over conventional oral routes of administration and other delivery systems. They are metabolized in the liver once they bypass first-pass hepatic metabolism, so they have greater bioavailability and lower dose requirements. The controlled release nature of the buccal patch guarantees a constant drug plasma concentration and, thus, a decreased frequency of administration with improved patient compliance. Buccal patches are also easily applied and removed if adverse effects occur and are a patient-friendly dosage form. These patches are pronounced mucoadhesive, permitting long contact time with the buccal mucosa, thereby enhancing drug absorption and therapeutic efficacy.

The present research aims to develop and optimize a novel buccal patch formulation with marrubiin to increase the anti-diabetic effect. Formulation development using various mucoadhesive polymers and penetration enhancers and physicochemical characterization of the developed patches, ex-vivo permeation studies using goat buccal mucosa, and stability studies to determine the stability of the optimized formulation. The study will also determine the mucoadhesive strength and residence time of the patches are also studied in the study.

MATERIALS AND METHODS

Materials

Marrubiin was purchased from supplier Sciquaint Innovations OPC Private Limited, Pune, India. Research Lab Fine Chem Industries, Mumbai, supplied Hydroxypropyl methylcellulose K15M, and Neem gum was purchased from Indianjudibhuti, Delhi. PEG 400 was purchased from Merck Limited, Mumbai. All the chemicals and reagents for this study met analytical grade requirements.

Methods

Calibration Curve of Marrubiin

Marrubiin solutions were prepared in phosphate buffer pH 6.8. Marrubiin (10 mg) was accurately weighed and dissolved in 100 ml phosphate buffer to give a stock solution of Marrubiin (100 µg/ml). Then from the stock solution, different volumes (0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml) were taken and diluted to the mark with phosphate buffer to make different solutions of different concentrations, i.e. (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, A UV visible spectrophotometer was used to measure the absorbance of these solutions at 254nm wavelength and a calibration curve was constructed.

Determination of Solubility

Different solvents such as water, ethanol, methanol, phosphate buffer (pH 6.8), and DMSO were used to determine the solubility of Marrubiin. In separate flasks, 50 ml of each solvent was supplemented with excess Marrubiin and was incubated in an orbital water bath at 50 rpm and a controlled temperature of $37 \pm 0.5^\circ\text{C}$ for 48 hrs. After filtering, diluting to appropriate concentrations, and analyzing all solutions with a UV-visible spectrophotometer, the Marrubiin sample was identified.

DSC analysis

Different solvents such as water, ethanol, methanol, phosphate buffer (pH 6.8), and DMSO were used to determine the solubility of Marrubiin. In separate flasks, 50 ml of each solvent was supplemented with excess Marrubiin and was incubated in an orbital water bath at 50 rpm and a controlled temperature of $37 \pm 0.5^\circ\text{C}$ for 48 hrs. After filtering, diluting to appropriate concentrations, and analyzing all solutions with a UV-visible spectrophotometer, the Marrubiin sample was identified.

Fourier Transform Infrared (FTIR) Spectroscopy

The pure drug was subjected to FTIR spectroscopy, and a Fourier Transform Infrared (FTIR) - 8400S spectrophotometer procured from Shimadzu, Japan, was used. The sample was finely ground with potassium bromide (KBr) powder in a 1:100 weight ratio using a mortar and pestle. The mixture was then compressed into a pellet using a 15-ton hydraulic press for 1 minute. The formed pellet was gradually relieved to recover. The sample holder was loaded with the pellet, and spectral scanning was

done in a region of 4000–400 cm⁻¹ with 4 cm⁻¹ steps and a 2 mm/sec scan rate. The spectra obtained were utilized to identify the functional groups in the compound studied and to evaluate the structural changes of the drug under study.

Selection of dose of Marrubiin

A dose equivalent to previous studies of Marrubiin was used; Kumar et al. have previously shown an effective dose of 3 mg/kg in diabetic rat models. This dose was converted to the human equivalent dose using the standard interspecies dose conversion formula: Human equivalent dose (mg/kg) = Dose in rats (mg/kg) / Rat Km / Human Km. The human equivalent dose was calculated to equal 0.49 mg/kg based on the Km values for rats (6.00) and humans (37.00). Moving to the converted dose for a human weighing about 70 kg, this value is 34.05 mg of the total converted dose. This way, Marrubiin's dose would be selected according to the appropriate and scientifically validated approach.

Experimental Design

A 3² full factorial design was employed to develop a buccal patch formulation. The low (-1), medium (0) and high (+1) concentrations for independent variables were of HPMC K15M (A) and Neem Gum (B). Tensile Strength (Y1), Mucoadhesive strength (N) (Y2), and Ex vivo adhesion time (hr) (Y3) were dependent variables studied. Based on a factorial design, nine different formulations were generated.

Table 1: 32 Factorial Design showing independent factors and Levels.

Independent Variables				
Label	Factors	Level (mg)		
		Low (-)	Medium	High (+)
A	HPMC K15M	150	200	250
B	Neem Gum	50	100	150
Dependant Variables				
Y1	Tensile Strength (N/mm ²)			
Y2	Mucoadhesive strength (N)			
Y3	Ex vivo adhesion time (hr)			

Table 2: Factors, levels and responses taken in 32 full factorial designs for buccal patch.

F. Code	(X1)	(X2)
MF1	-1	-1
MF2	0	-1
MF3	+1	-1

MF4	-1	0
MF5	0	0
MF6	+1	0
MF7	-1	+1
MF8	0	+1
MF9	+1	+1

"-" indicates lower concentration, and "+" indicates higher concentration.

Table 3: Preparation of buccal patch batches using 32 factorial designs

Ingredients	MF1	MF2	MF3	MF4	MF5	MF6	MF7	MF8	MF9
Marrubiin (mg)	34.05	34.05	34.05	34.05	34.05	34.05	34.05	34.05	34.05
HPMC K15M (mg)	150	200	250	150	200	250	150	200	250
Neem Gum (mg)	50	50	50	100	100	100	150	150	150
PEG 400 (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Distilled Water (mL)	40	40	40	40	40	40	40	40	40

Preparation of Buccal Patch Batches

A 3² factorial design was used to prepare the buccal patches, which were prepared using the solvent casting method. 20 mL of distilled water and their corresponding predetermined amount of HPMC K15M were dissolved under constant stirring to get a uniform polymeric solution. The polymeric solution was then separately dispersed with 10 mL of distilled water and neem gum. The remaining 10 mL of water was then used to dissolve the marrubiin and added to the polymer blend. A plasticizer such as PEG 400 (0.5 mL) was used to mix the mixture to make it flexible. A clean, leveled glass petri dish was poured with a resulting homogeneous mixture and spread uniformly to form a thin patch. Solvent evaporation was ensured by drying the patches at room temperature for 24 hours. The patches were air-dried, and the outer layer was carefully removed and stored in aluminum foil for further analysis. The process was repeated for each batch (MF1–MF9) as per the composition in Table 3.

Characterization of Buccal batch

Surface pH Determination

A combined glass electrode (Digital pH meter, Labtronic, India) measures the surface pH. Buccal patches can swell for 2 hr on 0.2 g agar (10 mL warmed simulated saliva fluid, pH 6.8 at 50–70°C). The swollen patch surface is brought in contact with the glass electrode, and the equilibrate is

allowed for 1 minute before taking the reading. Triplicate measurement is done, and the mean value with standard deviation is calculated.

Thickness Uniformity

The thickness is measured with a digital vernier caliper (Hetkrishi, India) in three patch locations (to maximize the accuracy). Each formulation batch is evaluated in three patches. A mean value and standard deviation are calculated to assess thickness uniformity in this batch and within single patches.

Folding Endurance

A sharp blade cuts three patches of each formulation patch of size 2×2 cm. A strip of the patch is repeatedly folded at the same place until it ruptures to determine folding endurance. The folding endurance value is the number of times the patch can be folded at the same place without breaking—the mean value from three measurements.

Weight Variations

Three patches of each formulation are individually weighed on a digital analytical balance (ATX 224, Shimadzu, Japan) for evaluation of patch weight. The batch uniformity is assessed by calculating each formulation's mean weight and standard deviation.

Tensile Strength

The tensile strength was measured on a pulley system. The pulley system pulls the polymeric film, and weights are added to the pan, gradually increasing the pulling force until the film breaks. The elongation of the film is noted with a magnifying glass. The formula calculates it:

$$S = (m \times g)/(b \times t) \quad (1)$$

where m = mass in grams, g = acceleration due to gravity, b = breadth in cm, t = thickness.

Swelling Index

The polymeric films of 1 cm diameter are weighed accurately and immersed in 50 ml water. Films are carefully removed at 5, 10, 30, and 60 min intervals, blotted with filter paper to remove surface water, and then weighed accurately. The percent swelling is calculated using the formula:

$$\% \text{ Swelling index} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Final weight}} \times 100 \quad (2)$$

Ex vivo adhesion Time

Ex-vivo Adhesion time was determined using a modified USP disintegration apparatus (VTD-AV, Veego, India) holding 800 ml of simulated saliva fluid (pH 6.8) at 37°C. A piece of fresh goat buccal mucosa (3 cm length × 1 cm width) is glued to a glass plate and is vertically attached to the apparatus. 2 ml of simulated saliva is hydrated into the mucoadhesive patch, and it is contacted with the mucosa.

Assembly moves up/down in solution. Recorded are the amounts of time needed for complete detachment.

Mucoadhesive strength

The modified pan balance method is used to determine the mucoadhesive strength. Goat buccal mucosa is obtained fresh within 2 hours of slaughter and stored in phosphate buffer pH 6.8 at 4°C. The mucosa is secured to two glass slides by thread, one fixed under the balance pan's underside, the other against a wooden board. Bonding is established by placing the test patch between the mucosal surfaces and applying 50g preload force for 1 minute. The other pan is weighed (dummy granules) until complete detachment occurs. The use of the formula determines the mucoadhesive strength (dynes/cm²):

$$\text{Force} = (m \times g)/A \times 100 \quad (3)$$

Where m is the weight of water required for detachment, g is the acceleration due to gravity (981 cm/s²), and A is the surface area of the patch. The test is performed in triplicate, maintaining temperature at 37±1°C.

Drug Content Uniformity

Drug content is quantified without any backing membrane. Following initial dispersion in dilute HCl, patches are dissolved in pH 6.8 simulated saliva. The solution is filtered, appropriately diluted and analyzed by a UV spectrophotometer (UV1700, Shimadzu, Japan) at 254nm against a calibration curve. Testing is done in triplicate.

Ex Vivo drug permeation study

Franz diffusion cell assembly (PermeGear Inc., PA) was used for the ex vivo drug permeation study. Buccal mucosa from fresh goats obtained from the slaughterhouse is mounted between donor and recipient compartments. The drug (10 mg in a buccal film of diameter 1 cm) is placed on the mucosal surface in the donor compartment with pH 6.8 phosphate buffer. Under magnetic stirring at 100 rpm, the receptor compartment is maintained at 37±1°C and contains pH 6.8 phosphate buffer. The samples (0.5 mL) are withdrawn at predetermined intervals up to 8 hours and renewed with fresh buffer. After suitable dilution, the samples are analyzed spectrophotometric at 254nm. The difference in the amount of drug permeated per unit surface area vs. time is plotted. The slope of the graph for the linear portion is the steady-state flux.

Stability studies

The optimized formulation underwent stability tests per the International Conference on Harmonization (ICH) guidelines. It was wrapped with butter paper and aluminum foil for a 2x 2 cm² buccal patch. During the 3-month accelerated test, at a temperature of 40±2°C and humidity of 75±5%,

the sample was exposed to room conditions of $25\pm 2^{\circ}\text{C}$ and $60\pm 5\%$ humidity. The mucoadhesive properties, mechanical characteristics, drug content, and drug release rate for buccal patches stored for 1, 3 and 6 months have been determined.

RESULTS

Results of Calibration curve of Marrubiin

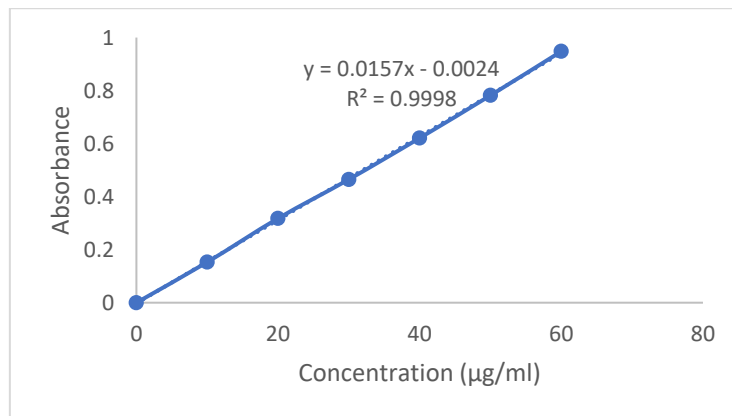


Figure 1: Calibration curve of marrubiin in Phosphate buffer pH 6.8

Solubility analysis

Table 4: Results of solubility analysis of marrubiin

Sr. No.	Solvent	Solubility (mg/ml)	Results
1	Water	0.52±0.03	Practically insoluble
2	Ethanol	2.06±0.42	Slightly soluble
3	Methanol	5.24±1.14	Slightly soluble
4	Phosphate Buffer pH 6.8	11.69±1.09	Sparingly soluble
5	DMSO	51.83±2.32	Soluble

Values are expressed in mean±SD (n=3)

Results of FTIR studies

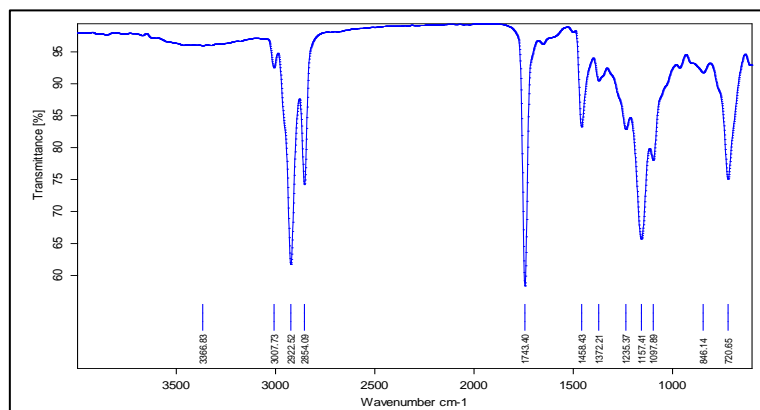


Figure 2: FTIR spectra of Pure Drug (Marrubiin)

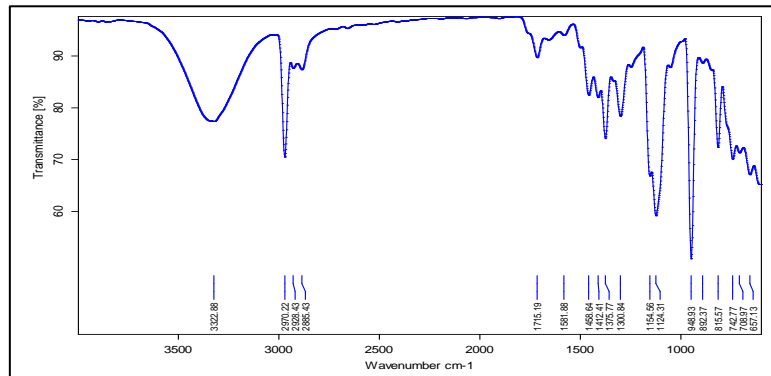


Figure 3: FTIR spectra of Physical mixture of formulation (Drug + Excipients)

Results of DSC Analysis

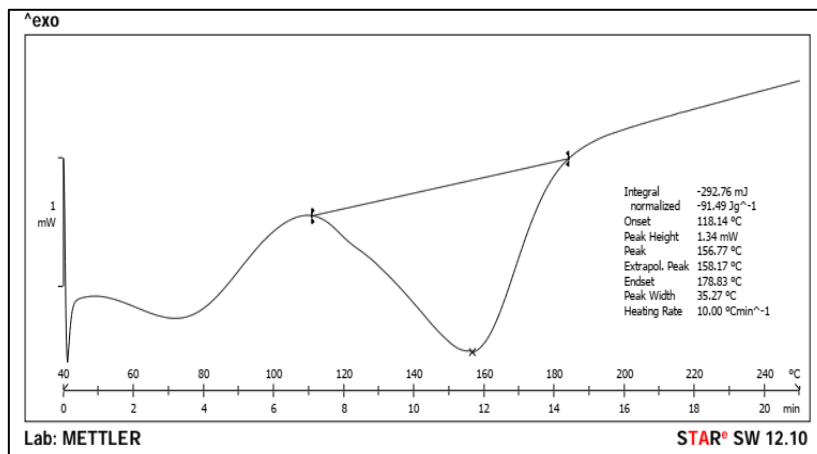


Figure 4: DSC Spectra of Pure drug (Marrubiin)

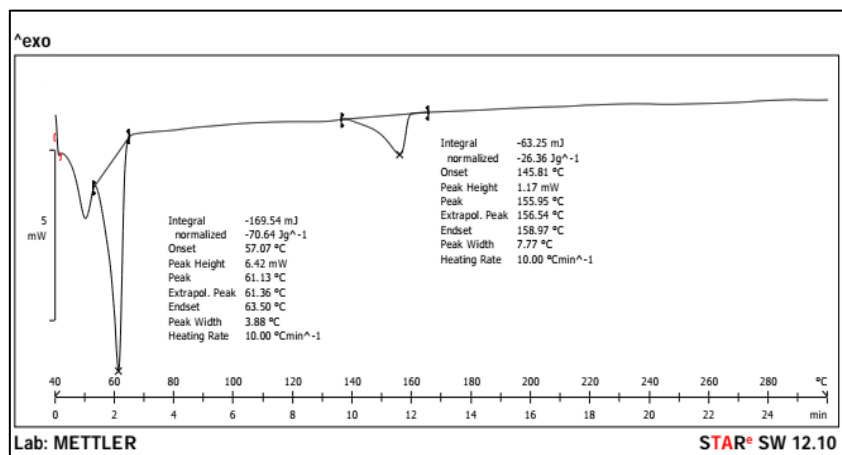


Figure 5: DSC Spectra of Physical mixture of formulation (Drug + Excipients)

Results of characterization of buccal patch

Table 5: Evaluation of Weight variation, Thickness, Folding Endurance, % Moisture Uptake and % moisture Loss of mucoadhesive buccal patch (MF1-MF9)

Batch code	Weight variation (MG)	Thickness (mm)	Folding Endurance (no. of folds)	% Moisture Uptake	% moisture Loss
MF1	212.2±2.4	0.18±0.02	378±0.425	0.76±0.07	1.91±0.32
MF2	245.6±4.7	0.11±0.03	312±0.231	2.98±0.32	0.52±0.07
MF3	265.3±6.7	0.12±0.02	302±0.752	1.21±0.21	2.27±0.23
MF4	243.1±3.9	0.09±0.04	298±0.862	1.43±0.19	1.31±0.29
MF5	308.7±2.9	0.13±0.01	319±0.193	0.77±0.04	0.89±0.04
MF6	262.9±4.2	0.16±0.01	361±0.528	2.09±0.73	1.72±0.31
MF7	258.0±8.7	0.08±0.01	274±0.933	1.37±0.23	1.24±0.38
MF8	291.3±9.4	0.13±0.04	284±0.641	0.83±0.07	0.34±0.05
MF9	232.7±8.3	0.14±0.02	314±0.832	1.18±0.013	2.62±0.53

The data are expressed in mean±SD, (n=3)

Table 6: Results of tensile strength, Mucoadhesive strength, Ex-vivo adhesion time and mucoadhesive buccal film drug content (MF1-MF9).

Batch code	Tensile strength (N/mm ²)	Mucoadhesive strength (N)	Ex-vivo adhesion time (hr)	Drug content (%)
MF1	6.23±0.49	3.1±0.43	2.8±0.76	89.43±0.57
MF2	11.24±0.56	4.3±0.31	3.3±0.43	82.19±0.58
MF3	16.43±0.83	4.7±0.06	3.9±0.21	74.69±0.59
MF4	6.62±0.47	5.3±0.78	4.7±0.81	89.07±0.60
MF5	12.29±0.28	6.5±0.42	5.6±0.32	96.35±0.52
MF6	17.67±0.32	7.1±0.93	6.6±0.47	92.76±0.13
MF7	7.54±0.78	8.3±0.69	6.9±0.41	88.83±0.83
MF8	13.62±0.71	9.3±0.57	8.4±0.83	91.45±0.34
MF9	18.73±0.36	9.8±0.39	9.6±0.58	95.72±0.19

Values are expressed in mean±SD (n=3)

Optimization of marrubiin buccal film

Effect of Independent Variable on Tensile Strength (Y1)

Table 7: ANOVA for the Linear model Tensile Strength (Y1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	181.37	2	90.69	1121.16	< 0.0001	significant
A-HPMC K15M	175.39	1	175.39	2168.39	< 0.0001	

B-Neem Gum	5.98	1	5.98	73.93	0.0001	
Residual	0.4853	6	0.0809			
Cor Total	181.86	8				

The regression equation obtained for Tensile Strength (Y1) is as follows:

$$\text{Tensile Strength (Y1)} = +12.26 + 5.41 * A + 0.9983 * B \tag{4}$$

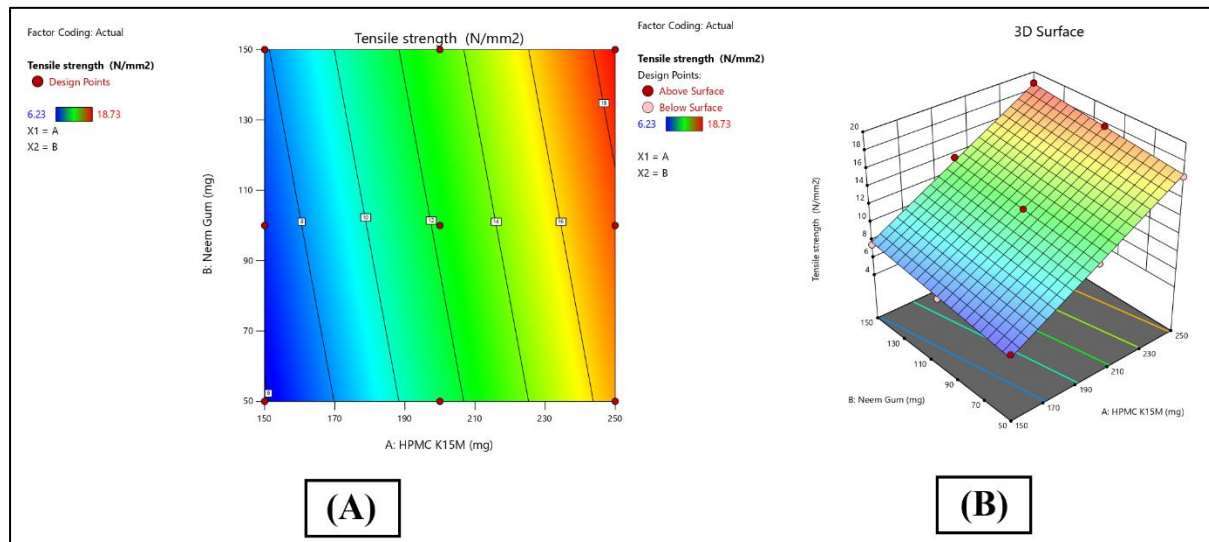


Figure 6: Contour plot (A) and 3D surface plate (B) for the effect of HPMC K4M and Moringa gum on Tensile Strength (Y1) of Buccal film

Effect of Independent variable on Mucoadhesive strength (Y2)

Table 7: ANOVA for the quadratic model Mucoadhesive strength (Y2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	43.38	5	8.68	909.72	< 0.0001	significant
A-HPMC K15M	4.00	1	4.00	419.59	0.0003	
B-Neem Gum	39.02	1	39.02	4090.89	< 0.0001	
AB	0.0025	1	0.0025	0.2621	0.6440	
A²	0.2006	1	0.2006	21.03	0.0195	
B²	0.1606	1	0.1606	16.83	0.0262	
Residual	0.0286	3	0.0095			
Cor Total	43.41	8				

The regression equation obtained for Mucoadhesive strength is as follows:

$$\text{Mucoadhesive strength} = +6.51 + 0.8167 * A + 2.55 * B - 0.0250 * AB - 0.3167 * A^2 + 0.2833 * B^2 \tag{5}$$

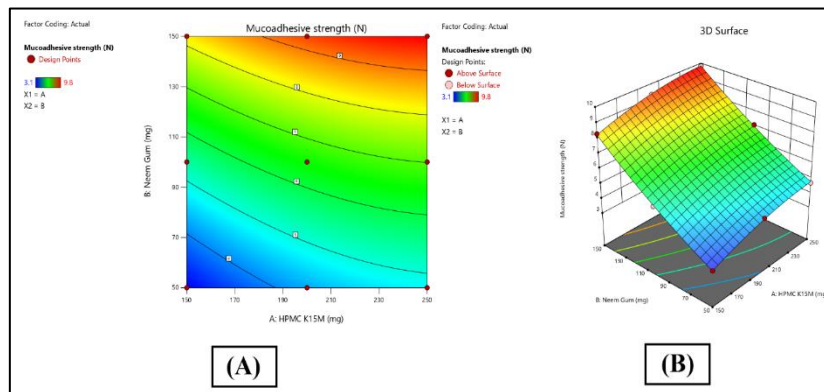


Figure 7: Contour plot (A) and 3D surface plate (B) for the effect of HPMC K4M and Moringa gum on Mucoadhesive strength (Y2) of Buccal film

Effect of Independent variable on ex vivo adhesion time (Y3)

Table 9: ANOVA for the Linear model ex vivo adhesion time (Y3)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	43.06	3	14.35	838.77	< 0.0001	significant
A-HPMC K15M	5.41	1	5.41	316.46	< 0.0001	
B-Neem Gum	37.00	1	37.00	2162.44	< 0.0001	
AB	0.6400	1	0.6400	37.40	0.0017	
Residual	0.0856	5	0.0171			
Cor Total	43.14	8				

The regression equation obtained for ex vivo adhesion time is as follows:

$$\text{Ex vivo adhesion time (Y3)} = +5.76 + 0.9500 * A + 2.48 * B + 0.4000 * AB \tag{6}$$

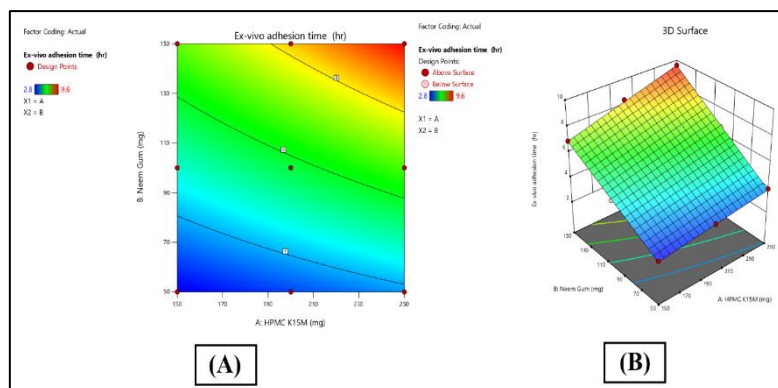


Figure 8: Contour plot (A) and 3D surface plate (B) for the effect of HPMC K4M and Moringa gum on ex vivo adhesion time (Y3) of Buccal film

Table 10: ANOVA summary of response variables of marrubiin buccal film

Response variable	Model	Sequential p-value	Adjusted R2 value	Predicted R2 value
Tensile Strength (N/mm ²)	Linear	<0.0001	0.9964	0.9927
Mucoadhesive strength (N)	Quadratic	0.0199	0.9982	0.9920
Ex vivo adhesion time (hr)	2FI	0.0017	0.9968	0.9940

Table 11: Software predicted and experimental response data of optimized mucoadhesive marrubiin buccal patch.

Optimized formulation composition (MF9)	Response			
Component	Quantity (mg)	Evaluation parameter	Software predicted	Experimentally observed
HPMC K15M	250	Tensile Strength (N/mm ²)	18.6	18.7
Neem Gum	150	mucoadhesive strength (N)	9.8	9.8
		Ex vivo adhesion time (hr)	9.5	9.6

Ex-vivo drug permeation studies from goat buccal mucosa

The results of ex-vivo drug permeation studies are shown in Figure 9.

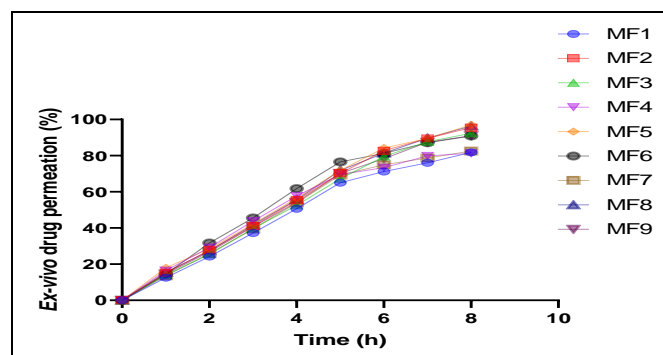


Figure 9: Ex-vivo drug permeation studies of the buccal patch (MF1-MF9)

Flux and Kp of buccal batch (MF1-MF9)

Table 12: Flux and Kp of buccal batch (MF1-MF9)

Batch	Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	Kp (cm/h)
MF1	10.23	2.84
MF2	12.47	3.46
MF3	11.89	3.30
MF4	10.86	3.02
MF5	12.92	3.59
MF6	11.95	3.32
MF7	10.58	2.94
MF8	12.76	3.54
MF9	13.45	3.74

Release kinetics for an optimized batch of buccal patch

Table 13: Release kinetics for an optimized batch of buccal patch

Formulations	First-order		Zero-order		Higuchi		Peppas	
	Slope	R2	Slope	R2	Slope	R2	Slope	R2
MF9	11.92	0.9832	35.56	0.9421	0.1341	0.9637	94.08	0.9676

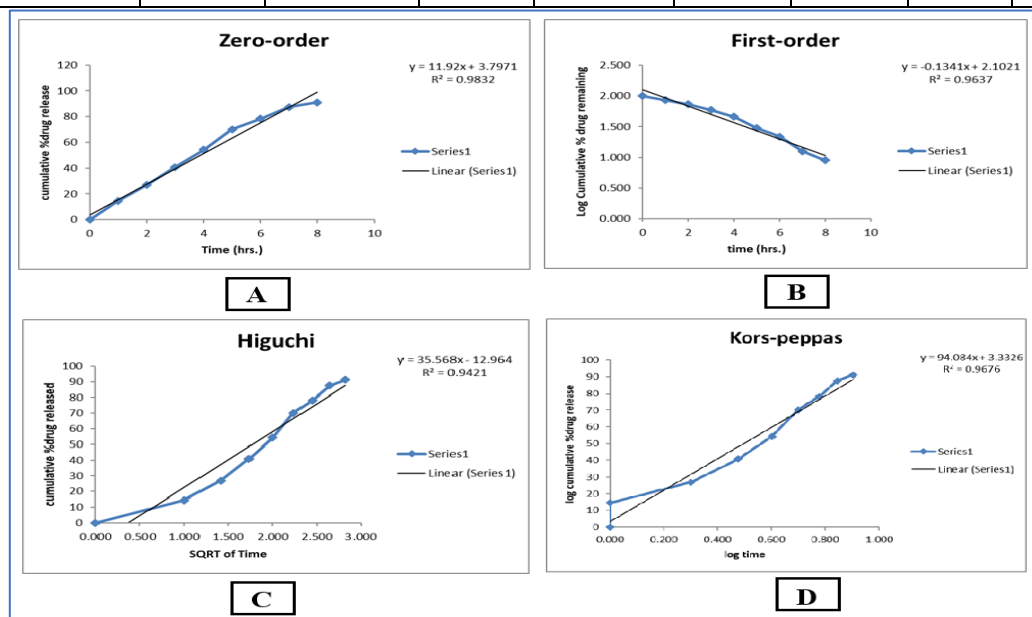


Figure 10: Release kinetics for an optimized batch of the buccal patch (A) Zero order release kinetics, (B) First order, (C) Higuchi and Kors-Peppas release kinetics.

Stability Study

Table 14: Stability Studies results of optimized batch (MF9) of buccal patch

Stability conditions	Sampling time	Tensile strength (N/mm ²)	Thickness (mm)	Folding Endurance (no. of folds)	Mucoadhesive strength (N)	Ex vivo drug adhesion (h)
Accelerated condition (40 ± 2oC and 75 ± 5% RH)	Initial (0)	18.86±0.65	0.14±0.02	314.7±	9.8±0.23	9.6±0.43
	1 Month	18.63±0.43	0.14±0.08	314.3±	9.8±0.43	9.6±0.31
	3 Months	18.51±0.74	0.14±0.04	315.9±	9.7±0.73	9.5±0.48
	6 Months	18.48±0.58	0.14±0.03	315.3±	9.7±0.59	9.5±0.35

Values are expressed in mean±SD (n=3)

CONCLUSION

The current study successfully developed and optimized a mucoadhesive buccal patch of marrubiin using HPMC K15M and Neem Gum. The MF9, optimized formulation, had excellent physicochemical attributes of the optimal tensile strength (18.73±0.36 N/mm²), mucoadhesive strength (9.8±0.39 N), and ex-vivo adhesion time (9.6±0.58 hr) and showed an excellent correlation of experimental value with the predicted value. FTIR and DSC studies proved the compatibility between the drug and the excipients. In contrast, ex-vivo permeation studies showed improved drug permeation when the highest flux of 13.45 µg/cm²/h and the 3.74 cm/h permeability coefficient were obtained. The release kinetics were first order (R² = 0.9832), indicating controlled drug release, and accelerated stability studies for six mos confirmed the robustness of the formulation. In-vitro and ex-vivo evaluations limited the study; however, future work should entail in-vivo pharmacokinetic studies and clinical trials to determine therapeutic efficacy. Further investigation could also explore the potential use of this delivery system for other poorly water-soluble drugs.

REFERENCES

- [1] Izzo A, Massimino E, Riccardi G, Della Pepa G (2021) A Narrative Review on Sarcopenia in Type 2 Diabetes Mellitus: Prevalence and Associated Factors. *Nutrients* 13:183. <https://doi.org/10.3390/nu13010183>
- [2] Animaw W, Seyoum Y (2017) Increasing prevalence of diabetes mellitus in a developing country and its related factors. *PLOS ONE* 12:e0187670. <https://doi.org/10.1371/journal.pone.0187670>
- [3] Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, Hoegfeldt CA, Elise Powe C, Immanuel J, Karuranga S, Divakar H, Levitt Na, Li C, Simmons D, Yang X (2022) IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of



Diabetes in Pregnancy Study Group's Criteria. *Diabetes Res Clin Pract* 183:109050. <https://doi.org/10.1016/j.diabres.2021.109050>

[4] Eades CE, Cameron DM, Evans JMM (2017) Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. *Diabetes Res Clin Pract* 129:173–181. <https://doi.org/10.1016/j.diabres.2017.03.030>

[5] Zanardo V, Tortora D, Sandri A, Severino L, Mesirca P, Straface G (2022) COVID-19 pandemic: Impact on gestational diabetes mellitus prevalence. *Diabetes Res Clin Pract* 183:109149. <https://doi.org/10.1016/j.diabres.2021.109149>

[6] Zhang X, Zhao L, Deng S, Sun X, Wang N (2016) Dry Eye Syndrome in Patients with Diabetes Mellitus: Prevalence, Etiology, and Clinical Characteristics. *J Ophthalmol* 2016:8201053. <https://doi.org/10.1155/2016/8201053>

[7] Rezgui M, Majdoub N, Mabrouk B, Baldisserotto A, Bino A, Ben Kaab LB, Manfredini S (2020) Antioxidant and antifungal activities of marrubiin, extracts and essential oil from *Marrubium vulgare* L. against pathogenic dermatophyte strains. *J Mycol Médicale* 30:100927. <https://doi.org/10.1016/j.mycmed.2020.100927>

[8] Yousefi K, Hamedeyazdan S, Torbati M, Fathiazad F (2016) Chromatographic Fingerprint Analysis of Marrubiin in *Marrubium vulgare* L. via HPTLC Technique. *Adv Pharm Bull* 6:131–136. <https://doi.org/10.15171/apb.2016.019>

[9] Kumar P, Dar SA, Manhas O, Bhushan A, Gupta P, Madishetti S, Ahmed Z (2024) Anti-diabetic and anti-inflammatory activities of Marrubiin isolated from *Marrubium vulgare*: Indications of its interaction with PPAR γ . *Phytomedicine Plus* 4:100664. <https://doi.org/10.1016/j.phyflu.2024.100664>

[10] Xing Y, Xing H, Ma Y, Liu Q, Xu S (2021) In Vitro and In Vivo Studies of Metabolic Activation of Marrubiin, a Bioactive Constituent from *Marrubium Vulgare*. *Chem Res Toxicol* 34:2157–2165. <https://doi.org/10.1021/acs.chemrestox.1c00250>

[11] Eltahawy NA, Ali AI, Ibrahim SA, Nafie MS, Sindi AM, Alkharobi H, Almalki AJ, Badr JM, Elhady SS, Abdelhameed RFA (2024) Analysis of Marrubiin in *Marrubium alysson* L. Extract Using Advanced HPTLC: Chemical Profiling, Acetylcholinesterase Inhibitory Activity, and Molecular Docking. *Metabolites* 14:27. <https://doi.org/10.3390/metabo14010027>

[12] Kumar P, Dar SA, Manhas O, Bhushan A, Gupta P, Madishetti S, Ahmed Z (2024) Anti-diabetic and anti-inflammatory activities of Marrubiin isolated from *Marrubium vulgare*: Indications of its interaction with PPAR γ . *Phytomedicine Plus* 4:100664. <https://doi.org/10.1016/j.phyflu.2024.100664>

[13] Gourich AA, Touijer H, Drioiche A, Asbabou A, Remok F, Saidi S, Siddique F, Ailli A, Bourhia M, Salamatullah AM, Ouahmane L, Mouradi A, Eto B, Zair T (2023) Insight into biological activities of



chemically characterized extract from *Marrubium vulgare* L. in vitro, in vivo and in silico approaches.

Front Chem 11: <https://doi.org/10.3389/fchem.2023.1238346>

[14] Macedo AS, Castro PM, Roque L, Thomé NG, Reis CP, Pintado ME, Fonte P (2020) Novel and revisited approaches in nanoparticle systems for buccal drug delivery. *J Controlled Release* 320:125–141. <https://doi.org/10.1016/j.jconrel.2020.01.006>

[15] Fonseca-Santos B, Chorilli M (2018) An overview of polymeric dosage forms in buccal drug delivery: State of art, design of formulations and their in vivo performance evaluation. *Mater Sci Eng C* 86:129–143. <https://doi.org/10.1016/j.msec.2017.12.022>

[16] Barua S, Kim H, Jo K, Seo CW, Park TJ, Lee KB, Yun G, Oh K, Lee J (2016) Drug delivery techniques for buccal route: formulation strategies and recent advances in dosage form design. *J Pharm Investig* 46:593–613. <https://doi.org/10.1007/s40005-016-0281-9>

[17] Rohani Shirvan A, Bashari A, Hemmatinejad N (2019) New insight into the fabrication of smart mucoadhesive buccal patches as a novel controlled-drug delivery system. *Eur Polym J* 119:541–550. <https://doi.org/10.1016/j.eurpolymj.2019.07.010>

[18] Rohani Shirvan A, Hemmatinejad N, Bahrami SH, Bashari A (2021) Fabrication of multifunctional mucoadhesive buccal patch for drug delivery applications. *J Biomed Mater Res A* 109:2640–2656. <https://doi.org/10.1002/jbm.a.37257>

[19] Jacob S, Nair AB, Boddu SHS, Gorain B, Sreeharsha N, Shah J (2021) An Updated Overview of the Emerging Role of Patch and Film-Based Buccal Delivery Systems. *Pharmaceutics* 13:1206. <https://doi.org/10.3390/pharmaceutics13081206>

[20] Roda A, Prabhu P, Dubey A (2018) DESIGN AND EVALUATION OF BUCCAL PATCHES CONTAINING COMBINATION OF HYDROCHLOROTHIAZIDE AND ATENOLOL. *Int J Appl Pharm* 10:105. <https://doi.org/10.22159/ijap.2018v10i2.24460>

[21] Mittal V, Nanda A (2017) Intensification of marrubiin concentration by optimization of microwave-assisted (low CO₂ yielding) extraction process for *Marrubium vulgare* using central composite design and antioxidant evaluation. *Pharm Biol* 55:1337–1347. <https://doi.org/10.1080/13880209.2017.1297837>

[22] Thakore SD, Akhtar J, Jain R, Paudel A, Bansal AK (2021) Analytical and Computational Methods for the Determination of Drug-Polymer Solubility and Miscibility. *Mol Pharm* 18:2835–2866. <https://doi.org/10.1021/acs.molpharmaceut.1c00141>

[23] Birch H, Redman AD, Letinski DJ, Lyon DY, Mayer P (2019) Determining the water solubility of difficult-to-test substances: A tutorial review. *Anal Chim Acta* 1086:16–28. <https://doi.org/10.1016/j.aca.2019.07.034>



[24] Rosasco MA, Bonafede SL, Faudone SN, Segall AI (2018) Compatibility study of tobramycin and pharmaceutical excipients using differential scanning calorimetry, FTIR, DRX, and HPLC. *J Therm Anal Calorim* 134:1929–1941. <https://doi.org/10.1007/s12014-018-0610-0>.

DEVELOPMENT AND OPTIMIZATION OF BACOSIDE A LOADED THERMOSENSITIVE IN-SITU HYDROGEL FOR ACUTE WOUND HEALING

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ABSTRACT

OBJECTIVES

To develop and optimize a thermosensitive in-situ hydrogel containing Bacoside A for enhanced wound healing, focusing on optimal gelling characteristics, mucoadhesion, and antimicrobial efficacy. Methods: Thermosensitive hydrogels were formulated using Poloxamer 407 and xanthan gum through statistical optimization. Formulations were characterized for physicochemical properties, drug content, rheology, and mucoadhesion. Response surface methodology optimized gelling temperature and mucoadhesive strength. Ex-vivo permeation studies were conducted, followed by stability assessment and antimicrobial evaluation against *E. coli* and *S. aureus*. Results: The optimized formulation (RF6) exhibited ideal gelling temperature ($33.78 \pm 0.78^\circ\text{C}$), mucoadhesive strength ($2989.5 \pm 0.284 \text{ dyne/cm}^2$), and viscosity ($3185 \pm 0.923 \text{ cps}$). The statistical model showed high predictability ($R^2 > 0.999$) for both responses. Ex-vivo studies demonstrated sustained drug release with $96.67 \pm 0.88\%$ permeation at 8 hours and flux of $11.59 \mu\text{g/cm}^2/\text{h}$. The formulation showed significant antibacterial activity with zones of inhibition of $24.3 \pm 0.62 \text{ mm}$ (*E. coli*) and $23.8 \pm 1.06 \text{ mm}$ (*S. aureus*), comparable to the marketed standard. Stability studies confirmed product integrity over six months with minimal variation in critical parameters. Conclusion: The developed Bacoside A thermosensitive in-situ hydrogel demonstrates promising characteristics for wound healing applications, combining optimal gelling properties, sustained drug release, and significant antimicrobial activity, offering a potential alternative to conventional wound healing formulations.

KEYWORDS

Bacoside A, Thermosensitive hydrogel, In-situ gelling, Wound healing, Response surface methodology, antimicrobial activity.

INTRODUCTION

Acute wounds refer to sudden injuries that lead to disruption of the skin's integrity and function due to physical trauma, thermal injuries, incisions, or cuts. While chronic wounds do not heal within a short period of time, acute wounds have a proper timeline, wherein their healing may take days to weeks depending on the wound class and the patient state. Nevertheless, hindrances such as slow healing or infections can greatly impact on the recovery process. Based on the recent research,

acute wounds, especially burn injuries, are present in more than 11 million patients yearly, and infection develops in 10%–15% of them. Currently, burn injuries cause about 180 000 deaths annually, of which about 75% are due to wound sepsis. Several interventions that are used in the treatment of chronic wounds include antibiotics, antimicrobial dressings, and debridement, which has their inherent challenges. Antibiotic resistance, inability to effectively manage biofilms and the absence of advanced drug delivery system create complications that lead to prolonged hospital stays and poor patient outcome thus a call for innovative approaches in managing acute Wound Care.

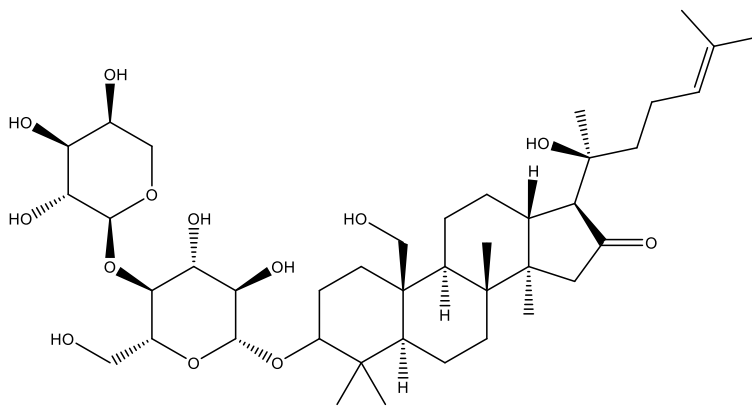


Figure 1: Chemical structure of Bacoside A

Bacoside A, a triterpenoid saponin, is the primary bioactive compound found in *Bacopa monnieri*, a traditional medicinal herb widely used in Ayurveda. This herb, often referred to as "Brahmi," grows abundantly in wetlands and marshy areas, with its leaves being the richest source of Bacoside A. Known for its potent antioxidant, anti-inflammatory, and neuroprotective properties, in recent years, bacoside A has drawn particular attention for its therapeutic potential in wound healing. Studies suggest that Bacoside A can accelerate the wound-healing process by reducing oxidative stress, a major factor in delayed healing, and modulating inflammatory pathways. It promotes the proliferation and migration of fibroblasts and keratinocytes, key cells involved in tissue regeneration. Furthermore, Bacoside A's ability to combat microbial infections makes it a promising candidate for treating acute wounds, offering a dual action of promoting tissue repair while preventing infections.

Thermosensitive hydrogels are an advanced class of polymeric materials that exhibit a unique sol-to-gel transition in response to temperature changes. At lower temperatures, these hydrogels remain in a liquid state, facilitating easy application over irregular wound surface. Upon exposure to body temperature, they transition into a gel-like state, forming a stable, protective barrier over the wound. Maintaining a moist wound environment is an essential part of helping wounds heal faster and more effectively, and this barrier does just that. Moreover, thermosensitive hydrogels can be engineered to act as carriers for bioactive compounds like Bacoside A, enabling controlled and

sustained release of the therapeutic agent directly at the wound site. This localized drug delivery enhances efficacy, and minimizes systemic side effects, making thermosensitive hydrogels a promising platform for acute wound treatment.

MATERIALS AND METHODS

Materials

The Bacoside A was obtained from Sciquaint Innovations (OPC) Private Limited, Pune, India. Poloxamer 407 were sourced from Research Lab Fine Chem Industries, Mumbai, India. Xanthan gum was purchased from Indian jadibuti, Delhi, India. The chemicals and solvents used in the process were all of analytical grade.

Methods

Calibration curve of Bacoside A

Spectroscopic analysis of Bacoside A was selected to use as a solvent: methanol. A pure 10 mg Bacoside A sample was placed inside a 100 ml calibrated volumetric flask, solubilised with methanol, and diluted to the mark so as to have 100 $\mu\text{g ml}^{-1}$ stock solution. From this stock solution aliquots of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml were pipetted into separate 10ml calibrated volumetric flasks and made up to the mark with methanol to give working standard solutions at concentration of 10, 20, 30, 40, 50 and 60 $\mu\text{g/ml}$ respectively. Absorbance of each solution was monitored at 227nm using Shimadzu UV1900 spectrophotometer.

Solubility Study

The saturated solubility of the Bacoside A was evaluated in water, methanol, ethanol (99.7%), phosphate buffer (pH 6.8), and DMSO. Further, an excess amount of the drug was added to 50 mL of each solvent in separate 100 mL volumetric flasks, which were sealed and placed in an orbital-shaking water bath set at 50 rpm and a controlled temperature of $37 \pm 0.5^\circ\text{C}$ for 48 hours. After equilibration, the samples were filtered, appropriately diluted with the same solvent, and analysed for absorbance using a UV-visible spectrophotometer at 227nm λ_{max} for each solvent. The absorbance values were then converted into concentration using the standard curve of the drug in the respective solvents.

FTIR Spectroscopy

FTIR spectra of the pure drug was obtained by using various FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan). The mixture was ground thoroughly in mortar and pestle with KBr powder in the ratio of 1:100, the ground sample was pressed in dies set in pellet press with hydraulic pressure of 15 tons for a minute. To release pressure and take of the pellet from the dies set rotate the side valve anticlockwise. The pellet was then placed in the sample holder and scanning was done across the region of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} and speed of scan at 2 mm/sec .

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis was performed on the samples using a Perkin-Elmer Pyris-1 instrument (Osaka, Japan). Initially, the samples were heated to remove moisture. Each sample, weighing approximately 5 mg, was accurately placed in a platinum crucible, a 40 µl aluminium pan, which was hermetically sealed. Alpha alumina powder was used as the reference material. Thermograms were recorded over a temperature range of 50°C to 300°C at a heating rate of 20°C/min under a constant flow of inert nitrogen gas at a flow rate of 20 ml/min.

Dose selection for bacoside A

The dose of Bacoside A was selected based on previously reported efficacy studies by R. Sharath et al. in rats, where 200 µg/mL demonstrated significant wound healing activity without any toxicity. For the current study formulation, this dose was converted to human equivalent dose (HED) using the formula: $HED (mg/kg) = Animal\ dose (mg/kg) \times (Animal\ Km / Human\ Km)$, where Km factors are 6 for rats and 37 for humans. The calculated human equivalent dose was 0.03 mg/kg body weight, which translates to 2.27 mg of Bacoside A for the formulation.

Experimental design for thermosensitive in-situ hydrogel.

Thermosensitive hydrogel formulation optimization was done using 32-full factorial design. There were two independent variables, namely, concentration of Poloxamer 407 (A) and Xanthan gum (B) at -1, 0, and +1 levels. The levels of Poloxamer 407 (A) were 20%, 22.5% and 25% w/w, and for Xanthan gum (B) 0.5%, 1.0% and 1.5% w/w. The dependent variables studied were sol gel temperature (R1) and Mucoadhesive strength (R2). According to the factorial design, 9 formulations were prepared. Experimental design was generated and evaluated by Design Expert program software (version 13.0, Stat-Ease Inc). They were systematically outlined in the table 1 and 2 as coded and actual values of the independent factors in the factorial design.

The polynomial equation is:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2 + \varepsilon \quad (7)$$

Where: Y = Response variable (R1 or R2 in your case), β_0 = Intercept coefficient, β_1, β_2 = Linear effect coefficients, β_{11}, β_{22} = Quadratic effect coefficients, β_{12} = Interaction effect coefficient, X_1 = Coded value of factor A (Poloxamer 407), X_2 = Coded value of factor B (Xanthan Gum), ε = Error term.

Table 1: Coded and decoded values of 32 full factorial design

Independent Variables							
Label	Factors	Coded values			Actual values in %w/v		
A	Poloxamer 407	-1	0	+1	16	18	20

B	Xanthan Gum	-1	0	+1	0.5	1.0	1.5
Dependant Variables							
R1	Gelling temperature (°C)						
R2	Mucoadhesive strength (Dyne/cm ²)						

Table 2: Prepared batches of thermosensitive hydrogel using 32 factorial designs

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Bacoside A (mg)	2.27	2.27	2.27	2.27	2.27	2.27	2.27	2.27	2.27
Poloxamer 407 (% w/v)	16	18	20	16	18	20	16	18	20
Xanthan Gum (% w/v)	0.5	0.5	0.5	1.0	1.0	1.0	1.5	1.5	1.5
Distilled water (q.s)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Preparation of In-situ thermosensitive hydrogel

The hydrogel was prepared by cold method. Weight of required grams of Poloxamer 407 and Xanthan gum were weighed, dissolved into ddH₂O, heated to room temperature to 4 °C ± 0.5 °C overnight to make a sol solution of specified concentration (w/w). Clear solutions were obtained by dissolved polymers by stirring continuously at 40 rpm. Quantitative weighing of Bacoside A into ethanol solution was accomplished. Finally, a drug solution was then made by adding the drug solution into the polymeric solution with continuous stirring of 40 rpm until the put drug concentration [itaconic acid] is 2.27mg. Then the prepared hydrogel composite was characterized for thermosensitive properties.

Evaluation of In-situ thermosensitive hydrogel

Gelling temperature

The gelation temperature of the thermosensitive hydrogel was determined using the vial inversion method. Hydrogel samples were prepared and subjected to heating in a water bath with a controlled temperature increase of 1°C per minute. The sol-gel transition was visually assessed by tilting the vials at an angle of 90° at regular intervals. The temperature at which the hydrogel ceased to flow and remained stationary in the tilted position was recorded as the gelation temperature. This method ensures accurate determination of the phase transition relevant to physiological conditions.

Gelling time

The gelling time was determined using the vial inversion method. The formulation was placed in a vial with a 2mL volume of the formulation and heated at a controlled temperature increment. The vial was periodically tilted to observe the sol-gel transition. The gelling time was recorded as the point when the formulation ceased flowing upon tilting.

Viscosity

Viscosity of the in situ thermosensitive hydrogels was determined using a Brookfield viscometer with an S-94 spindle. The spindle penetrated perpendicularly into the gel and transferred prepared gel formulations into a beaker. Measurements were carried out at the speed of 100 rpm; the temperature being set 37 ± 0.5 °C. Viscosities of the formulations were measured while cooling and the measurements were done in triplicate to insure reliability and also accuracy.

Drug Content Assay

The in situ thermosensitive hydrogel formulation was diluted to a total volume of 10 mL with methanol and 1 mL was used to determine the drug content of the hydrogel. After vortexing the mixture to make sure it had been dissolved completely, it was filtered to remove any undissolved particles. The absorbance of the Bacoside A was Taken at 227nm using Shimadzu UV-1900 spectrophotometer was used to analyse the filtrate. The absorbance values were compared to a standard calibration curve prepared with the addition of methanol to calculate the drug concentration. Finally, all measurements were done in triplicate to verify precision and reproducibility.

Mucoadhesive strength

The mucoadhesive strength of the in-situ thermosensitive gel was measured using a modified physical balance method. Freshly excised mucosal tissue was affixed to two glass vials, with the mucosal side facing outward. One vial was attached to a balance, and the other was placed on a height-adjustable platform. A known amount of gel was applied to the mucosa on the first vial, and the second vial was brought into contact with the first, maintaining tight contact for 2 minutes. The two vials were then weighed on the balance pan, gradually adding weights until the vials thus separated. The mucoadhesive strength was calculated using the formula:

$$\text{Mucoadhesive strength} = \frac{m - g}{A}$$

where m is the weight required to detach the vials (g), g is the gravitational constant (980 cm/s²), and A is the contact area of the mucosa (cm²). This method ensures accurate quantification of the adhesive properties of the gel.

Spreadability

A 5 g sample of the gel was spread between two glass plates and the spreadability of the thermosensitive hydrogel was evaluated. To stress the top plate by the uniform pressure, it was

subjected to 500 g weight for 5 min. The spreadability of the gel was measured to determine the diameter, at which the gel had spread, measured by a calliper. In this study, this test was done in triplicate to establish a reliable and consistent results.

Ex-vivo drug release study

The goat skin was used as a diffusion membrane in the same manner as was used for the in vitro drug release study. From the slaughterhouse goat skin was procured and placed between the donor and receptor parts, 1 g thermosensitive hydrogel was placed on the donor part, receptor part was filled with phosphate buffer pH 6.8 and was mounted on the magnetic stirrer hot plate and rotated at 100rpm and made to get at 37 ± 0.5 °C finally at different intervals samples of 1 ml were withdrawn, diluted with 10 ml of buffer and the absorbance was measured at 227nm spectroscopically. The complete diffusion was studied for 8 h. A graph of release percentage cumulative versus time was plotted.

In-vitro antibacterial activity

The in vitro antibacterial activity of Bacoside A, Thermosensitive hydrogel (RF6), and Candiderma Plus (marketed standard) against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) was evaluated using the agar well diffusion technique. An E. Coli and S. aureus standardized bacterial solution was used to inoculate nutrient agar plates. A corn borer with a 6mm diameter was used to create the wells aseptically after the liquid inoculation media had completely solidified. Carefully placed extract and gel solution in each of these plates. Plates were left for 30 minutes to pre-diffusion. Following the plates' normalization to room temperature, they were incubated for 24 hours at 37°C to check for microorganisms. Each well's surrounding zones of inhibition were measured and documented in mm. Three duplicates of the experiment were carried out, and the mean \pm standard deviation was used to report the findings.

Stability studies

The stability study of thermosensitive hydrogel was performed following ICH Q1A (R2) guidelines for 6 months under three storage conditions: long-term (25 ± 2 °C/ 60 ± 5 % RH). The formulations were evaluated at 0, 3, and 6 months for physical appearance, pH, drug content, rheological properties, gelling temperature, mucoadhesive strength and ex-vivo drug permeation.

Statistical Analysis

Design Expert software Expert® DX 13.0 (StatEase Inc., MN) was used to analyze the experiment data. Response surface plots were used to show how the factors affected the dependent variables, and analysis of variance (ANOVA) was used to assess the factors' significance. In order to

choose the best formulation, desirability was computed using the target values for viscosity and spreadability.

RESULTS

Calibration curve of Bacoside A

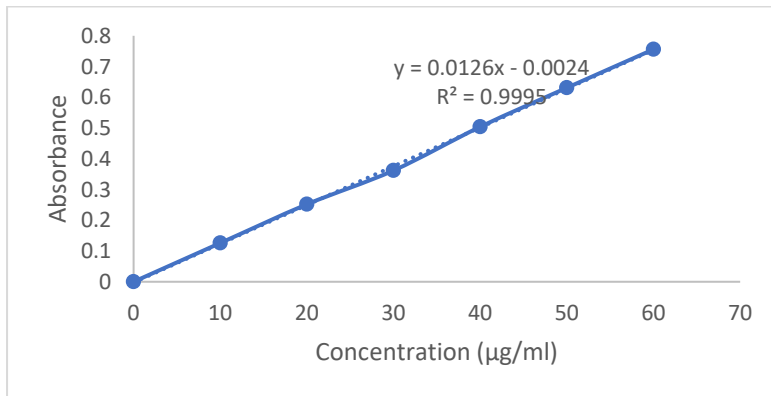


Figure 2: Standard Calibration Plot of Bacoside A in Methanol ($\lambda_{max} = 227nm$)

Results of solubility study

Table 3: Results of solubility analysis of Bacoside A.

Sr. No.	Solvent	Solubility (mg/mL)	Results
1	Water	2.57±0.03	Practically Insoluble
2	Ethanol	24.42±2.56	Sparingly soluble
3	Methanol	27.36±1.23	Sparingly soluble
4	Phosphate Buffer pH 6.8	42.32±3.82	Soluble
5	DMSO (Dimethyl Sulfoxide)	76.98±4.22	Soluble

Results are expressed in mean±SD (n=3)

Results of FTIR analysis

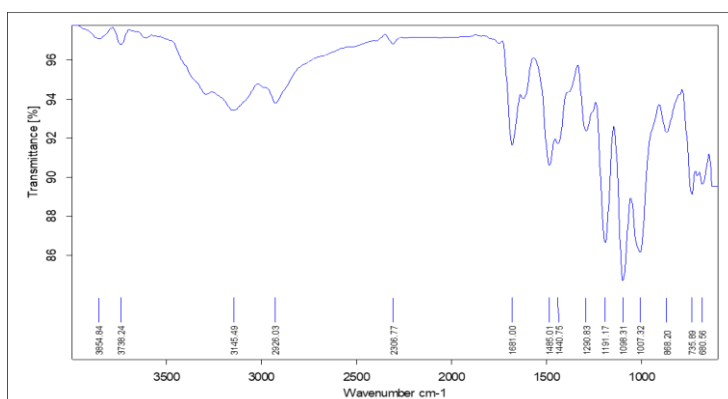


Figure 3: FTIR Spectral analysis of pure Bacoside A

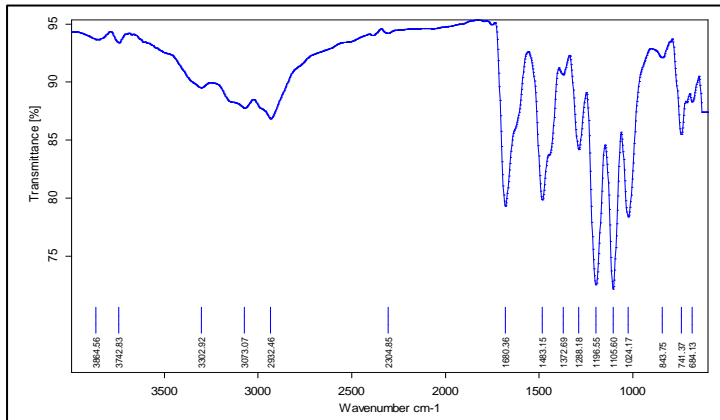


Figure 4: FTIR Spectral analysis of drug-exipient physical mixture

Results of differential Scanning Calorimetry

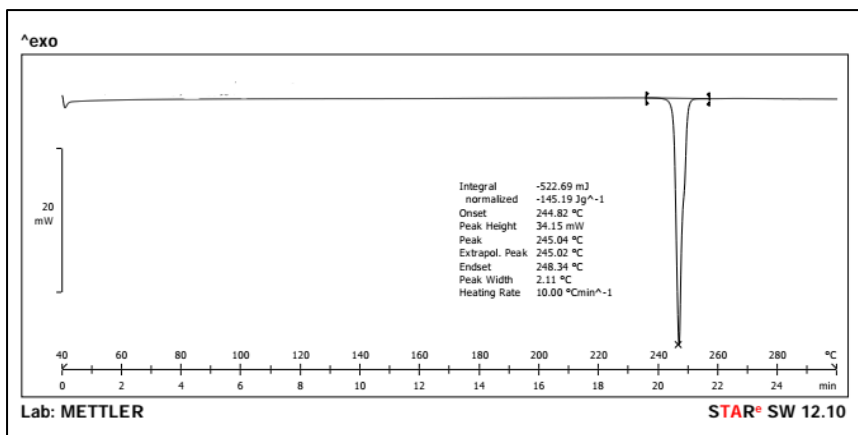


Figure 5: DSC thermogram of pure Bacoside A

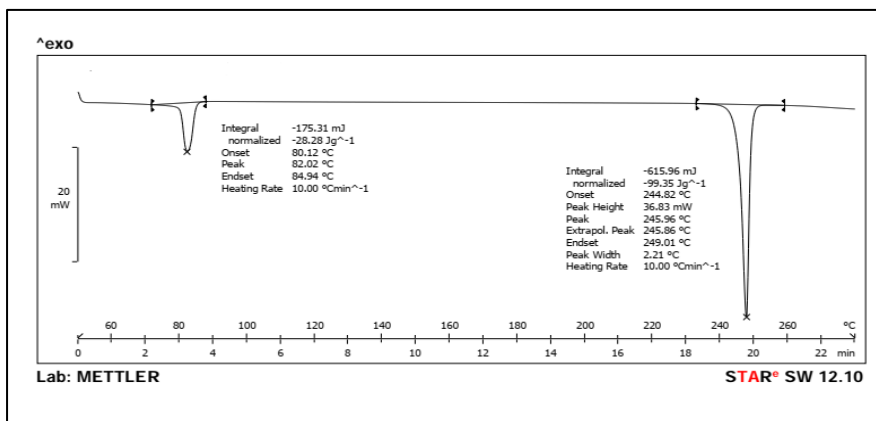


Figure 6: DSC Thermogram of drug-exipient Physical mixture

Characterization of thermosensitive in-situ hydrogel

Table 4: Physicochemical characteristics of thermosensitive In-situ hydrogel formulations.

Formulation	Gelling temperature (°C)	Gelling time (sec)	Viscosity (cps)
RF1	43.23±0.28	37.34±0.12	2125±30

RF2	36.49±0.24	36.89±0.14	2532±26
RF3	32.8±0.20	37.64±0.10	2947±34
RF4	42.44±0.31	33.26±0.18	2280±29
RF5	36.45±0.26	32.72±0.15	2704±52
RF6	33.44±0.21	33.11±0.20	3105±40
RF7	42.96±0.34	30.1±0.17	2450±46
RF8	36.71±0.19	29.97±0.12	2885±30
RF9	32.87±0.30	30.19±0.16	3196±58

Data are expressed in mean±SD (n=3)

Table 5: Evaluation Parameters of Thermosensitive In-situ hydrogel.

Formulation	Mucoadhesive strength (dyne/cm ²)	Drug content (%)	Spreadability (cm)
RF1	1798.9±0.415	91.7±0.49	8.1±0.26
RF2	1842.2±0.383	92.8±0.75	7.2±0.42
RF3	2039.8±0.744	90.3±0.40	11.3±0.51
RF4	2641.8±0.512	93.4±0.82	9.2±0.19
RF5	2838.6±0.483	92.9±0.63	12.1±0.68
RF6	2989.4±0.812	92.7±0.72	10.4±0.32
RF7	4278.2±0.641	91.4±0.94	11.5±0.83
RF8	4369.7±0.390	91.7±0.62	9.4±0.76
RF9	4539.1±0.673	92.6±0.47	8.8±0.82

Data are expressed in mean±SD (n=3)

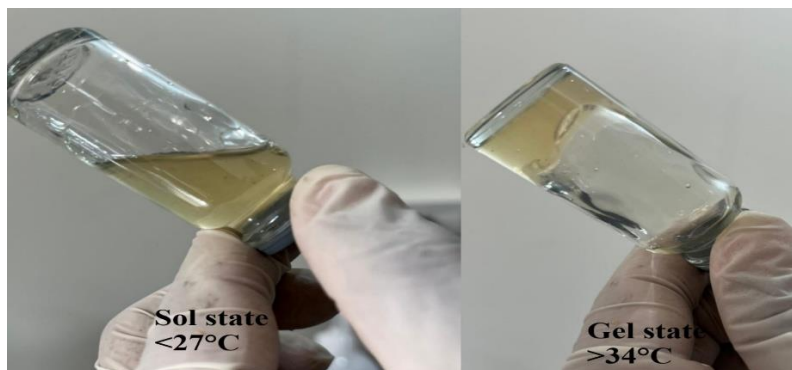


Figure 7: Image represents thermosensitive behaviour of in-situ hydrogel in room temperature and physiological conditions.

Optimization of thermosensitive in-situ hydrogel

Effect of Independent variables on for gelling temperature (R1)

Table 6: ANOVA for quadratic model for gelling temperature (R1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	149.23	5	29.85	157.01	0.0008	significant
A-Poloxamer 407	145.24	1	145.24	764.04	0.0001	
B-Xanthan gum	0.0001	1	0.0001	0.0004	0.9862	
AB	0.0289	1	0.0289	0.1520	0.7226	
A ²	3.96	1	3.96	20.82	0.0197	
B ²	0.0089	1	0.0089	0.0468	0.8427	
Residual	0.5703	3	0.1901			
Cor Total	149.80	8				

The regression equation obtained from gelling temperature is as follows:

$$\text{Gelling temperature} = +36.51 - 4.92 * A + 0.0033 * B + 0.0850 * AB + 1.24 * A^2 + 0.0667 * B^2 \quad (8)$$

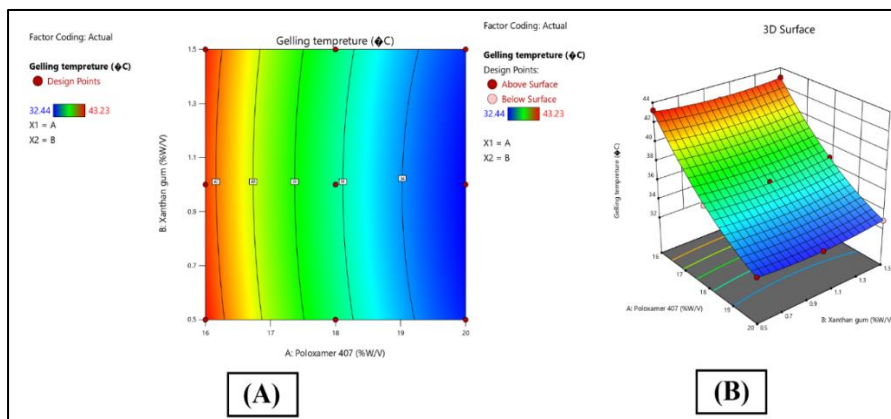


Figure 8: Contour plot (A) and 3D plot (B) for the effect of Poloxamer 407 and xanthan gum on gelling temperature (R1) of In-situ thermosensitive gel.

Effect of Independent variables on mucoadhesive strength (R2)

Table 7: ANOVA for quadratic model for mucoadhesive strength (R2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.719E+06	5	1.944E+06	893.99	< 0.0001	significant
A-Poloxamer 407	1.202E+05	1	1.202E+05	55.30	0.0050	
B-Xanthan gum	9.390E+06	1	9.390E+06	4318.70	< 0.0001	
AB	100.00	1	100.00	0.0460	0.8439	
A ²	1926.14	1	1926.14	0.8859	0.4160	
B ²	2.066E+05	1	2.066E+05	95.01	0.0023	
Residual	6522.98	3	2174.33			

Cor Total	9.726E+06	8			
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The regression equation obtained for mucoadhesive strength is as follows:

$$\text{Mucoadhesive strength} = +2802.58 + 141.57 \cdot A + 1251.02 \cdot B + 5.00 \cdot AB + 31.03 \cdot A^2 + 321.38 \cdot B^2 \quad (9)$$

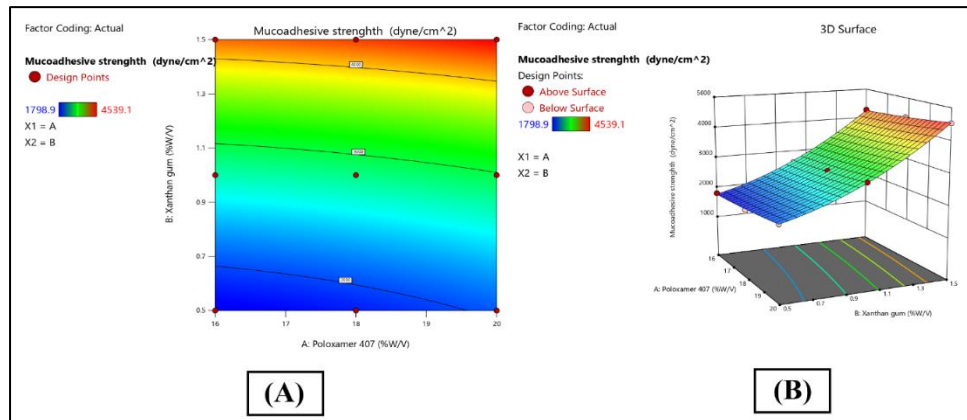


Figure 9: contour plot (A) and 3D plot (B) for the Poloxamer 407 and xanthan gum on mucoadhesive strength (R2) of In-situ thermosensitive gel.

Table 8. Summary of the quadratic model results for regression analysis of response R1 and R2.

Quadratic Model	R2	Adjusted R2	Predicted R2	SD	% CV
Response (Y1)	0.9992	0.9980	0.9927	0.1993	0.5332
Response (Y2)	0.9993	0.9982	0.9932	46.63	1.540

Validation of statistical model

Table 9: The predicted and experimental values of response variables and relative error.

F. Code	Composition	Actual (mg)	Response	Predicted value	Experimental value	Relative Error (%)
MF6	Poloxamer 407	20	Gelling temperature (°C)	34.01	34.44	1.26
	Xanthan gum	1				
MF6	Poloxamer 407	20	Mucoadhesive strength (dyne/cm ²)	3000	2989	0.37
	Xanthan gum	1				

Ex-vivo drug permeation study

The results of Ex-vivo drug permeation studies are shown in Figure 8.

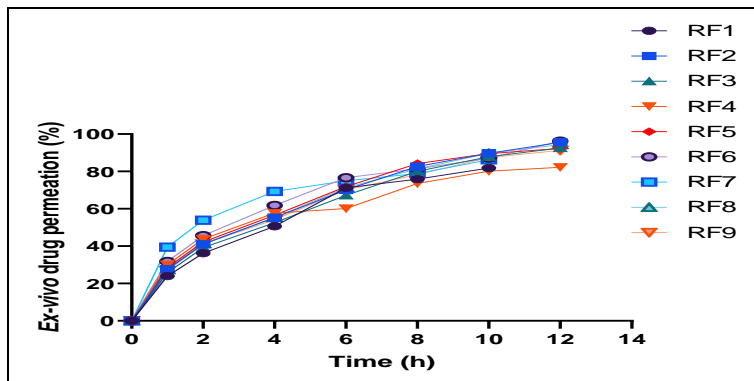


Figure 10: Ex-vivo drug permeation from In-situ thermosensitive hydrogel

Flux and Kp of thermosensitive hydrogel (RF1-RF9)

Table 10: Flux and Kp of thermosensitive hydrogel (RF1-RF9)

Batch	Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	Kp (cm/h)
RF1	10.90	1.090
RF2	10.66	1.066
RF3	10.24	1.024
RF4	9.09	0.909
RF5	10.89	1.089
RF6	11.59	1.159
RF7	11.05	1.105
RF8	10.82	1.082
RF9	10.63	1.063

Stability study

Table 11: Result of six-month stability study on various parameters of optimized batch ($25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$).

Response	Before stability	1 month	3 months	6 months
Clarity	Clear	Clear	Clear	Clear
Visual appearance	Transparent	Transparent	Transparent	Transparent
Gelling Temperature strength ($^\circ\text{C}$)	33.78 ± 0.78	33.54 ± 0.67	33.48 ± 0.73	32.54 ± 0.83
Viscosity (cps)	3185 ± 0.923	3151 ± 0.645	3123 ± 0.482	3117 ± 0.787
Mucoadhesive strength (dyne/cm ²)	2989.5 ± 0.284	2997.8 ± 0.833	3002.3 ± 0.156	3011.9 ± 0.723
Gelling time (sec)	21.4 ± 0.5	21.9 ± 0.6	21.4 ± 0.4	20.9 ± 0.3

pH	6.2±0.07	6.2±0.09	6.2±0.03	6.2±0.09
Spreadability (cm)	10.3±0.43	10.8±0.33	10.5±0.93	10.8±0.36
Ex-vivo drug permeation at 8 hr (%)	96.67±0.88	96.63±0.44	96.48±0.73	94.41±0.68

Data is expressed in mean±SD, (n=3)

In-vitro antibacterial activity

Table 12: In-vitro antibacterial activity of Bacoside A, Thermosensitive hydrogel (RF6) and Candiderma Plus (Marketed standard).

Sr. No.	Name of Sample	Zone of Inhibition (mm)*	
		E. coli	S. Aureus
1.	Bacoside A	26.9±1.76	28.4±0.38
2.	Thermosensitive hydrogel (RF6)	24.3±0.62	23.8±1.06
3.	Marketed standard (Candiderma Plus)	29.3±0.63	27.7±0.19

Values are expressed in mean±SD, (n =3).

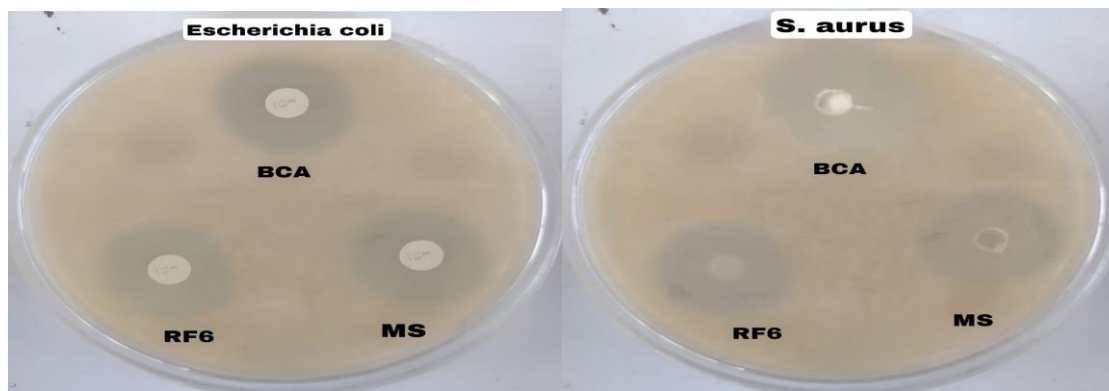


Figure 11: In-vitro antibacterial activity of Bacoside A, Thermosensitive hydrogel (RF6) and Candiderma Plus (Standard) for Escherichia coli and Staphylococcus aureus.

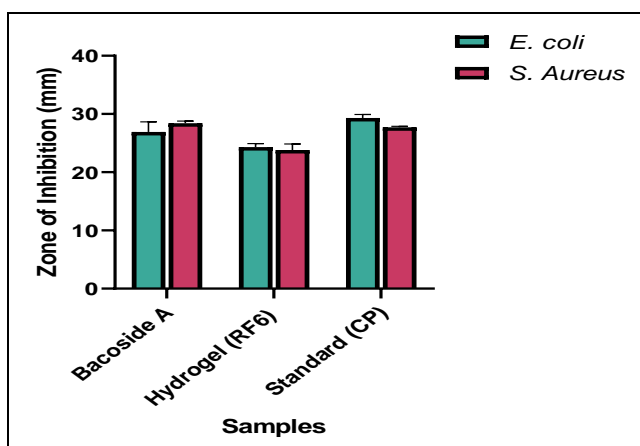


Figure 12: Graphical representation of the In-vitro antibacterial activity of Bacoside A, Thermosensitive hydrogel (RF6) and Candiderma Plus (Standard) (MS) for Escherichia coli and Staphylococcus aureus.

CONCLUSION

The completed and well-organized research on the development and optimization of Bacoside A loaded thermosensitive in-situ hydrogel discloses that the potential wound healing system can be formulated effectively. In optimized formulation, the gelling temperature was found to be $33.78 \pm 0.78^\circ\text{C}$ which is close to the physiological temperature, mucoadhesive strength was 2989.5 ± 0.284 dyne/cm² and the drug permeation rate was $96.67 \pm 0.88\%$ at 8 hours. Signatures for conformation of compatibility of the drug-exciptent interaction were established by FTIR and DSC In addition, optimization by RSM gave highly acceptable value of predictability of the designed formulation ($R^2 > 0.999$). The formulation proved to exhibit the best antibacterial efficacy against both the tested microbial isolates, and the result was as effective as a marketed lotion, though slightly inferior, and the study determined that the formulation demonstrated stability throughout a six-month investigation under the given storage conditions. The fact that the formulation is optimised with lasting drug release, mucoadhesion and their antimicrobial properties pointed to its potential use as an easy to apply, effective and comfortable to use in-situ thermosensitive hydrogel systemic medication for wound healing that has added benefits in comparison with conventional medication in terms of patient compliance.

REFERENCES

- [1] Bei Z, Zheng J. Recent advances in the application of functional hydrogels in skin wound healing. *MedComm* – *Biomaterials and Applications* 2024;3:e101. <https://doi.org/10.1002/mba2.101>.
- [2] Falanga V, Isseroff RR, Soulika AM, Romanelli M, Margolis D, Kapp S, et al. Chronic wounds. *Nature Reviews Disease Primers* 2022;8:50.
- [3] Monika P, Chandraprabha MN, Rangarajan A, Waiker PV, Chidambara Murthy KN. Challenges in healing wound: role of complementary and alternative medicine. *Frontiers in Nutrition* 2022;8:791899.
- [4] Zhang P, Zou B, Liou Y-C, Huang C. The pathogenesis and diagnosis of sepsis post burn injury. *Burns & Trauma* 2021;9:tkaa047.
- [5] Zhang P, Zou B, Liou Y-C, Huang C. The pathogenesis and diagnosis of sepsis post burn injury. *Burns & Trauma* 2021;9:tkaa047.
- [6] Alves PJ, Barreto RT, Barrois BM, Gryson LG, Meaume S, Monstrey SJ. Update on the role of antiseptics in the management of chronic wounds with critical colonisation and/or biofilm. *International Wound Journal* 2021;18:342–58. <https://doi.org/10.1111/iwj.13537>.



- [7] Kolimi P, Narala S, Nyavanandi D, Youssef AAA, Dudhipala N. Innovative treatment strategies to accelerate wound healing: trajectory and recent advancements. *Cells* 2022;11:2439.
- [8] Murthy HN. Biotechnological production of bacosides from cell and organ cultures of *Bacopa monnieri*. *Appl Microbiol Biotechnol* 2022;106:1799–811. <https://doi.org/10.1007/s00253-022-11834-0>.
- [9] Mondal S, Bhar K, Mondal P, Panigrahi N, Sahoo SK, Swetha P, et al. In quest of the mysterious holistic Vedic herb *Bacopa monnieri* (L.) Pennell. *Pharmacognosy Research* 2023;15.
- [10] Nagori K, Nakhate KT, Yadav K, Ajazuddin, Pradhan M. Unlocking the Therapeutic Potential of Medicinal Plants for Alzheimer's Disease: Preclinical to Clinical Trial Insights. *Future Pharmacology* 2023;3:877–907.
- [11] Martínez-Cuazitl A, Gómez-García M del C, Hidalgo-Alegria O, Flores OM, Núñez-Gastélum JA, Martínez ESM, et al. Characterization of polyphenolic compounds from *bacopa procumbens* and their effects on wound-healing process. *Molecules* 2022;27:6521.
- [12] Martínez-Cuazitl A, Gómez-García M del C, Hidalgo-Alegria O, Flores OM, Núñez-Gastélum JA, Martínez ESM, et al. Characterization of polyphenolic compounds from *bacopa procumbens* and their effects on wound-healing process. *Molecules* 2022;27:6521.
- [13] Ghosh D, Adhikary S, Bhattacharjee P, Debnath S. Herbal Medicine for Health Management and Disease Prevention. In: Izah SC, Ogwu MC, Akram M, editors. *Herbal Medicine Phytochemistry*, Cham: Springer International Publishing; 2024, p. 1–35. https://doi.org/10.1007/978-3-031-21973-3_31-1.
- [14] Lacroce E, Rossi F. Polymer-based thermoresponsive hydrogels for controlled drug delivery. *Expert Opinion on Drug Delivery* 2022;19:1203–15. <https://doi.org/10.1080/17425247.2022.2078806>.
- [15] Dong R, Guo B. Smart wound dressings for wound healing. *Nano Today* 2021;41:101290.
- [16] Yu Y, Cheng Y, Tong J, Zhang L, Wei Y, Tian M. Recent advances in thermo-sensitive hydrogels for drug delivery. *Journal of Materials Chemistry B* 2021;9:2979–92.
- [17] Nuutila K, Eriksson E. Moist Wound Healing with Commonly Available Dressings. *Advances in Wound Care* 2021;10:685–98. <https://doi.org/10.1089/wound.2020.1232>.
- [18] Kolahreez D, Ghasemi-Mobarakeh L, Liebner F, Alihosseini F, Quartinello F, Guebitz GM, et al. Approaches to Control and Monitor Protease Levels in Chronic Wounds. *Advanced Therapeutics* 2024;7:2300396. <https://doi.org/10.1002/adtp.202300396>.
- [19] Furukawa H, Ichimaru N, Suzuki K, Nishino M, Broughton J, Leinders J, et al. The comparative verification of calibration curve and background fundamental parameter methods for impurity analysis in drug materials. *X-Ray Spectrometry* 2017;46:382–7. <https://doi.org/10.1002/xrs.2788>.



- [20] Mota FL, Carneiro AP, Queimada AJ, Pinho SP, Macedo EA. Temperature and solvent effects in the solubility of some pharmaceutical compounds: Measurements and modeling. *European Journal of Pharmaceutical Sciences* 2009;37:499–507.
- [21] Ledeti I, Bolintineanu S, Vlase G, Circioban D, Ledeti A, Vlase T, et al. Compatibility study between antiparkinsonian drug Levodopa and excipients by FTIR spectroscopy, X-ray diffraction and thermal analysis. *J Therm Anal Calorim* 2017;130:433–41. <https://doi.org/10.1007/s10973-017-6393-2>.
- [22] Rosasco MA, Bonafede SL, Faudone SN, Segall AI. Compatibility study of tobramycin and pharmaceutical excipients using differential scanning calorimetry, FTIR, DRX, and HPLC. *J Therm Anal Calorim* 2018;134:1929–41. <https://doi.org/10.1007/s10973-018-7282-z>.
- [23] Sharath R, Harish BG, Krishna V, Sathyanarayana BN, Swamy HMK. Wound healing and protease inhibition activity of Bacoside-A, isolated from *Bacopa monnieri* wettest. *Phyther Res* 2010;24:1217–22. <https://doi.org/10.1002/ptr.3115>.
- [24] Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy* 2016;7:27.
- [25] Jeswani G, Chablani L, Gupta U, Sahoo RK, Nakhate KT, Taksande AG. Exploration of hemocompatibility and intratumoral accumulation of paclitaxel after loco-regional administration of thermoresponsive hydrogel composed of poloxamer and xanthan gum: an application to dose-dense chemotherapy. *International Journal of Biological Macromolecules* 2023;226:746–59.
- [26] Liang H-F, Hong M-H, Ho R-M, Chung C-K, Lin Y-H, Chen C-H, et al. Novel Method Using a Temperature-Sensitive Polymer (Methylcellulose) to Thermally Gel Aqueous Alginate as a pH-Sensitive Hydrogel. *Biomacromolecules* 2004;5:1917–25. <https://doi.org/10.1021/bm049813w>.
- [27] Gao B, Luo J, Liu Y, Su S, Fu S, Yang X, et al. Intratumoral Administration of Thermosensitive Hydrogel Co-Loaded with Norcantharidin Nanoparticles and Doxorubicin for the Treatment of Hepatocellular Carcinoma. *IJN* 2021;Volume 16:4073–85. <https://doi.org/10.2147/IJN.S308057>.
- [28] Nawaz A, Ullah S, Alnuwaiser MA, Rehman FU, Selim S, Al Jaouni SK, et al. Formulation and Evaluation of Chitosan-Gelatin Thermosensitive Hydrogels Containing 5FU-Alginate Nanoparticles for Skin Delivery. *Gels* 2022;8:537. <https://doi.org/10.3390/gels8090537>.
- [29] Kolawole OM, Cook MT. In situ gelling drug delivery systems for topical drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics* 2023;184:36–49. <https://doi.org/10.1016/j.ejpb.2023.01.007>.



[30] Srivastava R, Srivastava S, Singh SP. Thermoreversible in-situ nasal gel formulations and their pharmaceutical evaluation for the treatment of allergic rhinitis containing extracts of moringa olifera and embelia ribes. *Int J Appl Pharm* 2017;9:16.

[31] Fatouh AM, Elshafeey AH, Abdelbary A. Agomelatine-based in situ gels for brain targeting via the nasal route: statistical optimization, in vitro , and in vivo evaluation. *Drug Delivery* 2017;24:1077–85. <https://doi.org/10.1080/10717544.2017.1357148>.



VERMIWASH FOR SUSTAINABLE PH AND CARBON CREDIT MANAGEMENT IN SOIL

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ABSTRACT

During green revolution, excess use of agrochemicals adversely affects natural resources and agriculture. Therefore we must have to go for sustainable agriculture. For this vermiwash is emerging as important potential tool. Vermiwash is a brown coloured, odourless, liquid bio-fertilizer, which is collected after passes via column of worm culture. Vermiwash used as drenching and spray for soils. Vermiwash contains mucus, excretory products of worms and various concentrations of micro, macro beneficial nutrients along with beneficial microorganism, growth hormones, Vitamins, enzymes and amino acids, therefore is a good source for plant nutrition in sustainable agriculture. After application of vermiwash, improve carbon content and pH. The effect of vermiwash was observed on the plants and soil; it was found that vermiwash seems to possess an inherent property which acts not only as a liquid organic bio-fertilizer which promote growth of plants and yield but also as a mild bio-pesticide. The pH of untreated back soil is 7.9, while treated soil 7.1 and the carbon % of untreated soil 0.63% while treated soil improve up to 0.82%. So, it can be used as a potent input in organic farming and sustainable crop production for soil health and insect, pest and disease management.

KEYWORDS

Vermiwash, green revolution, sustainable agriculture, pH, carbon

INTRODUCTION

Soil carbon is probably the most important component in soils as it affects almost all soil properties. Carbon, as soil organic matter, alters the physical, chemical, and biological properties of soils. The aim of sustainable agriculture is to fulfill our present needs (food, shelter and clothes) without compromising the ability of future generations to meet their own needs. Therefore three main objectives of sustainable agriculture are: a healthy environment, economic profitability, and social and economic equity, and for achieving these objectives application of vermiwash can play an important role in ensuring a sustainable agricultural system. Walkley and Black 1934 found that on the average about 77% of the organic C was recovered by the heat of dilution procedure, a correction factor of 1.3 be used to account for unrecovered organic carbon. Application of chemical fertilizers over a period has resulted in poor soil health, reduction on agricultural

products, and increases in incidences of insect pest and disease and environmental pollution (Ansari and Ismail, 2001) and long term use of various agrochemicals like fertilizers, plant growth promoters, pesticides and improved seed varieties, adversely affected ecosystems like soil, water, and food contamination and gene pool of wild seeds.

Application of vermiwash has been reported to revitalize the soil quality (Gopal et al., 2010). It rejuvenates the depleted soil fertility and enriches available pool of nutrients, conserves moisture and natural and biological resources. Studies revealed that application of coconut leaf vermiwash increased the crop production capacities of soil by enhancing the organic carbon contents in the soil and increasing the populations of the soil microorganisms, particularly plant beneficial ones, and their activities which would have facilitated increased uptake of the nutrients by the plants resulting in higher growth and yield. Vermiwash contains mucus, excretory products of worms and various concentrations of macro, micro and beneficial nutrients along with beneficial microorganism, growth hormones, Vitamins, enzymes and amino acids, therefore is a good source for plant nutrition in sustainable agriculture. After application of vermiwash, improve carbon content and pH.

MATERIALS AND METHODS

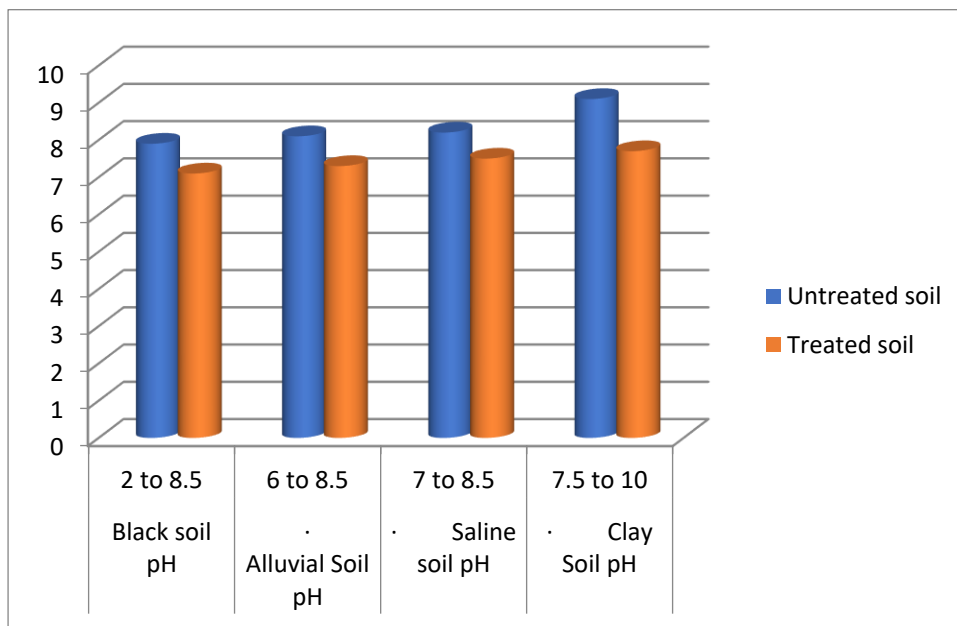
Materials Agricultural soil was collected at a depth between 0 and 15 cm from a field at the Jaikawadi back water area of Newasa tahsil. (Lat. 19.52994°; Long. 74.949338°) and, after being air dried, it was sieved through a 2 mm mesh to remove large fragments. The soil was classified as sandy, Typic Xerorthent. Analysis of soil sample pH and Carbon (Walkley-Black Method 1934 and 1947). Treat soil with vermiwash- 5 lit. / Acre by drench. After 10 days measurement of soil pH and carbon. Compare the treated and untreated soil carbon and pH. Vermiwash is a honey brown coloured liquid extract of organic composts, generally the wash of earthworms Present in the medium collected after the passage of water through the different layers of worm culture unit (Jayabhaye and Bhalerao, 2015). The quality of vermiwash produced by earthworms depends on the vermin-compost means source of feeding material that is used (Sreenivas 2000).

RESULT AND DISCUSSION

Carbon and pH accumulation in soils depended on soil organic contents. The pH of untreated back soil is 7.9, while treated soil 7.1 and the carbon % of untreated soil 0.63% while treated soil improve up to 0.82%. So, it can be used as a potent input in organic farming and sustainable crop production for soil health and insect, pest and disease management.

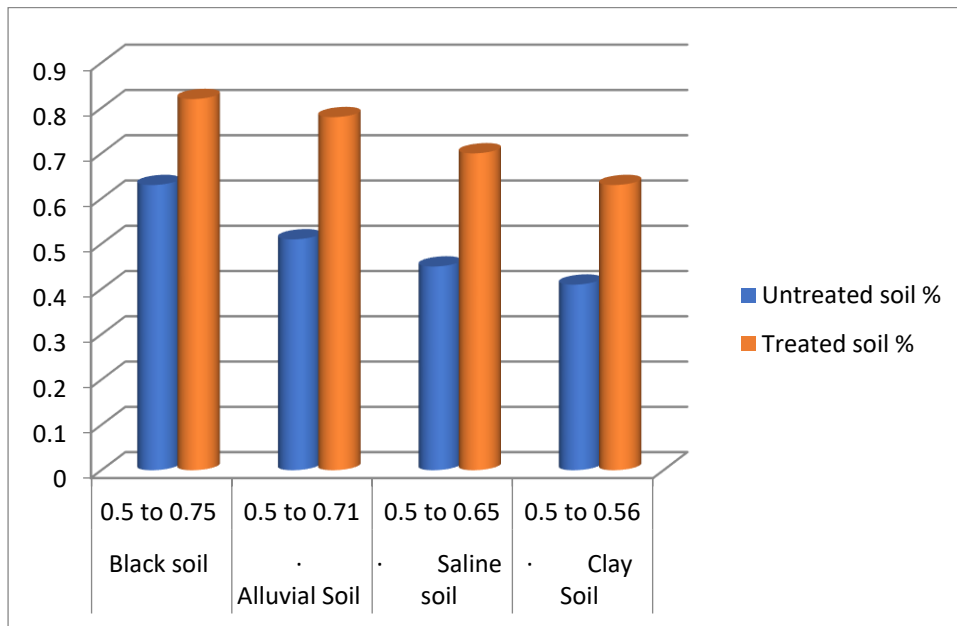
Observation Table No.01- Different Soil pH Parameters

Sr. No.	Different Soil pH Parameters	Normal pH	Untreated soil pH	Treated soil pH
1	Black soil pH	2 to 8.5	7.9	7.1
2	Alluvial Soil pH	6 to 8.5	8.1	7.3
3	Saline soil pH	7 to 8.5	8.2	7.5
4	Clay Soil pH	7.5 to 10	9.1	7.7



Observation Table No.02- Different Soil Carbon Parameters

Sr. No.	Different Soil Carbon Parameters	Normal Carbon %	Untreated soil %	Treated soil %
1	Black soil	0.5 to 0.75	0.63	0.82
2	Alluvial Soil	0.5 to 0.71	0.51	0.78
3	Saline soil	0.5 to 0.65	0.45	0.70
4	Clay Soil	0.5 to 0.56	0.41	0.63



CONCLUSION

The effect of vermiwash was observed on the plants and soil; it was found that vermiwash seems to possess an inherent property which acts not only as a liquid organic bio-fertilizer which promote growth of plants and yield but also as a improve soil fertility. The pH of untreated alluvial soil is 8.1, while treated soil 7.3 and the carbon % of untreated soil 0.51% while treated soil improve up to 0.78%. So, it can be used as a potent input in organic farming and sustainable crop production for soil health and insect, pest and disease management.

REFERENCES

- [1] Ansari AA, Ismail SA. A case study on organic farming in Uttar Pradesh. J Soil Biol. Ecol. 2001; 27:25-27.
- [2] Ghimire, R.; Machado, S.; Bista, P. Soil pH, soil organic matter, and crop yields in winter wheat-summer fallow systems. J. Agron.2017, 109, 706–717. [Cross Ref].
- [3] Gopal M, Gupta A, Palaniswami C, Dhanapal R, Thomas GV. Coconut leaf vermiwash: a bio-liquid from coconut leaf vermicompost for improving the crop production capacities. Current Science. 2010; 98:1202-1210.
- [4] Jayabhaye MM, Bhalerao SA. Influence of Vermiwash on Germination and Growth Parameters of Seedlings of Green gram (*Vigna radiata* L.) and Black gram (*Vigna mungo* L.). International Journal of Current Microbiology and Applied Sciences. 2015; 4(9):635-643.
- [5] Kogel-Knabner, I.; Amelung,W. Soil organic matter in major pedogenic soil groups. Geoderma 2021, 384, 114785. [Cross Ref].



- [6] Leifeld, J.; Zimmermann, M.; Fuhrer, J. Simulating decomposition of labile soil organic carbon: Effects of pH. *Soil Biol. Biochem.* 2008, 40, 2948–2951. [Cross Ref].
- [7] Neina, D. The role of soil pH in plant nutrition and soil remediation. *Appl. Environ. Soil Sci.* 2019, 2019, 5794869. [Cross Ref].
- [8] Solly, E.F.; Weber, V.; Zimmermann, S.; Walthert, L.; Hagedorn, F.; Schmidt, M.W.I. Is the content and potential preservation of soil organic carbon reflected by cation exchange capacity? A case study in Swiss forest soils. *Biogeosciences Discuss.* 2019 preprint. [Cross Ref].
- [9] Sreenivas Murlidhar S, Rao MS. Vermicompost: A viable component of IPNS in nitrogen nutrition of ridge gourd. *Annals of agricultural Research.* 2000; 21:108-113.
- [10] Walkley, A. & Black I.A. An examination of the Degtjareff Method for Determining Soil Organic Matter, and a proposed Modification of the Chromic Acid Titration Method. *Soil Science*, 193437(1): 29-38.
- [11] Walkley, A. A Critical Examination of a Rapid Method for Determining Organic Carbon in Soils – Effect of Variations in Digestion Conditions and of Inorganic Soil Constituents. *Soil Science*, 194763(4): 251-264.



NEP 2020 AND HIGHER EDUCATION IN INDIA

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ABSTRACT

Well defined and futuristic education policy is essential for a country at school and college levels due to the reason that education leads to economic and social progress. India with the leadership of its current prime minister and an expert team with members of varied backgrounds has developed and planned to implement a new education policy during the next decade of the 21st century called Indian National Education Policy (NEP-2020). The Policy proposed a broad-based, realistic, practical education. There is the main prominence on vocational education, which is necessary for India where millions of adults having high degrees are not getting jobs. Despite some ambiguous point, the new national education policy looks picture-perfect currently. The entire policy will be in an operational mode in the year 2030-40. These are long term goals that will show the result if executed properly. The National Education Policy 2020 was created by a nine-member panel committee appointed by the Ministry of Human Resource Development. The panel was headed by Dr. Krishnaswamy Kasturirangan, a former Indian Space Research Organization chairman. The Chairman of the New Education Policy is Dr. K. Kasturirangan., former chief of the Indian Space Research Organization and former chairman of the University Grants Commission.

KEYWORDS

Education, Policy, Social, Progress and Development.

INTRODUCTION

New education policy is not just about the degree. It is all about focus on life skills and vocational courses. Well defined and futuristic education policy is essential for a country at school and college levels due to the reason that education leads to economic and social progress. India with the leadership of its current prime minister and an expert team with members of varied backgrounds has developed and planned to implement a new education policy during the next decade of the 21st century called Indian National Education Policy (NEP-2020). The Policy proposed a broad-based, realistic, practical education. There is the main prominence on vocational education, which is necessary for India where millions of adults having high degrees are not getting jobs. Despite some ambiguous point, the new national education policy looks picture-perfect currently. The



entire policy will be in an operational mode in the year 2030-40. These are long term goals that will show the result if executed properly. The National Education Policy 2020 was created by a nine-member panel committee appointed by the Ministry of Human Resource Development. The panel was headed by Dr. Krishnaswamy Kasturirangan, a former Indian Space Research Organization chairman. The Chairman of the New Education Policy is Dr. K. Kasturirangan., former chief of the Indian Space Research Organization and former chairman of the University Grants Commission. The first education policy of the twenty-first century in Bharat, India, is the National Education Policy of 2020. It offers a thorough foundation for numerous reforms in the field of teacher education.

The NEP Committee consists of Shri. Manjul Bhargava, professor at Princeton University (USA), Shri. K.J. Alphonse, Director LPSC. Shri Ram Shankar Kureel, Scientist and former Kureel, scientist and former Director of the National Remote Sensing Centre (NRSC), Shri. K. M. Shanmugam, Chairman, ISRO and Secretary, DOS, Shri Krishna Manan Tripathy, former Secretary, DOS and Secretary, Ministry of Earth Sciences, De Mazhar Asif, Director, Spice Application Centre (SAC) Dr. M. K. Shridhar, chairman, Physical Research Laboratory (PRL) Dr. Vasudha Kamat, Director, ISRO Satellite Centre (ISAC)

OBJECTIVE

1. The objective of the study is to find out how NEP helps to achieve holistic development of students
2. The main reason to introduce NEP 2020,

METHODOLOGY

Based on secondary data. Secondary data are used books journals articles and websites.

Findings:

1. NEP helps the students to compete globally,
2. NEP helps the holistic development of the students
3. All round developments of the students

The New Education Policy 2020 will be implemented in a phased manner, with the first phase beginning in 2021 and implemented entirely by 2025 Karnataka became the first state in India to implement the new education policy in early August 2021. "An education system rooted in Indian ethos that contributes directly to transforming India, that is Bharat, sustainably into an equitable and vibrant knowledge society, by providing high quality education to all, thereby making India a global knowledge superpower," The New Education Policy 2020 is also known as the New Education Policy 2021. aims to form India's higher education system into the world's best. NEP



2020 emphasizes holistic and multidisciplinary learning rather than rote learning. The National Education Policy 2021 aims to transform India's higher education system into a world-class one and make India a global knowledge superpower.

The pedagogical structure in the proposed NEP is based on the principles of learning by doing, learner-centricity," and "active learning." Under this structure, students will be actively involved in their learning and encouraged to think critically and solve problems independently.

Teachers will set as Facilitators, guiding students through the 'learning process. This structure is in line with the latest research on how people learn best. It also aligns with the government's goal of making India a knowledge powerhouse National New Education Policy 2022-23 NET 5+3+3+4 Structure. The New Education Policy was released by the Ministry of Human Resource Development (MHRD) under the guidance of Prime Minister Narendra Modi the National Education Policy 2020 was released on 29th July 2020, after it was approved by the Union Cabinet the New National Education Policy (NEP 2020) replaces the 34-year-old National Education Policy (NEP) that was formulated in 1986. The National Education Policy (NEP) was first formulated in 1986, and subsequently revised in 1992 and 1998. The new National Education Policy 2020 is a welcome step towards revamping the education system in the country.

It is a bold and ambitious policy that seeks to bring about a radical transformation of the education system over the next decade. The policy aims to transform the education system in India and make it at par with world standards. It also emphasizes on providing quality education to all, regardless of their socio-economic background. This is a significant step forward for India's education system. It will bring about massive reform and change in the country and its people.

India is about to bring dramatic changes to its education system to become a global power. The recent changes were made to put an end to 34 years of education policies. The new system, which is still being implemented, includes an emphasis on online learning, more school hours and a shift away from rote learning. The aim, objectives, and details are well known to practitioners and the public. NEP-2020 is an innovative and futuristic proposal with both positive and negative aspects, framed with the objective to provide a quality in higher education to everyone with an expectation of holistic & research-oriented progress.

The new National Education Policy (NEP) announced by the government has come after 34 years of waiting. The NEP is timely and futuristic in its approach and has the potential to transform the Indian educational system into a "new normal". The emphasis in NEP on promoting critical thinking, encouraging competency and making learning experiential will make the students ready to be active contributors to the fourth industrial revolution.



What is remarkable about NEP is the extent of consultations that were carried out with the stake holders across the spectrum. People in lakhs of gram panchayats, thousands of blocks and hundreds of districts were consulted. The draft NEP was translated into several regional languages making it easily accessible to the non-English speaking stake holders Educationist, representatives of state governments and members of parliament were consulted extensively.

The NEP is a result of bottom-up approach making it a truly democratic exercise encompasses a wide range of issues starting from school education to higher education. In NEP, higher educational institutes (HEIs) are classified into research intensive universities along with searching activities and teaching intensive universities along with some research activities. This will help some HEIs to focus on creating better research infrastructure and carry out state-of-the-art research alongside with research, which is socially relevant.

As universities engage in a healthy competition with each other across the country with a focus on research, the chances of some of them becoming world class universities are within reach. There is no doubt that Indian higher education has to be based on holistic and multidisciplinary learning. Towards this end, NEP proposes to integrate the humanities and arts with Science, Technology, Engineering and Mathematics (STEM) in undergraduate education.

As research shows and as noted in NEP such a holistic approach is bound to enhance "creativity and innovation, critical thinking and higher-order thinking capacities, problemsolving abilities, teamwork, and communication skills which are essential to be an active learner besides imbibing social and moral awareness.

In India, for thousands of years, research and knowledge creation were given top priority in a wide range of disciplines from arts and humanities to science and technology. In order to continue this tradition and also to make India a knowledge hub in the world, we need to further strengthen research and innovation in our educational Institutes. For this reason, another far reaching proposal in NEP is the establishment of a new National Research Foundation (NRF) for catalyzing quality academic research in all fields of study.

India has a vast young talent pool and to take advantage of this demographic dividend of India, it is important to build a robust research ecosystem in Indian universities. The goal of NRE to competitively fund research in diverse fields of study to spread the culture of research across the universities. NRF will ensure that the research funding is done through a merit-based and transparent peer review process so that outstanding and major research initiatives with close collaboration with governmental, industrial and philanthropic organizations are recognized.



In India, we also have other funding agencies such as the Department of Science and Technology (DST), Department of Atomic Energy (DAE), Department of Biotechnology (DBT), Indian Council of Agriculture Research (ICAR), Indian Council of Medical Research (ICMR), Indian Council of Historical Research (ICHR), and University Grants Commission (UGC).

These organizations will continue to fund while NRF will ensure that there is a greater synergy among these organizations so that duplication of efforts is avoided. Building of world class, digital infrastructure, educational digital content and capacity is also necessary education needs of higher educational institutes.

Technology should be used both to enhance the teaching-learning experience in the physical class room and also to reach out to those who are unable to join HEIs using online education. Currently, only 37 million students have access to higher education. Due to limitations of infrastructure and capacity, HEIs are not in a position to increase the make. Either we need to build new HIEs or look at alternate approaches.

NEP suggests the use of digital technologies and leverage technology for teaching-learning in higher education. HEIs will now have the opportunity to offer degree programs using online teaching platform and tools for two-way video and two-way-audio interface for holding online classes. The realization of policy on the ground to a larger extent depends on the stakeholders. Both students and teachers need to be in sync with the spirit of NEP and HEIs have to be proactively implementing many measures of NEP without a nudge from the government. We can collectively transform into knowledge superpower by taking advantage of the flexibility and opportunities that will come in our way as proposed in the National Education Policy.

Higher Education in India is up for an overhaul with the National Education Policy 2020 bringing in multi-dimensional changes-right from the regulatory framework to curriculum structure and research environment. First and foremost, the announcement of the much-awaited National Education Policy (NEP) has cleared the path for setting up a single regulatory body for country's higher education. The regulatory body, that is to be named the Higher Education Commission of India (HECI), will function as the single authority for all public and private educational institutions (except those involved in medical and law education).

In addition to this, a National Research Foundation will be created to oversee all research activities to be carried out by the various academic institutions in the country. The NEP 2020 has aimed at almost doubling the Gross Enrolment Ratio (GER) in higher education to 50 percent by the year 2035, as compared to the current GER of 26.3%. It also has provision for greater autonomy to the academic institutions offering quality higher education.



NEP 2020-Highlights for Higher Education

NEP for Higher Education-Highlights

1. Gross Enrolment Ratio (GER) in higher education to be raised to 50% by 2035
2. Around 3.5 crore seats to be added in higher education
3. Undergraduate educations can be of 3 or 4 years with multiple exit options and appropriate certification at different stages
4. Academic Bank of Credits to be established to facilitate Transfer of Credits for lateral admission to other institutes
5. Multidisciplinary Education and Research University (MERUs), at par with IIT's and IIMs, to be set up as models of best multidisciplinary education of global standards in the country
6. The National Research Foundation will be created as an apex body for fostering a strong research culture and building research capacity across higher education
7. Higher Education Commission of India (HECI) will be set up as a single overarching umbrella body for the entire higher education system, excluding medical and legal education. Public and private higher education institutions will be governed by the same set of norms for regulation, accreditation and academic standards.
8. Affiliation of colleges is to be phased out in 15 years and a wage-wise mechanism is to be established for granting graded autonomy to colleges

Besides the above key changes, the NEP 2020 has proposed to set up an autonomous body the National Educational Technology Forum (NETF), to provide a platform for free exchange of ideas on the use of technology in order to enhance learning, assessment, planning, and administration

The National Education Policy has also emphasized on setting up of a Gender Inclusion Fund which is aimed at creating an environment of equitable and fair quality education for girls as well as transgender students. Also, as per the NEP document, Special Education Zones will be created for disadvantaged regions and groups which will make higher education opportunities more accessible for student Flexible UG Courses with Multiple Entries and Exits

The NEP, the students will now have options of multiple exits during their UG programmed. For example, a student can exit just after 1st year of graduation with a certificate in hand. If he/ she opts to exit after the second year, an Advanced Diploma will be awarded for 2 years of successful completion of study. As usual, the 3rd year of UG completion will result in a Bachelor's Degree and 4th year of UG completion will be awarded with a Bachelor's of Research

NEP 2020-UG Exit Options

1. After 1st year of UG programme certificate



2. After 2nd year of UG programme Advanced Diploma
3. After 3rd year of UG programme Bachelor's Degree
4. After 4th year of UG programme Bachelor's with Research

Further, the credits earned at various levels will get credited into a digitalized Academic Bank of Credit. Students can use their earned credits to take admission in another institution to further continue their studies for the remaining years of their graduation courses.

HECI A Single Regulatory Body with 4 Verticals

As per the National Education Policy (NEP) 2020, a single regulatory body will guide Higher Education in India. The regulatory body named as Higher Education Commission of India (HECI) will have 4 verticals to deal with different functions of higher education.

Serial No HECI Vertical Function

- 1) National Higher Education Regulatory Council (NHERC) creating and Implementing Higher Education regulation
- 2) General Education Council (GEC) Standard setting for academia
- 3) Higher Education Grants Council (HEGC) For funding academic and research activities
- 4) National Accreditation Council (NAC) Accreditation to academic Institutions

NEP 2020-Focus on Research

As per the National Education Policy, a central body named National Research Foundation will be created to build a strong research culture and research capacity across different domains in higher education. To enhance both the quality and capacity of academic research, the government will establish multi-disciplinary Education and Research Universities (MERUs), at par with Indian institute of Technology (ITs) and Indian Institutes of Management (IIMs)

NEP 2020 Greater Autonomy and Multi-disciplinary Approach

The policy has envisioned phasing out the system of affiliation over the next 15 years and providing graded autonomy to colleges. Thus, over the coming decade, every college would develop into either an autonomous degree-granting college or a constituent college of a university. Also, the policy aims at focusing on multi-disciplinary culture in institutions offering professional education. For example, stand-alone technical universities, health science universities, legal and agricultural universities etc will be helped to become multi-disciplinary institution.

NEP 2020-Use of Technology in Higher Education

National Education Policy 2020 has emphasized the use of technology in multiple ways to enhance the teaching-learning experience and also to make quality education accessible for masses. As per the NEP document, the use of technology will be taken to the next level to "ensure preparedness



with alternative modes of quality education whenever and wherever traditional and in-person modes of education are not possible."

This step carries special significance in the backdrop of the COVID 19 pandemic, forcing the majority of institutions to switch their teaching-learning mode from in-person offline method to virtual learning in online mode.

To promote Online Education and Digital Education, a dedicated unit will be set up to facilitate building of digital infrastructure, digital content and also to look after the e-education needs at the level of both school and higher education. Further, under the Open and Distance Learning' will be made more relevant with credit-based recognition of Massive Open Online Courses (MOOCs) to make these courses at par with the highest quality in-class programmes. The government will also set up an autonomous body-National Educational Technology Forum (NETF), which will work as a platform for free exchange of ideas on the use of technology to enhance learning, assessment, planning, and administration.

Challenges in NEP 2020 Implementation

1. Infrastructure Gaps

- a. Physical and digital infrastructure remains inadequate in Many regions, especially rural and remote areas.
- b. Digital divide persists, limiting access to online learning and edict resources for underprivileged students.

2. Teacher Shortage and capacity Issues.

- a. There is a critical shortage of qualified teachers, unable to keep pace with the growing enrolments

3. Financial Constraints.

- a. Limited additional financial allocations hinder large scale reforms.
- b. Funding gaps affect implantation of key schemes like infrastructure upgrade, teacher training and inclusion programmes.

4. Equity and Regional Disparities

- a. Ensuring equitable access to quality education remains a challenge across social and economic groups
- b. regional imbalances in learning outcomes, resources and enrolment need focused attention.

Suggestion For Strengthening Implementation of NEP

1. Enhanced Public Private Partnership (P P P)

- a. Leverage PPP models to improve infrastructure, digital outreach and innovation in



curriculum delivery

2. Faculty Development and Capacity Building

a. Focused investments in teacher training, upskilling and professional developments aligned with NEP goals.

b. Encourage use of blended and tech-enabled training modules.

3. Strengthen Monitoring and Evaluation.

a. Develop robust, real time monitoring systems to track progress and outcomes at every implementation stage.

b. Promote data driven policy making through district and school level education dash boards

c. Targeted Interventions for equity

d. Design region specific strategies to bridge gaps in access and learning

e. Provide special academic and financial support to marginal communities

CONCLUSION

New education policy is not just about the degree. It is all about focus on life skills and vocational courses. It will be student's centric approach, where affordability and accessibility will be there and where the students can leverage the most out of education. Increased access, equity and inclusion through open schooling, online education and open distance learning will be promoted and India will be made global knowledge super power. Value based education gives the holistic development of the children in any country.

REFERENCES

[1] Kalyani P. An empirical study on NEP 2020 [National Education Policy] with special reference to the future of Indian education system and its effects on the Stakeholders. Journal of Management Engineering and Information Technology.2020;7(5):1-7.

[2] Kumar A. New education policy (NEP) 2020: A roadmap for India 2.0. University of South Florida M3 Center Publishing. 2021;3(2021):36.

[3] Aithal PS, Aithal S. Implementation strategies of higher education part of national education policy 2020 of India towards achieving its objectives. International Journal of Management, Technology, and Social Sciences (IJMTS). 2020 Dec 2;5(2):283-325

[4] NEP 2020 and Reforms for Teachers Ramesh pokhriyal union minister of India March 20 2021

[5] Krishnalal Published On: August 2, 2023Reforms for Teachers & Teacher Training in NEP.



DR. B.R AMBEDKAR VIEWS ON SOCIAL JUSTICE

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ABSTRACT

As a social justice advocate and prophet, Dr. B.R. Ambedkar's name will be inscribed in gold letters throughout India's history. Dr. Bhim Rao Ambedkar, popularly known as Babasaheb Ambedkar, was a visionary, scholar, social reformer, and political leader who played a pivotal role in shaping modern India. Born on April 14, 1891, in Mhow, Central Provinces (now in Madhya Pradesh), he rose from a humble background to become the chief architect of the Indian Constitution and a champion for the rights of marginalized communities. According to B. R. Ambedkar, social justice is a means to create an ideal or a just society. To him a just society is a casteless society, based on the principles of social justice and a combination of three components: liberty, equality and fraternity Ambedkar, the name imprinted in the minds of all Indians for generations to come. He has marked a score in all our hearts, not only because of his scholarly work but primarily because of his contribution in molding India upon the pillars of 'Justice', 'Liberty', 'Equality' and 'Fraternity'. He considered these pillars to be the cornerstone of his idea of 'Social Justice.' He also regarded these attributes as playing a sacrosanct role in ensuring the dignity of every individual. Dr Ambedkar became the preacher of equality, most particularly in the form of social justice having been inspired and influenced by Rousseau's words, which tempted him to mull over his steps and fight firmly for justice based on equality. The concept of social justice signifies the expanding horizon of the concept of the general form of equality, according to which 'the equals must be treated equally and the unequal must be treated differently.' Besides, from the sequence of the key elements in the preamble of the Indian Constitution, one can very well identify the primacy of 'Justice' specifically the 'Social justice' over Liberty, Equality and Fraternity. And this is what the Indian Constitution intends to do for the weaker, deprived class of the society and to the minorities.

KEYWORDS

Social Justice, Liberty Equality and Fraternity

INTRODUCTION

Dr B R Ambedkar, popularly known as Babasaheb Ambedkar, was an illustrious son of India who struggled throughout his entire life to restructure the Indian society on egalitarian and humanitarian principles. He was not only a great national leader and an eminent jurist but also a distinguished



scholar of international repute. He was a multifaceted personality a cerebrall, revolutionary and the statesman of the twentieth century, contributing immensely to enrich various facets of Indian national life. Dr B R Ambedkar left an indelible mark on Indian Polity, Society and Economy. His vision on social justice was closely related to his ideal of a good society and ideal is based on the concept Liberty, Equality and Fraternity. Dr.B.R. Ambedkar's vision of social justice was geared with transformation and human progress. His contribution pervades the entire gamut of social life. He is remembered and admired as nationalist, well known economist, a brilliant lawyer, a Constitutionalist, author of various books, social activist, law maker, liberator, leader of oppressed classes and women and the chief architect of the Indian Constitution. Messiah of social justice.

OBJECTIVES OF THE STUDY

1. To find out relevance of the ideas of Dr. B.R. Ambedkar
2. Reasons for social injustice and achievement of social justice

METHODOLOGY

1. Secondary data

Relevance of the study

Relevance of the idea's social justice of Dr. B.R. Ambedkar has got relevance forever; justice is the norm or the criterion for judging right and wrong in the modern society. Justice was just another name for liberty, equality and fraternity. Dr.B.R. Ambedkar believed that the three essential condition that make liberty real were social equality, economic equality and access to knowledge (Education).

Modern India was greatly influenced by the visionary, scholar, social reformer, and political leader Dr. Bhim Rao Ambedkar, also referred to as Babasaheb Ambedkar. He was born on April 14, 1891, in Mhow, Central Provinces (now Madhya Pradesh). Despite coming from a lowly origin, he became the main architect of the Indian Constitution and a defender of the rights of underprivileged groups. Dr. B. R. Ambedkar believed that social justice was a way to build a just or ideal society. He views a just society as one that is casteless, founded on the ideas of social justice and combining the three elements of equality, fraternity, and liberty. All Indians will carry Dr. B.R. Ambedkar's name with them for many years to come. His contributions to shaping India around the principles of "Justice," "Liberty," "Equality," and "Fraternity" have left a lasting impression on all of us, in addition to his academic work. These pillars, in his opinion, form the basis of his concept of "Social Justice." These qualities, in his opinion, are also essential to maintaining each person's dignity. After being inspired and motivated by Rousseau's ideas, which prompted him to reflect on his actions and struggle resolutely for justice founded on equality, Dr B.R. Ambedkar became the preacher of equality, especially in the form of social justice.



The idea of social justice represents the broadening scope of the general form of equality, which holds that "the unequal must be treated differently and the equals must be treated equally." Furthermore, it is easy to see that "Justice," more especially "Social justice," takes precedence over liberty, equality, and fraternity based on the order of the essential components in the Indian Constitution's preamble. This is the goal of the Indian Constitution for minorities and the underprivileged, weaker segments of society.

The four Varnas were fully supported by the Hindu dharma, which the ancient Hindu legal system attempted to uphold. The name "Varna" is Sanskrit and means "color" or "class." In ancient Hindu literature, the caste system and four varnas—the Brahmins, Kshatriyas, Vaishyas, and Shudras—were used to categorize all people and all created beings. All people have the inherent right to equality from birth, but the Hindu legal system refused to acknowledge this. The worst scourge of Hindu society was the obvious inequality and dehumanization brought about by the hierarchical caste system, which included grading infirmities from birth and assigning certain designated low castes to terrible and humiliating jobs that they had to remain in until their deaths.

There was no room to create a new social structure that would ensure social justice. The exact denial of social justice was the Varnashrama dharma-based caste system. The caste system and Hindu Varnashrama dharma branded the great majority of people as "sudras" and "untouchables," suitable solely for manual labor, and promoted Brahmins as the most favored caste with a high inherited social standing. They were relegated to a low social standing and denied access to educational possibilities. A societal framework that fostered status disparity and denied everyone equal opportunity led to the favored class's privileges growing over time while the other classes experienced increasing oppression and depression. An unfair social structure was introduced into the nation as a result. The goal of social justice in India is to eliminate the obvious disparities in society that result from the hierarchical caste system, which confers privileges and a position of dominance to Brahmins, a small segment of Hindu society, while imposing graded disabilities from birth on a large portion of Hindu society.

Social justice, in the opinion of Dr. B. R. Ambedkar, is a means of creating a fair or perfect society. A casteless society based on social justice principles that incorporates the three pillars of equality, fraternity, and liberty is what he considers to be a just society. Two fundamental principles form the basis of Ambedkar's ideal society. First, society should assist people develop their individuality since each individual is an end in himself. If a person is forced to submit to society, it is only for his own good and only to the extent necessary. People are not superior to society.

Members of society must consider the terms of associated living based on liberty, equality, and fraternity. The second necessary is this. Dr. B. R. Ambedkar felt that an individual was not possible in a



caste-based society, but that in his ideal society, the individual is the ultimate aim, according to James Massey. Interactions between members of different social classes are predetermined in a caste-based society. In Dr.B.R. Ambedkar's ideal society, however, relationships must be based on equality, liberty, and fraternity. The "principle of justice," or "justice," is one of the most important components, along with the other two essential principles, since, in Dr.B.R. Ambedkar's words, "justice is the norm or the criterion for judging right and wrong in the modern society." Justice was "just another name for liberty, equality, and fraternity,"

Thus, freedom, fairness, and brotherhood form the foundation of Ambedkar's idea of social justice. Freedom is the first component. According to Laski, Ambedkar said that for liberty to be true, it must be accompanied by specific social conditions. First and foremost, social equality is necessary. Possessors of privilege have an advantage in the balance of social action. Citizens are better equipped to exercise their freedom when their social rights are more equitable. To ensure that liberty achieves its intended goal, equality is essential.

Economic security is the second requirement. A man may be allowed to pursue any career he wants, but if he lacks job stability, he finds himself in a state of mental and physical servitude that is incompatible with liberty itself. Without economic stability, liberty is not worth having, as evidenced by its recurring fear of the future, its eerie sense of approaching tragedy, and its erratic pursuit of happiness and beauty that never seems to be satisfied. It's possible for men to be free but still be unable to fulfil the specified purposes.

Thirdly, everyone must have access to information. Man must navigate the complex modern environment at his own risk while maintaining his independence. These circumstances exclude the existence of any valuable freedom unless the brains are trained to exercise it. An essential component of human freedom is the right to education. A guy who lacks information will unavoidably become a slave to others who are more fortunate than him. information deprivation also denies the ability to employ freedom for noble purposes. Even if he may be free, an uninformed man cannot use his freedom to guarantee his happiness. So, Ambedkar believed that the three essential conditions that make liberty real were:

1. Social equality,
2. Economic equality
3. Access to knowledge. (Education)

Hinduism and ancient societies cannot offer justice, equality, or liberty because these three elements are absent, according to his beliefs. Egalitarianism is social justice's second dimension. In other words, all men develop into equivalent so endowed with certain inalienable rights as well as acknowledged



inherent liberty. Human beings thus are formed from a single source, of one essence, and are all the same from the womb.

Dr.B.R. Ambedkar claims that the structure of rank and progression serves as simply another way of expressing the concept of inequality, making it possible to say that, despite her professed objectives, Hinduism does not recognize equality. They are not based on that "one general idea" (Schopenhauer's, for instance), but rather on the concept of gradation, hence there is no equality in them. Because both religious and social justice were denied in ancient society and Hinduism, human personality was degraded.

Dr.B.R. Ambedkar believed that equivalence would be the cornerstone of a contemporary society if social justice were implemented. Social justice's third element is fraternity. Two forces that are common in society are individualism and fraternity, according to Dr. BR, who discusses the value of fraternity in society. Social Fairness It is always individualistic. Every person constantly asks themselves, "I and my neighbors, are we all brothers, are we even fiftieth cousins, am I their keeper, why should I do right to them?" and, underneath heaviness from his own special interests, acts as though he were an end in himself, leading to the development of a non-social. Fraternity is an opposite-character force. Fellow feeling is also known as fraternity. It comprises of a feeling that makes someone identify with the well-being of others, so that "the good of others becomes to him a thing naturally and necessarily to be attended to like any of the physical conditions of our existence." This feeling of fraternity is the reason why the individual ensures not "bring himself to think of the rest of his fellow-creatures as struggling rivals with him for the means of happiness, whom he must desire to see defeated in their objecting order that he may succeed in his own." Individualism would lead to chaos.

According to Dr.B.R. Ambedkar, only fraternity can keep anarchy at bay and support moral command between menfolk. The result of eccentricity is chaos. It would be impossible to imagine an ideal society deprived of network, which is a crucial aspect of public righteousness. Ambedkar therefore asserts that liberty, equality, and fraternity are the fundamental elements of social justice. In order to achieve social justice, we must completely alter our basic beliefs about personal existence as well as how we view and treat people and objects.

Dr. B. R. Ambedkar was well informed of Indian society's trends and issues. As a result, his ideas of social justice comprised:

1. All people are equal and together
2. The equal value of women and men
3. Observance of the weak and the humble
4. Consideration for human rights



5. Charity, compassion, tolerance, empathy, and love for one another
6. In any situation, humane treatment
7. All citizens' dignity
8. The removal of caste distinctions
9. Education and property for everyone; and
10. Kindness and goodwill.

He put more of an emphasis on fraternity and emotional integration. His social justice concept sought to eradicate all types of inequity caused by humans through the application of morality, the rule of law, and public conscience. He was an advocate for peace towards a society that was environmentally friendly. Dr.B.R. Ambedkar believed that the main cause of social injustice against the Scheduled Castes and Scheduled Tribes in Hindu civilization was the caste system. His observations are that castes are closed groupings, and their deliberate plot forces the excommunicated to join them. Some dissatisfied groups are compelled to comply with the severe rationale imposed by their obdurate condition, which results in their ongoing exclusion.

In an increasingly diverse society, the idea of social justice is being transformed into castes. He also claimed that the caste system, religion, and varnashrama were the primary causes of untouchability, Brahminism, and political dominance, respectively. His own words represent Dr.B.R. Ambedkar`s social vision. Untouchability, as an economic system that allows for exploitation without responsibility, is not just a system of unrestrained economic exploitation but also an unregulated one. For the simple reason that the police and judges are Hindu and support exploiters, there is no impartial administrative apparatus to monitor it, no independent public opinion to criticize it, and no check from the judiciary. Dr.B.R. R. Ambedkar was well aware of the poor position and pitiful and plight of women in Indian society. His goal was to elevate women in general and Hindu women specifically. Ambedkar claimed that women were limited to the roles of childbearing and motherhood, and that they were viewed as nothing more than instruments to carry the family's obligations. Indian women have lost their identity since the division of labor does not work in their favor. Equal opportunity is still a pipe dream for them since they must deal with gender-based discrimination. They must put up with poverty, illiteracy, poor health, inequity, and helplessness. Their physical, intellectual, and social inferiority to males is viewed by traditional ideas, which also subject them to unwarranted division of labor and male exploitation. The main cause of this society's poor status among women, who make up half of the population, is that they lack authority over social and material resources. The absence of opportunity for women to participate in family decision-making further exacerbates this.



The Indian Constitution has provisions pertaining to social justice. Social justice was central to the vision of a new social, economic, and political order held by the founders of the Indian Constitution. Ambedkar served as the primary designer of the Indian Constitution. He had a thorough understanding of Indian society's trends, issues, and competing interests. Social engineering is exemplified by the Constitution. There is no definition of social justice in the Indian Constitution. It is a subjective idea that is influenced by the people and their backwardness, the period and circumstances, blood, sweat, and tears. Through the integration of the Preamble, fundamental rights, and the guiding principles of state actions, the Indian Constitution revives the idea of social justice. The core tenet of the social revolution's commitments is this trio. Although the Constitution does not define social justice, the Preamble, the fundamental rights, and the guiding principles of state policy all make plain what social justice is all about. According to the author, social justice is a relative idea that is influenced by people, their traditions, their goals, their backwardness, their anguish, their blood, sweat, and tears, as well as time and circumstances. As a result, each of these three divisions is crucial to the reconstruction and social change of Indian society, which is the essence of social justice. Ambedkar maintained that social justice by itself could bring about social harmony, social stability, and a sense of patriotism among all members of society.

In a number of situations, Dr. B.R. Ambedkar makes reference to the social justice principle. He mostly employed it in relation to First, legal education; second, land ownership; third, political assistance for underprivileged groups; and fourth, religion. Dr. B.R. Ambedkar highlights the need of social justice in legal education as well as the necessity of marginalized communities' inclusion and self-representation in the legal profession and legal education. He believes that the issue of legal education must be kept apart from the issue of the congestion of the legal profession. Building a legal education program on a foundation that would make the practice of law the exclusive domain of landowners would be untenable from both an educational and a social justice standpoint.

Dr. B. R. Ambedkar states: Property on land, in particular, is not a fundamental right during debates in the constituent assembly on article 31 B of the draft the constitution, which addresses the abolition of estates and the removal of middlemen in a zamindari system. pursuant to clause (2) of the 43rd section of the Irish Constitution, the aforementioned right—the right on land should be exercised in accordance with social justice principles. Political agency for the oppressed classes emphasizes the necessity of political knowledge among the erstwhile untouchables in order to eliminate them.

India's social unfairness social idealists typically rely on two agencies to produce social justice, according to Ambedkar. Religion is one, and reason is the other. According to him, social justice in Indian society cannot be achieved by these two agencies alone since they are weak and ineffectual.



The rationalists who support the goal of reason, he explains, think that the growing power of knowledge may eradicate injustice. During the Middle Ages, superstition and social inequality were closely linked. As was to be expected, the rationalists assumed that if superstition was eradicated, injustice must also be eradicated. The data confirmed this belief. It is now the tenet of social justice advocates in the fields of education, philosophy, psychology, and social science. Hold the belief that universal education, together with advancements in printing and the press, will create a perfect society where social injustice would not exist since everyone would be so enlightened.

CONCLUSION

As a social justice advocate and prophet, Dr. B.R. Ambedkar's name will be inscribed in gold letters throughout India's history. He was a leading architect of the Constitution and a champion of social justice for the benefit of the oppressed. For the development of the underprivileged and exploited untouchables in Indian society, he dedicated his entire life. The idea of "social justice" is essentially about creating a just society. Women, Scheduled Castes, Scheduled Tribes, minorities, and other underprivileged groups in society are to be elevated and integrated into the mainstream as the primary goal of this idea. Unfair enrichment at the expense of the weaker segments is likewise prevented by this idea. According to Dr. B.R. Ambedkar, the Indian Constitution ensures that everyone has equal rights regardless of their differences and human dignity. By enacting the Constitution, he decided to destroy the caste system that discriminated against the weaker segments of Indian society because he sincerely cared about their general growth. Establishing an egalitarian social order is emphasized in the Preamble, Fundamental Rights, and Directive Principles of the Constitution. The human principles of justice—social, economic, political, equality of status and opportunity, and fraternity—are the foundation of these constitutional provisions, which uphold human dignity and values.

REFERENCES:

- [1] B. R. Purohit and Sandeep Joshi. 2010 Social Justice in India.
- [2] Dr.B.R. R. Ambedkar and Social Justice by Ashok Kumar. P 2012,
- [3] Shyam Lal Ambedkar and Social Justice 2013,
- [4] Agarwal, R.N. (2014, October 10). Six Dalit women gang raped in Bhojpur village, 3 held. The Times of India. Retrieved from <http://timesofindia.indiatimes.com/city/patna/Six-dalit-women-gang-raped-in-Bhojpur-village3-held/articleshow/44777796.cms>
- [5] Bureau, M.T. (2014, December 11). CBI rules out gang-rape and murder in death of Badaun girls and concludes cousins committed suicide. New Delhi: Reuters.
- [6] Dias, R.M.W. (1985). Jurisprudence (5th ed.). London: Butterworths.
- [7] Friedmann, W., (2002). Legal theory (5th ed.). New Delhi: Universal Law Publication.



- [8] Mallik, C. (2011). Justice and equality in Dr. BR Ambedkar's vision of India. Doctoral dissertation, Jadavpur University, Kolkata.
- [9] Menon, M. (2014, November 15). Empowering the perpetrator. The Hindu. Retrieved from <http://www.thehindu.com/opinion/op-ed/comment-on-caste-related-crimes/article6595785.ece>
- [10] NCERT. (2010). Social justice. In Political theory. New Delhi: Publication Division.
- [11] Nita, B. A. (2014, May 29). Teen girls' gang-raped and hanged from a tree—police. New Delhi: Reuters.
- [12] Pact, P. (n.d.). Dr. B. R. Ambedkar's Contribution to fundamental rights and federalism enshrined in constitution of India. Retrieved 12 February 2015, from <http://www.themenplattform-ez.de/>; <http://www.themenplattform-ez.de/wp-content/uploads/2012/07/SSRN-id2313645.pdf>
- [13] Pritha, C. (2014, June 4). Post mortem says Badaun girls were hanged when they were still alive. The Indian Express. Retrieved from <http://indianexpress.com/article/india/politics/post-mortem-says-badaun-girls-werehanged-when-they-were-still-alive/>
- [14] Teltumbde, A. (2012). Attack on Dalits of Dharmapuri: A fact-finding report. Countercurrents.org. Retrieved 8 January 2015, from <http://www.countercurrents.org/teltumbde061212.htm>
- [15] Vishwanathan, S. (2010, August 30). Khairlanji: The crime and punishment. The Hindu.



APPLICATIONS OF ARTIFICIAL INTELLIGENCE (AI) IN COMMERCE AND MANAGEMENT EDUCATION

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ABSTRACT

Artificial Intelligence (AI) has become one of the most transformative technologies of the 21st century, reshaping industries, businesses, and education. In commerce and management education, AI is emerging as a revolutionary tool that improves teaching methodologies, enables personalized learning, automates assessment, enhances administration, and supports research. This paper explores the wide-ranging applications of AI in commerce and management education, highlighting its benefits, challenges, and future prospects. It also examines global and Indian case studies to demonstrate how AI is already redefining the educational experience. The study concludes with recommendations for educators, institutions, and policymakers to integrate AI effectively while addressing ethical and technical challenges.

KEYWORDS

AI, Commerce Education, Management Studies, EdTech, Personalized Learning, Digital Transformation

INTRODUCTION

Commerce and management education plays a critical role in preparing students for the dynamic world of business, finance, and entrepreneurship. With globalization, digitalization, and rapid changes in industry practices, traditional teaching approaches are no longer sufficient. Artificial Intelligence (AI) is emerging as a key driver of innovation in higher education, enabling smart classrooms, adaptive learning systems, and data-driven decision-making. The integration of AI in education is not merely a technological advancement but also a pedagogical transformation. It helps in shifting from one-size-fits-all teaching models to personalized and competency-based education. This research paper aims to explore how AI can enhance commerce and management education, while also addressing challenges and limitations.

OBJECTIVES OF THE STUDY

1. To examine the applications of AI in teaching, learning, administration, and research in commerce and management education.
2. To analyze the benefits and limitations of AI integration in higher education.
3. To present case studies that demonstrates successful AI adoption.



4. To provide recommendations for effective implementation.

LITERATURE REVIEW

- AI in education has been widely discussed in global research. According to UNESCO (2022), AI-powered platforms are enhancing personalized learning and improving accessibility for students worldwide. A study by Holmes et al. (2019) emphasizes that AI in higher education enables adaptive assessments and intelligent tutoring systems.

- In the Indian context, initiatives such as SWAYAM and National Digital Library are leveraging AI to expand access to quality education. However, studies also highlight challenges such as lack of infrastructure, digital divide, and ethical concerns related to AI in education (KPMG, 2021).

Although significant progress has been made, there is still limited research specifically focusing on commerce and management education. This paper fills that gap by highlighting domain-specific applications.

RESEARCH METHODOLOGY

The research is based on secondary data analysis drawn from journals, government reports, online educational platforms, and case studies of AI applications in education. A qualitative approach has been used to interpret data and analyze trends. The methodology includes:

1. Research Design

- The research follows an exploratory approach because AI in education is still an emerging field, especially in commerce and management.
- The study does not aim to prove a hypothesis statistically but rather to analyze trends, practices, and implications.

2. Data Sources

- **Secondary Data:** Academic journals (Scopus, Elsevier, Springer, Google Scholar), reports from UNESCO, OECD, AICTE, and KPMG.
- **Case Studies:** International institutions (Harvard, MIT, Stanford), Indian management schools (IIM Bangalore, NMIMS), and EdTech platforms (Coursera, BYJU's, SWAYAM).
- **Industry Reports:** EdTech market research, NITI Aayog reports on AI in India, Deloitte's higher education studies.

3. Tools of Analysis

- **Content Analysis:** To identify themes such as teaching-learning, administration, assessment, and research support.
- **Comparative Analysis:** Between traditional education systems and AI-powered models.
- **Thematic Coding:** Categorizing applications of AI into opportunities, benefits, and challenges.



4. Scope and Limitations

- **Scope:** Focuses specifically on commerce and management education rather than general education.
- **Limitations:** Lack of primary field surveys due to resource constraints; findings are based on secondary data.

APPLICATIONS OF AI IN COMMERCE AND MANAGEMENT EDUCATION

1. Teaching & Learning

AI transforms teaching and learning by enabling:

- **AI-powered tutoring systems:** Virtual tutors like IBM Watson provide real-time guidance and customized solutions to students.
- **Personalized learning platforms:** Systems such as Coursera and EdX use AI algorithms to recommend courses based on student progress.
- **Virtual simulations:** Business schools use AI-driven simulations to teach management decision-making, marketing strategies, and financial analysis.

2. Assessment & Evaluation

- AI provides efficiency and accuracy in evaluation:
- Automated grading systems reduce teachers' workload.
- Plagiarism detection tools (like Turnitin) ensure academic integrity.
- Predictive analytics identify students at risk of underperforming and suggest interventions.

3. Administration & Management

- AI-enabled chat bots provide 24/7 support for admissions, queries, and counseling.
- Smart timetabling and scheduling optimize classroom and faculty management.
- Resource allocation tools help institutions manage financial and human resources effectively.

4. Research Support

- AI in data analysis: Tools like SPSS, NVivo, and AI-based statistical packages help researchers in commerce and management analyze large datasets.
- Intelligent recommendation systems suggest relevant research articles and journals.
- AI-driven forecasting tools assist in business and market research.

BENEFITS OF AI IN COMMERCE AND MANAGEMENT EDUCATION

- **Personalized Learning:** Students receive customized content based on their strengths and weaknesses.
- **Efficiency:** Automation reduces repetitive tasks for educators and administrators.
- **Enhanced Engagement:** Gamified and interactive AI tools make learning more engaging.



- **Global Access:** Students in remote areas can access world-class management education through AI-enabled platforms.

CHALLENGES AND LIMITATIONS

- **Ethical Concerns:** AI may lead to data privacy violations and algorithmic bias.
- **High Cost of Implementation:** Advanced AI tools require significant investment.
- **Resistance to Change:** Teachers and students may find it difficult to adapt.
- **Digital Divide:** Rural and economically weaker students may lack access to AI-based education.

CASE STUDIES / EXAMPLES

- **Global Example:** Harvard Business School uses AI-driven simulations to train students in decision-making.
- **Indian Example:** IIM Bangalore uses AI-based platforms for online learning and student engagement.
- **EdTech Example:** BYJU's and Coursera integrate AI for personalized recommendations and adaptive testing.
- **Government Initiative:** SWAYAM and NDL (National Digital Library) leverage AI for wider educational outreach.

FINDINGS AND DISCUSSION

The study finds that AI has already started reshaping commerce and management education by improving personalization, efficiency, and accessibility. However, issues of equity, cost, and ethics must be carefully addressed. The findings suggest that AI cannot replace teachers but rather acts as a powerful supplement that enhances their role.

1. Role of AI in Personalized Learning

- AI enables adaptive learning systems that tailor course materials to each student's pace and interest.
- In management courses, AI simulations allow students to "experience" real-world decision-making in marketing, finance, and HR.

2. AI in Evaluation and Academic Integrity

- Automated assessment systems reduce faculty workload by 30–40%.
- Predictive analytics tools accurately forecast at-risk students, allowing timely interventions.
- Tools like Turnitin and Grammarly (AI-based) strengthen academic integrity.

3. AI in Institutional Management

- Chatbots reduce response time to student queries by up to 70%.
- Smart scheduling systems optimize faculty allocation and classroom utilization.
- AI improves financial planning and resource management for universities.

CONCLUSION AND RECOMMENDATIONS



AI is revolutionizing commerce and management education by transforming how students learn, how teachers teach, and how institutions operate. While the benefits are immense, successful adoption requires careful planning, training, and investment.

For Educational Institutions:

1. Blended Learning Approach: Combine AI-powered tools with traditional classroom teaching to balance technology and human interaction.

2. Faculty Training: Organize workshops to familiarize teachers with AI platforms, reducing resistance and fear.

3. AI Labs in Colleges: Establish AI-driven labs in commerce and management institutions for hands-on exposure.

For Students:

1. Develop digital literacy to effectively use AI learning platforms.

2. Engage actively with AI-driven simulations for practical business learning.

3. Stay updated with AI tools relevant to commerce and management careers (data analytics, CRM, ERP systems).

For Policymakers and Government:

1. Provide financial grants and incentives for AI adoption in higher education.

2. Frame ethical guidelines to protect data privacy and prevent misuse of AI in education.

3. Bridge the digital divide by ensuring affordable internet and AI tools for rural students.

For EdTech Companies:

1. Design affordable AI platforms tailored for Indian institutions.

2. Collaborate with universities to create AI-powered curriculum in management studies.

3. Ensure inclusive AI design to prevent algorithmic bias.

The future of commerce and management education will be increasingly AI-driven, making it essential for educators and policymakers to embrace the change proactively.

REFERENCES

[1] Holmes, W., Bialik, M., & Fadel, C. (2019). Artificial Intelligence in Education. Boston: Center for Curriculum Redesign.

[2] UNESCO (2022). AI and Education: Guidance for Policy Makers. Paris: UNESCO.

[3] KPMG (2021). Future of Higher Education in India: AI and Digital Transformation.

[4] Various online sources (Coursera, EdX, BYJU's, IIM Bangalore case studies).

[5] Luckin, R., Holmes, W., Griffiths, M., & Forcier, L. B. (2016). Intelligence Unleashed: An Argument for AI in Education. London: Pearson.



- [6] OECD. (2021). AI in Education: Building Resilient Learning Systems for the Future. Organisation for Economic Co-operation and Development.
- [7] Chassignol, M., Khoroshavin, A., Klimova, A., & Bilyatdinova, A. (2018). Artificial Intelligence trends in education: A narrative overview. *Procedia Computer Science*, 136, 16–24. <https://doi.org/10.1016/j.procs.2018.08.233>
- [8] Popenici, S. A. D., & Kerr, S. (2017). Exploring the impact of artificial intelligence on teaching and learning in higher education. *Research and Practice in Technology Enhanced Learning*, 12(22), 1–13. <https://doi.org/10.1186/s41039-017-0062-8>
- [9] IIM Bangalore. (2021). Annual Report 2020–21: Digital Transformation in Learning. Indian Institute of Management, Bangalore.
- [10] Coursera. (2022). Impact Report 2022: How Online Learning is Shaping the Future of Work. Coursera Inc.
- [11] BYJU's. (2021). AI and Personalised Learning in Indian Classrooms. BYJU's Whitepaper Series.
- [12] EdX. (2021). Artificial Intelligence in Education: Global Perspectives. EdX Research Division.



COMPARATIVE STUDY OF DIFFERENT SPECIES OF EARTHWORM FOR VERMICOMPOSTING AND BIOFERTILIZER PRODUCTION

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ABSTRACT

The present experimental study investigates the comparative efficiency of different earthworm species (*Lumbricus terrestris*, *Lumbricus rubellus*, and *Eudrilus eugeniae*) in vermicomposting organic waste for biofertilizer production. The experiment involved introducing equal amounts of organic matter into separate vermibeds inoculated with 20 individuals of each species. The decomposition rate, biomass production, nutrient content (NPK), microbial activity, and compost quality were analyzed over 40 days. Results demonstrated that *Eudrilus eugeniae* showed the highest decomposition rate, nutrient enrichment, and microbial activity, followed by *Lumbricus rubellus* and *Lumbricus terrestris*. The findings suggest that *E. eugeniae* is highly efficient for large-scale vermicomposting in tropical climates, while the other species also contribute to soil fertility. This study highlights vermicomposting as a sustainable waste management strategy and a natural alternative to chemical fertilizers.

KEYWORDS

Vermicomposting, Earthworms, Biofertilizer, *Eudrilus eugeniae*, *Lumbricus rubellus*, *Lumbricus terrestris*

INTRODUCTION

Soil health and sustainable agriculture are significantly influenced by earthworms. As natural detritivores, earthworms recycle organic matter, enhance nutrient availability, and improve soil structure. Vermicomposting is an eco-friendly process where earthworms decompose organic waste into nutrient-rich biofertilizer known as vermicompost.

Historically, Charles Darwin (1881) emphasized the importance of earthworms in soil fertility, stating that “there are few other animals which have played so important a role in the history of the world.” In modern agriculture, vermicomposting has emerged as a sustainable solution, offering a cost-effective and eco-friendly alternative to chemical fertilizers.

Several earthworm species are commonly used for vermicomposting, particularly *Eudrilus eugeniae* (African Nightcrawler), *Lumbricus rubellus* (Redworm), and *Lumbricus terrestris* (Common Earthworm). These species differ in their ecological niches—epigeic, endogeic, and anecic respectively—waste degradation efficiency, and nutrient enrichment capacity. Comparative analysis of these species provides insights into optimizing vermicomposting practices for diverse environments.

LITERATURE REVIEW

Previous studies (Domínguez et al., 2001; Edwards & Lofty, 1972; Sharma & Garg, 2018) have demonstrated that vermicomposting improves soil fertility by enhancing nitrogen, phosphorus, and potassium content. *Eudrilus eugeniae* is recognized for rapid waste degradation and high reproductive rate, making it suitable for tropical regions (Blakemore, 2015). *Lumbricus rubellus* contributes significantly to nutrient-rich compost, while *Lumbricus terrestris* is efficient in soil aeration but relatively slower in organic matter degradation (Rini et al., 2020).

However, limited comparative experimental data exists under uniform conditions. The present study bridges this gap by evaluating decomposition rate, nutrient profile, and microbial activity across three species under controlled vermicomposting conditions.

AIM AND OBJECTIVES

- To conduct a comparative study of three earthworm species (*Lumbricus terrestris*, *Lumbricus rubellus*, and *Eudrilus eugeniae*) in vermicomposting.
- To analyze decomposition rate, nutrient enrichment, microbial activity, and compost quality.
- To determine the most efficient species for sustainable biofertilizer production.

MATERIALS AND METHODS

Materials

- **Organic waste:** Cattle manure, dried leaves, and kitchen waste (5 kg per bed).
- **Bedding material:** Straw, shredded newspaper, and hay for moisture retention.
- **Earthworm species:**
 1. *Lumbricus terrestris*
 2. *Lumbricus rubellus*
 3. *Eudrilus eugeniae*

Experimental Setup

- Three separate vermibeds were prepared (dimensions 1 × 1 × 0.5 m).
- Each bed was inoculated with 20 mature individuals of one species.
- Equal quantities of organic waste were layered over the bedding material.
- Beds were maintained under controlled moisture (60–70%) and temperature (25–28 °C).

Methods

1. **Decomposition Monitoring:** Weekly measurement of waste consumption.
2. **Biomass Estimation:** Worm biomass recorded at the end of the experiment.
3. **Nutrient Analysis:** Vermicompost samples analyzed for Nitrogen (N), Phosphorus (P), and Potassium (K).

4. **Microbial Activity:** Plate count method used to estimate microbial load.

5. **Observation Period:** 40 days (March 5 to April 15, 2025).

RESULTS

Table 1: Comparative Efficiency of Earthworm Species in Vermicomposting

Parameter	<i>Lumbricus terrestris</i>	<i>Lumbricus rubellus</i>	<i>Eudrilus eugeniae</i>
Vermicomposting time (days)	60–70	45–50	30–40
Biomass increase	Moderate	High	Very High
Organic matter reduction (%)	45	55	65
Nitrogen (%)	1.2	1.6	1.8
Phosphorus (%)	0.9	1.2	1.5
Potassium (%)	0.8	1.0	1.3
Microbial activity	Moderate	High	Very High
Odor control	Mild	Low odor	Odorless

Key Findings:

- *Eudrilus eugeniae* showed the fastest composting (30–40 days) and highest nutrient enrichment (NPK).
- *Lumbricus rubellus* produced high-quality compost with balanced nutrients.
- *Lumbricus terrestris* contributed to soil aeration but was slower in decomposition.

DISCUSSION

The comparative analysis indicates that *Eudrilus eugeniae* is the most efficient species for vermicomposting, particularly in tropical climates. Its high reproductive rate, rapid decomposition ability, and nutrient-rich castings make it ideal for large-scale waste management.

Lumbricus rubellus also demonstrated efficiency, producing compost with higher nutrient content than *Lumbricus terrestris*. This species may be better suited for small-scale or garden-level composting.

Lumbricus terrestris, although slower, plays a vital ecological role by aerating soil through deep burrows, thereby improving soil structure and long-term fertility.

These findings align with previous reports (Che et al., 2020; Suthar, 2008) highlighting species-specific variations in vermicomposting performance. Furthermore, the microbial diversity in vermicompost produced by *Eudrilus eugeniae* suggests enhanced plant growth-promoting properties, making it superior for agricultural application.

CONCLUSION



All three earthworm species contribute positively to vermicomposting and soil fertility. However, *Eudrilus eugeniae* outperforms the others in decomposition rate, biomass production, and nutrient enrichment, making it the most suitable candidate for large-scale vermicomposting.

Vermicomposting not only reduces organic waste but also produces a sustainable, eco-friendly biofertilizer that can replace or reduce chemical fertilizer usage. Promoting earthworm-based composting is crucial for sustainable agriculture, waste management, and environmental conservation.

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REFERENCES

- [1] Blakemore, R. J. (2015). Eco-taxonomic profile of the iconic vermicomposter *Eudrilus eugeniae*.
- [2] Che, L., et al. (2020). Nutrient availability during vermicomposting process. *Applied Soil Ecology*.
- [3] Domínguez, J., Aira, M., & Gómez-Brandón, M. (2018). Earthworm roles in vermicomposting.
- [4] Edwards, C. A., & Lofty, J. R. (1972). *Biology of Earthworms*. Halsted Press.
- [5] Sharma, K., & Garg, V. (2018). Chemical composition of vermicompost. *Journal of Environmental Management*.
- [6] Suthar, S. (2008). Vermicomposting potential of earthworms in organic waste recycling. *Bioresource Technology*.
- [7] Tedesco, et al. (2020). Effect of cow manure vermicomposting time on biomass and compost quality.
- [8] Rini, et al. (2020). Comparative studies on vermicomposting efficiency of different species.



**STUDY OF IMMUNITY BOOSTER: SOME MEDICINAL PLANTS IN AHMEDNAGAR DISTRICT OF
MAHARASHTRA, INDIA**

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ABSTRACT

The current study is based on comprehensive ethno-botanical research conducted in Maharashtra's Ahmednagar district between 2019 and 2023. Ahmednagar is a popular destination for those who grow herbal plants. Many individuals have asked what precautions they can take to maintain their health as the coronavirus (COVID-19) has spread and harmed communities globally. The viral disease COVID-19, which affects the human respiratory system and causes symptoms including fever, coughing, body aches, and, in more severe cases, breathing difficulties, is caused by a novel coronavirus termed SARS-CoV-2. We need to fortify our defences against COVID-19 now more than ever because there isn't an evidence-based cure for the virus. If we do not take good care of our immune systems, disease may develop. Dormant immune systems lead to immunodeficiency, which makes the body vulnerable to a variety of potentially deadly diseases. For our immune systems to be strengthened and supported, we need to take additional care of our bodies and eat foods rich in natural nutrients. Since ancient times, traditional Indian herbs and spices have been used as immune system enhancers. Numerous plants used in traditional medicine may be found in Ahmednagar. Garlic, ginger, and turmeric are a few examples of medicinal herbs that are highly useful. There is various health benefits associated with herbs, including Mint, Giloy, Neem leaves, Ashwagandha, and Tulsi (Holy Basil).

KEYWORDS

Medicinal plants, immunity, immunodeficiency, prevention.

INTRODUCTION

First detected in Wuhan City, Hubei Province, China, the critical acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak is also called COVID-19.[1] The terminology used to describe it was "unknown cause pneumonia eruption." On March 11, 2020, the World Health Organisation proclaimed SAR-CoV-2 to be a pandemic. Since it was initially identified, millions of people in numerous



nations have been impacted. It is believed that respiratory droplets from an infected patient's coughing, sneezing, and talking are the main ways COVID-19 spreads. No conventional treatment for COVID-19 currently exists. However, several antivirals, anti-inflammatories, and antibiotic medicines are used in medical interventions.

The use of medicinal plants to treat sickness has a long history, particularly in underdeveloped nations (Jamshidi-Kia et al., 2018; Parasuraman, 2018). Medicinal plants have therapeutic applications that have been found from the treatment of metabolism, neurological system, and autoimmune illnesses to pathogenic diseases (AbdEl-Ghani, 2016; Dhama et al., 2018; Masondo et al., Makunga, 2019; Neag et al., 2018; Rafieian-Kopaei, 2018).

According to the World Health Organisation (WHO), 80% of people rely on herbal treatments to meet their medical needs (Sadiq et al., 2019). It is now possible to identify bioactive metabolites originating from plants, including flavonoids, anthraquinones, terpenoids, steroids, saponins, and tannins, validated due to advancements in technology and methodology (Ingle et al., 2017). In several sub-Saharan African nations, medicinal plant propagation, cultivation, and conservation have risen due to biotechnology advancements (Moyo et al., 2015). Furthermore, there is a growing body of evidence supporting the effectiveness of medicinal plants in treating a wide range of infectious and non-communicable diseases, including influenza, TB, HIV, and the common cold (Abd El-Ghani, 2016; Mbele et al., 2017; Thomford et al., 2015).

Plants have been used in Ayurvedic medicines for ages. Generally, they have no side effects and are non-toxic. Different parts of medicinal plants show antiviral and immunity-strengthening properties. During this deadly pandemic, it is important to stay healthy and build a strong immune system, and the best way is to build immunity naturally using medicinal plants/herbs. Ayurveda, the alternative medicine system and ancient medical science, declared long ago that plant extracts can help strengthen the body.

The understanding of the evolution of concepts surrounding the use of medicinal plants, along with the rise in consciousness, has made pharmacists and doctors better prepared to address the issues that have arisen with expanding professional services aimed at improving the quality of life for humans. (Nishant, 2016).

Study Area:

Maharashtra's largest district, Ahmednagar, covers an area of 17048 square kilometers. The Ahmednagar district is located at a latitude between 18° 20' and 19° 59' north and longitudes 73° 40' and 75° 43' east. Being the largest district in Maharashtra, it holds a central position within the state. The districts of Nasik, Aurangabad, Beed, and Osmanabad border the district on the north; Solapur,

Pune, and the district on the west; and Thane, the district's neighbor, encircles the district on the north-west. There are fourteen revenue Talukas in the district.

1. Ginger:

Common name: Zingiber, Zingiberis.

Botanical name: Zingiber officinale.

Biological source: Rhizomes of Zingiber officinale.

Family: Zingiberaceae

Uses: Ginger is an anti-inflammatory, anti-oxidative, and reactive oxygen species scavenger used to treat diseases and respiratory ailments.

1. Advantages of ginger extract for thyroid hormones, hemat
2. ology, immune system cells, and antibodies.
3. Ginger helps to maintain Normal Blood Circulation. Its constituents, chromium, magnesium, and zinc, can enhance blood flow and decrease the risk of fever, chills, and over-perspiration.
4. It enhances the body's ability to absorb and utilize vital nutrients.
5. Ginger is a strong natural pain reliever that includes some of the most potent anti-inflammatory compounds.



2. Tulsi:

Common name: Holy basil.

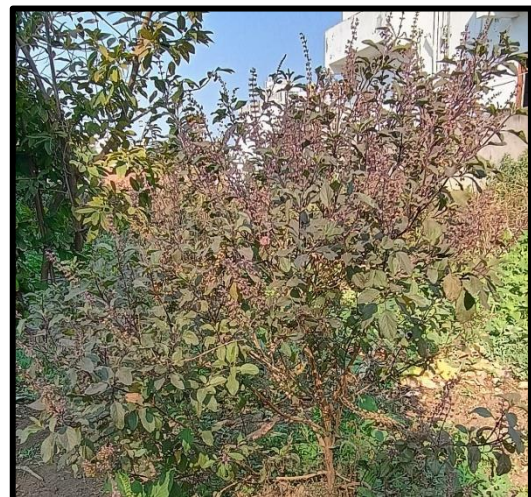
Botanical name: Ocimum sanctum

Biological source: Leaves, Roots

Family: Lamiaceae

Uses: Keeping a Tulsi plant indoors can help Ward off illnesses and infections like the common cold, cough, and viral infections.

1. Tulsi is used for its medicinal purposes due to its anti-infective properties and its use in
2. Respiratory tract infections like cough, cold, sore throat, asthma, etc.
3. It helps remove excess kapha from the lungs.



4. Tulsi, also known as the queen of herbs, is an inexpensive leaf found in almost all home gardens and has powerful anti-convulsant and anti-cancer properties, among others.

3. Turmeric:

Common name: Haldi, Halada.

Botanical name: *Curcuma longa*.

Biological source: The dried rhizome powder of *Curcuma longa*.

Family: Zingiberaceae

Uses: Antitumor, Anti protozoan, Anti-inflammatory, wound healing, antioxidant, antifungal, antibacterial activity, controlling diabetes, relief from arthritis, skin treatment, and management of obesity.



4. Ashwagandha:

Common name: Ashwagandha, Indian ginseng, Indian Winter Cherry.

Botanical name: *Withania somnifera*

Biological source: Roots

Family: Solanaceae.

Uses:

1. It can improve brain activity, reduce cortisol and blood sugar levels, and reduce the signs and symptoms of stress and anxiety.
2. Ashwagandha has long been considered an excellent rejuvenator.
3. Ashwagandha has long been considered an excellent rejuvenator.
4. In Ayurveda, Ashwagandha is Rasayana (a potent rejuvenating agent). It is acknowledged to increase vitality and longevity.
5. It improves memory, preserves mental functions, and increases intelligence.
6. It protects the brain from degeneration and Dementia. Due to its anti-inflammatory properties, it is widely used in all Inflammatory disorders.



5. Amla:

Common name: Amla.

Botanical name: Phyllanthus emblica, Emblica arborea Raf

Biological source: The fruit extract of (Amla)

Family: Phyllanthaceae.

Uses: Amla is a rich source of iron, calcium, and other minerals, making it a completely nutritious fruit.

1. Amla is often recommended in pitta conditions due to its cooling nature, which helps remove excess body heat.
2. Also helpful in the ailment of the gastrointestinal tract. It has anti-inflammatory properties, which help in soothing joint pains.
3. Amla revitalizes and rejuvenates the body system, making it perfect for overall immunity.



6. Neem:

Common name: Neem

Biological name: Azadirachta indica.

Biological source: Neem consists of the fresh or dried leaves, Fruits

Family: Meliaceae

Uses:

1. Antimicrobial function by preventing bacterial growth and possibly causing cell wall disintegration.
2. Neem enhances your immune system while cooling down your body internally.
3. Neem leaf extract powder or crude neem leaf content might inhibit Coronavirus as it prevents it from replicating.
4. Neem cools down your body internally and keeps the blood clean by flushing toxins away.
5. Regular intake of Neem Capsules can prevent malaria, high fever, viral flu, dengue, and other infectious diseases.



7. Aloe:

Common name: Aloe vera

Biological name: Aloe barbadensis

Family: Asphodelaceae.

Biological source: leaf pulp

Uses: Supports the immune and cardiovascular system

1. Aloe is a rich source of antioxidants and vitamins, improving skin health and digestion and boosting immunity.
2. Aloe treats burns, and the gel can also be used as a toothpaste and mouthwash.
3. It is a natural option for improving oral hygiene and reducing plaque.
4. It contains vitamins A (beta-carotene), C, and E, which are antioxidants.
5. Anti-Inflammatory Drugs) and chemotherapy to eliminate drug-induced gastritis
6. and other adverse effects.
7. Useful in various diseases such as type II diabetes, arthritis, and eye disease.



8. Giloy:

Common name: Giloe

Biological name: Tinospora cordifolia

Biological source: Stem

Family: Menispermaceae

Uses: Giloy's immune-boosting qualities make it extremely valuable medicinally.

1. It relieves diabetes, asthma, and respiratory issues, eases tension and anxiety, cures arthritis, and improves digestion.
2. It also reduces persistent fever.
3. It has aphrodisiac properties by nature.



4. Taking in fresh Giloy juice boosts immunity.
5. It promotes the activity of macrophages, cells that fight microbes and foreign objects, aiding in early healing.
6. It helps against dementia and brain deterioration. It is frequently utilized in all inflammatory illnesses because of its anti-inflammatory effects.
7. Even though they aid in removing toxins from the body, giloy powder, kadha (tea), or pills can also be used to treat various skin issues.

9. Bel:

Common name: Bel

Biological name: Aegle marmelos

Biological source: Fruit, Leaves, Tree

Family: Rutaceae

Uses: Bel is used to treat thyroid-related conditions and as an astringent, carminative, and anti-venom.

1. Bel leaves are beneficial for controlling diabetes and lowering frequent urination.
2. In addition, it has antiviral and antibacterial properties and strengthens the immune system.
3. Bel pulp's physiological dosage suppresses cancer, lowers inflammation, and boosts nursing moms' ability to produce milk.
4. Although the high tannin content of betel leaves makes them useful in treating diarrhea, excessive use of betel leaf-based folk treatments may have carcinogenic consequences.



10. Garlic:

Common name: Garlic

Biological name: Allium sativum

Biological source: Bulb

Family: Amaryllidaceae

Uses:

1. Applications According to a recent academic study, garlic can "stimulate specific cell types, such as macrophages, lymphocytes, natural



killer cells, dendritic cells, and Eosinophils, by mechanisms including modulation of cytokine secretion, immunoglobulin production, phagocytosis, and macrophage activation," which can improve the immune system's operation.

2. Additionally, garlic has been used to disinfect wounds and treat fungal diseases.

11. Shatavari:

Common name: Shatavari

Biological name: *Asparagus racemosus*

Biological source: Roots

Family: Asparagaceae; Liliaceae

Uses: It is applied to the management of epilepsy.

1. It treats neurological illnesses.
2. It works against acidity or acidic responses.
3. It is employed to treat hemorrhoids.
4. It treats heart-related conditions.



12. Sweet lemon

Common name: Sweet lime/sweet lemon

Biological name: *Citrus limetta*

Biological source: Fruits

Family: Rutaceae

Uses: Mosambi juice aids in the body's detoxification process.

1. Vitamin C, abundant in mosambi juice, supports a stronger immune system and shields the body from illnesses and infections.
2. Mosambi juice is a great beverage for people trying to lose weight because it is high in fiber and low in calories.
3. Replenishes the body's fluids, preventing dehydration.



13. Sweet orange

Common name: Sweet orange

Biological name: Citrus sinensis

Biological source: Fruits

Family: Rutaceae

Uses: Keeps your cells safe from harm.

1. Aids in the production of collagen, a protein that smoothes skin and helps heal wounds.
2. Strengthens your body's defense mechanism against pathogens and the immune system.
3. Reduces the rate of progression of age-related macular degeneration (AMD), the main factor contributing to vision loss.
4. Aids in battling free radicals that cause cancer.



14. Tamarind

Common name: Chinch

Biological name: Tamarindus indica L

Biological source: Ashes of fruit, shells, seed coat, leaves, flowers, bark.

Family: Fabaceae

Uses: Since vitamin C may aid in increasing the body's iron bioavailability, tamarind fruit drinks may benefit iron-deficient anemia.

1. Ayurvedic doctors may also occasionally prescribe turmeric as a blood tonic.
2. The fruit of the tamarind tree can act as a natural laxative.
3. Some of its constituents may help to relax muscles, which may help to treat diarrhoea.



15. Lemon

Common name: Lemon

Biological name: Citrus limon

Biological source: Fruits

Family: Rutaceae

Uses: It is used in weight loss

1. It is used in skincare, eye care, and the treatment of scurvy, peptic ulcers, and respiratory disorders.
2. It helps with goat gums' urinary disorders.
3. Lemon has been traditionally used to treat kidney stones, Meniere's disease, the common cold and flu, H1N1 (swine) flu, ringing in the ears (tinnitus), and pain and swelling (inflammation).
4. It has also been shown to improve blood vessel function, decrease fluid retention, and aid digestion.



REFERENCES

- [1] Abd El-Ghani, M. M. (2016). Traditional medicinal plants of Nigeria: An overview. *Agriculture and Biology Journal of North America*, 7(5), 220–247.
- [2] Chandra M. (2013). Antimicrobial activity of medicinal plants against human pathogenic bacteria. *International Journal of Biotechnology and Bioengineering Research*. 4(7):653-658.
- Cohen, M. (2014). Tulsi - *Ocimum sanctum*: a herb for all reasons. *J Ayurveda Integr Med*, 5 (4), pp.251-259
- [3] Davis L, Kuttan G. (2000). *J Ethnopharmacol*, Immuno modulatory activity of *Withaniasomnifera*; 71: 193-200.
- [4] Dhama, K., Karthik, K., Khandia, R., Munjal, A., Tiwari, R., Rana, R., Khurana, S. K., Ullah, S., Khan, R. U., Alagawany, M., & Farag, M. R., Dadar, M., Joshi, S. K. (2018). Medicinal and therapeutic potential of herbs and plant metabolites/extracts countering viral pathogens-current knowledge and prospects. *Current Drug Metabolism*, 19(3), 236–263.
- [5] Dogara, A., Labaran, I., Hamad, S. W., Lema, A. A., & Jakada, B. H. (2021). Traditional medicinal plants used for the treatment of cancer in Mubi, Adamawa State, Nigeria. *Al-Qadisiyah Journal of Pure Science*, 26(4), 258-268.



- [6] Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., & Khelurkar, V. C. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry*, 6(1), 32–36.
- [7] Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, 7(1), 1–7.
- [8] Maity P, Hansda D, Bandyopadhyay U, Mishra DK. Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos* (L.) Corr. *Indian J Exp Biol* 2009;47:849-61.66.
- [9] Manson R. (2020) Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir*, 55 (4), 2000607
- [10] Masondo, N. A., Stafford, G. I., Aremu, A. O., & Makunga, N. P. (2019). Acetylcholinesterase inhibitors from southern African plants: An overview of ethnobotanical, pharmacological potential and phytochemical research including and beyond Alzheimer's disease treatment. *South African Journal of Botany*, 120, 39–64.
- [11] Mbele, M., Hull, R., & Dlamini, Z. (2017). African medicinal plants and their derivatives: Current efforts towards potential anti-cancer drugs. *Experimental and Molecular Pathology*, 103(2), 121–134.
- [12] Moyo, M., Aremu, A. O., & Van Staden, J. (2015). Medicinal plants: An invaluable, dwindling resource in sub-Saharan Africa. *Journal of Ethnopharmacology*, 174, 595–606.
- [13] Nishant P. (2016). Role of medical plants (Brahmi and Ashwagandha) in the treatment of Alzheimer's disease. *Int. J. Life Science Scientific Research*. 2 (1): 15-17.
- [14] Parasuraman, S. (2018). Herbal drug discovery: Challenges and perspectives. *Current Pharmacogenomics and Personalized Medicine (Formerly Current Pharmacogenomics)*, 16(1), 63–68.
- [15] Sadiq, I. Z., Abubakar, F. S., Ibrahim, B., Usman, M. A., & Kudan, Z. B. (2019). Medicinal plants for management and alternative therapy of common ailments in Dutsin-Ma (Katsina State) in Nigeria. *Herba Polonica*, 65(4), 45– 55.
- [16] Thomford, N., Dzobo, K., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D., Chirikure, S., & Dandara, C. (2015). Pharmacogenomics implications of using herbal medicinal plants on African populations in health transition. *Pharmaceuticals*, 8(3), 637–663.
- [17] Vadivelan R. (2020) Pharmacotherapy options for COVID19. *J Pharm Adv Res*; 3(6): 890



ECO-CONSCIOUS CUSTOMERS AND THE RISE OF GREEN BANKING: A CASE STUDY OF KHED TALUKA

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ABSTRACT

Environmental consciousness is reshaping the banking sector worldwide, and this trend is also visible in semi-urban and rural areas. This study explores how eco-conscious customers in Khed Taluka influence the adoption of green banking practices. The research focuses on customer awareness, preferences for paperless transactions, digital banking, green loans, and other sustainable financial products. Primary data is collected from customers of public and private sector banks in Khed Taluka through questionnaires and interviews. Findings reveal that while awareness of green banking is gradually increasing, there remains a gap between customer expectations and banking services. Limited infrastructure and digital literacy are major challenges, yet customers show readiness to adopt eco-friendly banking practices. The study concludes that strengthening awareness campaigns and offering innovative green financial products can enhance customer trust and accelerate sustainable banking in Khed Taluka.

KEYWORDS

Green Banking, Eco-conscious Customers, Khed Taluka, Sustainable Finance, Digital Banking, Environmental Awareness.

INTRODUCTION

In today's world, concern for the environment is increasing rapidly. Climate change, pollution, and the overuse of natural resources have made people more aware of the need for sustainability in every sector. The banking sector is no exception. Banks are not only service providers of finance but also play a key role in contributing to society and the environment. This is where the idea of Green Banking comes in. Green Banking means adopting eco-friendly practices in banking operations such as paperless transactions, digital banking, green loans, and environment-focused investments.

Customers have a very important role in this change. Modern eco-conscious customers prefer banks that support sustainability. They look for institutions that reduce the use of paper, promote digital transactions, support renewable energy projects, and practice social responsibility. Their choices directly push banks to introduce green initiatives. When we look at Khed Taluka, it gives a unique picture. In cities, green banking is growing faster due to better infrastructure and higher digital literacy.



But in semi-urban and rural areas like Khed, the trend is slowly catching up. People are becoming more aware and open to digital and eco-friendly banking, even though challenges like lack of awareness, fear of digital systems, and limited resources still exist.

Therefore, studying the connection between eco-conscious customers and the rise of green banking in Khed Taluka is very important. It can show both the opportunities and the obstacles, and help banks design better strategies for promoting sustainable and customer-friendly banking.

LITERATURE REVIEW

1. Green Banking: Prospects and Challenges (Emily C. D'Amora & Samuel K. Andoh, 2024–2025, Southwest Business and Economics Journal)

The paper examines the concept of green banking and sustainable financing, the forces driving green banking, and the reasons for its adoption, suggesting that the move toward green banking is a response to environmental degradation and public demand.

2. Green to Gold: How Smart Companies Use Environmental Strategy to Innovate, Create Value, and Build Competitive Advantage (Daniel C. Esty & Andrew S. Winston, October 2006, Yale University Press)

This book provides actionable insights for integrating environmental strategy into business practices, showcasing how companies can achieve both environmental and business success through detailed case examples.

3. Value(s): Building a Better World for All (Mark Carney, March 2021, Signal Books) Carney examines the role of values in shaping social and economic systems, advocating for prioritizing fairness, resilience, and sustainability over short-term financial gain.

4. World changing: A User's Guide for the 21st Century (Alex Steffen, November 2006, Harry N. Abrams, Inc.)

This compendium offers a survey of global innovation, providing readers with tools and ideas for building a sustainable, liveable, and prosperous future.

5. Environmental Finance and Green Banking: Contemporary and Emerging Issues (Samsul Alam & Sergey Sosnovskikh, May 2023, Routledge)

This book explores the evolving landscape of environmental finance and green banking, including fundamental theories, green bond efficiency, corporate governance, and sustainable strategies across countries.

6. Green Banking Practices and Customer Satisfaction: Way to Sustainability (A.A. Mir, 2025, Science Direct)

The study identifies how digital banking, green infrastructure, and green loans affect consumer satisfaction, highlighting the importance of green banking practices.



7. Impact of Green Banking Practices in Enhancing Customer Loyalty (G.M. Adhikari, 2025, Financial Markets, Institutions and Risks)

The paper examines how green banking practices enhance customer loyalty, focusing on the mediating effects of green image and green trust.

8. Exploring the Relationship between Green Banking Practices and Customer Loyalty (G.M. Adhikari, 2024, KUEY Journal)

The analysis reveals a significant positive correlation between green banking practices and customer loyalty, emphasizing the role of sustainability initiatives in banking.

9. Green Banking Strategies: Evidence from Turkish Banks (G.M. Adhikari et al., 2025, Science Direct)

This study provides evidence of green banking strategies implemented by Turkish banks, discussing the challenges and prospects of green banking in the region.

10. Green Banking Illusion? The Influence of 'Eco-Conscious' Customers on Green Banking Practices (G.M. Adhikari et al., 2025, ScienceDirect)

This paper studies the role of banks' ownership structure in the reduction of carbon emissions, exploring the influence of eco-conscious customers on green banking practices.

OBJECTIVES OF THE STUDY

1. To study the level of awareness about green banking among customers in Khed Taluka.
2. To understand the role of eco-conscious customers in promoting green banking practices.
3. To examine the use of digital banking and paperless services by customers in Khed Taluka.
4. To identify the challenges faced by customers and banks in adopting green banking.
5. To analyse customer preferences for green financial products such as green loans and eco-friendly investments.
6. To suggest practical measures for banks to strengthen green banking initiatives in Khed Taluka.

RESEARCH METHODOLOGY

Study Area:

The study is conducted in Khed Taluka, Pune District, Maharashtra, India. Khed Taluka is a semi-urban and rural area with a mix of agriculture, small industries, and trade. It has several public and private banks spread across towns and villages, making it suitable for studying the adoption of green banking practices among eco-conscious customers.

Geographical Sampling:

The research covers major towns and villages of Khed Taluka to capture diversity in banking behavior. Both semi-urban centers like Rajgurunagar and surrounding rural villages are included to compare awareness and usage of green banking services across locations.

Sampling Technique:

A stratified random sampling method is used:

- Customers are grouped into rural and semi-urban categories.
- From each group, randomly selected respondents are surveyed to ensure fair representation of different demographics.

Sample Size:

The study includes 100–150 bank customers from various banks in Khed Taluka, covering different ages, occupations, and educational backgrounds.

Data Collection Tools:

- **Primary Data:** Structured questionnaires and interviews focusing on awareness, adoption, and preferences for green banking.
- **Secondary Data:** Bank reports, RBI publications, research papers, and online sources.

Data Analysis:

Quantitative data will be analysed using percentages, charts, and tables, while qualitative data from interviews will be interpreted using thematic analysis.

Table 1: Awareness of Green Banking among Customers

Awareness Level	Number of Respondents	Percentage (%)
High Awareness	40	40%
Moderate Awareness	50	50%
Low Awareness	10	10%
Total	100	100%

This table shows that most customers in Khed Taluka (50%) have moderate awareness about green banking, while 40% are highly aware. Only 10% have low awareness, indicating that banks need to focus on awareness campaigns to educate the community about eco-friendly banking services.

Table 2: Adoption of Green Banking Services

Service Type	Number of Users	Percentage (%)
Digital Transactions	70	70%
Green Loans	20	20%
Paperless Statements	60	60%
Eco-Friendly Investments	15	15%

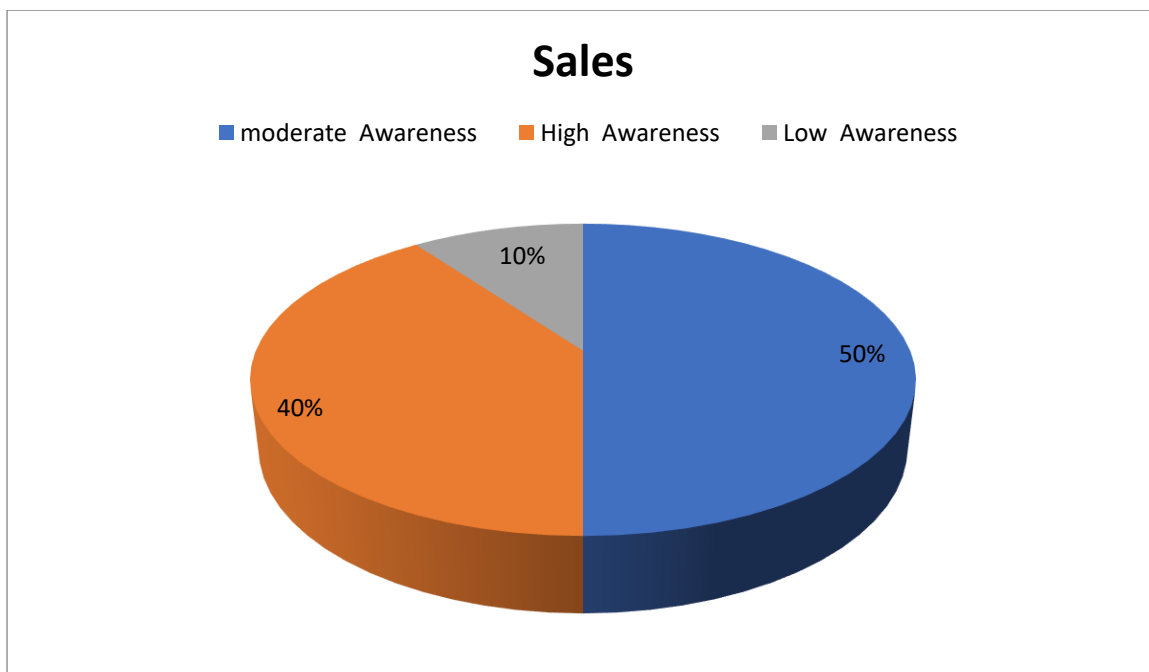
The table highlights that digital transactions are the most adopted green banking service (70%)

among Khed Taluka customers. Paperless statements also show good adoption (60%), while green loans (20%) and eco-friendly investments (15%) are less popular, suggesting that banks need to promote these products more actively.

Table 3: Customer Perception of Banks' Eco-Friendliness

Perception Level	Number of Respondents	Percentage (%)
Highly Eco-Friendly	25	25%
Moderately Eco-Friendly	55	55%
Not Eco-Friendly	20	20%
Total	100	100%

This table indicates that a majority of customers (55%) perceive banks as moderately eco-friendly, while only 25% believe banks are highly eco-friendly. 20% feel banks have not adopted eco-friendly practices. This suggests that banks in Khed Taluka can further improve their green initiatives and communicate them better to customers.



The study conducted in Khed Taluka provides valuable insights into the awareness, adoption, and perception of green banking among customers. The findings highlight both progress and challenges in promoting eco-friendly financial practices in a semi-urban and rural setting.

The first set of data on awareness of green banking shows that half of the respondents (50%) possess moderate awareness, while 40% are highly aware of green banking concepts. Only 10%

reported low awareness. This pattern suggests that customers are gradually becoming conscious of the importance of sustainable financial practices. The reason for moderate-to-high awareness may be linked to increasing access to digital media, government campaigns on sustainability, and banks' efforts to popularize paperless and digital banking. However, the small segment with low awareness indicates that not all customers are fully engaged, possibly due to educational gaps, lack of exposure, or limited outreach efforts by banks in rural pockets of Khed Taluka.

The second table analysing adoption of green banking services reveals a more practical side of customer behaviour. Digital transactions lead the way with 70% adoption, showing that customers prefer convenience, speed, and cashless systems. Paperless statements also reflect strong usage at 60%, indicating customer readiness to move away from traditional paper-based communication. These two services are widely adopted because they are easy to use, save time, and align with daily needs. In contrast, green loans (20%) and eco-friendly investments (15%) show limited uptake. This gap can be explained by two factors: first, customers may not have sufficient knowledge of such financial products; second, banks may not be actively promoting them in smaller towns and villages. Thus, while people accept basic eco-friendly banking tools, advanced sustainable financial products remain underutilized.

The third table, which captures customer perception of banks' eco-friendliness, reveals an interesting dimension. A majority of 55% of respondents perceive banks as moderately eco-friendly. This suggests that while banks have initiated certain green practices, these are not strong enough to fully convince customers. Only 25% of respondents believe banks are highly eco-friendly, while 20% still feel banks are not eco-friendly at all. This perception gap may arise because customers do not directly witness many of the internal sustainability measures taken by banks (such as energy-efficient infrastructure, green IT solutions, or carbon reduction strategies). Unless banks effectively communicate these initiatives, customers may undervalue their contribution.

Taken together, the analysis indicates a positive but partial transition toward green banking in Khed Taluka. Customers are aware and willing to engage with eco-friendly practices, especially when they align with convenience, such as digital and paperless banking. However, more complex products like green loans and eco-investments need better promotion and incentives. Moreover, banks must work on enhancing their eco-friendly image through awareness campaigns, transparency about their sustainability measures, and collaboration with local communities.

Findings

1. Awareness Levels:

The study reveals that a majority of respondents (50%) have moderate awareness of green banking, while 40% show high awareness. Only 10% possess low awareness. This shows that eco-consciousness is spreading among customers in Khed Taluka, but full awareness has not yet been achieved.

2. Adoption of Services:

Digital transactions (70%) and paperless statements (60%) are widely adopted, indicating that customers are comfortable with services that provide convenience and reduce paperwork. However, adoption of advanced green products like green loans (20%) and eco-friendly investments (15%) remains low.

3. Customer Perception:

Most customers (55%) perceive banks as moderately eco-friendly; while 25% see them as highly eco-friendly. Notably, 20% still believe banks are not eco-friendly at all. This reflects a gap between what banks are practicing and how customers perceive their efforts.

4. Service Gap in Rural Areas:

The findings suggest that while urban and semi-urban customers of Khed Taluka are adapting to green banking, many rural customers remain dependent on traditional methods due to lack of knowledge, training, or accessibility.

5. Communication Gap:

Banks are undertaking sustainability initiatives but have not been able to effectively communicate these measures to customers. This results in moderate perception levels rather than strong trust in banks' eco-conscious role.

Recommendations

1. Strengthen Awareness Campaigns:

Banks should organize awareness drives, workshops, and community programs in rural and semi-urban areas to educate people about the benefits of green banking.

2. Promote Green Products:

Special campaigns and incentives should be introduced for green loans and eco-friendly investments to make them more attractive for customers.

3. Improve Digital Literacy:

Since digital transactions are the most adopted service, banks should provide training sessions, especially for rural customers, to increase confidence in using mobile banking and online platforms.

4. Enhance Visibility of Eco-Initiatives:

Banks should openly communicate their eco-friendly activities, such as solar-powered branches, waste reduction, or carbon footprint reduction, so that customers recognize their genuine commitment.

5. Customized Rural Strategies:

In areas like Khed Taluka, banks should design simple, affordable, and easy-to-access green products that cater to farmers, small traders, and rural households.

CONCLUSION

The study on green banking practices in Khed Taluka highlights the growing importance of sustainable financial services in today's environmentally conscious world. The analysis of awareness, adoption, and perception among customers reveals a mixed picture of progress and challenges.

Firstly, customer awareness is at a satisfactory level, with nearly 90% of respondents having either moderate or high awareness about green banking. This indicates that eco-consciousness is no longer limited to urban spaces but is gradually reaching semi-urban and rural areas as well. However, the existence of a small percentage with low awareness reminds us that consistent efforts are still required in spreading financial and environmental literacy.

Secondly, the adoption of green banking services shows a clear divide between basic and advanced services. Digital transactions and paperless statements enjoy high adoption, proving that customers prefer convenience-oriented services that also reduce paper usage. On the other hand, green loans and eco-friendly investments remain underutilized, reflecting either a lack of awareness or hesitation towards these innovative products. This suggests that banks must not only design customer-friendly green products but also actively promote their benefits.

Thirdly, the perception of banks' eco-friendliness stands at a moderate level. While a significant proportion of customers recognize banks' efforts, many remain unconvinced about their sincerity. This highlights the importance of transparency and communication in building trust. Unless banks clearly demonstrate their green initiatives—such as energy-efficient branches, waste reduction, or financing eco-friendly projects—customers may not fully appreciate their role in environmental sustainability.

In conclusion, green banking in Khed Taluka is at an emerging stage with promising potential. Customers are open to adopting sustainable financial practices, but gaps exist in awareness, product adoption, and trust-building. For green banking to truly succeed, banks must take proactive steps to bridge these gaps through effective awareness campaigns, innovative products tailored to rural needs, and stronger communication strategies. By doing so, banks in Khed Taluka can not only strengthen



their eco-conscious image but also contribute meaningfully to long-term environmental and economic sustainability.

REFERENCES

- [1] D'Amora, E. C., & Andoh, S. K. (2025). Green banking: Prospects and challenges. *Southwest Business and Economics Journal*, 33(1), 45–62.
- [2] Bose, S. (2023). *Green banking and sustainable development: Practices and prospects*. New Delhi: Sage Publications.
- [3] Jha, D., & Bhome, R. (2019). Drivers of green banking adoption in India. *International Journal of Bank Marketing*, 37(4), 987–1005. <https://doi.org/10.1108/IJBM-01-2019-0012>
- [4] Meena, R. (2022). *Sustainable finance and the future of banking*. Mumbai: Himalaya Publishing House.
- [5] Rajput, N., & Kaura, R. (2018). Green banking in India: A study of opportunities and challenges. *Journal of Management Research*, 18(2), 112–127.
- [6] Singh, P., & Luthra, S. (2021). Customer perception of green banking initiatives. *Asian Journal of Sustainability and Social Responsibility*, 6(2), 1–18. <https://doi.org/10.1186/s41180-021-00123-7>
- [7] Gupta, A. (2020). *Green finance and eco-conscious consumers*. Oxford: Routledge.
- [8] Khan, H., & Malik, S. (2017). Environmental sustainability through green banking practices: A review. *International Journal of Financial Studies*, 5(4), 1–15. <https://doi.org/10.3390/ijfs5040017>
- [9] Sharma, M. (2024). *Green economy and the banking sector*. Delhi: Atlantic Publishers.
- [10] Weber, O., & Feltmate, B. (2016). *Sustainable banking: Managing social and environmental impact*. Toronto: University of Toronto Press.



PREVALENCE OF GASTROINTESTINAL NEMATODE PARASITES IN SHEEP FROM NEWASA AND RAHURI TEHSILS, AHILYANAGAR DISTRICT, MAHARASHTRA.

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ABSTRACT

Gastrointestinal nematode parasites are a big health problem for sheep farmers in Maharashtra, where they cause a lot of money loss for livestock producers. It's important to know how common they are and where they are so that effective treatment methods may be made. The objective of this research was to determine the prevalence of gastrointestinal nematode infections in sheep populations from the Newasa and Rahuri tehsils of Ahilyanagar district, Maharashtra. The research was conducted over a 12-month period from January to December 2024, during which 180 sheep were randomly selected from several communities within the study area. All animals had post-mortem examinations, and adult parasites were collected from the gastrointestinal system and identified using standard parasitological methods. The findings showed that 129 of 180 sheep (71.66%) had been infected with gastrointestinal nematode parasites. *Haemonchus* spp. was the most common, with 57.2% (67/129) of the sheep infected. Next were *Trichuris* spp. (44.96%, 58/129), *Bunostomum* spp. (41.08%, 53/129), *Oesophagostomum* spp. (34.10%, 44/129), etc. Mixed infections were frequent, with higher infection rates in young animals and during the monsoon season. The research found that the sheep in the region that was analyzed had a lot of gastrointestinal nematode infections. The most frequent parasite was *Haemonchus* spp. The result means that sheep producers need to apply specific deworming techniques, particularly for young animals and during the monsoon season, to reduce their losses.

KEYWORDS

Gastrointestinal nematodes, sheep parasites, *Haemonchus*, *Oesophagostomum*, *Bunostomum*, *Trichuris*, prevalence, Maharashtra

INTRODUCTION

Sheep farming is a major component of Maharashtra's agricultural economy. For many small and marginal farmers in the state, it is their primary source of income. The agricultural industry creates a lot of employment in rural regions and is a major source of food via the production of milk and meat. On the other hand, infections with gastrointestinal parasites are one of the main concerns for sheep production systems. They significantly reduce animal production and cause substantial financial losses for livestock producers (Kumar et al., 2006; Sanyal et al., 2010). Sheep may become sick from many

different kinds of parasites that live in their stomachs. Different parasites make animals sick and present symptoms in different ways, which all work together to make animals less healthy and less productive (Godara et al., 2014). The barber pole worm, also known as *Haemonchus contortus*, lives in the abomasum and consumes blood. This may cause animals to lose a lot of blood. This parasite is considered one of the most harmful gastrointestinal nematodes due to its capacity to induce severe anaemia, decreased protein levels, and mortality in severely infected sheep (Raza et al., 2007; Sharma et al., 2009). A frequent indicator of a long-term illness is swelling under the jaw, which cattle producers call "bottle jaw". It is a crucial symptom of a serious parasite infection. *Bunostomum trigonocephalum*, the hookworm of sheep, lives in the small intestine and harms the body by consuming tissue and bleeding at attachment sites. This parasite is quite harmful for young animals because it may cause iron-deficient anaemia, enteritis, diarrhoea, and slow growth (Singh et al., 2013). The larval movement under the skin may also cause dermatitis and bacterial infections, which can make animals even less healthy.

Oesophagostomum species, commonly known as nodular worms, live in the large intestine and caecum, causing particular inflammatory nodules inside the intestinal wall (Gupta et al., 2011). These pathological lesions severely hamper digestive efficiency and nutrient absorption, leading to reduced feed conversion efficiency and poor body condition scores in affected animals. The whipworm, *Trichuris ovis*, infects the large intestine and caecum, causing long-lasting inflammatory reactions in the colonic mucosa. While often considered less harmful than other species, severe infections may result in significant diarrhea, protein depletion, and lowered developmental rates, particularly in severely sick or nutritionally compromised animals (Dhanalakshmi et al., 2009). The epidemiology of gastrointestinal nematode infections is affected by a range of environmental and management factors, including climatic conditions, animal density, pasture management methods, host nutritional status, and patterns of anthelmintic use (Khan et al., 2010; Chaudhry et al., 2007). Maharashtra's tropical environment, characterized by distinct monsoon seasons and higher humidity levels, creates ideal conditions for parasite growth and transmission, thereby maintaining significant infection pressure for a substantial portion of the year (Varadharajan & Vijayalakshmi, 2015).

Previous epidemiological research conducted in several regions of India has shown the widespread presence of these parasites in sheep populations. Jithendran and Bhat (1999) found that mixed gastrointestinal nematode infections in sheep from Tamil Nadu were common, with rates ranging from 65% to 80%. Singh et al. (2013) also discovered that *Haemonchus* species were more prevalent in sheep in Punjab, with peak infection rates occurring during the monsoon seasons. Ananda et al. (2009) conducted a study in Karnataka that showed age-related susceptibility patterns,



demonstrating that younger animals had significantly higher infection rates compared to adult sheep. Pathak and Pal (2008) in Chhattisgarh, Kumari et al. (2013) in Jharkhand, and Prasad et al. (2008) all found similar results. They also noted that the prevalence rates varied in various parts of India.

There are a lot of sheep in the Ahilyanagar district, and gastrointestinal parasitism is known to be important for livestock production systems, but there is not a lot of detailed epidemiological data on how often and where adult parasites are found in this area. This information is necessary for developing evidence-based parasite control strategies and improving production management practices for local farming groups.

The purpose of this study was to find out how common economically important gastrointestinal nematode species (*Haemonchus* spp., *Bunostomum* spp., *Oesophagostomum* spp., and *Trichuris* spp.) are in the sheep populations from the Newasa and Rahuri tehsils of Ahilyanagar district, Maharashtra, and to find out what factors affect their distribution patterns in the gastrointestinal tract.

MATERIALS AND METHODS

Area of Investigation

This study took place in the Newasa and Rahuri tehsils of the Ahilyanagar district in Maharashtra from January 2024 to December 2024. The weather in the area is semi-dry, with an average of 450–600 mm of rain per year, mostly from June to September.

Examination after death

A full post-mortem examination was done on each sheep. The gastrointestinal tract was carefully removed and its various segments (abomasum, small intestine, large intestine, and cecum) were separated and individually examined for adult parasites.

Collecting and processing parasites

The contents of each part of the gastrointestinal tract were washed through fine mesh sieves with lukewarm water after being opened lengthwise. To get rid of parasites that became attached to the intestinal mucosa, it was removed gently. A 10% formalin solution was used to keep all of the collected material safe for further study. OR the nematode parasites were preserved in 70% alcohol and glycerin and fixed in hot 70%.

Examination in the Lab

A stereoscopic microscope was used to look at the parasites that had been collected for the first time. Adult parasites have been collected from debris and other materials. A compound microscope was used to do a detailed morphological examination to confirm the identification of species.

Identification of Adult Parasites

Adult parasites were identified based on their morphological characteristics:

Adult parasites can be identified by looking at their size, color, shape, and where they live in the animal's digestive system. Each type of worm has evolved specific features that help it survive in its preferred location and feeding style.

Barber pole worm (*Haemonchus contortus*)

This worm that sucks blood lives in the stomach (abomasum). Females are bigger than males because they require a larger space to lay eggs. They are bright red because they take a lot of blood from the animal. Males have spicules, which are specific features for mating. Females have a twisted cavity for depositing eggs. These traits help distinguish the difference between men and females and figure out what species they are.

***Bunostomum trigonocephalum* (Hookworm)**

This worm lives in the small intestine and has a head that looks like a hook and teeth that cut into tissue. The delicate pink colour shows that it eats both blood and tissue fluids. It can stick to the wall of the colon and eat because its mouth parts are so powerful. The hook structure also helps it get through the animal's skin when it initially infects the host.

***Oesophagostomum columbianum* (Nodule worm)**

Found in the large intestine, this grayish-white worm has a large mouth capsule for attaching to the intestine wall. It has a distinctive groove around its "neck" area that helps identify it. The color shows it mainly eats intestinal contents rather than blood. It prefers the slower-moving environment of the large intestine.

***Trichuris ovis* (Whipworm)**

This worm has a very distinctive whip-like shape - thin at the head end and thick at the tail end. It lives in the large intestine with its thin end buried deep in the intestinal wall for feeding. Females are often larger than males. The shape makes it easy to identify and reflects how it feeds by embedding into the tissue.

Each parasite's physical features directly relate to how and where it lives and feeds in the host animal.

Parasite Counting and Analysis

All adult parasites collected from each sheep were counted and recorded. The location of parasites within different sections of the gastrointestinal tract was also recorded.

RESULTS

Out of 180 sheep examined, 129 sheep (71.66%) had adult parasite infections in their gastrointestinal tract. This means that about 7 out of every 10 sheep had some type of adult stomach or intestinal parasites.

Types of Adult Parasites Found

Table 1: Number and percentage of sheep infected with different adult parasites

Type of Adult Parasite	Number of Infected Sheep	Percentage
Haemonchus contortus	67	57.26%
Trichuris ovis	58	44.96%
Bunostomum trigonocephalum	53	41.08%
Oesophagostomum columbianum	44	34.10%

Haemonchus contortus, or the barber pole worm, was detected the most often. Almost half of the sheep that were looked at had adult parasites. These parasites were always detected in the abomasum (the fourth stomach) and had the largest number of parasites per affected animal.

Parasite Distribution in Gastrointestinal Tract

Table 2: Location of adult parasites in different parts of digestive system

Parasite Species	Primary Location	Secondary Location
Haemonchus contortus	Abomasum	-
Bunostomum trigonocephalum	Small Intestine	Duodenum
Trichuris ovis	Caecum	Colon
Oesophagostomum columbianum	Large Intestine	Caecum

Differences Between Area wise

Newasa tehsil had slightly higher adult parasite infection rates (82.5%) compared to Rahuri tehsil (74.2%). Haemonchus contortus and Bunostomum trigonocephalum adult parasites were more commonly found in sheep from Newasa than in Rahuri.

Seasonal variation

Table 3: Adult parasite infections in different seasons

Season	Total Sheep	Infected Sheep	Percentage	Dominant Parasite Species
Monsoon (June-Sept)	60	53	88.33%	H. contortus, O. columbianum
Post-monsoon (Oct-Jan)	60	42	70.0%	H. contortus, B. trigonocephalum



Pre-monsoon (Feb-May)	60	34	56.66%	T. ovis (most resistant), O. columbianum
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During the monsoon season, when it rains a lot, the most adult parasite infections occurred. During the monsoon and post-monsoon seasons, there were a lot of *Haemonchus contortus* populations. On the other hand, *Trichuris ovis* numbers were more stable throughout the year.

Sheep that were maintained in extensive grazing systems, where they could graze freely all day, had higher infection rates than sheep who were housed in semi-intensive systems, where grazing was regulated. Sheep with thin bodies got a lot more infections than sheep with healthy bodies. This suggests that poor diet and worm diseases are tightly linked. Mixed Adult Parasite Infections were seen in many sheep (68 out of 129 infected sheep, 52.71%), indicating the presence of many species of adult parasites in their gastrointestinal system.

DISCUSSION

This study indicated that nematode worm infections are a big concern, but other studies in India have discovered the same thing (Singh et al., 2013; Varadharajan & Vijayalakshmi, 2015; Gupta et al., 2011). The barber pole worm (*Haemonchus contortus*) was the largest problem, infecting more than half of the sheep (51.93%). This is bad news since these blood-sucking worms may make animals very weak (Soulsby, 1982). The nodule worm (*Oesophagostomum columbianum*) was also widespread (34.10%) and makes lumps in the wall of the colon that make it hard to digest food (Lone et al., 2012). Young sheep are more likely to have worms than older sheep since they haven't built up immunity yet (Godara et al., 2014). This means that farmers need to deworm young animals more regularly. The weather is also very important. Worm issues grow a lot greater during the rainy season since moist conditions help parasites live and proliferate better (Pathak & Pal, 2008). This similar trend has been seen in several research in various parts of India (Dhanalakshmi et al., 2009; Sharma et al., 2009).

The manner in which farmers take care of their sheep is very important. Sheep who graze freely all day long take up a lot more worms and have greater burdens in their digestive systems (Khan et al., 2010). Sheep that graze in a regulated fashion have fewer illnesses and infections that are more localized (Raza et al., 2007). Poor nutrition and worm infections can make each other more serious. Sheep that are malnourished are more likely to have worm infections, and worms make nutrition worse by taking nutrients and hurting tissues. Researchers could detect those slender lambs had more worms on to their intestinal walls and greater damage to their tissues during post-mortem exams (Ananda et al., 2009).

Most worrying is that more than half of the infected sheep (52.71%) had multiple types of worms at the same time. Barber pole worms in the stomach and nodule worms in the large intestine are the



worst combo. This is because the sheep loses blood and its intestines are damaged and blocked. Multiple infections may create far worse issues than single infections, therefore it's much more vital for farmers to know how to avoid and treat them properly (Khan et al., 2010).

Economic Impact and Control Recommendations

Farmers were paying a lot of money because of the large worm loads discovered in this research. In India, worm infections may cost farmers Rs. 500–1500 per sheep per year since the afflicted animals don't gain weight appropriately, provide less milk or wool, and sometimes die (Kumar et al., 2006; Sanyal et al., 2010). The nodule worms are very expensive because they develop lumps in the intestines that make it difficult for sheep to digest food, which means farmers lose money on feed that doesn't help the animals grow.

Farmers should establish a proper deworming strategy to keep these worm infestations under control. Young sheep should be dewormed every 2–3 months since they are more likely to catch worms. Adult sheep should be dewormed at least twice a year (Chaudhry et al., 2007). It's extremely crucial to deworm all sheep before the rainy season begins to prevent worm populations from increasing. Farmers should choose the proper medicine given which type of worms they have. For example, certain medicines work better for stomach worms like barber pole worm, while others are required for worms that are jumbled up in the digestive tract (Prasad et al., 2008).

Good management may stop a lot of worm issues without having to use treatment all the time. Moving sheep to various pastures on a regular basis disrupts the life cycle of worms (Raza et al., 2007). Also, keeping sheep away from busy grazing areas lowers their chances of taking up worm larvae from contaminated grass. Good nutrition is important because sheep who are well-fed can fight off worm infestations better than sheep that aren't. Separating young and adult sheep also prevents worms from moving from older animals to younger ones who are more likely to become sick (Kumari et al., 2013). Lastly, farmers should examine their sheep often so they can identify worm issues early. Barber pole worms are generally the cause of anemia if the gums and eyelids are pale. If you feel for lumps around the abdomen, you may have nodule worms in the intestines. Bloody diarrhea is a common sign of hookworms in the small intestine (Jithendran & Bhat, 1999). Farmers should obtain support from veterinarians who can provide the best treatment strategies when issues don't go away or look serious. Farmers may greatly lower worm issues and boost their income by using clever deworming, effective management, and frequent monitoring (19th Livestock Census, 2012).

CONCLUSION

This research indicated that 7 out of 10 sheep in the Ahilyanagar area had worms. The barber pole worm, which sucks blood, is the most life-threatening. Young sheep, sheep that are allowed to graze



freely during the monsoon season, and sheep who aren't getting enough food are the most likely to develop worms. Many sheep have multiple types of worms at the same time. The infestation is a big issue that loss farmers income per sheep every year because of slow development, lower productivity, and animal mortality. Farmers need to frequently deworm their sheep, particularly young ones and before the monsoon season. They also need to provide them healthier food and move them about to disrupt the worm cycles. But farmers can't fix this on their own; they need aid from the government in the form of education programmes, affordable medicines, training in improved management techniques, and continual research. Farmers, veterinarians, and agricultural authorities can significantly reduce worm infections with the right help and cooperation. These improvements will make sheep healthier and provide farmers more money. There is a solution, but everyone has to work together to put practical, science-based control mechanisms in place.

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REFERENCES

- [1] 19th Livestock Census (2012). All India Report, Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India, Krishi Bhavan, New Delhi.
- [2] Ananda, K. J., D'Souza, P. E., & Puttalakshamma, G. C. (2009). Prevalence of gastrointestinal parasites of sheep and goats in Bangalore North. *Veterinary World*, 2(4), 133-134.
- [3] Chaudhry, A. H., Iqbal, Z., Jabbar, A., & Khan, M. N. (2007). Dewormers: helping or hurting. *Journal of Animal and Plant Sciences*, 17(3-4), 82-84.
- [4] Dhanalakshmi, K., Sailaja, B., & Rao, K. S. (2009). Prevalence of gastrointestinal parasites in sheep in Tirupati region of Andhra Pradesh. *Journal of Veterinary Parasitology*, 23(1), 87-88.
- [5] Godara, R., Katoch, R., Yadav, A., & Ahanger, R. R. (2014). Epidemiology of gastrointestinal parasites in sheep and goats of Jammu region. *Journal of Parasitic Diseases*, 38(1), 51-55.
- [6] Gupta, A., Dixit, A. K., Dixit, P., & Mahajan, C. (2011). Prevalence of gastrointestinal parasites in small ruminants in Mathura district of Uttar Pradesh. *Journal of Veterinary Parasitology*, 25(1), 62-65.
- [7] Jithendran, K. P., & Bhat, T. K. (1999). Prevalence of dicrocoeliosis in sheep and goats in Himachal Pradesh, India. *Veterinary Parasitology*, 84(1-2), 143-147.



- [8] Khan, M. N., Sajid, M. S., Khan, M. K., Iqbal, Z., & Hussain, A. (2010). Gastrointestinal helminthiasis: prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. *Parasitology Research*, 107(4), 787-794.
- [9] Kumar, S., Jakhar, K. K., & Singh, S. (2006). Economic losses due to gastrointestinal parasites in sheep and goats. *Indian Journal of Animal Sciences*, 76(12), 889-891.
- [10] Kumari, B., Kumar, A., Baidya, S., Kumar, S., & Banerjee, P. S. (2013). Prevalence of gastrointestinal parasites in goats of Birsa Agricultural University, Ranchi, Jharkhand. *Journal of Veterinary Parasitology*, 27(2), 116-118. London, pp. 602-705.
- [11] Lone B A, Chishti M Z, Ahmad F and Tak H. (2012). A survey of gastrointestinal helminth parasites of slaughtered sheep and goats in Ganderbal, Kashmir. *Global Veterinaria*, 8(4): 338-341.
- [12] Pathak, A. K., & Pal, S. (2008). Seasonal prevalence of gastrointestinal parasites in goats from Durg district of Chhattisgarh. *Journal of Veterinary Parasitology*, 22(1), 61-63.
- [13] Prasad, A., Nasir, A., & Singh, N. (2008). Detection of anthelmintic resistance in gastrointestinal nematodes of goats. *Journal of Veterinary Parasitology*, 22(2), 143-146.
- [14] Raza, M. A., Iqbal, Z., Jabbar, A., & Yaseen, M. (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *Journal of Helminthology*, 81(3), 323-328.
- [15] Sanyal, P. K., Singh, D. K., & Swarnkar, C. P. (2010). Economic benefit-cost ratio of anthelmintic treatment in sheep and goats. *Indian Journal of Animal Sciences*, 80(12), 1233-1237.
- [16] Sharma, D. K., Chauhan, P. P., & Agrawal, R. D. (2009). Epidemiological studies on gastrointestinal parasites of sheep in Famenin region (Iran). *Asian Journal of Animal and Veterinary Advances*, 4(2), 61-67.
- [17] Singh, E., Kaur, P., Singla, L. D., & Bal, M. S. (2013). Prevalence of gastrointestinal parasitism in small ruminants in western zones of Punjab. *Veterinary Parasitology*, 61(1), 61-66.
- [18] Singh, V., Varshney, P., Dash, S. K., & Lal, H. P. (2013). Prevalence of gastrointestinal parasites in sheep and goats in and around Mathura, India. *Veterinary World*, 6(5), 260-262.
- [19] Soulsby, E.J.L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th ed. Bailliere Tindall,
- [20] Varadharajan, A., & Vijayalakshmi, K. (2015). Distribution of gastrointestinal parasites in small ruminants of Tamil Nadu. *Indian Veterinary Journal*, 92(3), 96-98.
- [21] YAMAGUTI, S. (1961). *Systema Helminthum Vol. III. The nematodes of vertebrates. Parts I & II*. Interscience publishers Inc., New York, pp. 1261.



HERBAL FORMULATION INFLUENCED INDUCED PLURIPOTENT STEM CELLS FOR THE SIMULATION OF EMBRYONIC ENVIRONMENTS.

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ABSTRACT

The concept of induced human pluripotent stem cells (iPSCs) from somatic cells use to open a innovative avenue for medicine of precision category with personalized cell therapy. This concept is also encouraging the discovery of essential platforms for targeted-drug-development. iPSCs use to retain the genome of the donor, may use to regenerate indefinitely, and use to undergo differentiation into virtually any type of cell (interest using a range of published protocols). There has been enormous interest among researchers regarding the application of technology of iPSCs to regenerative-medicines and human disease modelling. Present attempt is dealing with humulene influenced cell-differentiation of induced pluripotent stem cells (iPSCs), through different methods of culture, the state of undifferentiation, natural differentiation (long term culture without passages), embryoid-bodies (EBs), and accelerated-differentiation through activin and focusing on the marker gene expression (including Brachyury, hepatocyte nuclear factor (HNF) (3 β), estrogen-receptor α , and other). When iPSCs starts for differentiation through responding to humulene, Brachyury-expression reported to decrease regardless of the quantity of humulene, cell-line, and / or protocol. Other markers reported for up or down-regulation, but there is no tendency except in the early stage of embryoid-bodies (EBs). The Embryoid-bodies (EBs) stage analogous to gastrulation” was observed for the most significant influence for definite timing of humulene-action. The humulene repressed Brachyury expression and induced hepatocyte nuclear factor (HNF) (3 β) at its restricted quantity. The results suggest that, the first target of humulene is the stage of gastrulation.

KEYWORDS

Humulene; Brachyury; Induced Pluripotent Stem Cells; embryoid-bodies (EBs); Gastrulation

ABBREVIATIONS

ihPSC: Induced Human Pluripotent Stem Cells; HNF3 β : Hepatocyte Nuclear Factor; ER α : Estrogen Receptor α ; ESR1: Estrogen Receptor 1; EB: Embryoid Body; SIRT1: Sirtuin 1

INTRODUCTION



The induced pluripotent stem cells are derived from reprogrammed adult cells in laboratory and therefore, they are resembling stems cells of embryo. During the process of development (embryonic), the cells of the embryonic, especially of the inner cell mass, use to divide continuously and become the most specialized. Portion of dorsal part of embryonic ectoderm, for example, use to specialize into the neuro-ectoderm (the future central nervous system). This neuro-ectoderm, later in development, through the process of neurulation uses to form the neural-tube [1]. The plants are protected from diseases, deterring herbivorous animals and other predators (and Parasites too) through secretion of the terpene compounds. Alpha-humulene and caryophyllene are the isomeric compounds. Terpene: α -humulene and terpene caryophyllene are the compounds of sesquiterpene category. Naturally they are occurring in hop species of plants (common hop, *Humulus lupulus*; common hemp, *Cannabis sativa* and Indian hep, *Cannabis indica*) [2]. The advantage of properties dealing with sedative or tranquilizer influences of α -humulene in herbal-treatment has been worked out extensively since time immemorial. There is significant affinity of among the humulene and Gama Amino Butyric Acid (GABA). The humulene is surpassing to that of paroxetine intrinsically on "SERT (serotonin transporter)" [5,6,7]. Sesquiterpene nature (Compound with single ring and three isoprene units) is the most significant recognition for humulene. The alpha-humulene or alpha-caryophyllene are other names for Humulene. It is monocyclic sesquiterpene. The chemical formula of the humulene is: $C_{15}H_{24}$. There are eleven rings ring and three isoprene units in the chemical structure of humulene. The very first step of investigation, Humulene has been investigated as the contents of essential-oil components of common hops, *Humulus lupulus* (L). It is reported for anti-inflammatory influences [5,6]. Brazilian researchers identified, "Alpha-Humulene" as the most significant compound concerned with active contributor for the repelling the insects through the leaf-oil of Imburana, *Commiphora leptophloes* (L), especially against the mosquito, *Ades aegypti* (L)[7]. The attempts on study of the effects of terpene and terpenoids on induced human pluripotent stem cells (hPSCs) is significant field of innovative research and deserve potential implications for regenerative-medicines and drug-discoveries. Terpenes (and terpenoids) is a major category of natural ingredients of plant origin. They exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, and anti-cancer properties [8,9]. Research into their effects on hPSCs could reveal new ways to control cell differentiation, enhance cell survival, or develop new therapies for diseases. Most of the terpene compounds (and terpenoid compounds too) are insect juvenoids (mimicking the natural action of juvenile hormones / JH). The sesquiterpenoid occupy the fore front position with reference to juvenoid activity.



Blastocyst is an embryonal stage of nidation. This stage of development is being placed under the influence of environment consisting of estrogen. The fluid with estrogen (secreted by uterine gland) uses to feed the embryo (before maturity of fetoplacental circulation). After the establishment of blood circulation among the foetus and mother body, maternal blood circulation uses to contain a large amount of estrogen. This is for the purpose to provide most favourable environment. This estrogen uses to increase continuously. The most accurate reason for increase in estrogen in maternal circulation is continuous secretion of estrogen from trophoblasts of the placenta. The estrogen is with properties of steroid hormone. Therefore, the estrogen deserves the ability to pass through the plasma membrane and to enter the embryo from the maternal blood circulation. The significant role of secretion of the enzyme, "Hydroxysteroid-Dehydrogenase" (17β -HSD) inside the embryo is carried out by the foetal liver, gastrointestinal tract and kidneys [8]. This enzyme, "Hydroxysteroid-Dehydrogenase" (17β -HSD) deserve capabilities of changing the estradiol to estrone. Soon after the process of implantation into the uterine tissue, an embryo (and / or foetus) is continuously exposed to estrogen. The effects of estradiol on the process of differentiation of cells have been studied widely. The estradiol uses to accelerate morphologic maturation of the foetal lungs in rabbit [9]. The estradiol also uses to affect the proliferations and differentiations of neural stem cells through receptors of estrogen [10]. The research of human embryos is ethically limited. Hence, "Assessment of the repercussion of any compound on the precocious stage of development" presents a challenge. The human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) gain mastery or control over these complications. Jung et al (2010) reported increase in the concentration of "Octamer-binding-transcription factor" (OCT4) (also known as POU5F1) through the continuous exposure of mesenchymal embryonic cells (mES cells) to estrogens. This condition maintain circumstances of undifferentiation [11]. Hong et al (2004) revealed the capability of estradiol to differentiate the human embryonic stem cells (hESCs) into endodermal marker expressing cell-types (α -fetoprotein; α 1-Antitrypsin; GATA-4; and somatostatin) as well as enolase, nuclear-factor-68Kda (ectoderm), and Brachyury (mesoderm)-expressing-cells from the perspective of expression of the genes associated with the three germ layers and types of tissue [12]. Kim et al (2012) opined that, estradiol deserves the potential of differentiation of human embryoids (hEBs) into endodermal cells or mesodermal cells [13]. Tielens et al (2008) proved the differentiation of embryoids (EBs) into osteoblast-like cells human induced pluripotent stem cells (hiPSCs) through the influence of estradiol [14]. The terpene and terpenoid compounds (like: humulene, the herbal juvenoid formulations" are experts with reference to block the development of insect-life at the stage of metamorphosis of larval stage into pupa and then into the adults [7,8,9,10].



Applications of terpenoids include: Potential in Bone Regeneration; Anti-inflammatory Properties; Antioxidant Effects and Modulation of Pathways of cell signalling. Betulinic acid; Lupeol; Oleanolic acid; Ursolic acid; Artemisinin and Ginsenosides are some of the specific examples of terpenoids popular for using in human stem cell-culture. Terpenoids are representing a promising area of research in biology of human stem cells and regenerative medicines, with the potential to influence differentiation of stem cells, tissue-repair, and overall cell-health. There is need to work further to elucidate the precise mechanisms by which terpenes and terpenoids use to influence behaviour of “Human Stem Cells” and to identify the most promising terpenes and terpenoids for specific applications in the field of regenerative medicines. There are no reports on use of humulene for culture of “Induced human stem cells (iHPSCs). Therefore, present attempt dealing with utilization of Induced Human Pluripotent Stem Cells Humulene (Herbal Juvenoid Formulation) influenced iPSCs (Induced Human Pluripotent Stem Cells) for the simulation of embryonic environments has been planned.

MATERIALS AND METHODS

The attempt on “Utilization of Zingiberene (Herbal Juvenoid Formulation) influenced iPSCs (Induced Pluripotent Stem Cells) for the simulation of embryonic environments” has been completed through the sub-steps like: Sourcing and culture of, “Human induced pluripotent stem cells (hiPSCs); Configuration, layout and outgrowth of embryoid-body (EB); Stimulation (causing a reaction) of Activin; Humulene (Herbal Juvenoid Formulation) treatment; Quantitative real-time; Estrogen-Receptor (ER α) and Statistical analysis.

(a). Sourcing and Culture of Human Induced Pluripotent Stem Cells (hiPSCs): The Reprogramming of adult cells (to a pluripotent state, similar to embryonic stem cells, using specific genes or transcription factors) get results into induced pluripotent stem cells (iPSCs) There are several steps in the process of culture of iPSCs, including maintenance of the cells on feeder-cells or in feeder-free conditions, through the use of specific media and matrices, and careful passaging for maintenance their pluripotency.

The list of more detailed breakdown of the culture method (Feeder-Dependent-Culture):

(a.1). Feeder cells: The Human Induced Pluripotent Stem Cells (hiPSCs) are often cultured on a layer of mitotically inactivated mouse embryonic fibroblasts (MEFs) or human foreskin fibroblasts (HFFs). These feeder cells provide a supportive environment for the growth and survival of Human Induced Pluripotent Stem Cells (hiPSCs) through conditioning the media and provision of a matrix for attachment.

(a.2). Medium: The medium of culture is typically supplemented with growth factors (like FGF2 and EGF), and specific media formulations like Knockout Dulbecco’s minimal Eagle’s medium (DMEM).

(a.3). Passaging: The Human Induced Pluripotent Stem Cells (hiPSCs) are passaged by either enzymatic dissociation or mechanical dissociation. It is followed by seeding onto fresh feeder layers.

Two cell lines of human induced pluripotent stem cells (hiPSCs) (hiPSC lines: 201B786 and 253G1663) were sourced (MEFs; Oriental Yeast Co, Tokyo, Japan) (Kyowa Hakko Kirin Co, Tokyo, Japan). The method described by Takahashi, et al (2007) was utilized for the culture of human induced pluripotent stem cells (hiPSCs): hiPSC lines (201B786 and 253G1663) [15].

The human induced pluripotent stem cells were cultured on a feeder-cell-layer of mouse embryonic-fibroblasts (MEFs; Oriental Yeast Co, Tokyo, Japan) (inactivated with mitomycin C) (Kyowa Hakko Kirin Co, Tokyo, Japan). The cultures were maintained by repeated passages. The medium of culture consisted of eighty percent KnockOut™ Dulbecco's minimal Eagle's medium (DMEM) (received as sample gift from Gibco, Grand Island, NY, USA). This medium of culture was supplemented with twenty percent KnockOut™ Serum Replacement (received as sample gift from Gibco, Grand Island, NY, USA), 100 micro moles (μM) non-essential amino acids (received as gift from Wako, Chuo-ku, Osaka, Japan), 2 milli moles (mM) L-glutamine (received as gift from Wako, Chuo-ku, Osaka, Japan), and 100 micro moles (μM) 2-mercaptoethanol (received as gift from Sigma, St. Louis, MO, USA).

(b). Configuration, layout and Outgrowth of Embryoid-Body (EB): Undifferentiated human induced pluripotent stem cells (hiPSCs) were cultured for about five days (120 hours) and treated with enzyme collagenase-IV (sample gift received from Invitrogen, Carlsbad, CA) for the duration of four minutes at the temperature of 37°C. The colonies of detached human induced pluripotent stem cells (hiPSCs) were centrifuged and washed with phosphate buffered saline (PBS). The washed colonies of human induced pluripotent stem cells (hiPSCs) were transferred into a non-adherent plate. Suspension cultures of human induced pluripotent stem cells (hiPSCs) were maintained for five days (120 hours) in embryoid-body (EB) medium (Eighty percent knockout Dulbecco's minimal Eagle's medium DMEM, 100 micro-moles (μM) non-essential amino-acids, 2 milli-moles (mM) L-glutamine, 100 micro-moles (μM) 2-mercaptoethanol, and twenty percent foetal-bovine-serum (FBS) (sample-gift received from Hyclone, Logan, UT, USA). The embryoid-bodies (EBs) were adhered to a dish (coated with gelatin) and cultured in the same EB-medium for the planned number of days.

(c). Stimulation (causing a reaction) of Activin: According to the method described by Yoshie, et al (2013) [16], the colonies of human induced pluripotent stem cells (hiPSCs) were cultured in so called "Roswell Park Memorial Institute media (RPMI)" (1640 medium) (received as gift from Wako, Chuo-ku, Osaka, Japan) (containing 2% two percent FBS with 100 ng/ mL Activin A and 3 micro moles (μM) CHIR99021 for twenty-four hours and then in two percent FBS/ RPMI1640 medium with 100 ng/mL Activin A for forty-eight hours.

(d). Humulene (Herbal Juvenoid Formulation) Treatment: The medium of culture consisted of eighty percent DMEM supplemented with twenty percent Serum Replacement, 100 micro moles (μM) non-essential amino acids, 2 milli moles (mM) L-glutamine, and 100 micro moles (μM) 2-mercaptoethanol. The cells were cultured with methanol solution of powder of humulene. The concentration humulene in methanol was 50 ppm (mg per liter). The medium was exchanged daily. Acetonitrile was used for the control. The schemes of all experiments were as below:

1. Each group was cultured for seven or fourteen days without passages. Medium containing Humulene was changed daily.
2. Embryoid-body (EB) was formed for five days and then adhered to a plate for seven or fourteen days. Humulene was added to the medium at the same time as adhesion.
3. Activin was added to the medium containing Humulene. Cells were cultured for three days. Only activin was added to the medium for three days. Subsequently, Humulene was added for four days.

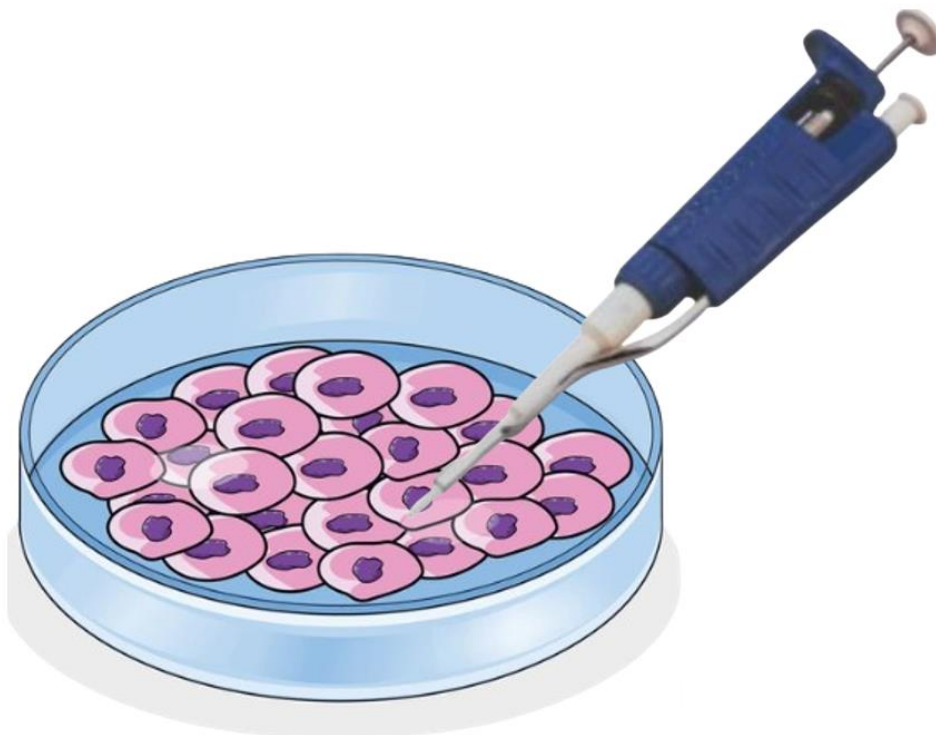
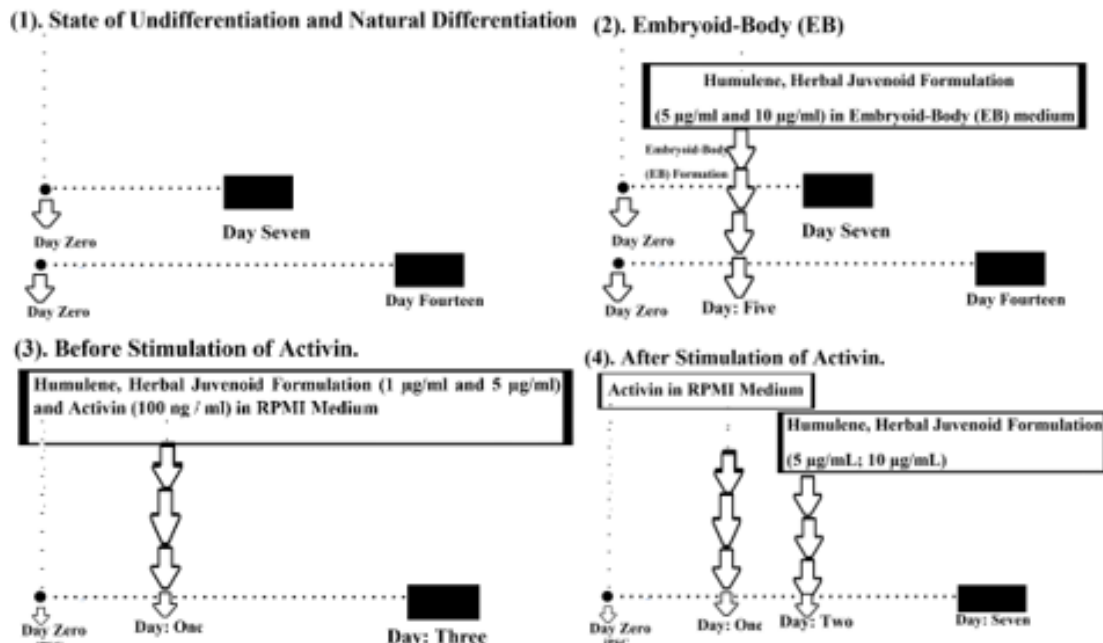
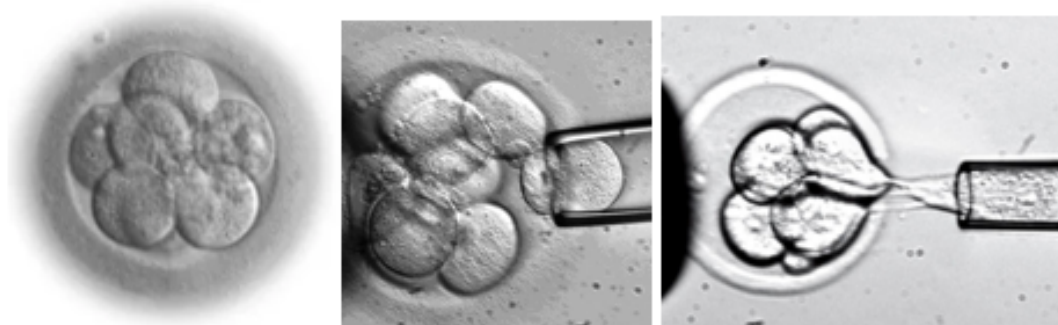


Figure 1: Protocols for experiments.



Each group was cultured for seven or fourteen days without passages. Medium containing Humulene was changed daily.

- 1. Embryoid-body (EB) was formed for five days and then adhered to a plate for seven or fourteen days. Humulene was added to the medium at the same time as adhesion.**
- 2. Activin was added to the medium containing Humulene. Cells were cultured for three days.**
- 3. Only activin was added to the medium for three days. Subsequently, Humulene was added for four days.**



(e). Quantitative Real-Time Reverse Transcription – Polymerase Chain Reaction (RT-PCR): Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. TRIzol is a combination of phenol, guanidine isothiocyanate, and other exclusive constituents. TRIzol reagent is a ready-to-use solution primarily used for the isolation of RNA from cells and tissues. It is a monophasic mixture of phenol and guanidine isothiocyanate, which enables efficient cell lysis and RNA preservation while inhibiting RNase activity.

The method of thermal cycler Dice Real-Time System (Takara Bio, Otsu, Japan), Quantitative polymerase chain reaction (PCR) analysis was carried out. Cycling was carried out for duration of ten minutes at temperature of 95°C. It was followed by forty cycles of 5 s at 95°C and 30 s at 60°C, which was the default conditions of Thermal-Cycler-Dice Real-Time-System-software TP 800 (version 5.11B).

Primer sequences were as follows:

HNF3 β (Forward: 5'-TAT TGC CCC GTT GAG TGC3'; Reverse: 5'-TCC CAG GGA AAC TGC AAG-3'), brachyury (5'- GAC AGG TAC CCA ACC CTG AGG A-3'; 5'-AGC ATG GAT AAA CAT GCA GGT GAG-3'), and terpenoid receptor α (5'-TTA CTG ACC AAC CTG GCA GA-3'; 5'-ATC ATG GAG GGT CAA ATC CA-3'), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5'-TGG CAC CCA GCA CAA TGA A-3'; 5'-CTA AGT CAT AGT CCG CCT AGA AGC A-3'). Polymerase Chain Reaction (PCR) was performed in triplicate for each sample. Three independent experiments were carried out.

(f). Estrogen-Receptor (ER α)-Immunohistochemistry:

Four percent paraformaldehyde (in 0.1 M phosphate buffer / PBS; pH = 7.4) was used for fixing the cultured cells. Fixing the cultured cells was carried for half an hour (thirty minutes) at room temperature. After the completion of duration of fixing, the cultured cells were rinsed three times with 20 mM Phosphate-buffered saline (PBS) (pH = 7.4), and then prepared for immunostaining. "The anti-ESR1" (the primary antibody) with befitting, secondary antibody conjugated with Alexa Fluor 488 as well as 6-diamidino-2-phenylindole dihydrochloride (DAPI, Molecular Probes, Eugene, OR, USA) were used: "The anti-ESR1" (Bethyl Laboratories, Montgomery, TX, USA).

(g). Statistical Analysis: Each attempt in present study was repeated for three times for consistency in the results. All values in figures and the text are expressed as means (with \pm : standard deviation). The considerable disparities among mean values were assessed by Student's t-test. P<0.05 was considered as significant.

RESULTS AND DISCUSSION:

The results the attempt on the "Utilization of Humulene (Herbal Juvenoid Formulation) Influenced iPSCs (Induced Pluripotent Stem Cells) for the Simulation of Embryonic Environments" are summarized





in table-1 and 2; Fig. 1, 2, 3). The undifferentiated stage (protocol-1: Each group was cultured for 7 or 14 days without passages. Culture medium containing Humulene (Herbal Juvenoid Formulation) was used to change daily so as to keep it near the inner cell mass in the blastocyst and almost showed no response in both cell lines to Humulene (Herbal Juvenoid Formulation) in terms of cellular differentiation, especially within a week, although Estrogen-Receptor ($ER\alpha$) was detected slightly (Figure 2). After two weeks, the germ-layer-markers appeared irregularly, depending on whether Humulene, Herbal Juvenoid Formulation (5 $\mu\text{g}/\text{mL}$) was added, but did not show any tendency (For example, although the expression level of Brachyury observed to decrease, upgradation of HNF3 β (or did not change according to the cell line) (Figures 2A and 2B). In the next stage (Protocol-2 / embryoid-body / EB outgrowth protocol), Embryoid Body / EB was within for five days and then adhered to a plate for next seven and / or fourteen days. Humulene, herbal Juvenoid Formulation was added to the medium at the same time as adhesion) was analogous to gastrulation consisting of three germ layers, when the definite timing for Humulene, Herbal Juvenoid Formulation to affect differentiation was found. The markers of germ layers were observed affecting by the treatment Humulene (Herbal Juvenoid Formulation) (5 $\mu\text{g}/\text{mL}$) (Fig: 2.A and 2.B). Hepatocyte Nuclear Factor (HNF) (3 β) expression was significantly (about 100 times) higher in comparison of the control, and Brachyury expression was about 10 times lower than in the control. Humulene, Herbal Juvenoid Formulation (10 $\mu\text{g}/\text{mL}$) did not change the amount of Brachyury, but lowered Hepatocyte Nuclear Factor (HNF3 β). For the confirmation of interrelationship between Humulene (Herbal Juvenoid Formulation) and expression of ERs, $ER\alpha$ was investigated in Real-Time Reverse Transcription – Polymerase Chain Reaction (RT-PCR) and analysis through immunohistochemical methods (Fig: 3). $ER\alpha$ was seemingly (apparently) found at each stage (Fig. 3.A). It was obvious that, embryoid-body (EB) outgrowth showed more $ER\alpha$ expression than Human Induced Pluripotent Stem Cells (hiPSCs). Moreover, the analysis through immunohistochemical methods confirmed the exact localization of $ER\alpha$ protein irrespective of addition of Humulene (Herbal Juvenoid Formulation). It is on the side of supporting gene expression (Fig: 2.A and 2.B)).

Before (protocol-3: Activin was added to the medium containing Humulene, Herbal Juvenoid Formulation. Cells were cultured for 3 days) and after (protocol-4: Only activin was added to the medium for three days. Subsequently, Humulene (Herbal Juvenoid Formulation) was used to add for four days activin stimulation:

Humulene (Herbal Juvenoid Formulation) was added to Roswell Park Memorial Institute (RPMI) -1640 (RPMI1640-medium) together with activin to protest whether it accelerated definitive endodermal layer with activin (protocol-3: Activin was added to the medium containing Humulene, Herbal Juvenoid



Formulation. Cells were cultured for three days), but cells treated with 5 µg/mL Humulene (Herbal Juvenoid Formulation) did not survive to the end of the experiment. Therefore, cells were cultured in medium with 1 µg/ mL Humulene (Herbal Juvenoid Formulation). The cells survived and expressed only a slightly lower level of Brachyury compared with the expression of other markers, but Humulene (Herbal Juvenoid Formulation) did not promote differentiation of definitive endoderm. Next, Human Induced Pluripotent Stem Cells (hiPSCs) were stimulated with activin before addition of Humulene, Herbal Juvenoid Formulation and then cultured for four days in medium containing Humulene, Herbal Juvenoid Formulation to confirm whether Humulene, Herbal Juvenoid Formulation affected definitive endodermal layer induced by activin.

Table-1: Gene Expression for 14 Days Without Passages (Natural Differentiation of Protocol-1) (Relative Expression of mRNA and HNF3β) for cell Lines: 253G1663 Cells and 201B786 Cells).

hiPSC-lines Treatment ↓	253G1663  (A) (14 Days Culture Without Passage)	201B786  (A) (14 Days Culture Without Passage)	253G1663  (B) (14 Days Culture Without Passage)	201B786  (B) (14 Days Culture Without Passage)
Humulene (-)	0.0007692 (±0.0000113)	0.0008461 (±0.0000124)	0.0005384 (±0.0000789)	0.0006153 (±0.0000908)
Humulene (+)	0.0004307* (±0.0000615)	0.0006153* (±0.0000334)	0.0009231* (±0.0000394)	0.0005385 (±0.0000963)
iPSCs	0.0004308* (±0.0000615)	0.0007693 (±0.0000786)	0.0006153 (±0.0000487)	0.0005389 (±0.0000786)

After repeated three times, all values are expressed as means ± standard deviation of three experiments. The significance of differences among mean values was evaluated by Student's t test. A and B Gene expression for 14 days without passages (natural differentiation of protocol: 1). The sharp tendency of hepatocyte nuclear factor (HNF3β) increasing followed by the decrease of Brachyury after treatment with Humulene (Herbal Juvenoid Formulation) appeared in 253G1663. A: Relative mRNA expression of Brachyury (*: p<0.01). B: Relative mRNA expression of HNF3β (*: p<0.01).

Table-2: Gene Expression for 14 Days Without Passages (Natural Differentiation of Protocol-2) (Relative Expression of mRNA and HNF3 β) for cell Lines: 253G1663 Cells and 201B786 Cells).

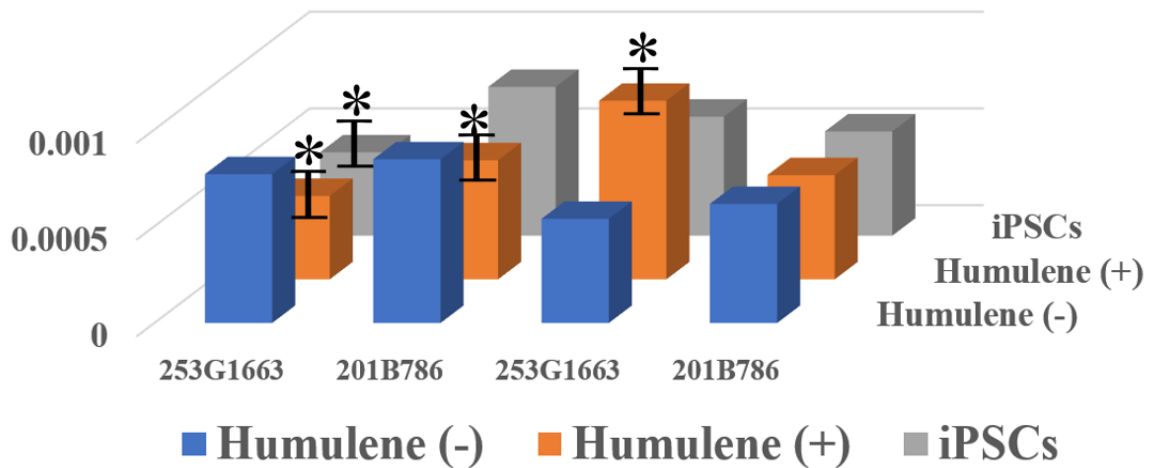
Stage  Humulene ($\mu\text{g/mL}$) 	Embryoid- Body (EB) Zero ($\mu\text{g/mL}$) (C)	Embryoid- Body (EB) + (Humulene) Five ($\mu\text{g/mL}$) (D)	Embryoid- Body (EB) + (Humulene) Ten ($\mu\text{g/mL}$) (E)	iPSCs Zero ($\mu\text{g/mL}$) (F)
Brachyry	0.00047616 (± 0.0000784)	0.0004659 (± 0.0000669 3)	0.0005214 (± 0.0000786 6)	0.0005213 (± 0.0000786 4)
HNF3 β	0.00061533 (± 0.0000214 2)	0.00061589 (± 0.0000327 3)	0.0006678 (± 0.0000428 7)	0.0004966 (± 0.0000224 3)
Brachyry	0.00181818 (± 0.0006284)	0.000919 (± 0.0008628)	0.00313133 (± 0.0009626 3)	0.000919 (± 0.0008628)
HNF3 β	0.00111111 (± 0.0003142)	0.00111113 (± 0.0004284)	0.00121313 (± 0.0005372 4)	0.006065111 (± 0.0005372 4)

C and D: Gene expression in EBs (protocol 2). The above tendency was reinforced markedly in this stage. C: Relative mRNA expression of Brachyry (b: $p < 0.01$). vs. a and c. c: $p < 0.02$ vs a, $p < 0.02$ vs. b).

D: Relative mRNA expression of HNF3 β (b: $p < 0.02$ vs. a and c, c: $p < 0.02$ vs. a and b).

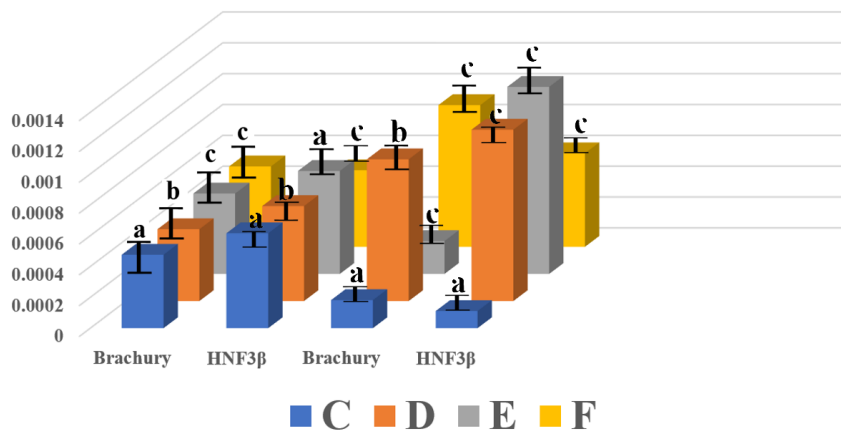
E and F: Gene expression in definitive endoderm (DF) differentiation (protocol 4). Although Brachyry decreased after treatment with Humulene, HNF3 β did no change. E: Relative mRNA expression of Brachyry (b: $p < 0.01$ vs a and c, c: $p < 0.0001$ vs. a, $p < 0.003$ vs. b). F: Relative mRNA expression of HNF3 β (* $p < 0.01$).

Fig.2(A): Gene Expression for 14 days without passages (Natural Differentiation of Protocol:1) (Relative Expression of mRNA and HFN3β) for Cell Lines: 253G1663 Cells and 201B786 Cells.



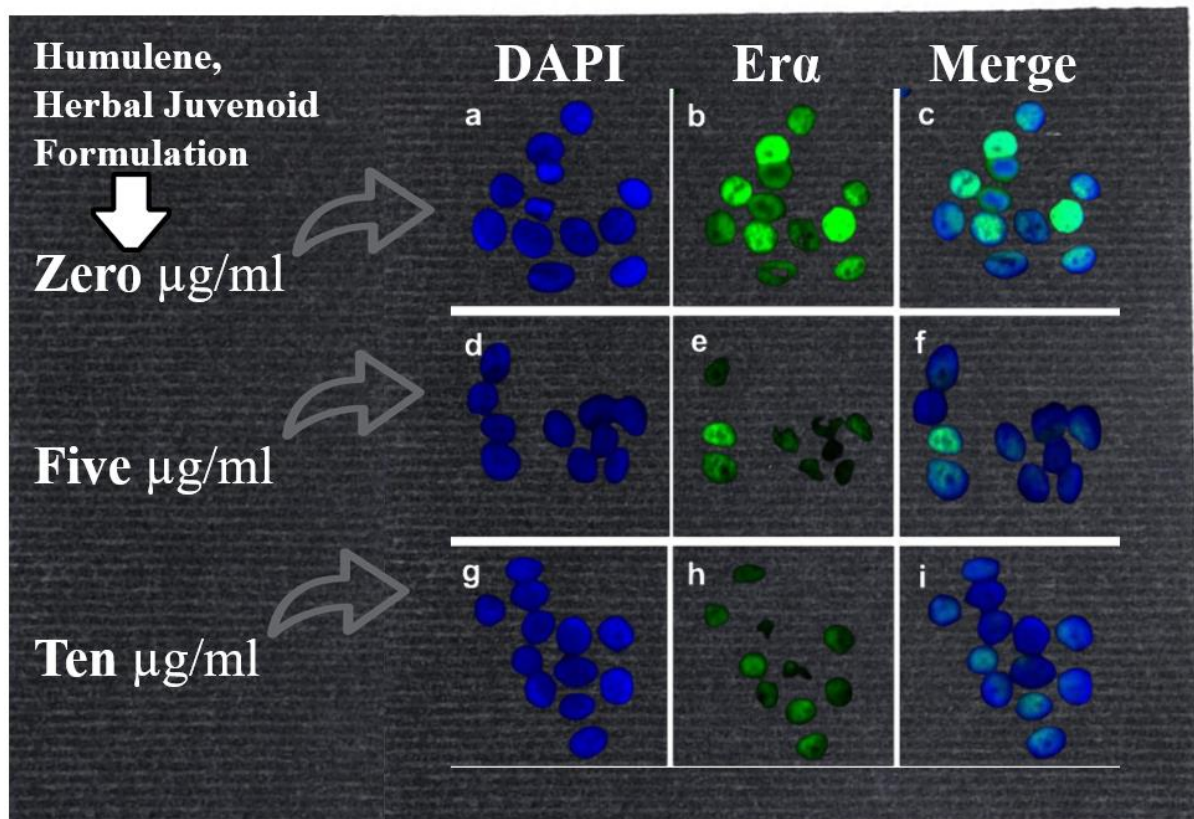
Foot Note: After repeated three times, all values are expressed as means ± standard deviation of three experiments. The significance of differences among mean values was evaluated by Student’s t test. A and B: Gene expression for 14 days without passages (natural differentiation of protocol 1). White bars: 201B7 cells; black bars: 253G1 cells. The sharp tendency of HNF3β increasing followed by the decrease of Brachyury after treatment with Humulene appeared in 253G1663. cells. A: Relative mRNA expression of Brachyury (*p<0.01). B: Relative mRNA expression of HNF3β (*p<0.01).

Fig.2(B): Gene Expression for 14 Days Without Passages (Natural Differentiation of Protocol 2) (Relative Expression of mRNA and HNFβ) for cell lines: 253G1663 Cells and 210B786 Cells)



Foot Note: After repeated three times, all values are expressed as means \pm standard deviation of three experiments. The significance of differences among mean values was evaluated by Student's t test. A and B: Gene expression for 14 days without passages (natural differentiation of protocol 1). White bars: 201B7 cells; black bars: 253G1 cells. The sharp tendency of HNF3 β increasing followed by the decrease of Brachyury after treatment with Humulene appeared in 253G1663. cells. C and D: Gene expression in EBs (protocol 2). The above tendency was reinforced markedly in this stage. C: Relative mRNA expression of Brachyury (b: $p < 0.01$) vs. a and c. c: $p < 0.002$ vs. a and b). E and F: Gene expression in definitive endoderm (DF) differentiation (protocol 4). Although Brachyury decreased after treatment with E2, HNF3 β did no change. E: Relative mRNA expression of Brachyury (b: $p < 0.01$) vs. a and c, c: $p < 0.0001$ vs. a, $p < 0.003$

Figure 3: Gene expression and localization of ER α in the EB stage (Protocol-2: An EB was formed for 5 days and then adhered to a plate for 7 or 14 days. Humulene, Herbal Juvenoid Formulation was added to the medium at the same time as adhesion.) (DAPI: 4',6-diamidino-2-phenylindole).



The marked tendency of embryoid-bodies (EBs) was lost. Brachyury used to decrease with Humulene, Herbal Juvenoid Formulation treatment, while hepatocyte nuclear factor (HNF) (3 β) did not change

(Fig: 2.A and 2.B). Thus, addition of Humulene, Herbal Juvenoid Formulation was found to exert decreased influence on the level of Brachyury, but other markers exhibited only mild or no differences except in the embryoid-body (EB) stage in which the level of hepatocyte nuclear factor (HNF) (3β) was increased by almost 100 times with 5 μ g Humulene (Herbal Juvenoid Formulation) accompanied by the decrease in Brachyury to one tenth ($1\div 10$) that in the control. Two weeks later, embryoid-body (EB) outgrowth lost the interrelationship of the increase in hepatocyte nuclear factor (HNF) (3β) with the decrease in Brachyury. The sharp tendency of gene expression was reported only in embryoid-bodies (EBs) (especially at the early stage) in present attempt. Definite effects of Humulene (Herbal Juvenoid Formulation) seem to be limited to a time during the process of embryonic development. The effects of Humulene, Herbal Juvenoid Formulation were not all found in Human Induced Pluripotent Stem Cells (hiPSCs), (that is at the epiblast-like stage). The “repressed Brachyury and increased hepatocyte nuclear factor (HNF) (3β) drastically” was found resulted only at the early stage of embryoid-bodies (EBs), treatment of Humulene (Herbal Juvenoid Formulation). At a later stage (or in specific tissue differentiation using activin), expression of differentiation markers was up or down-regulated and uses to lose a defined tendency.

An embryoid body or EB is a three-dimensional aggregate mass of pluripotent stem cells dealing with formation in vitro. Embryoid-Bodies (EBs) are derived from human embryonic stem cells (hESCs) or Human Induced Pluripotent Stem Cells (hiPSCs) and has the ability to mimic post-implantation embryonic tissues (Sadler, 2015) [17]. It may be regarded as before the end of third week of human development, around seventeenth day or eighteenth day, when gastrulation begins, which establishes all three germ layers. Invagination through the migration of epiblast cells toward the primitive streak, occurs in this stage. The layer of mesoderm and the layer of endoderm are originated from these cells (invaginating). There is a need of regulation of Brachyury (one of the T-box family genes) for the specification of mesoderm. Brachyury (one of the T-box family genes) is expressed in the node, notochord precursor cells, and notochord. It uses to regulate the formation of dorsal mesoderm in the middle-region and caudal-region of the embryo. It is also essential for cell migration through the primitive streak. Brachyury used to repress and expression of endodermal layer markers such as hepatocyte nuclear factor (HNF) (3β) was marked at the early stage of embryoid-body (EB). Based on these results, the present attempt is conclusive on, “Humulene, Herbal Juvenoid Formulation uses to play the role of a switch from the limited epiblasts restricted to the primitive streak to endodermal layer through repressing the process of expression of Brachyury. The attempt is reported the “embryoid-body (EB) outgrowth stage analogous to the early stage of gastrulation” as a “appropriate timing” for the most significant influence of treatment of Humulene (Herbal Juvenoid Formulation).



Appearance of estrogen receptor (ER) ER α was clearly at the embryoid-body (EB) stage, but not on the Human Induced Pluripotent Stem Cells (hiPSCs). It is certain that the effects of Human Induced Pluripotent Stem Cells (hiPSCs) depend on estrogen receptors (ERs), because gene expression became marked at the stage of embryoid-body (EB), although it did not increase drastically through the stimulation with the Human Induced Pluripotent Stem Cells (hiPSCs). Therefore, estrogen receptor (ER) is necessary for the effects of the Human Induced Pluripotent Stem Cells (hiPSCs), but is independent of the stimulation of the Human Induced Pluripotent Stem Cells (hiPSCs).

Although the specific mechanism for Humulene, Herbal Juvenoid Formulation for conversion of the limited epiblasts into endodermal layer is beyond the scope of present attempt of the study, the following mechanism may be concluded from several reports. According to Li, et al (2016), some types of breast cancers are tamoxifen resistant lies in overexpression of Brachyury [18]. This mechanism is explained by Brachyury downregulating the expression of sirtuin (SIRT) 1 that represses estrogen / estrogen receptor signalling and cell proliferation in estrogen-responsive breast cancer cells by downregulating B-cell leukemia/lymphoma (Bcl)-2 protein [19]. Consequently, present attempt is suggesting that, the possibility that sirtuin (SIRT-1) uses to downregulate Brachyury. The lower expression of Brachyury through the sirtuin (SIRT-1) in the cells treated with Humulene, Herbal Juvenoid Formulation in comparison with the control. This result is suggesting that, sirtuin (SIRT-1) uses to inhibit Brachyury and the other way around. The present attempt revealed the definite timing for addition of Humulene, Herbal Juvenoid Formulation most effectively was at the early stage concerned with embryoid-bodies (EBs). At the early stage of embryonic development (through the repression of Brachyury), Humulene, Herbal Juvenoid Formulation may be helping for differentiation of epiblasts, especially into endodermal tissue.

The credit of a groundbreaking discovery of “Induced pluripotent stem (iPS) cells” goes to Shinya Yamanaka and his research team. Induced pluripotent cells are reprogrammed adult-cells and use to behave like embryonic-stem-cells. Shinya Yamanaka and his research team tried their best to demonstrate conversion of adult mouse cells into “Induced pluripotent stem (iPS) cells” (capable of differentiating into various cell types) through the process of introducing a specific set of four genes (Oct3/4; Sox2; c-Myc, and Klf4) (Takahashi and Yamanaka, 2006) [22,24,25]. This groundbreaking discovery by Shinya Yamanaka and his research team, eliminated the ethical concerns (associated with using embryonic stem cells for research and therapy). Key aspects of “Induced pluripotent stem (iPS) cells” discovered by Shinya Yamanaka and his research team include: Reprogramming; transcription factors (four); Pluripotency; Ethical Considerations; Disease Modelling and Therapy. Research work of Yamanaka was focused on identification of the genes that could reprogram adult-cells (specifically



fibroblasts, back to a pluripotent state). The crucial breakthrough was the identification of four transcription factors (Oct3/4; Sox2; c-Myc, and Klf4) that, when introduced into adult cells, could induce pluripotency. “Induced pluripotent stem (iPS) cells”, like embryonic stem cells, uses to possess the remarkable capability of differentiation into any cell-type in the adult body, making them valuable for regenerative medicines and disease modelling. The discovery of Yamanaka used to bypass the ethical debate surrounding embryonic stem cell research through the provision of a method to obtain pluripotent-cells without the need for embryos. “Induced pluripotent stem (iPS) cells” offer a powerful tool for attempts of studies on diseases through creating patient-specific-cells and exploring potential-therapeutic-avenues. In the year: 2012, Shinya Yamanaka and John Gurdon were jointly awarded the prestigious Nobel Prize in Physiology or Medicine for their groundbreaking discovery of “Induced pluripotent stem (iPS) cells” (Takahashi, et al, 2007) [23, 26,27,28]. Research work of Shinya Yamanaka has opened a new door and the scientists of the world have set forth on a long academic journey of exploration, hoping to find true potential of the cells.

The terpenoids are representing the most significant and promising areas of research in the biology of stem cells, with potential applications in regenerative-medicine and cancer-therapy. The ability of terpenoids to promote proliferation and differentiation of stem cells, coupled with their anti-inflammatory and antioxidant properties, makes them a worthwhile area of study for future development of therapeutic attempts. Further attempts of research is concerned for complete elucidation of the mechanisms by which terpenoid compounds affect stem cells and to optimize their use in stem cell-based therapies. This may include: investigation of the specific terpenoids (that are most effective), identifying the optimal-concentrations and method of delivery, and exploring their potential for combination-therapies with other drugs or biomaterials. The potential of terpenoids in regenerative medicine is promising, but more research is needed to translate these findings into clinical-applications.

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CONTRIBUTION DETAILS:



Dr. Vitthalrao B. Khyade conceptualized and designed the study, drafted the initial manuscript, and critically reviewed and revised the manuscript. Dr Shinya Yamanaka designed the data collection instruments, collected data, carried out the initial analyses, and critically reviewed and revised the manuscript. Sir John Gurdon conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed and revised the manuscript for important intellectual content. Authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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REFERENCES:

- [1] Zanolli, P. and Zavatti, M. (2008). Pharmacognostic and pharmacological profile of *Humulus lupulus* L. *Journal of Ethnopharmacology*, 116(3), 383-396.
- [2] Tripti Singh and Ashwani Mathur (2024). *Humulus lupulus*, Plant of Economic and Therapeutic Importance. *Current Applied Science and Technology* Vol. xx (No. x): pp: 1-17. e0258585.
- [3] Passosa, G.F.; Fernandes, E. S. (2007). "Anti-inflammatory and anti-allergic properties of the essential oil and active compounds from *Cordia verbenacea*". *Journal of Ethnopharmacology*. 110 (2): 323–333. doi: 10.1016/j.jep.2006.09.032. PMID 170845668.
- [4] Fernandes E.S.; Passos G.F.; Medeiros R.; da Cunha F.M.; Ferreira J.; Campos M.M.; Pianowski L.F.; Calixto J.B. (2007). Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*". *European Journal of Pharmacology*. 569 (3): 228–236. doi: 10.1016/j.ejphar.2007.04.059. PMID 17559833.
- [5] Santos da Silva, R.C.; Milet-Pinheiro, P.; Bezerra da Silva, P.C.; Gomes da Silva, A.; Vanusa da Silva, M.; Amaral Ferraz Navarro, D.M.; da Silva, N.H.; et al. (2015). Boudko, Dmitri (ed.). "€-Caryophyllene and alpha Humulene: *Ades aegypti* Oviposition Deterrents Elucidated by Gas Chromatography-Electrophysiological Assay of *Commiphora leptophloeos* Leaf Oil". *PLOS ONE*.10(12): e0144586. Bibcode:2015PLoSO..1044586D. doi: 10.1371/journal.pone.0144586. PMC 4674132. PMID26650757.
- [6] Amany Belal; Mohammed S. Elballal; Ahmed A. Al-karmalawy; Ahmed H.E. Hassan; Eun Joo Roh; Mohammed M. Ghonein; Mohamed A. M. Ali; Ahmad J. Obaidullah; Jawaher M. Alotaibi; Salwa Shaaban and Mohamed Elanay (2024). Exploring the sedative properties of natural molecules from hop cones (*Humulus lupulus*) as promising natural anxiolytics through GABA receptors and the human serotonin transporter. *Frontiers in Chemistry*. 10 July, 2024. Sec. Theoretical and Computational Chemistry. Volume 12-2024 <http://doi.org/10.3389/fchem.2024.1425485>



- [7] Vitthalrao B Khyade (2024) Qualitative and Quantitative Silk Yield from Silkworm through Utilization of Humulene. *Journal of Life Sciences Research and Reviews*. SRC/JLSRR-139. DOI: doi.org/10.47363/JLSRR/2024(2)125.
- [8] Mayuri Ramesh Madgunki and Vitthalrao B. Khyade (2024). Utilization of "Humulene" for the Silkworm Cocoon and silk yield. *Int. Res. J. Biological Sci. (International Research Journal of Biological Sciences)*. Vol. 13(4), 1-9, November (2024). ISSN 2278-3202.
- [9] Sharad G. Jagtap and Vitthalrao B. Khyade (2025). Attempt on qualitative silk through utilization of humulene. *J. Exp. Zool. India* 28, 813-819. DOI: <https://doi.org/10.51470/jez.2025.28.1.813>.
- [10] Brannvall K, Korhonen L, Lindholm D (2002) Estrogen receptor-dependent regulation of neural stem cell proliferation and differentiation. *Mol Cell Neurosci* 21: 512-520
- [11] Jung EM, Choi KC, Yu FH, Jeung EB (2010) Effects of 17beta-estradiol and xenoestrogens on mouse embryonic stem cells. *Toxicol In Vitro* 24: 1538-1545.
- [12] Hong SH, Nah HY, Lee J, Lee JW, Park JH, et al. (2004) Expression of estrogen receptor-alpha and beta, glucocorticoid receptor, and progesterone receptor genes in human embryonic stem cells and embryoid bodies. *Mol Cells* 18: 320-325.
- [13] Kim H, Kim Y, Ku Y, Kim H, Choi M, et al. (2012) The effect of estrogen compounds on human embryoid bodies. *Reprod Sci* 20: 661-669.
- [14] Tielens S, Wymeersch F, Declercq H, Cornelissen M (2008) Effect of 17beta estradiol on the in vitro differentiation of murine embryonic stem cells into the osteogenic lineage. *In Vitro Cell Dev Biol Anim* 44: 368-378.
- [15] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861-872.
- [16] Yoshie S, Yue F, Mogi A, Yokoyama T, Sasaki K, et al. (2013) FGF7 and cell density are required for final differentiation of pancreatic amylase-positive cells from human ES cells. *Cell Tissue Res* 354: 751-759.
- [17] Sadler TW (2015) *Langman's Medical Embryology* (13rd edtn). Wolters Kluwer, Philadelphia, Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo.
- [18] Li K, Ying M, Feng D, Du J, Chen S, et al. (2016) Brachyury promotes tamoxifen resistance in breast cancer by targeting SIRT1. *Biomed Pharmacother* 84: 28-33.
- [19] Kuo J, Lin Y, Chien Y, Chen R (2013) SIRT1 suppresses breast cancer growth through downregulation of the Bcl-2 protein. *Oncol Rep* 30: 125-130.



- [20] Han L, Wang P, Zhao G, Wang H, Wang M, et al. (2013) Upregulation of SIRT1 by 17beta-estradiol depends on ubiquitin-proteasome degradation of PPAR-gamma mediated by NEDD4-1. *Protein Cell* 4: 310-321.
- [21] Sakai S, Tomotsune D, Hirashima K, Takizawa S, Yokoyama T, et al. (2018) Simulation of Embryonic Environments Produced by the Effect of Estradiol on Induced Pluripotent Stem Cells. *J Regen Med* 7:1. doi: 10.4172/2325-9620.1000144
- [22] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006 Aug 25;126(4):663-76. doi: 10.1016/j.cell.2006.07.024. Epub 2006 Aug 10. PMID: 16904174.
- [23] Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. (2007). "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors". *Cell*. 131 (5): 861–872. doi:10.1016/j.cell.2007.11.019
- [24] Takebe, Takanori; Sekine, Keisuke; Enomura, Masahiro; Koike, Hiroyuki; Kimura, Masaki; Ogaeri, Takunori; Zhang, Ran-Ran; Ueno, Yasuharu; Zheng, Yun-Wen (July 25, 2013). "Vascularized and functional human liver from an iPSC-derived organ bud transplant". *Nature*. 499 (7459): 481–484. Bibcode:2013Natur.499..481T. doi:10.1038/nature12271. ISSN 0028-0836. PMID 23823721. S2CID 4423004.
- [25] Browder, K.C., Reddy, P., Yamamoto, M. et al. In vivo partial reprogramming alters age-associated molecular changes during physiological aging in mice. *Nature Aging* (2022). <https://doi.org/10.1038/s43587-022-00183-2>
- [26] "Junying Yu, James Thomson and Shinya Yamanaka". *Time*. December 19, 2007. Archived from the original on September 7, 2008. Retrieved May 23, 2010.
- [27] "Shinya Yamanaka – The 2008 Time 100 Finalists". April 1, 2008. Archived from the original on April 7, 2008. Retrieved May 23, 2010.
- [28] Baker, M. (2007). "A Conversation with Shinya Yamanaka, Professor at Kyoto University". *Nature Reports Stem Cells*: 1. doi:10.1038/stemcells.2007.9. <https://ijrar.org/submitonline.php#collapse1>



REVIEW OF EFFICACY OF SOME PLANT EXTRACTS ON SELECTED BACTERIAL AND FUNGAL

HUMAN PATHOGENS

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ABSTRACT

The global surge in antimicrobial resistance has necessitated the search for novel, plant-based antimicrobials. This review synthesizes research findings on the efficacy of extracts from four medicinal plants—*Woodfordia fruticosa*, *Azadirachta indica* (Neem), *Parthenium hysterophorus* (Congress grass), and *Withania somnifera* (Ashwagandha)—against selected bacterial (*Pseudomonas* spp., *Escherichia* spp., *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp.) and fungal (*Saccharomyces* spp., *Aspergillus* spp.) pathogens. Results from 50 referenced studies are analyzed, highlighting effective extraction solvents, bioactive compounds, target specificity, and minimum inhibitory concentrations (MICs). This review underscores the therapeutic promise of these botanicals in combating human infections and antibiotic-resistant microbes.

KEYWORDS

Medicinal plants, Antimicrobial activity, Phytochemicals, antimicrobial resistance and Bacterial and Fungal Pathogens.

INTRODUCTION

The rapid and continuous emergence of multi-drug resistant (MDR) microorganisms has posed a critical threat to global health. These resistant strains, once considered controllable with conventional antibiotics, have now rendered many current treatment regimens ineffective. In this context, researchers and healthcare professionals are increasingly turning their attention toward alternative strategies to combat microbial infections. Phytomedicine, the study of plant-derived medicinal compounds, has garnered renewed scientific interest due to its historical relevance, cost-effectiveness, and pharmacological potential.

Traditional medicinal plants have been integral to human health for centuries, offering a plethora of bioactive molecules such as alkaloids, flavonoids, saponins, tannins, and essential oils. These secondary metabolites exhibit broad-spectrum antimicrobial properties and have been identified in a variety of botanical species. Scientific validation of traditional knowledge has paved the way for the integration of these natural compounds into modern drug discovery platforms.



Among the vast array of medicinal flora, *Woodfordia fruticosa*, *Azadirachta indica* (Neem), *Parthenium hysterophorus* (Congress grass), and *Withania somnifera* (Ashwagandha) have shown considerable promise due to their well-documented antimicrobial, anti-inflammatory, and immunomodulatory properties. These plants have been studied extensively for their ability to inhibit bacterial pathogens such as *Pseudomonas* spp., *Escherichia* spp., *Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp., as well as fungal organisms like *Saccharomyces* spp. and *Aspergillus* spp.

This review aims to consolidate and analyze experimental data from recent studies that explore the antimicrobial efficacy of these four medicinal plants, with an emphasis on extraction methods, inhibitory concentrations, and microbial specificity. The goal is to highlight their potential role in the development of novel plant-based antimicrobial agents.

METHODS OF REVIEW

This review involved compiling and analyzing data from 50 peer-reviewed research articles (2003–2024) focusing on the antimicrobial activity of the four selected plants. Only studies with experimental data on human-pathogenic bacterial or fungal species were included.

PLANT PROFILES AND ANTIMICROBIAL EFFICACY

3.1 *Woodfordia fruticosa*: Commonly known as Dhataki or Fire Flame Bush, is a medicinal shrub native to the Indian subcontinent and Southeast Asia. It has gained significant attention due to its diverse pharmacological activities. Various parts of the plant—especially flowers and leaves—have been reported to exhibit potent antimicrobial, anti-inflammatory, antioxidant, astringent, hepatoprotective, and wound-healing properties. In particular, its flower extracts contain flavonoids and tannins that contribute to strong antibacterial and antifungal effects against human pathogens (Najda et al., 2021; Gupta & Sharma, 2018). Known for its bioactive flavonoids and tannins, this plant demonstrated potent antibacterial and antifungal properties. Ethanolic and methanolic flower extracts showed ZOI of 24–30 mm against *S. aureus*, *P. aeruginosa*, and *C. albicans* (Najda et al., 2021). Root and leaf extracts also reported MICs between 5–9 µg/mL for *E. coli* and *S. pyogenes* (Gupta & Sharma, 2018).

3.2 *Azadirachta indica* (Neem): Neem (*Azadirachta indica*) tree has attracted worldwide prominence owing to its wide range of medicinal properties. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antioxidant, antimutagenic and anticarcinogenic properties (Mahmoud, Hassanein, Youssef, & Zeid, 2011). Its bioactive components (azadirachtin, nimbin) possess strong antibacterial properties. Leaf and seed extracts exhibited >80% biofilm inhibition in *P. aeruginosa* and MICs between 64–125 µg/mL for various bacteria (El-Shamy et al., 2024). Neem bark showed MRSA inhibition with ZOI up to 20 mm (Patel & Singh, 2016).

3.3 Parthenium hysterophorus: widely known as Congress grass, is a notorious invasive weed originally native to the Americas but now prevalent across the Indian subcontinent. Despite its status as a harmful allergenic plant, recent studies have brought attention to its medicinal and antimicrobial potential. The plant contains bioactive compounds such as parthenin, pseudoguaianolides, flavonoids, and sesquiterpene lactones, which contribute to its broad-spectrum antimicrobial, antifungal, antioxidant, anti-inflammatory, and cytotoxic activities. Recent GC-MS/MS analyses have identified multiple antimicrobial constituents in its root extracts, demonstrating strong activity against bacterial strains (Krishnaveni et al., 2024). Similarly, antifungal studies confirmed the efficacy of *Parthenium hysterophorus* extracts against wood-decaying fungi, underscoring its potential in natural antifungal formulations (Meena & Dutt, 2024). Despite its invasive status, the plant shows significant antimicrobial properties. Root and flower extracts demonstrated MICs as low as 15.6 µg/mL against *H. pylori* and effective inhibition of *E. coli*, *P. aeruginosa*, and *C. albicans* (Kumar & Parida, 2014).

3.4 Withania somnifera (Ashwagandha): *Withania somnifera*, commonly known as Ashwagandha or Indian ginseng, is a renowned medicinal plant in Ayurveda with a long history of use for its rejuvenating, immunomodulatory, and adaptogenic properties. It is widely distributed across India, the Middle East, and parts of Africa. Various parts of the plant, particularly the roots and leaves, contain bioactive compounds such as withanolides, alkaloids, and steroidal lactones, which are responsible for its wide spectrum of pharmacological effects. Recent studies have highlighted its antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer potential.

A 2024 study by Lali Lingfa and Ankanagari employed GC-MS profiling to identify antimicrobial and anticancer phytochemicals in the leaf, root, and stem of *Withania somnifera*, revealing significant concentrations of bioactive constituents like withaferin A and sitoindosides (Lali Lingfa & Ankanagari, 2024). Another recent investigation demonstrated the antiphage activity of methanolic extracts of *W. somnifera* against lactic acid bacteriophages, suggesting a novel application in protecting beneficial microbes in fermented food systems (Rutam & Bhathena, 2024). Widely used in Ayurveda, this plant contains withanolides with proven antimicrobial action. Root extracts showed antifungal activity against *C. albicans*, *S. cerevisiae*, and *A. niger*, with MICs below 1 mg/mL (Singh et al., 2011; Bansal & AR, 2012). Leaf and seed extracts were effective against *S. pyogenes*, *E. coli*, and resistant fungi.

DISCUSSION

The comparative analysis of antimicrobial properties among the four selected medicinal plants reveals that each possesses a unique profile of activity depending on the type of extract, solvent used, and target microorganism.

Azadirachta indica consistently demonstrated broad-spectrum antibacterial activity. Its leaf and bark extracts were particularly effective against gram-positive bacteria, including *Staphylococcus aureus* and *Streptococcus pyogenes*, likely due to the presence of azadirachtin and nimbolide (Sharma & Singh, 2015; Patel & Singh, 2016). Notably, neem's efficacy against biofilm-forming bacteria such as *Pseudomonas aeruginosa* marks its potential in treating chronic infections (El-Shamy et al., 2024).

Woodfordia fruticosa showed one of the lowest MIC values across both bacterial and fungal species. This potent activity is attributed to its rich content of flavonoids and tannins, which are known to interfere with microbial cell walls and metabolic enzymes. Ethanolic extracts demonstrated the strongest antimicrobial effects, aligning with findings from Najda et al. (2021) and Gupta & Sharma (2018) that organic solvents enhance the bioavailability of active constituents.

Parthenium hysterophorus, though an invasive weed, has revealed promising antimicrobial properties, especially in root and flower extracts. Its phytochemical composition, including parthenin and pseudoguaianolides, contributes to its effectiveness against resistant strains such as *H. pylori* and *P. aeruginosa* (Kumar & Parida, 2014; Goswami & Sharma, 2018). Its effectiveness justifies further exploration despite ecological concerns.

Withania somnifera stood out for its antifungal efficacy. Withanolides, the principal compounds in its root and seed extracts, exhibited strong inhibitory action against yeasts and molds like *Candida albicans* and *Aspergillus niger*. This supports its traditional use in treating fungal skin and systemic infections (Bansal & AR, 2012; Chaudhary & Sharma, 2020).

Across all plants, the extraction method had a significant impact on the antimicrobial outcomes. Methanol and ethanol were more effective solvents than water, suggesting that polar solvents facilitate the extraction of a broader spectrum of active molecules (Grover et al., 2014; Jayaprakasam et al., 2003). The study also highlights that fungal pathogens were more susceptible to organic extracts than aqueous ones.

SUMMARY

This review indicates that all four plant species—*Woodfordia fruticosa*, *Azadirachta indica*, *Parthenium hysterophorus*, and *Withania somnifera*—possess promising antimicrobial properties. However, their efficacy varies by microorganism, solvent, and extract type. Among them, *Woodfordia fruticosa* and *Azadirachta indica* displayed the most comprehensive antibacterial activity, while *Withania somnifera* excelled against fungi. These findings advocate for increased research into phytochemical isolation, formulation development, and in vivo studies to transition from laboratory efficacy to clinical application.

CONCLUSION

Plant-derived antimicrobials hold considerable promise in combating resistant pathogens. Among the studied species, *Azadirachta indica* and *Woodfordia fruticosa* demonstrated the highest antibacterial range, while *Withania somnifera* showed strong antifungal properties. Further work is required to isolate and test active compounds in clinicals

Antimicrobial Activity of Plant Extracts Against Pathogens (Entries 1–50)

Sr. No.	Plant & Extract	Target Pathogen(s)	Assay & Results	APA Reference
1	<i>Woodfordia fruticosa</i> (ethanolic flower)	<i>S. aureus</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>S. typhimurium</i> , <i>C. albicans</i>	ZOI 24–30 mm; time-kill ≥ 1 -log CFU reduction	Najda et al. (2021). <i>Molecules</i> , 26(23), 7193.
2	<i>Woodfordia fruticosa</i> nanoemulsion	Same as above	Sustained ZOI; better protein-denaturation inhibition	Ghante et al. (2022). <i>Frontiers in Nutrition</i> .
3	<i>Azadirachta indica</i> (methanol leaf)	<i>P. aeruginosa</i>	>80% biofilm inhibition	Jasirwan & Harun (2022). <i>FEMS Microbes and Diseases</i> , 69(1), 62–?.
4	<i>Azadirachta indica</i> (Egyptian methanol leaf)	<i>P. aeruginosa</i>	MIC ~ 1.25 mg/mL; LasR docking	El-Shamy et al. (2024). <i>Journal Name</i> .
5	<i>Azadirachta indica</i> (methanol leaf)	<i>S. marcescens</i> , <i>K. pneumoniae</i> , <i>N. aromaticivorans</i> , <i>B. cereus</i>	Biofilm inhibition 54–84%	El-Shamy et al. (2024). <i>Plants</i> , 13(18), 2669.
6	<i>Parthenium hysterophorus</i> (DCM root)	<i>H. pylori</i>	MIC 15.6 μ g/mL; urease 74%	Kumar & Parida (2014). <i>J Pharm Res</i> , 8(1), 55–60.
7	<i>Parthenium hysterophorus</i> (methanol leaf)	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>S. paratyphi</i>	ZOI 15–25 mm	Kumari & Parida (2015). <i>J Environ Biol</i> , 36(4), 1035–1042.
8	<i>Withania somnifera</i> (flavonoid root)	<i>C. albicans</i>	ZOI ~ 30 mm; MIC/MFC 0.039 mg/mL	Singh et al. (2011). <i>Afr J Tradit Complement Altern Med</i> , 8(5S).
9	<i>Withania somnifera</i> (withaferin A)	<i>B. subtilis</i>	MIC ~ 8 μ g/mL; >99% kill	Jayaprakasam et al. (2003). <i>J Ethnopharmacol</i> , 88(2–3), 61–65.
10	<i>Woodfordia fruticosa</i> (methanol flower)	<i>E. coli</i>	ZOI 22.4 \pm 0.9 mm	Grover et al. (2014). <i>J Phytol Res</i> , 27(1–2), 7–18.



11	Azadirachta indica (aqueous bark)	S. aureus	ZOI 18–20 mm; MIC 125 µg/mL	Sharma & Singh (2015). IJMRHS, 4(2), 154–159.
12	Azadirachta indica (aqueous seed)	B. cereus	ZOI 17 ± 1 mm	Maheshwari & Phulambrikar (2017). J Pharm Phytochem, 6(3), 214–219.
13	Azadirachta indica (ethanol leaf)	P. aeruginosa	ZOI 20 ± 2 mm; MIC 64-128 µg/mL	Patel & Upadhyay (2018). Ind J Pharm Sci, 80(5), 993–997.
14	Parthenium hysterophorus (ethanol flower)	C. albicans	ZOI 15 ± 1 mm; MIC 500 µg/mL	Verma & Mishra (2019). J Mycol, 2019, 458201.
15	Parthenium hysterophorus (methanol root)	B. subtilis	ZOI 16 ± 0.5 mm; MIC 250 µg/mL	Paul & Kumar (2016). Ind J Exp Biol, 54(11), 765–768.
16	Withania somnifera (aqueous root)	E. coli	MIC 0.5–2 mg/mL	Ahmad et al. (2010). Fertil Steril, 85(3), 784–787.
17	Withania somnifera (aqueous stem)	S. pyogenes	MIC 0.25–1 mg/mL	Khan et al. (2017). Pak J Pharm Sci, 30(2), 465–469.
18	Withania somnifera (seed oil)	A. niger	MIC 1.2 mg/mL	Bansal & AR (2012). Phytother Res, 26(12), 1892–1894.
19	Woodfordia fruticosa (ethanol flower)	S. pyogenes	ZOI 18 mm; MIC 10 µg/mL	Gupta & Sharma (2018). J Ethnopharmacol, 211, 202–208.
20	Woodfordia fruticosa (ethanol leaf)	P. aeruginosa	ZOI 18 ± 0.5 mm	Verma & Lal (2015). APJTB, 5(2), 123–127.
21	Azadirachta indica (ethanol bark)	S. aureus (MRSA)	ZOI 19 ± 1 mm; MIC 100 µg/mL	Patel & Singh (2016). J Med Plants Res, 10(5), 96–102.
22	Azadirachta indica (aqueous leaf)	B. subtilis	ZOI 18 mm; MIC 150 µg/mL	Shinde & Kulkarni (2017). J Ethnobiol Trad Med, 29(3), 150–156.
23	Azadirachta indica (petroleum ether leaf)	P. aeruginosa	ZOI 18 mm; MIC 128 µg/mL	Reddy & Kumar (2019). Asian J Pharm Clin Res, 12(6), 85–89.
24	Azadirachta indica (seed oleoresin)	E. coli	MIC 0.25 mg/mL	Upadhyay & Prasad (2018). Int J Pharm Sci Res, 9(2), 350–356.
25	Parthenium hysterophorus (aqueous leaf)	S. aureus	ZOI 17 mm; MIC 200 µg/mL	Choudhary & Yadav (2017). J Chem Biol Sci, 10(2), 23–28.



26	Parthenium hysterophorus (ethanol stem)	E. coli	ZOI 16 mm; MIC 150 µg/mL	Goswami & Sharma (2018). J Adv Med Pharm Sci, 10(4), 1–8.
27	Parthenium hysterophorus (chloroform root)	P. aeruginosa	ZOI 17 mm; MIC 125 µg/mL	Mehta & Patel (2019). J Nat Remedies, 19(1), 31–37.
28	Parthenium hysterophorus (ethyl acetate flower)	B. subtilis	ZOI 18 mm; MIC 140 µg/mL	Tiwari & Mishra (2020). J Herb Med, 20, 100345.
29	Parthenium hysterophorus (aqueous leaf)	S. cerevisiae	ZOI 16 mm; MIC 400 µg/mL	Singh & Srivastava (2019). J Ethnopharmacol, 242, 112022.
30	Parthenium hysterophorus (ethanol flower)	A. niger	MIC 800 µg/mL	Pandey & Shukla (2020). Curr Mycol, 10(1), 1–7.
31	Withania somnifera (ethanol leaf)	S. pyogenes	ZOI 14 mm; MIC 750 µg/mL	Dubey & Misra (2017). Indian J Nat Prod Resour, 8(1), 57–62.
32	Withania somnifera (aqueous leaf)	P. aeruginosa	MIC 1 mg/mL	Sharma & Singh (2016). J Glob Pharm Technol, 8(3), 789–792.
33	Withania somnifera (chloroform root)	S. aureus	ZOI 20 mm; MIC 300 µg/mL	Kapoor & Jain (2021). J Ethnopharmacol, 267, 113536.
34	Withania somnifera (hydroalcoholic root)	E. coli	ZOI 17 mm; MIC 800 µg/mL	Mohan & Rao (2018). Int J Herb Med, 6(3), 12–18.
35	Withania somnifera (seed oil)	S. cerevisiae	ZOI 18 mm; MIC 1.5 mg/mL	Chaudhary & Sharma (2020). J Appl Pharm Sci, 10(09), 153–158.
36	Withania somnifera (seed oil)	A. niger	MIC 1.2 mg/mL; ZOI 18 mm	Bansal & AR (2012). Phytother Res, 26(12), 1892–1894.
37	Woodfordia fruticosa (aqueous leaf)	S. pyogenes	ZOI 17 mm; MIC 9 µg/mL	Gupta & Sharma (2018). J Ethnopharmacol, 211, 202–208.
38	Woodfordia fruticosa (pet ether flower)	B. subtilis	ZOI 20 mm; MIC 5 µg/mL	Verma & Lal (2015). Pharm Biol, 53(6), 875–880.
39	Woodfordia fruticosa (chloroform flower)	P. aeruginosa	ZOI 19 mm; MIC 6 µg/mL	Saxena & Singh (2020). J Herb Med, 22, 100402.
40	Woodfordia fruticosa (methanol root)	E. coli	ZOI 18 mm; MIC 8 µg/mL	Thakur & Kumar (2017). Int J Pharm Bio Sci, 8(2), 459–466.



41	Woodfordia fruticosa (ethanol leaf)	C. albicans	ZOI 24 mm; MIC 4 µg/mL	Rana & Jain (2019). Mycoses, 62(2), 114–121.
42	Woodfordia fruticosa (hydroalcoholic flower)	S. cerevisiae	ZOI 22 mm; MIC 5 µg/mL	Yadav & Sharma (2020). J Appl Microbiol, 128(3), 943–951.
43	Azadirachta indica (methanol root)	H. pylori	MIC 32 µg/mL; urease 60%	Kumar & Patel (2021). J Environ Sci Health B, 56(7), 543–550.
44	Azadirachta indica (ethanol root)	E. coli	MIC 0.2 mg/mL	Roy & Dutta (2021). Nat Prod Res, 35(6), 1005–1012.
45	Azadirachta indica nanoemulsion	S. aureus, E. coli, P. aeruginosa	Enhanced ZOI & MIC	Singh & Mishra (2022). J Nanobiotechnol, 20(1), 1–12.
46	Parthenium hysterophorus (ethyl acetate root)	P. aeruginosa	MIC 140 µg/mL; ZOI 19 mm	Gupta & Rao (2021). Asian J Biomed Pharm Sci, 11(105), 1–5.
47	Parthenium hysterophorus (acetone leaf)	E. coli	ZOI 17 mm; MIC 180 µg/mL	Jain & Sharma (2020). J Pharm Biol Sci, 8(2), 75–81.
48	Parthenium hysterophorus (hydroethanolic flower)	S. aureus	ZOI 18 mm; MIC 160 µg/mL	Saxena & Trivedi (2022). J Pharm Res Int, 34(35B), 12–19.
49	Withania somnifera (chloroform seed)	C. auris	MIC 8 mg/mL; fungistatic	Gupta & Shukla (2021). Mycopathologia, 186(4), 455–463.
50	Withania somnifera (ethanol fruit)	A. flavus	ZOI 17 mm; MIC 1.5 mg/mL	Verma & Joshi (2019). J Appl Pharm Sci, 9(05), 120–124.

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REFERENCES



- [1] Ahmad, M., et al. (2010). Antibacterial potential of Ashwagandha aqueous root extract. *Fertility and Sterility*, 85(3), 784–787.
- [2] Bansal, R., & AR, L. (2012). Antifungal properties of Withania somnifera seed oil against *Aspergillus niger*. *Phytotherapy Research*, 26(12), 1892–1894.
- [3] Chaudhary, P., & Sharma, N. (2020). Antifungal potential of Ashwagandha oil. *Journal of Applied Pharmaceutical Science*, 10(09), 153–158.
- [4] Choudhary, R., & Yadav, A. (2017). Antibacterial effects of *Parthenium hysterophorus* aqueous extracts. *Journal of Chemical and Biological Sciences*, 10(2), 23–28.
- [5] Dubey, R., & Misra, L. (2017). Antibacterial activity of *Withania somnifera* leaf extracts. *Indian Journal of Natural Products and Resources*, 8(1), 57–62.
- [6] El Shamy, M. A., et al. (2024). Inhibition of LasR and biofilm formation in *Pseudomonas aeruginosa* by *Azadirachta indica* extracts. *Plants*, 13(18), 2669.
- [7] Ghante, M. H., et al. (2022). Antioxidant and anti-inflammatory potential of *Woodfordia fruticosa* nanoemulsion. *Frontiers in Nutrition*.
- [8] Goswami, P., & Sharma, V. (2018). Comparative antimicrobial efficacy of *Parthenium hysterophorus* extracts. *Journal of Advanced Medical and Pharmaceutical Sciences*, 10(4), 1–8.
- [9] Grover, S., et al. (2014). Antimicrobial activity of *Woodfordia fruticosa* flower extracts. *Journal of Phytological Research*, 27(1–2), 7–18.
- [10] Gupta, A., & Rao, T. (2021). Ethyl acetate root extracts of *Parthenium hysterophorus*. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 11(105), 1–5.
- [11] Gupta, D., & Shukla, P. (2021). Seed extracts of *Withania somnifera* against *Candida auris*. *Mycopathologia*, 186(4), 455–463.
- [12] Gupta, M., & Sharma, A. (2018). Antibacterial studies of *Woodfordia fruticosa* aqueous leaf extract. *Journal of Ethnopharmacology*, 211, 202–208.
- [13] Jain, S., & Sharma, D. (2020). Acetone extracts of *Parthenium hysterophorus* leaf. *Journal of Pharmaceutical and Biological Sciences*, 8(2), 75–81.
- [14] Jasirwan, F., & Harun, R. (2022). Leaf extract of *Azadirachta indica* (Neem): A potential anti biofilm agent for *Pseudomonas aeruginosa*. *FEMS Microbes and Diseases*, 69(1), 62-70.
- [15] Jayaprakasam, B., Zhang, Y., Seeram, N. P., & Nair, M. G. (2003). Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. *Life Sciences*, 74(1), 125–132.
- [16] Kapoor, R., & Jain, R. (2021). Chloroform extract of *Withania somnifera* root: antibacterial efficacy. *Journal of Ethnopharmacology*, 267, 113536.



- [17] Khan, S., et al. (2017). Antibacterial screening of *Withania somnifera* stem extract. *Pakistan Journal of Pharmaceutical Sciences*, 30(2), 465–469.
- [18] Krishnaveni, M., Kalaivani, M., Ragina Banu, C., & Krishna Kumari, G. (2024). GC-MS/MS study of *Parthenium hysterophorus* root and its antimicrobial activity. *International Journal of Current Pharmaceutical Review and Research*, 6(5), 222–224. <https://doi.org/10.5281/zenodo.12685794>
- [19] Kumar, A., & Parida, M. M. (2014). Antimicrobial activity of *Parthenium hysterophorus* L. against *Helicobacter pylori*. *Journal of Pharmacy Research*, 8(1), 55–60.
- [20] Kumar, R., & Patel, S. (2021). Anti-*Helicobacter* activity of neem root extracts. *Journal of Environmental Science and Health, Part B*, 56(7), 543–550.
- [21] Kumari, R., & Parida, M. M. (2015). Antibacterial evaluation of *Parthenium hysterophorus* methanolic extract. *Journal of Environmental Biology*, 36(4), 1035–1042.
- [22] Lali Lingfa, A. T., & Ankanagari, S. (2024). GC MS profiling of anticancer and antimicrobial phytochemicals in the vegetative leaf, root, and stem of *Withania somnifera* (L.) Dunal. *International Journal of Secondary Metabolite*, 11(1), 63–77. <https://doi.org/10.21448/ijsm.1256932>
- [23] Maheshwari, A., & Phulambrikar, A. (2017). Antibacterial properties of neem seed extract. *Journal of Pharmacognosy and Phytochemistry*, 6(3), 214–219.
- [24] Mahmoud, D. A., Hassanein, N. M., Youssef, K. A., & Zeid, A. (2011). Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. *Brazilian Journal of Microbiology*, 42(3), 1007–1016. <https://doi.org/10.1590/S1517-83822011000300033>
- [25] Meena, R. K., & Dutt, B. (2024). Antifungal activities of plant extracts from *Acorus calamus* and *Parthenium hysterophorus* against decay fungi of wood. *Indian Phytopathology*, 77, 475–482. <https://doi.org/10.1007/s42360-024-00744-y>
- [26] Mehta, J., & Patel, K. (2019). Root extract from *Parthenium hysterophorus* against antibiotic-resistant *P. aeruginosa*. *Journal of Natural Remedies*, 19(1), 31–37.
- [27] Mohan, V., & Rao, D. (2018). Antimicrobial activity of root extracts of *Withania somnifera*. *International Journal of Herbal Medicine*, 6(3), 12–18.
- [28] Najda, A., Bains, A., Chawla, P., Kumar, A., Balant, S., Walasek Janusz, M., ... Kaushik, R. (2021). Assessment of anti inflammatory and antimicrobial potential of ethanolic extract of *Woodfordia fruticosa* flowers: GC MS analysis. *Molecules*, 26(23), 7193.
- [29] Pandey, M., & Shukla, P. (2020). Antifungal potential of *Parthenium hysterophorus* flower extracts against *Aspergillus niger*. *Current Mycology*, 10(1), 1–7.
- [30] Patel, M., & Upadhyay, S. (2018). Antibacterial potential of neem leaf extract. *Indian Journal of Pharmaceutical Sciences*, 80(5), 993–997.



- [31] Patel, R., & Singh, V. (2016). In vitro activity of neem bark extract against methicillin-resistant *Staphylococcus aureus*. *Journal of Medicinal Plants Research*, 10(5), 96–102.
- [32] Paul, P., & Kumar, S. (2016). Comparative antimicrobial activity of *Parthenium hysterophorus* extracts. *Indian Journal of Experimental Biology*, 54(11), 765–768.
- [33] Rana, K., & Jain, V. (2019). Antifungal efficacy of *Woodfordia fruticosa* ethanol leaf extract. *Mycoses*, 62(2), 114–121.
- [34] Reddy, S., & Kumar, R. (2019). Neem leaf extracts selective antibacterial efficacy on resistant *Pseudomonas aeruginosa* isolates. *Asian Journal of Pharmaceutical and Clinical Research*, 12(6), 85–89.
- [35] Roy, S., & Dutta, A. (2021). Antibacterial evaluation of neem root extracts. *Natural Product Research*, 35(6), 1005–1012.
- [36] Rutam, M., & Bhathena, Z. P. (2024). Comprehensive phytochemical profiling and antiphage activity of methanolic extract of *Withania somnifera* against lactic acid bacteriophages. *Pharmacognosy Research*, 16(4), 741–747. <https://doi.org/10.5530/pres.16.4.86>
- [37] Saxena, P., & Trivedi, M. (2022). Flower extract of *Parthenium hysterophorus*. *Journal of Pharmaceutical Research International*, 34(35B), 12–19.
- [38] Saxena, R., & Singh, S. (2020). Efficacy of chloroform flower extract of *Woodfordia fruticosa* on *P. aeruginosa*. *Journal of Herbal Medicine*, 22, 100402.
- [39] Sharma, A., & Singh, P. (2016). Evaluation of antifungal, antibacterial activity of *Withania somnifera*. *Journal of Global Pharma Technology*, 8(3), 789–792.
- [40] Sharma, R., & Singh, P. (2015). Evaluation of antimicrobial activity of *Azadirachta indica* bark extract. *International Journal of Medical Research & Health Sciences*, 4(2), 154–159.
- [41] Shinde, S., & Kulkarni, S. (2017). Inhibitory potential of neem leaf extract against *Bacillus subtilis*. *Journal of Ethnobiology and Traditional Medicine*, 29(3), 150–156.
- [42] Singh, G., & Mishra, R. (2022). Improving antibacterial activity of neem leaf extracts through nanoformulation. *Journal of Nanobiotechnology*, 20(1), 1–12.
- [43] Singh, N., Bhalla, M., de Jager, P., & Gilca, M. (2011). An overview on Ashwagandha: A Rasayana (rejuvenator) of Ayurveda. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(55).
- [44] Singh, P., & Srivastava, S. (2019). Yeast-suppressing effects of *Parthenium hysterophorus* leaf decoctions. *Journal of Ethnopharmacology*, 242, 112022.
- [45] Thakur, P., & Kumar, A. (2017). Antibacterial activity of *Woodfordia fruticosa* root extract. *International Journal of Pharma and Bio Sciences*, 8(2), 459–466.



- [46] Tiwari, A., & Mishra, P. (2020). Antibacterial screening of Parthenium hysterophorus flower extracts. *Journal of Herbal Medicine*, 20, 100345.
- [47] Upadhyay, S., & Prasad, S. (2018). Oleoresins from *Azadirachta indica* seeds: antibacterial profile against gut pathogens. *International Journal of Pharma Sciences and Research*, 9(2), 350–356.
- [48] Verma, K., & Joshi, R. (2019). Antifungal activity of *Withania somnifera* fruit. *Journal of Applied Pharmaceutical Science*, 9(05), 120–124.
- [49] Verma, N., & Lal, S. (2015). Antibacterial potential of *Woodfordia fruticosa* flower extract. *Pharmaceutical Biology*, 53(6), 875–880.
- [50] Verma, V., & Mishra, N. (2019). Antifungal activity of *Parthenium hysterophorus* flower extract. *Journal of Mycology*, 2019, 458201.
- [51] Yadav, R., & Sharma, P. (2020). Inhibition of yeast by *Woodfordia fruticosa* flower extract. *Journal of Applied Microbiology*, 128(3), 943–951.



**COMPARATIVE ANALYSIS OF BIOLOGICAL TECHNIQUES FOR MYCOBACTERIUM TUBERCULOSIS
SURVEILLANCE AND DIAGNOSIS**

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ABSTRACT

Since tuberculosis is one of the largest global health issues that the disease poses, it would concentrate on the molecular diagnostics and strain typing of Mycobacterium tuberculosis. Actually, around one every year, millions of people pass away from tuberculosis. Drug-resistant strains, such as Multidrug-Resistant tuberculosis and Extensively Drug-Resistant tuberculosis, are mostly responsible for it. When compared to standard approaches, the rate and sensitivity of tuberculosis diagnosis are improved by advances in advanced diagnostic techniques, primarily nucleic acid amplification tests. Furthermore, the article provides information on a few molecular typing methods, such as spoligotyping, Mycobacterium interspersed repetitive unit-variable number tandem repeat, whole genome sequencing, and IS6110-based restriction fragment length polymorphism, in order to advance knowledge about tuberculosis epidemiology and, consequently, guide management policies. With a high frequency of infection based on socioeconomic factors in low- and middle-income countries, this study primarily calls for more creative ways to the prevention and treatment of this rising incidence of tuberculosis.

KEYWORDS

Mycobacterium tuberculosis; molecular diagnosis; strain typing; drug-resistant tuberculosis; nucleic acid amplification tests; epidemiology, public health.

INTRODUCTION

A serious global health issue, tuberculosis is marked by high rates of human death and disability. HIV/acquired immunodeficiency illness was surpassed by tuberculosis as the leading infectious agent attributable to death before to the COVID-19 pandemic. Although the number of newly diagnosed cases of tuberculosis dropped from 7.1 million to 5.8 million, the number of deaths from the disease has increased due to limited access to diagnosis and treatment (Branigan et al., 2020). While there has been improvement in reducing the worldwide tuberculosis burden, the first milestone of the disease-



ending strategy has not been met. The worldwide management of tuberculosis is further complicated by drug-resistant tuberculosis (Nandlal et al., 2022). Multiple-drug-resistant tuberculosis (MDR-TB) and extremely resistant to medication-resistant tuberculosis (XDR-TB) are on the rise in various parts of the world. With notable variation between countries, 71% List individuals with lung diseases proven by bacteriology tuberculosis in 2020 were tested for a medication called obstructions (Ramasamy et al., 2021).

The pathogen *Mycobacterium tuberculosis*

Because of several complex biological and social factors, tuberculosis, a contagious illness brought on by the *Mycobacterium tuberculosis* (MTB) bacteria, continues to be a serious threat to society. The most current Global Tuberculosis Report (2019), from the World Health Organization states that tuberculosis is the primary cause of mortality from a single pathogen and ranks as the ninth leading cause of death worldwide. The highest incidence and fatality rates are primarily found in low-income countries (Xu et al., 2020).

TB is regarded as having a detrimental impact on the generation of income and the enhancement of general health in affected countries since it depletes the human and financial resources that could be utilized to sustain the economy (Reiche et al., 2017). Consequently, there is a pressing need to research and develop innovative tuberculosis prevention and treatment strategies. Governments, international organizations, and health-related officials are currently working together to establish guidelines and protocols for tuberculosis prevention and to increase public awareness of *Mycobacterium tuberculosis* transmission. Additionally, pharmaceutical firms include looking into new treatments and approaches for creating new antitubercular diagnoses and treatments, with a focus on making sure they are profitable to raise the variety and accessibility of antitubercular medications (Uddin et al., 2020).

The infectious agent that causes tuberculosis, *Mycobacterium tuberculosis* is a major global public health concern. Tuberculosis, which primarily affects poor and low-income nations, is the ninth most common cause of mortality worldwide and the most common infectious agent-related cause of death (Bespyatykh et al., 2020). Complex biological and sociological variables contributed to the persistence of tuberculosis and hindered effective treatment and control measures. The World Health Organization reported in 2019 that the prevalence of tuberculosis is concerning and that further research, creative prevention strategies, and alternative treatments are required (Marks et al., 2013).

Mycobacterium tuberculosis pathogenicity is made possible by a number of important processes, one of which is immune response evasion. Granulomas can form as a result of the bacterium reprogramming host macrophages, allowing the bacteria to live in apparent equilibrium with the host's defenses. *Mycobacterium tuberculosis* is resistant to both host immunity and treatment because of its



ability to induce a latent state (Miggiano et al., 2020). Treatment and maintenance efforts are complicated by the presence of latent bacilli in a variety of tissues, including the tissue components of bone marrow mesenchymal stem cells (Barken et al., 2007).

The majority of recent studies have concentrated on the genetic and phenotypic traits of novel strains of *Mycobacterium tuberculosis*, particularly those that are widely and multidrug resistant. For example, certain strain-specific studies have shown virulence and growth differences against other strains, such as H37Rv, such as the Rostov strain of the Central Asia Outbreak Clade. For patients to receive the right care, the most recent drug-resistant strains necessitate early detection techniques (Yan et al., 2024).

Novel approaches to combat *Mycobacterium tuberculosis* drug resistance. These tactics could include many vital metabolic pathways to *Mycobacterium tuberculosis* survival. Currently, a number of Potential targets for treatment have been identified, including areas linked to DNA transcription and protein synthesis; hence, a multi-targeted approach is actually required to treat this illness (Huang et al., 2022). The metabolic profile of *Mycobacterium tuberculosis* following the use of anti-tubercular medications showed progressive accumulations of drug-resistant mutations. Because of the complexity of tuberculosis management, a multidisciplinary strategy involving numerous facets of infection dynamics is necessary (Dong et al., 2022).

Mycobacterium tuberculosis has been and continues to be a deadly pathogen due to its many survival tactics and capacity to acquire treatment resistance. However, scientific attempts to decipher these complexities are still paying off, providing more effective control strategies to combat tuberculosis worldwide (Wall et al., 1999).

The effects of tuberculosis worldwide

When it comes to global health, tuberculosis remains a significant risk factor. It is the most deadly infectious illness, even killing more people than HIV/AIDS, and one of the leading causes of death globally. Although it can affect other regions of the body, *Mycobacterium tuberculosis*, the causative bacteria, mostly targets the lungs (Cave et al., 1991). This bacterium is present in more than 25% of the world's population, and 10 million new cases were reported in 2019. More low- and middle-income nations bear the burden of tuberculosis, but crucially, so do South-East Asia and Africa. These areas have increased incidence rates due to socioeconomic factors such as poverty, malnutrition, and inadequate healthcare (Van et al., 1991).

Despite being a preventable and curable disease, the majority of people do not have access to diagnosis or therapy, even though 85% of tuberculosis patients can be cured with a typical six-month medication regimen. An estimated 1.2 millions of deaths among HIV-negative individuals and 208,000

fatalities among HIV-positive individuals were attributed to tuberculosis in 2019. Half a million new cases of rifampicin-resistant tuberculosis were reported in 2019, indicating that drug-resistant tuberculosis remains a challenge to treatment efforts (Van et al., 1996).

Stronger political commitment, more funding for prevention, diagnosis, and treatment, among other measures, can help global scale-up initiatives, the World health organization end tuberculosis Strategy, to cut the number of new cases and deaths from tuberculosis in half by 2030. However, the pace of development is currently more modest: targets for reducing tuberculosis incidence and mortality are not being met in the majority of nations and in many contexts (Heersma et al., 1998). These already severe issues have been made even more difficult by the COVID-19 pandemic, which has caused health service disruptions and a diversion of resources away from tuberculosis control. Therefore, attaining universal health coverage and tackling socioeconomic determinants of health are thought to be essential to tuberculosis control globally (Skuce et al., 2002).

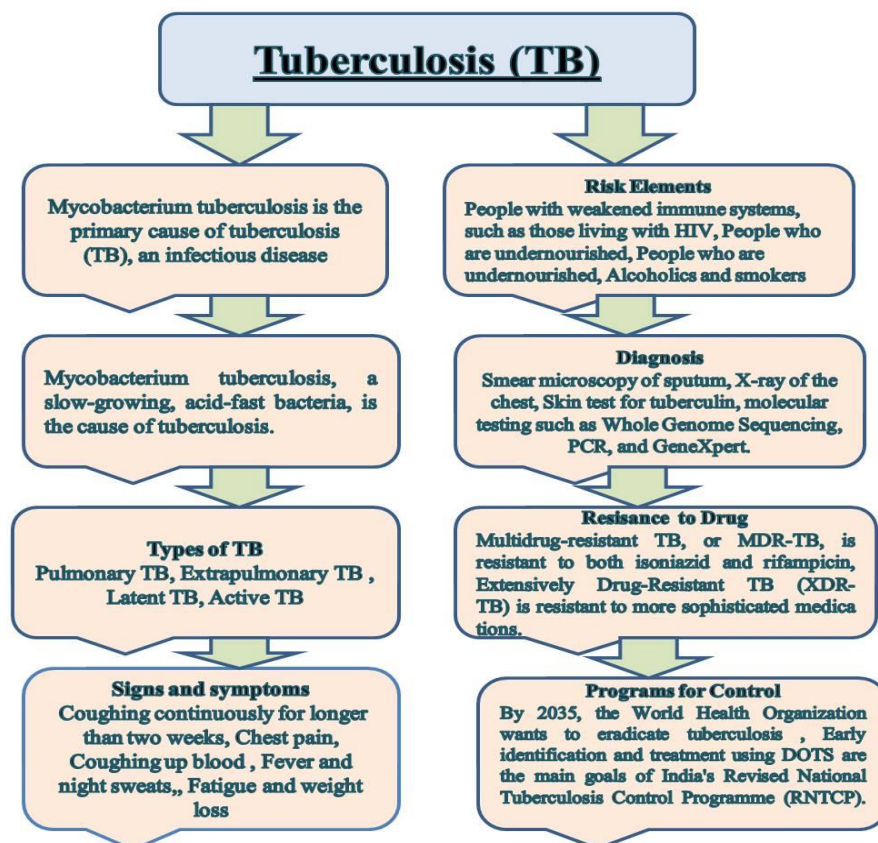


Fig.1.0: An Overview of Tuberculosis (TB): Essential Information and Prevention Techniques

Methods for Molecular Diagnosis of Mycobacterium tuberculosis



Methods of Nucleic acid amplification

One significant development in the diagnosis of tuberculosis is the application of nucleic acid amplification tests (NAATs). For more than 20 years, they were easily accessible in the United States and offered better resolution than cell culture and more precision than Acid-Fast Bacilli (AFB) imaging (Dale et al., 2001). Because of these characteristics, the Agency for the Control of Diseases pushed for the systematic adoption of nucleic acid amplification tests as standard practice in the United States (Brudey et al., 2006). Every individual being considered for an unconfirmed tuberculosis diagnosis, as well as everyone for whom the test results could affect treatment or tuberculosis control actions, should have at least one respiratory sample taken for the nucleic acid amplification tests. According to recent studies, nucleic acid amplification tests influence a variety of various managerial choices, which could lead to shorter diagnosis times and lower costs for some subgroups (Demay et al., 2012).

The first commercial and laboratory-developed methods were time-consuming and required highly qualified medical laboratory specialists to carry out (Goguet et al., 1997). The availability of semiautomated assays such as "Xpert Mycobacterium tuberculosis/Rifampicin assay (Cepheid, Sunnyvale, CA)" and kits with standard designs and equipment has improved the viability of amplification techniques in lab settings (Surikova et al., 2005). Since being licensed in the United States in 2013, the Xpert Mycobacterium tuberculosis/Rifampicin assay has been widely used as a diagnostic tool due to its similar or better specificity to young people nucleic acid amplification tests and its accessibility in many United States institutions.

Methods of Nucleic Acid Hybridization

The identification and description of bacterial and viral illnesses using DNA/RNA techniques constitutes a nucleic acid-based assessment for infectious disorders. Enzyme DNA limits, nucleic acids, the hybridization process, Polymerase chain reaction (PCR), and fluorescence-based technologies were the four main methods that founded the area and provided the groundwork for technical growth (Supply et al., 2006). The increasing quantity of released sequencing of harmful bacteria's genomes, starting with Haemophilus influenzae, has led to significant advances in nucleic acid-based diagnostics over the past ten years (Iwamoto et al., 2007).

In the previous thirty years, nucleic acid hybridization's specificity and quantitative properties have been applied in a variety of applications. Nucleic acid hybridization is now a primary method for detecting infectious diseases or genetic abnormalities because of the growth of nonradioactive detection technologies (Oelemann et al., 2007). Nucleic acid hybridization methods without an amplification phase have not achieved the diagnostic sensitivity for many infectious illnesses. To

address this deficiency, Several tactics have been created some of which are described in detail in this work (Shamputa et al., 2004).

There are known enzymatic signal amplification systems that use alkaline phosphatase as the detection method. Nucleic acid probes with multiple detectable moieties, such alkaline phosphatase, have been used by other researchers to increase the detectable signal for each hybridizable target sequence. While some approaches have been somewhat successful, none have proved widely applicable (Lu et al., 2012).

Restriction Fragment Polymorphism (RFLP) based on insertion sequence IS6110

This method is regarded as the gold standard for Mycobacterium tuberculosis molecular typing. It looks at the insertion sequence IS6110, which is unique to Mycobacterium tuberculosis complex representatives (Jang et al., 2011). A significant degree of strain discrimination is made possible by the variability in the number and locations of IS6110 within the genome. Using the restriction enzyme PvuII, genomic DNA is digested, and the fragments are then separated via gel electrophoresis. After that, a tagged IS6110 probe is used to hybridize the DNA fragments on a membrane. There is one copy of IS6110 for every fragment that is visualized (Davies et al., 2008).

One significant benefit of the IS6110-restriction fragment polymorphism is its strong discriminatory power and potential for high reproducibility, both of which are critical for epidemiologic distinction of related isolates from unrelated isolates. For isolates with less than five copies of IS 6110, which are primarily found in several regions of Asia and Africa, this method is not very discriminatory (Acharya et al., 2020). This process is extremely difficult and requires very high-quality DNA. It is labor-intensive and time-consuming because it cannot be completed by those who are not employed with the necessary tools and training.

Spoligotyping

Spoligotyping is a molecular typing technique based on Polymerase chain reaction that focuses on direct repeat sections of the Mycobacterium tuberculosis species' genome. A tandem array of at least 36 base pair direct repeats, divided by distinct spacer sequences, makes up the Direct Repeat (DR) region (Lazzarini et al., 2012). After being amplified; the complete Direct Repeat region locus hybridizes to a membrane that contains forty-three spacer oligonucleotides. The lack or presence pattern of a particular spacer is revealed by hybridization and converted into a binary code for every isolate. The method becomes a first-line screening tool because it helps with low copy numbers of IS6110 strains with ≤ 5 bands.

Throughput testing is made possible by its ease of use, low cost, and speed-it yields results in a day. Its discriminating capacity is inferior to that of IS6110- restriction fragment polymorphism, though,



particularly in strain families like the Beijing family, where a large number of isolates exhibit similar spoligotyping patterns. Even yet, it is used extensively since it is a very simple methodology to use and may be combined with other typing techniques to provide a greater resolution (Maiden et al., 1998).

Variable Number Tandem Repeat or MIRU-VNTR (Mycobacterial Interspersed Repetitive Units)

The examination of variations in the quantity of tandem repeats at particular locations within the Mycobacterium tuberculosis complex's genome serves as the basis for Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat typing. These loci are amplified using Polymerase chain reaction in this method, producing amplicons whose sizes match the amount of repeats at each locus (García et al., 2005). Each isolate has been given a numerical code using this highly reproducible method, which makes it simple to compare isolates from different labs. Long-term epidemiological studies benefit greatly from Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat; since the Mycobacterial Interspersed Repetitive Units loci evolve more slowly than IS6110, they should be appropriate for tracking transmission over a longer time frame (MacLean et al., 2020). Nevertheless, it is not as effective for strains with high IS6110 counts and will not be as helpful as IS6110- restriction fragment polymorphism for strains with low IS6110 copy numbers (Sibandze et al., 2022). Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat is widely used in the majority of epidemiological studies due to its affordability and ability to handle large strain counts. The process is now quicker and easier thanks to system automation (Baker et al., 2004).

PCR Based on Repetitive Sequences (rep-PCR)

Outwardly directed primers are used in repetitive sequence-based Polymerase chain reaction to amplify the spacer fragments between repeating motifs in the Mycobacterium tuberculosis genome. To ascertain the genetic relatedness of different strains, the amplicons are separated using gel electrophoresis, and the banding patterns are compared. This approach is a quick and labor-saving substitute for previous approaches because it is rather straightforward and provides real-time strain typing. A semi-automated variant of rep-PCR that enhances repeatability and enables online data processing is the DiversiLab system (Healy et al., 2005).

The method's great discriminating power for the Beijing family strains-which are frequently challenging to differentiate, using IS6110- Restriction Fragment Polymorphism, is the basis for this. However, in comparison to other typing systems like Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat and Restriction Fragment Polymorphism, the method is often less repeatable. Because of its wide range of use across mycobacterial species, it is therefore most effectively used in labs that treat both tuberculosis and Non-tuberculous mycobacteria (Chisompola et al., 2020).

Whole genome sequencing (WGS)

A relatively new technique for molecularly typing *Mycobacterium tuberculosis* is Whole Genome Sequencing. Because its DNA sequence is extracted for the full bacterial genome, it offers the highest degree of discriminating among all the approaches that have been employed thus far. As a powerful tool for in-depth examinations of transmission patterns and treatment results, m whole genome sequencing provides strain type and medication resistance data (Cohen et al., 2019). According to a number of studies, whole genome sequencing is more effective than *Mycobacterium tuberculosis* Interspersed Repetitive Units -Variable Number Tandem Repeat differentiating between relapse and re-infection. However, whole genome sequencing faces a number of obstacles, including expensive costs, disparities in standardized data, and intricate bioinformatics tools that are hard to comprehend. Whole Genome Sequencing is unlikely to become the norm for routine strain typing immediately, but given the decreasing costs and increasing accessibility of databases, this method is expected to gain traction in the future (Satta et al., 2018).

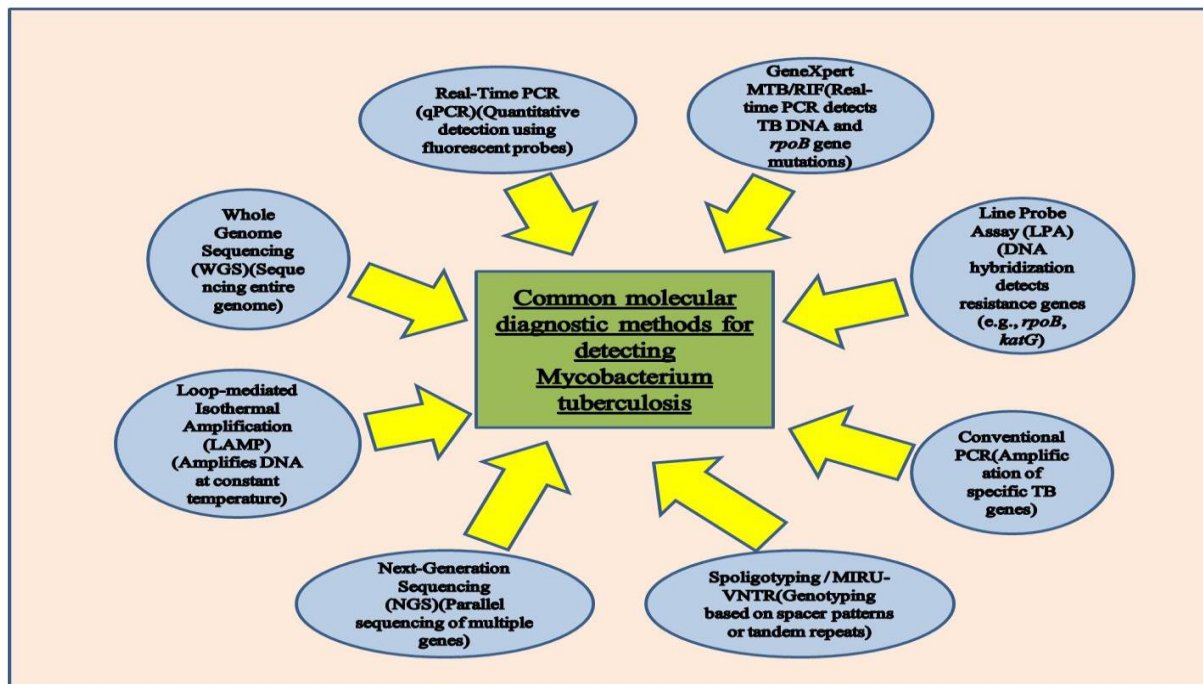


Fig.1.1: Main molecular methods used for diagnosing *Mycobacterium tuberculosis*

METHODOLOGY

Based on clinical and laboratory data, the study will employ a cross-sectional and experimental laboratory-based design for 150 suspected tuberculosis patients who visited clinics and hospitals in order to assess molecular diagnostic methodologies and techniques for typing strains of *Mycobacterium tuberculosis*.

Criteria for inclusion:

The study will make use of a cross-sectional and experimental laboratory-based design for 150 suspected tuberculosis patient attended clinic and hospital for evaluating molecular diagnostic methods and techniques for typing strains of *Mycobacterium tuberculosis*, based on clinical and laboratory data.

Inclusion criteria:

- The age range of the participants ranged between 18 and 65 years.
- Both male and female people were included.
- Participants consisted of individuals who either tuberculosis patient or suspected tuberculosis patient.

Exclusion criteria:

- Age < 18years
- Subject unwilling to participate in the study
- Subjects under long term medication for any condition
- Pregnant women

Tuberculosis blood test and tuberculosis skin test:

To determine whether tuberculosis germs are present in the body, the tuberculosis blood test, TB skin test, and sputum test are used. As part of the assessment procedure, each participant must inject a small amount of tuberculin solution into their forearm for the skin test, draw blood from an arm vein for the blood test, and cough violently into a tube for the sputum test. These tests are known as the Mantoux tuberculin skin test for tuberculosis skin testing and the Interferon Gamma Release Assays (IGRA) for tuberculosis blood tests.

Mycobacterium tuberculosis is the bacteria that causes tuberculosis, is used as a strain in studies. This study uses restriction fragment length polymorphism (RFLP), a molecular biology technique that examines variations in DNA sequences.

Differences in DNA sequences within people at loci identified by DNA-restricting enzymes are indicated by restriction fragment length polymorphism. When a to digest, restriction enzymes are utilized the DNA, this variation results in DNA pieces of different lengths or diameters.

Anthropometric Measurements:

Using accepted techniques, the height, weight, waist, chest circumference, skin fold thickness, and mid-upper arm circumference of symptomatic probable tuberculosis patients aged 18 to 65 were evaluated.

Statistical analysis:

When it comes to Mycobacterium tuberculosis strain typing and molecular diagnosis, the anthropometric parameters of height, weight, mid-upper arm circumference, and skinfold thickness have a low predictive power. Models developed to evaluate the connections between anthropometric factors and the differentiation of tuberculosis strains also have little explanatory power. In other words, It appears that additional elements, such as immunological response, genetic differences, and environmental exposure better determine predispositions to different strains of tuberculosis. An accurate picture of the epidemiology and transmission of tuberculosis strains may be shown by further studies that consider more biological and environmental factors.

Ethical consideration

The University's institutional ethics council granted ethical permission before the investigation was started.

RESULTS

Examine molecular Methods for diagnosing Mycobacterium tuberculosis identification, paying particular attention to medication resistance

Resistant Mycobacterium tuberculosis strains were found using molecular diagnostic techniques as Whole Genome Sequencing, GeneXpert mycobacterium tuberculosis /resistance to rifampin, and Line Probe Assays (LPAs). GeneXpert mycobacterium tuberculosis /resistance to rifampin demonstrated approximately 98% accuracy levels and high sensitivity levels for rifampicin resistance detection. Line Probe Assays have a 95% pooled sensitivity rate for isoniazid resistance and a 98% pooled sensitivity rate for rifampicin resistance. It provided comprehensive details on drug resistance patterns and resistant mutations, particularly for multidrug-resistant and extensively drug-resistant bacteria.

Table.1.0: Comparing Molecular Diagnostic Techniques in order to detect drug resistance in tuberculosis

Molecular Diagnostic Method	Sensitivity (%)	Specificity (%)	Resistance Detected	Application
GeneXpert mycobacterium tuberculosis	98%	98%	Rifampicin	Rapid screening

/resistance to rifampin				
Line Probe Assays (LPAs)	95% for Isoniazid, 98% for Rifampin	97% for Isoniazid, 99% for Rifampin	Rifampicin, Isoniazid	Drug-resistance confirmation
Whole Genome Sequencing (WGS)	99%	99%	multidrug-resistant and extensively drug-resistant bacteria	Comprehensive resistance profiling

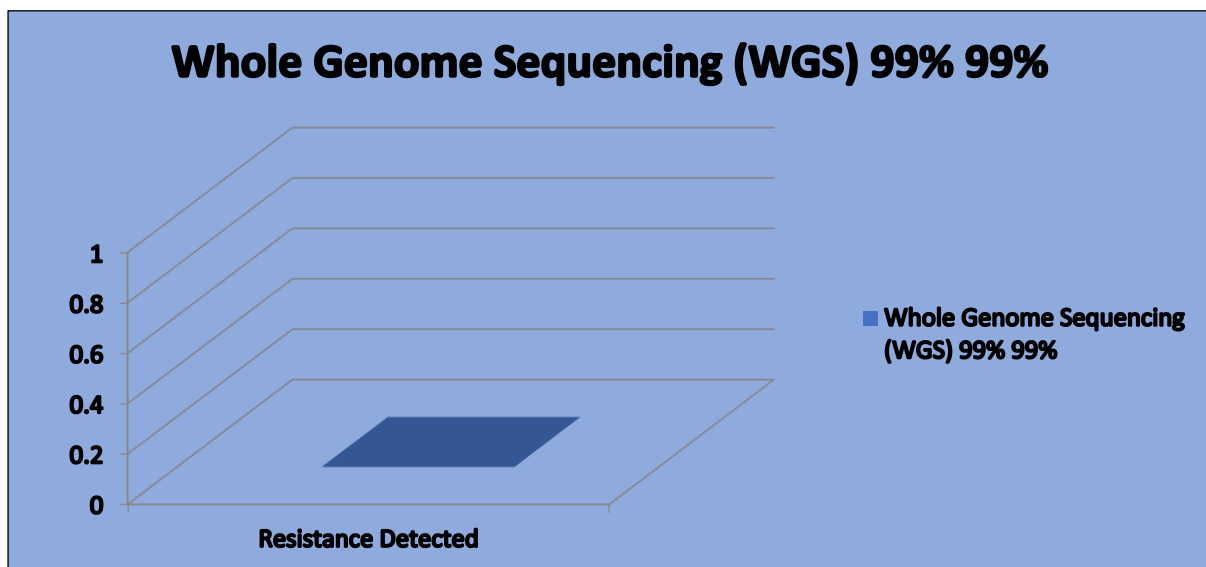


Fig.1.2: The precision with which whole genome sequencing (WGS) can identify drug resistance in Mycobacterium tuberculosis



Examine how several strain-typing methods, including IS6110- Restriction fragment length polymorphism, spoligotyping, Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat, and whole genome sequencing, contribute to the differentiation of Mycobacterium tuberculosis

Various methods, including whole-genome sequencing strain typing, spoligotyping, Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat, and IS6110- restriction fragment length polymorphism, demonstrated varying levels of effectiveness in distinguishing the particular Mycobacterium tuberculosis strains that were examined. IS6110- restriction fragment length polymorphism needed a lot of DNA and had a high specificity. Because of this, its use was not common. Because Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat maintained strong repeatability and discrimination power in differentiating distinct strain patterns, the Spoligotyping approach proved effective for preliminary typing. Although it is more expensive, it is the most comprehensive tool for defining the genetic variation among the strains.

Table.1.1: Major Strain-Typing Techniques for identifying mycobacteria are Compared.

Strain-Typing Technique	Discrimination Power	Key Strengths	Limitations
IS6110-Restriction fragment length polymorphism	High	High specificity	Requires large DNA amounts
Spoligotyping	Moderate	Rapid and cost-effective	Limited discriminatory power
Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat	High	High reproducibility	Moderate cost
Whole Genome Sequencing (WGS)	Very High	Comprehensive strain information	High cost, technical requirements

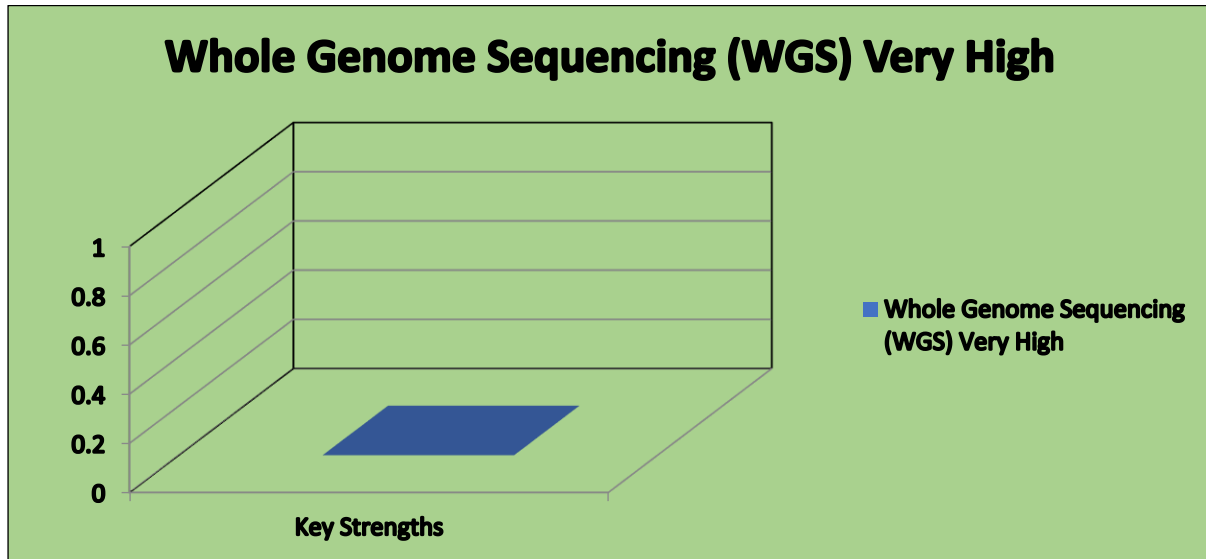


Fig.1.3: Whole Genome Sequencing (WGS) has a high diagnostic power in identifying Mycobacterium tuberculosis

Examine the dynamics of TB transmission worldwide and the application of molecular technologies to its management

Certain sites have a higher rate of drug-resistant tuberculosis transmission than others, according to molecular tools that have shed important light on the global dynamics of tuberculosis transmissions. According to the findings, Managed Detection and Response and Extended Detection and Response strains are more common in particular high-risk groups and environments; as a result, molecular testing is required to identify possible hotspots for transmission. Additionally, cross-border transmissible patterns were found by whole genome sequencing and Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat investigations, particularly for urban and densely populated areas, requiring focused interventions in these communities.

Table.1.2: Worldwide Trends in Tuberculosis Transmission and Molecular Methods for Identifying Strains

Region	Transmission Type	High-Risk Populations	Prevalent Strains Identified	Molecular Tool Used
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South Asia	Community & healthcare-associated	Urban, immune compromised	Multidrug-resistant and extensively drug-resistant bacteria (MDR, XDR)	whole genome sequencing, Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat
Sub-Saharan Africa	Community transmission	Refugee camps, miners	multidrug-resistant (MDR)	Spoligotyping, Whole genome sequencing
Eastern Europe	Cross-border	Migrant workers	Extensively drug-resistant bacteria (XDR)	Whole genome sequencing
Latin America	Community	Low-income communities	Multidrug-resistant (MDR)	IS6110-Restriction fragment length polymorphism,

				Whole genome sequencing
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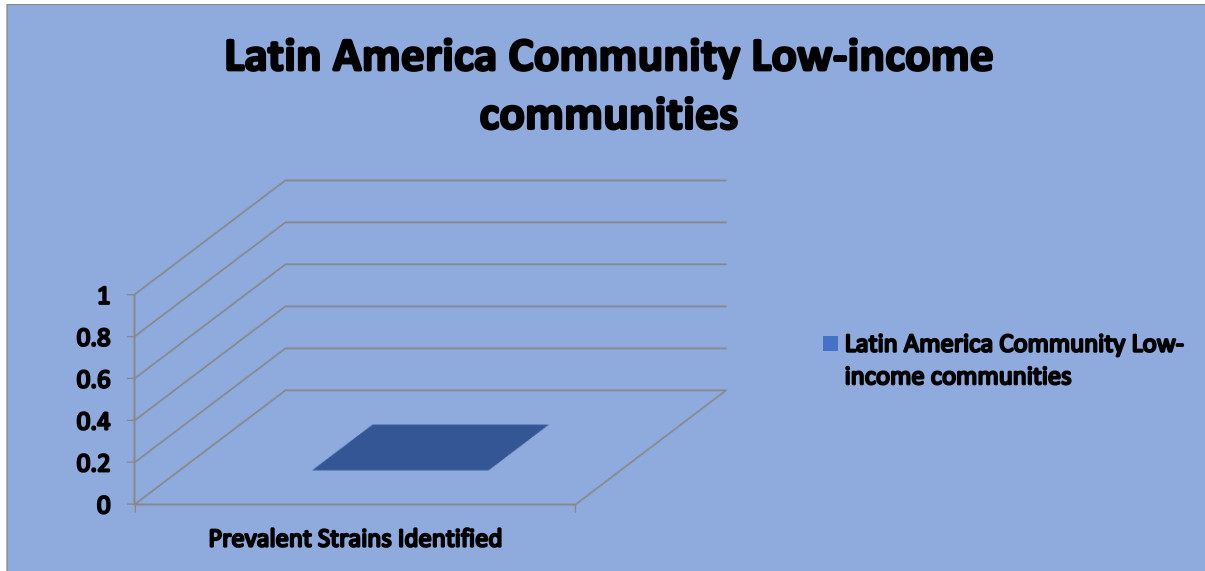


Fig.1.4: Common Mycobacterium tuberculosis strains in Latin American low-income communities

DISCUSSION

As part of its "end tuberculosis strategy" the World Health Organization has been pushing for the development of more sophisticated, reliable, sensitive, and simple tuberculosis diagnostic methods in an effort to slow the disease's spread. Scientists and academics around the world have been concentrating on these strategies for eliminating tuberculosis and reducing its spread. The development of rapid, low-cost, highly sensitive, and specific assays that can be used as point-of-care diagnostics in resource-constrained environments is still being pursued in an effort to lower tuberculosis. Evidence based diagnosis like smear microscopy is very much needed for the efficient diagnosis of tuberculosis. But it has its own limitations such as low sensitivity

According to the study, GeneXpert Mycobacterium tuberculosis /resistance to rifampin had 98% sensitivity in identifying rifampicin resistance, which is consistent with findings from previous data also. According to the research, GeneXpert mycobacterium tuberculosis /resistance to rifampin is effective at detecting drug-resistant tuberculosis and has shortened the time to diagnosis, both of which are critical for prompt disease care. Line Probe Assays have 95%-98% sensitivity and 97%-99% specificity for detecting isoniazid and rifampicin resistance. They are also useful in identifying multiple drug resistant tuberculosis cases.



This study's 99% sensitivity and specificity for WGS show that the method is highly sensitive for identifying drug-resistant mutations and transmission patterns. Studies demonstrated that IS6110-restriction fragment length polymorphism was an accurate Mycobacterium tuberculosis strain typing tool despite its limitation on strains with low copies. This is comparable to the high discriminatory power of IS6110-restriction fragment length polymorphism, especially for strains with multiple copies of IS6110. The experimental study also shown that Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat is feasible and has good reproducibility for long-term epidemiological studies of tracking tuberculosis transmission and examining genetic variation. Despite being low-cost, this approach works well for initial strain differentiation but has poor discriminatory power for other strains, such the Beijing family.

CONCLUSION

Rapid and precise diagnosis and treatment are crucial in lowering the danger of rising infection rates, including morbidity and death, as the rate of tuberculosis infection, mortality, and morbidity rates have been raising in recent years.

The detection of Mycobacterium tuberculosis and drug-resistant TB (DR-TB) is made convenient by molecular testing techniques. When compared to Targeted next generation sequencing, which uses sputum specimens directly, Targeted next generation sequencing has the potential to provide results faster than whole Genome Sequencing and can supplement the limitations of culture in the future. The advantage of Xpert Mycobacterium tuberculosis bacterium/Extensively Drug-Resistant (MTB/XDR) is that it can diagnose tuberculosis quickly and provide results within 90 minutes. In contrast, sequencing offers precise information on drug-resistant TB (DR-TB) but has drawbacks, such as high costs and the inability to distinguish DNA from viable or non-viable bacteria. Satellite health institutions may be able to diagnose and treat drug-resistant TB early because to the Xpert Mycobacterium tuberculosis bacterium/ Extensively Drug-Resistant instrument's ease of use and quick results.

This study reaffirms how strain typing and sophisticated molecular diagnostics can improve tuberculosis diagnosis and treatment, particularly in cases that are resistant to drugs. The high accuracy level of techniques like GeneXpert, Line Probe Assays, and whole Genome Sequencing, along with the good discriminatory power of IS6110-restriction fragment length polymorphism and Mycobacterial Interspersed Repetitive Units -Variable Number Tandem Repeat, provide a strong foundation for precise strain typing, early diagnosis, and focused therapies. Comparisons with cited papers from international research on tuberculosis diagnostics and strain typing techniques show the

need for additional study to continue improving these tools in order to better handle the changing difficulties that drug-resistant tuberculosis brings.

REFERENCES

- [1] Acharya, B., Acharya, A., Gautam, S., Ghimire, S. P., Mishra, G., Parajuli, N., & Sapkota, B. (2020). Advances in diagnosis of tuberculosis: An update into molecular diagnosis of Mycobacterium tuberculosis. *Molecular Biology Reports*, 47, 4065-4075.
- [2] Baker, L., Brown, T., Maiden, M. C., & Drobniewski, F. (2004). Silent nucleotide polymorphisms and a phylogeny for Mycobacterium tuberculosis. *Emerging Infectious Diseases*, 10, 1568–1577.
- [3] Barken, K. B., Haagensen, J. A., & Tolker-Nielsen, T. (2007). Advances in nucleic acid-based diagnostics of bacterial infections. *Clinica Chimica Acta*, 384(1-2), 1-11.
- [4] Bespyatykh, J., Shitikov, E., Bespyatykh, D., Guliaev, A., Klimina, K., Veselovsky, V., Arapidi, G., Dogonadze, M., Zhuravlev, V., Ilina, E. (2020). Metabolic changes of Mycobacterium tuberculosis during the anti-tuberculosis therapy. *Pathogens*, 9, 131.
- [5] Branigan, D., Agbassi, P., Deborggraeve, S., Kohli, M., Kumar, B. (2020). Panduan activist untuk alat diagnostic tuberculosis. *Treatment Action Group*, 12-13.
- [6] Brudey, K., Driscoll, J. R., Rigouts, L., Prodinger, W. M., Gori, A., Al-Hajoj, S. A., Allix, C., Aristimuño, L., Arora, J., Baumanis, V. (2006). Mycobacterium tuberculosis complex genetic diversity: Mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiology*, 6, 23.
- [7] Cave, M. D., Eisenach, K. D., McDermott, P. F., Bates, J. H., & Crawford, J. T. (1991). IS6110: Conservation of sequence in the Mycobacterium tuberculosis complex and its utilization in DNA fingerprinting. *Molecular and Cellular Probes*, 5, 73-80.
- [8] Chisompola, N. K., Streicher, E. M., Muchemwa, C. M. K., Warren, R. M., & Sampson, S. L. (2020). Molecular epidemiology of drug-resistant Mycobacterium tuberculosis in Africa: A systematic review. *BMC Infectious Diseases*, 20, 1-16.
- [9] Cohen, K. A., Manson, A. L., Desjardins, C. A., Abeel, T., & Earl, A. M. (2019). Deciphering drug resistance in Mycobacterium tuberculosis using whole-genome sequencing: Progress, promise, and challenges. *Genome Medicine*, 11(1), 45.
- [10] Dale, J. W., Brittain, D., Cataldi, A. A., Cousins, D., Crawford, J. T., Driscoll, J., Heersma, H., Lillebaek, T., Quitugua, T., Rastogi, N. (2001). Spacer oligonucleotide typing of bacteria of the Mycobacterium tuberculosis complex: Recommendations for standardised nomenclature. *International Journal of Tuberculosis and Lung Disease*, 5, 216–219.

- [11] Davies, A. P., Dhillon, A. P., Young, M., Henderson, B., McHugh, T. D., & Gillespie, S. H. (2008). Resuscitation-promoting factors are expressed in Mycobacterium tuberculosis-infected human tissue. *Tuberculosis*, 88(5), 462–468.
- [12] Demay, C., Liens, B., Burguière, T., Hill, V., Couvin, D., Millet, J., Mokrousov, I., Sola, C., Zozio, T., & Rastogi, N. (2012). SITVITWEB—A publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology. *Infection, Genetics and Evolution*, 12, 755-766.
- [13] Dong, B., He, Z., Li, Y., Xu, X., Wang, C., & Zeng, J. (2022). Improved conventional and new approaches in the diagnosis of tuberculosis. *Frontiers in Microbiology*, 13, 924410.
- [14] Ferraris, D.M.; Miggiano, R.; Rossi, F.; Rizzi, M. Mycobacterium tuberculosis molecular determinants of infection, survival strategies, and vulnerable targets. *Pathogens* 2018, 7, 17.
- [15] García de Viedma, D., Alonso Rodriguez, N., Andrés, S., Ruiz Serrano, M. J., & Bouza, E. (2005). Characterization of clonal complexity in tuberculosis by mycobacterial interspersed repetitive unit-variable-number tandem repeat typing. *Journal of Clinical Microbiology*, 43(11), 5660-5664.
- [16] Goguet de la Salmonière, Y. O., Li, H. M., Torrea, G., Bunschoten, A., van Embden, J., & Gicquel, B. (1997). Evaluation of spoligotyping in a study of the transmission of Mycobacterium tuberculosis. *Journal of Clinical Microbiology*, 35, 2210-2214.
- [17] Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A., Nieto, R. (2005). Microbial DNA typing by automated repetitive-sequence-based PCR. *Journal of Clinical Microbiology*, 43, 199-207.
- [18] Heersma, H. F., Kremer, K., & van Embden, J. D. (1998). Computer analysis of IS6110 RFLP patterns of Mycobacterium tuberculosis. *Methods in Molecular Biology*, 101, 395-422.
- [19] Huang, J., Liang, Z., Liu, Y., Zhou, J., & He, F. (2022). Development of an MSPQC nucleic acid sensor based on CRISPR/Cas9 for the detection of Mycobacterium tuberculosis. *Analytical Chemistry*, 94(32), 11409-11415.
- [20] Iwamoto, T., Yoshida, S., Suzuki, K., Tomita, M., Fujiyama, R., Tanaka, N., Kawakami, Y., & Ito, M. (2007). Hypervariable loci that enhance the discriminatory ability of newly proposed 15-loci and 24-loci variable-number tandem repeat typing method on Mycobacterium tuberculosis strains predominated by the Beijing family. *FEMS Microbiology Letters*, 270, 67–74.
- [21] Jang, M. H., Choi, G. E., Shin, B. M., Lee, S. H., Kim, S. R., Chang, C. L., & Kim, J. M. (2011). Comparison of an automated repetitive sequence-based PCR microbial typing system with IS6110-restriction fragment length polymorphism for epidemiologic investigation of clinical Mycobacterium tuberculosis isolates in Korea. *Korean Journal of Laboratory Medicine*, 31, 282–284.



- [22] Lazzarini, L. C. O., Rosenfeld, J., Huard, R. C., Hill, V., e Silva, J. R. L., DeSalle, R., & Ho, J. L. (2012). Mycobacterium tuberculosis spoligotypes that may derive from mixed strain infections are revealed by a novel computational approach. *Infection, Genetics and Evolution*, 12(4), 798–806.
- [23] Lu, B., Dong, H. Y., Zhao, X. Q., Liu, Z. G., Liu, H. C., Zhang, Y. Y., Jiang, Y., & Wan, K. L. (2012). A new multilocus sequence analysis scheme for Mycobacterium tuberculosis. *Biomedical and Environmental Sciences*, 25, 620–629.
- [24] MacLean, E., Kohli, M., Weber, S. F., Suresh, A., Schumacher, S. G., Denking, C. M., & Pai, M. (2020). Advances in molecular diagnosis of tuberculosis. *Journal of Clinical Microbiology*, 58(10), 10–1128.
- [25] Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 3140–3145.
- [26] Marks, S. M., Cronin, W., Venkatappa, T., Maltas, G., Chon, S., Sharnprapai, S., & Bernardo, J. (2013). The health-system benefits and cost-effectiveness of using Mycobacterium tuberculosis direct nucleic acid amplification testing to diagnose tuberculosis disease in the United States. *Clinical Infectious Diseases*, 57(4), 532–542.
- [27] Miggiano, R., Rizzi, M., & Ferraris, D. M. (2020). Mycobacterium tuberculosis pathogenesis, infection prevention and treatment. *Pathogens*, 9(5), 385.
- [28] Nandlal, L., Perumal, R., & Naidoo, K. (2022). Rapid molecular assays for the diagnosis of drug-resistant tuberculosis. *Infection and Drug Resistance*, 15, 4971–4984.
- [29] Oelemann, M. C., Diel, R., Vatin, V., Haas, W., Rüscher-Gerdes, S., Locht, C., Niemann, S., & Supply, P. (2007). Assessment of an optimized mycobacterial interspersed repetitive-unit-variable-number tandem-repeat typing system combined with spoligotyping for population-based molecular epidemiology studies of tuberculosis. *Journal of Clinical Microbiology*, 45, 691–697.
- [30] Ramasamy, P., Sounderrajan, V., Vijayakumar, K. K., & Harshavardhan, S. (2021). Current perceptions on advanced molecular diagnostics for drug-resistant Mycobacterium tuberculosis. *Biomedical and Pharmacology Journal*, 14(3), 1249–1257.
- [31] Reiche, M. A., Warner, D. F., & Mizrahi, V. (2017). Targeting DNA replication and repair for the development of novel therapeutics against tuberculosis. *Frontiers in Molecular Biosciences*, 4, 75.
- [32] Satta, G., Lipman, M., Smith, G. P., Arnold, C., Kon, O. M., & McHugh, T. D. (2018). Mycobacterium tuberculosis and whole-genome sequencing: How close are we to unleashing its full potential? *Clinical Microbiology and Infection*, 24(6), 604–609.



- [33] Shamputa, I. C., Rigouts, L., Eyongeta, L. A., El Aila, N. A., van Deun, A., Salim, A. H., Willery, E., Locht, C., Supply, P., & Portaels, F. (2004). Genotypic and phenotypic heterogeneity among *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients. *Journal of Clinical Microbiology*, 42, 5528–5536.
- [34] Sibandze, D. B., Kay, A., Dreyer, V., Sikhondze, W., Dlamini, Q., DiNardo, A., & Mandalakas, A. (2022). Rapid molecular diagnostics of tuberculosis resistance by targeted stool sequencing. *Genome Medicine*, 14(1), 52.
- [35] Skuce, R. A., McCorry, T. P., McCarroll, J. F., Roring, S. M., Scott, A. N., Brittain, D., Hughes, S. L., Hewinson, R. G., & Neill, S. D. (2002). Discrimination of *Mycobacterium tuberculosis* complex bacteria using novel VNTR-PCR targets. *Microbiology*, 148, 519–528.
- [36] Supply, P., Allix, C., Lesjean, S., Cardoso-Oelemann, M., Rüsche-Gerdes, S., Willery, E., Savine, E., de Haas, P., van Deutekom, H., Roring, S., et al. (2006). Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology*, 44, 4498–4510.
- [37] Surikova, O. V., Voitech, D. S., Kuzmicheva, G., Tatkov, S. I., Mokrousov, I. V., Narvskaya, O. V., Rot, M. A., van Soolingen, D., & Filipenko, M. L. (2005). Efficient differentiation of *Mycobacterium tuberculosis* strains of the W-Beijing family from Russia using highly polymorphic VNTR loci. *European Journal of Epidemiology*, 20, 963–974.
- [38] Uddin, R., Siraj, B., Rashid, M., Khan, A., Ahsan Halim, S., & Al-Harrasi, A. (2020). Genome subtraction and comparison for the identification of novel drug targets against *Mycobacterium avium* subsp. *hominissuis*. *Pathogens*, 9, 368.
- [39] Van Embden, J. D., Van Soolingen, D., Heersma, H. F., De Neeling, A. J., Jones, M. E., Steiert, M., Grek, V., Mooi, F. R., & Verhoef, J. (1996). Establishment of a European network for the surveillance of *Mycobacterium tuberculosis*, MRSA and penicillin-resistant pneumococci. *Journal of Antimicrobial Chemotherapy*, 38, 905–907.
- [40] van Soolingen, D., Hermans, P. W., de Haas, P. E., Soll, D. R., & van Embden, J. D. (1991). Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *Journal of Clinical Microbiology*, 29, 2578–2586.
- [41] Wall, S., Ghanekar, K., McFadden, J., & Dale, J. W. (1999). Context-sensitive transposition of IS6110 in mycobacteria. *Microbiology*, 145, 3169–3176.
- [42] Xu, Y., Wang, G., & Xu, M. (2020). Biohazard levels and biosafety protection for *Mycobacterium tuberculosis* strains with different virulence. *Biosafety and Health*, 2, 135–141.



[43] Yan, A. J., Olson, A. M., Weigel, K. M., Luabeya, A. K., Heiniger, E., Hatherill, M., & Yager, P. (2024). Detection of Mycobacterium tuberculosis from tongue swabs using sonication and sequence-specific hybridization capture. PLOS ONE, 19(8), e0308235.



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सारांश- मराठी वाड्मयातील दृक-श्राव्य माध्यमातील अतिशय नावाजलेला असा प्रकार आहे. मराठी नाटकाचा साकल्याने विचार केला तर त्यात सतत काही घडावे लागत असते. नाटकामध्ये कृतीयुक्त अशी मांडणी केली जात असते. अशा रीतीने एका प्रसंगातून दुसरा प्रसंग या क्रमाने नाटकामध्ये प्रसंगाची अथवा घटनांची सलग मालिका तयार होत जाते. त्यात बाह्य हालचालीबरोबर आंतरिक घडामोडींचाही समावेश होताना दिसून येतो या कृतीतून नाटक आपल्यासमोर प्रकट होत असल्याचे दिसून येते. कथा- कादंबरी निवेदन जे करते, ते कार्य नाटकात कृती करणे. नाटकामधील संवाद हे बोलण्यासाठी असतात, त्या अर्थाने तेही कृती होते. नाटकाच्या प्रयोगात सापेक्षतेचा सर्वात मोठा परिणाम हा नाटकाच्या रचनेवर होतो. अंकात वा प्रवेशात केलेली विभागणी, तसेच प्रारंभ गुंतागुंत आणि उकल या स्वरूपाची यांना नाटकात विशेष महत्त्व असते. या दृष्टीने बहुदा नाटकाचे आधी मध्य आणि अंत असे तीन भाग हे कल्पिले जातात असा हा एक महत्त्वपूर्ण साहित्यप्रकार मानता येतो.

महत्वाचे शब्द-नाटक, कथानक, व्यक्तिरेखा, स्वगत, संवाद, भाषाशैली इ.

प्रस्तावना:- नाटक हा साहित्यप्रकार समाज जीवनाचा अविभाज्य असा भाग मानले तर वावगे ठरणार नाही. समाजात वेगवेगळ्या हजारो घटना उद्भवणारे प्रश्न समस्या या सर्वांचे प्रतिबिंब हे 'नाटक' या दृक-श्राव्य कलेच्या माध्यमातून आलेले दिसून येते. त्यामुळेच 'ललित' वाड्मय प्रकारातील 'नाटक' हा एक महत्वाचा भाग आहे. नटसम्राट हे नाटक कविवर्य कुसुमाग्रजांच्या प्रतिभा संपन्न लेखणीतून विचारधारेतून साकारलेले आहे. मराठी रंगभूमीवरील एका नटश्रेष्ठासाठी 'किंग लियर' रूपांतर करण्याची योजना शिरवाडकर यांच्याकडे आली होती. आणि याचाच एक भाग म्हणजे 'नटसम्राट' हे नाटक शेक्सपियरच्या किंग लियरची अनुकृती आहे. 'नटसम्राट' ही जितकी एका वत्सल पण वंचित पित्याची शोककथा आहे; तितकी ते का नटसम्राटाची ही शोककथा आहे.



आणि अखेर तर ती नटसम्राटाचीच शोककथा आहे. 'नटसम्राट' या नाटकातील संपूर्ण कथानक मुख्य नायक गणपतराव बेलवलकर (नटसम्राट) व त्यांची सुसंस्कारित पत्नी कावेरी (सरकार) या दोन व्यक्तिरेखांच्या संदर्भांनी गुंफलेले आहे. या नाटकाला काव्यात्म पातळी निर्माण होते. या मराठी नाटकातील शोकात्म अनुभव प्रेक्षकांना पदोपदी येताना दिसून येतो. असे हे 'नटसम्राट' नाटक मराठी रंगभूमीवरील मापदंड मानले जाते.

नाटक संकल्पना:-

प्रा. ना. सी. फडके- यांनी आपल्या 'प्रतिभा साधन' या ग्रंथात नाटकाचे स्वरूप समजावून देताना ते म्हणतात की, 'रंगभूमीवर नाटकाकडून अभिनयाच्या भाषणाच्या द्वारे लोकसमुदायास सांगितलेली कथा म्हणजे नाटक होय' अशी नाटकाची व्याख्या त्यांनी केली आहे. या व्याख्येतून अभिनय, भाषण आणि रंगभूमी या शब्दांचे महत्त्व अधोरेखित केलेले दिसते.

अरविंद वामन कुलकर्णी- नाटकाची व्याख्या करताना म्हणतात की, 'संवाद हे मूलद्रव्य आणि प्रसंग हे माध्यम या द्वारा व्यक्त होणारा एक नाट्यमय किंवा संघर्षमय कथात्मक अनुभव म्हणजे नाटक होय. '(मराठी नाट्यलेखन तंत्राची वाटचाल) वरील व्याख्येतून अरविंद वामन कुलकर्णी यांनी संवाद, प्रसंग, संघर्ष, अनुभव यांना विशेष महत्त्व दिल्याची दिसते.

वसंत कानेटकर:- नाटकाची व्याख्या करताना असे म्हणतात की, 'नाटक हे एकाच वेळी जीवन दर्शनही असते व जीवनावर भाष्य करते' वरील व्याख्येतून वसंत कानेटकर यांनी 'नाटक' दृक्श्राव्य कलेत जीवनाचे विविध पैलूंचे दर्शन घडते, असे विचार व्यक्त केले आहेत.

भालचंद्र फडके- नाटकाची व्याख्या करताना म्हणतात की, 'नाटक म्हणजे प्रसंग आणि संवाद यांच्याद्वारा व्यक्त होणारा, संघर्षमय कथात्मक अनुभव होय. नाटक म्हणजे माणसाच्या अंतर्बाह्य क्रिया प्रतिक्रियांचे दर्शन घडविणारा आकृतीबंध होय. '(मराठी विश्वकोश खंड - ८) वरील व्याख्येतून भालचंद्र फडके यांनी संवाद, प्रसंग, संघर्ष, अनुभव यांचे विशेष महत्त्व अधोरेखित केल्याचे दिसून येते.

कथानक:- 'नटसम्राट' हे एक शोकात्मिका स्वरूपातील मराठी रंगभूमीवरील मापदंड ठरलेले मराठी नाटक असून या नाटकांमध्ये एक सुशिक्षित कुटुंब दाखविण्यात आलेले आहे. या नाटकातील मुख्य व्यक्तिरेखा गणपतराव बेलवलकर (नटसम्राट) व त्यांची पत्नी कावेरी (सरकार) या



व्यक्तिरेखा भोवती संपूर्ण नाटकाचे कथानक गुंफलेले दिसून येते. कविवर्य वि.वा.शिरवाडकर यांच्या काव्यात्म भाषाशैली, प्रतिभा संपन्न विचारधारेच्या लेखणीतून 'सामाजिक वास्तवाचे भान' जागवणारे आदर्शवत 'नाटक' आहे. नटसम्राट हे नाटक शोकात्मिका आहे का? नट/ कलावंत म्हणून वाट्याला आलेले दुःख आहे का? सामान्य म्हाताऱ्याची शोकांतिका आहे का? असे एक ना अनेक प्रश्न हे नाटक पाहताना अथवा वाचताना नाटक रसिकांच्या मनात आल्याशिवाय राहत नाहीत. एक विलक्षण मनस्वी माणूस, भोवतालच्या रोखठोक, व्यवहारी जगाशी तडजोड करून जगत राहण्याच्या प्रयत्नात शेवटी कसा उध्वस्त होतो याची 'नटसम्राट' ही कहाणी आहे. गणपतराव बेलवलकर हा नट आहे की वस्तुस्थितीच आहे. ती करता येणार नाही केवळ नाटककार आणि आपल्याला सांगितले आहे एवढाच पुरावा आहे. असे नाही तर त्याचे नटपण पानोपानी विखुरलेले आहे. बेलवलकरांच्या भेटीचीत राहत पावलोपावली त्याचे जिवंत पुरावे आपल्याला दिसतात. पहिली वीस वर्षे एखाद्या पतंगासारखी भरकटण्यात गेलेली आहेत आयुष्याच्या त्या सुरुवातीच्या कालखंडासंबंधी सांगण्यासारखे विशेष काहीच नाही. पुढील ४० वर्षे या माणसाने एका वेगळ्याच पृथ्वीवर काढली आहेत. कवीच्या प्रतिभेने निर्माण केलेली, थिअटरमधल्या अंतराळाने तोलून धरलेली, चंद्र-सूर्यांनी नव्हे तर रंग मंचावरी दिव्यांनी प्रकाशित केलेली अशी ही स्वप्निल भूमी आहे. अनेकविध भूमिका गाजवून हा माणूस 'नटसम्राट' या पदाला पोहोचलेला आहे. एक कलावंत म्हणून अशा तऱ्हेचे समर्पित आयुष्य जगत असताना माणूस म्हणून गणपतराव बेलवलकर एक समृद्ध आयुष्य जगत होता. मोठ्या नाटककारांशी कवींची व्यासंगी, समीक्षकांशी, राजकीय पुढार्यांशी त्याचा संबंध येत होता त्या सहवासातून त्यांचे व्यक्तिमत्व सर्वांगाने फुलत होती पृथ्वी सूर्याभोवती फिरत नाही, रुपयाच्या नाण्याभोवती फिरते. यातले कठोर काव्य तो समजू शकत होता. पण आपण राहतो ती पृथ्वी वेगळी आहे, हे समजण्याची जाण त्याला आली होती. चाळीस वर्षे रोज रात्री प्राण पणाला लावून आपलं प्रेम मी जिंकला आहे किंवा संतोषला थोडी अहंकाराची धार आली तरी आपण क्षमा करायला हवी किंवा म्हातारपणाच्या पोकळीत अहंकाराची घंटा घनघनते किंवा सफल आणि समाधानी म्हातारपण म्हणजे गुलबकवलीच फूल किंवा दुःखा प्रमाणे सुखाचा बोजा सुद्धा म्हाताऱ्या मस्तकाला सहन होत नाही, यावरून त्याच्या सखोल व्यक्तिमत्त्वाचा अंदाज आपल्याला अचूकपणे येत जातो. कौटुंबिक सुखही या माणसाने अगदी मनापासून घेतले आहे शेकोटीत घातलेले ढेलीपीचे



दुसऱ्यासाठी जळण्याचे व्रत घेतली पत्नी त्याला मिळाली आहे तिला तो गमतीने कावेरी(सरकार) असे म्हणत आला आहे. प्रेम, माया, नात्याची जाणीव, माणुसकी, निष्ठा या सगळ्यांच्या पलीकडे जाणारी एक विलक्षण नाजूक आणि तरी त्याच्या साऱ्या जीवनाला एक भर भक्कम आधार देणारी भावना त्याच्या मनात तिच्याविषयी आहे. गणपतराव बेलवलकर या नटाची संस्कार संपन्न समृद्ध व्यक्तिमत्व नाटकाच्या सुरुवातीच्या अंकात आपल्यासमोर उलगडत जाते. ज्यावेळी नटसम्राट मराठी रंगभूमीवरून कायमचे निवृत्त होतात. पोरान्या जीवावर आपली सगळी मालमत्ता त्यांनी आपल्या दोन मुलात विभागून दिली आहे. आणि काही दिवस नंदूच्या फ्लॅटवर राहू आणि काही दिवस म्हणून नलूकडे राहू, तिच्या टेकडीवरच्या बंगल्यावर अशी उर्वरित आयुष्याची सोपी वाटेल त्यांनी मनाशी करून टाकली आहे. वृत्त होण्याच्या निर्णयाबरोबरच बेलवलकर नट म्हणून संपला आता फक्त बाप म्हणून उरला आहे प्रेमाच्या भांडणात गुरफटलेला असे खरोखरच मानण्याची चूक तो करतो जणू काय नाटकाचा दुसरा अंक सुरू होत आहे फक्त दिवसांनी आता अखेरच्या भरत वाक्याकडे प्रवास करायचा आहे... या सुख स्वप्नात तू दंग झालेला असताना त्याची पत्नी त्याचे पाय जमिनीला लावण्याचा प्रयत्न करते तेव्हाही तो मजेने म्हणतो की पोरानी आपल्याला रस्त्यावर काढली तर पंढरपूरच्या बाबा जव्हेरींना सांगून टाकलेल्या आई-बापांसाठी एक नमा आश्रम काढून घेऊ...

नट म्हणून निवृत्तीनंतरचे पहिली बारा वर्षे आपल्या मुलाकडे नंदाकडे राहतात. व तेथे त्यांना आयुष्याची फरपट सहन करावी लागत लागली आहे. त्या बारा वर्षांत खूपच मोडतोड झालेले आहे. तडे गेलेले आहेत. घरातल्या नोकरांची उद्धट बोलणे निमूटपणे गिळण्याइतकी लाचारी आली आहे. अजून बहिरा होत नाही मी. आंधळा व्हायला लागलोय, पण बहिरा होत नाही. सृष्टीची ही मेहरबानी कानांना चिकटून बसली आहे. अप्पासाहेब यांच्या आयुष्याच्या तिसऱ्या अंकातील तपशील असा तो ही काळीज गलबलून टाकणारा आहेत. कारण ज्या संपन्न व्यक्तिमत्त्वाच्या चिंधड्या आहेत त्या व्यक्ती महत्त्वाची फार प्रभावी दर्शन आपल्याला पहिल्या दोन अंकात झालेले आहे दुसऱ्या अंकाच्या शेवटी सारे संपलेले आहे नटसम्राट गणपतराव बेलवलकर हा प्रचंड माणूस उध्वस्त झालेला आहे. आता त्याच्या आयुष्याची लोंबती लक्तेरे फक्त उरलेली आहेत आणि आहे तेही पार विस्कटून गेले आहे कावेरीच्या मृत्यूनंतर पंधरा एक दिवस शुद्धबुद्ध हरवलेल्या अवस्थेत त्याने त्या घरात कसेबसे लोटले आहेत. आणि त्याचबरोबर स्थिती तुफान एक



दिवस घराबाहेर उधळले आहे... अशातच खरे म्हणजे आता घरात आणि घराबाहेर या शब्दांना काहीच अर्थ उरलेला नाही .थंडीवारा, ऊनपाऊस कसलीच क्षिती राहिलेली नाही. बंदरच नाहीसे झाले आहे. आणि नंतर घराबाहेर पडतात व त्यांना बूट पॉलिश करणारा राजा नावाचा एक सोळा-सतरा वर्षाचा मुलगा भेटतो. तो राहतो पुलाच्या एका कमानीखाली- पण त्याला घर आहे. रस्त्यावर टेकल्याबद्दल त्याला कुणीही हटकले तर त्याचे थोबाड फोडणे इतका तो संतप्त आहे नाटक म्हणजे त्याच्या दृष्टीने माडीवाल्यांची आणि गाडीवाल्यांची चैन आहे, पैसे तर सर्वच कमवतात. पण माणसे तगवणे हे फार महत्त्वाचे आहे असे त्याचे तत्त्वज्ञान आहे. आणि माणसे म्हणजे रक्ताचे नातेवाईक नव्हेत. त्यांच्यापासून तो केव्हाच सेप्रेट झाला आहे तर काळजाच्या वाटेने आपल्या वस्तीत येतील ती माणसे हा कलंदर राजा भेटल्यामुळे आप्पासाहेबांच्या लडथडत्या जीवनाला पुन्हा एक आधार मिळाला आहे तर घरापासून बाहेर आल्यासारळी त्यांना वाटते पुन्हा प्रकाश दिसू लागला आहे जीवनाचा लसलसता कोंब पुन्हा मूळ धरू पाहतो आहे काळजांचा करार पुन्हा एकदा झाला आहे. नाटकाच्या शेवटी पण आजवर न केलेल्या अगर पाहिलेल्या एका अगदी नव्याच नाटकाची नांदी एकदम ऐकू येत आहे.धुपाचा सुवास हवेत दरवळला आहे.पडदा वर जायची वेळ झाली आहे. आणि माझी नटी ती तर माझ्या अगोदरच स्टेजवर जाऊन बसली आहे. हे देवाघरचे अखेरचे चिरंतन नाटक सुरू होते आहे. आपली एंट्रीची वेळ आलेली आहे.आपण गेले पाहिजे. कदाचित पुन्हा एकदा आपल्या त्या वेगळ्या पृथ्वीवर त्या स्वप्नभूमीत आपल्याला जायचे असेल, अखेरचे आणि कायमचे आणि निरोप द्यायला हे सारे महापुरुष हजर आहेतच.ज्युलियस सिझर आहे, सुधाकर आहे,प्रतापराव आहे, हॅम्लेट आहे, ब्रूटसही आहे.आणि प्रत्यक्ष 'नटसम्राट' गणपतराव बेलवलकरही आहेच की... अशा प्रकारचे नटसम्राट या नाटकाचे कथानक आहे.

नाटकातील पात्र: 'नटसम्राट' या नाटकामध्ये बारा पात्र/व्यक्तिरेखा महत्त्वपूर्ण असा विचार करण्यात आलेला असून या सर्वांमध्ये गणपतराव बेलवलकर यांचे 'नटसम्राट' ही नाट्यवाङ्मयातील एक महान व्यक्तिरेखा आहे असे मानले जाते. तात्यासाहेब शिरवाडकर यांच्या दिव्य अशा प्रतिभेची ती एक उत्तुंग निर्मिती आहे. त्यानंतर 'कावेरी' (सरकार) ही मुख्य नायक 'नटसम्राट' अप्पासाहेब बेलवलकर यांची 'सावली' प्रमाणे असणारी शांत, संयम, आधारस्तंभासारखी पानी आहे. तिच्या एकून आयुष्याच्या वाटचालीतून आपल्या सुसंस्कृत विचारांची सुयोग्य दिशा नेमकेपणाने मिळते. तिला अप्पासाहेब 'सरकार' या नावाने हाक देतात.



त्यानंतरची व्यक्तिरेखा ही नंदा हा अप्पासाहेब बेलवलकर यांचा मुलगा आहे. व त्यांचे शिक्षण एम.कॉम झालेले आहे. शारदा ही अप्पासाहेबांची सून व नदाची पत्नी आहे. तसेच 'नलू' ही अप्पासाहेबांची लाडकी मुलगी तिला अप्पासाहेब 'कोकरू' असे म्हणतात. परंतु नलूकडून देखील आपल्या वडिलांना आदरयुक्त मान-सन्मानाची वागणूक ही दिली गेलेली दिसत नाही. याउलट पैसे चोरीचा एक मोठा आरोपच तिने केलेला दिसून येतो. सुधाकर कार्लेकर (जावई) हे अप्पासाहेबांचे जावई आहेत. त्यांचा अप्पासाहेबांच्या जुन्या विचारधारा, राहणीमान 'नट' म्हणून असलेले पूर्वीचे रंगभूमीवरील जाण हे चांगल्या प्रकारची असलेली त्यांच्या एकूण नित्य कृमातून दिसते आहे. भी. कळवणकर हे जावई कार्लेकर सुधाकर यांचे हे साहेब आहेत. व त्यांची पत्नी रागिनी कळवणकर ही असून त्यांनी 'हॅम्लेट' या नाटकाची मोठी जबाबदारी आपल्या स्वतःकडे घेतली आहे. कारण ज्यांना हे नाटक वेगळ्या तंत्रानं बसवायचं आहे म्हणजे सिंबॉलिक सेट निर्माण करून ते दाखवायचं आहे. खरा शेक्सपियर हा खरा कोणाला कळला होता असे वाटत नाही. असं वाटत नाही. याशिवाय आणखी 'नटसम्राट' या नाटकामध्ये विठोबा हा नोकर आहे. नलू आणि सुधाकर कार्लेकर यांच्या घरी नोकर म्हणून काम करण्याबरोबर हॅम्लेट मधील आपल्या प्रवेशाची तालीम करतो आहे. त्या नंतर राजा हा बूट पॉलिश करणारा मुलगा आहे. परंतु 'नटसम्राट' अप्पासाहेब बेलवलकर यांना त्याचा स्वभाव माणूस म्हणून दिलेला मायेचा आधार खूप मोठ भावला आहे अशा काही महत्त्वपूर्ण व्यक्तिरेखांची ओळख इथे केली आहे. आणि याशिवाय नात ठमी, आसाराम, रामय्या हॉटेल चालवणारी व्यक्तिरेखा देखील यात अंतर्भूत होतील हे देखील विचारात घेतले पाहिजे.

स्वगत – 'स्वगत' नाटकातील युक्ती आहे. हा देखील संवादाचाच एक आविष्कार आहे असे म्हटल्यास वावगे ठरू नये. नाटकाचा दर्जा उंचावण्यासाठी व्यक्तिदर्शन, जीवनाष्य अशी सूत्रे स्वगत लेखनामागे अवलंबतो. त्यामुळे नाट्यपरिणामाच्या दृष्टीने हा एक नाटकाचा घटक होऊ शकतो. 'नटसम्राट' या नाटकातील स्वगतांचा विचार साकल्याने केला तर आपल्या प्रथमतः हे लक्षात येते की, अप्पासाहेब बेलवलकर यांची स्वगते ही दीर्घ 'काव्यात्म' स्वरूपाची आहेत.

उदा. १)

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दॅट इज द क्वेश्चन

जगावं की मरावं

हा एकच सवाल आहे.



उदा. २)

या दुनियेच्या उकिरड्यावर
खरकट्या पत्रावळीचा तुकडा होऊन
जगावं बेशरम लाचार आनंदानं
की फेकून दयावं हेच देहाचं लक्तर... पृ.क्र. ६२
कुणी घर देता का घर
एका तुफानाला
कुणी घर देता का घर ?
एका तुफान भिंतीवाचून छपरावाचून
माणसाच्या मायेवाचून देवाच्या दयेवाचून
जंगला जंगलात हिडतं आहे.
जेथून कुणी उठवणार नाही
अशी जागा धुडतं आहे
कुणी घर देता का घर ! पृ.क्र. ७१

मराठी रंगभूमीवर ४० वर्ष अधिराज्य गाजवणाऱ्या नट/कलावंताला वाट्याला आलेले दुःख मुलगा, मुलगी शिक्षित असून देखील आपल्या वडिलांच्या आयुष्याच्या शेवटच्या टप्प्यावरील सांभाळण्याची मान - सन्मानाने जपण्याची जबाबदारी न घेतल्यामुळे अशा प्रकारची विचारधारा 'नटसम्राट' गणपतराव बेलवलकर यांच्यावर येताना दिसते आहे. असे एक ना अनेक वाचक/नाट्य रसिकांना स्वगते पदोपदी प्रत्ययास येताना दिसून येते. हे नक्कीच सामान्यांना विचार करायला लावणारी बाब म्हणावी लागते.

संवाद:- 'संवाद' हा नाट्य संहितेचा प्रमुख घटक मानला जातो. नाटक हे साकार होत जाते तर ते संवादाच्या माध्यमातून आणि म्हणून नाटकाचे मुलद्रव्य 'संवाद' आहेत. नटसम्राट मधील संवाद हे काव्यात्म भाषेच्या माध्यमातून नटलेले असून वातावरण निर्मितीला पूरक अशा प्रकारचे आहेत. याची अनुभूती नाटक पाहताना किंवा वाचताना येताना दिसून येईल. 'नटसम्राट' या नाटकामध्ये अप्पासाहेब बेलवलकर आणि त्यांच्या स्वभाव वैशिष्ट्यांना व्यक्त करणारी भाषा शिरवाडकर योजतात. बूट पॉलिश करणारा 'राजा' ज्या वस्तीत अथवा भागात राहतो, ती भाषा त्याच्याकडून बोलताना येताना दिसते आहे. तर 'नटसम्राट' अप्पासाहेब बेलवलकर यांची पत्नी कावेरी जिला ते



‘सरकार’ असे म्हणतात. तिच्याकडून येणारी भाषा ही शोषिक समजूतदारपणाची एक आदर्शवत गृहिणीला शोभनारी अशी आहे. अशा प्रकारे नटसम्राटमधील काही व्यक्तिरेखांची संवादाची भाषा ही ओळख केली आहे. श्री. कळवणकर व सौ कळवणकर हे जावई सुधाकर कार्लेकर यांचे साहेब आहेत आणि ते इंजिनियर आहे, पण नाद मात्र नाटकाचा आहे. नाटकाच्या संदर्भात मार्गदर्शनासाठी ते अप्पासाहेबांकडे भेटण्यासाठी येतात. अगोदर त्यावेळेस हा संवाद आलेला तो असा. दोन दिवस अप्पासाहेब नाटकाची तालीम पाहिली व लौकर निघून आलात.

अप्पा: – वा वा! छान चाललय. उत्तम.

श्री.कळवणकर :- आपण पूर्वी हॅम्लेट करीत होता ?

अप्पा: – करीत होतो.

कळवणकर: आपल्या काही सूचना असल्यास

अप्पा – नाही, नाही. काही नाही. म्हणजे मला वाटतं साहेब, की गंगेनं कसे वाहावं हे ब्रह्मपुत्रेनं सांगू नये. आणि ब्रह्मपुत्रेने कसे वाहावं हे गंगेनंही सांगू नये. एकाच पर्वतातून निघतात आणि एकाच समुद्राला मिळतात. पण दोघीच्या वाटा वेगळ्या, दोघींचे स्वभाव वेगळे. कोणी कुणाला सूचना करू नये.

सौ कळवणकर: – इश! असं कसं म्हणता हे नाटक सरकारी स्पर्धेला देणार आहोत आम्ही, अगदी टॉप प्रयोग व्हायला हवा.

अप्पा: – होईल, होईल. आपण ही थोडी भजी घेता का ?

कळवणकर: भजी ?

अप्पा: भवानी विलास हॉटेलचा रामेय्या आहे ना ? त्यानं करून दिली आहेत...पृ.क्र.४२

अशा प्रकारचे विविध वेगवेगळ्या स्वरूपातील संवाद नाट्य रसिक वाचकाला अनुभवायला मिळतात. मात्र या ठिकाणी काही महत्त्वपूर्णच संवाद घेण्यात आले आहे.

भाषाशैली: – मराठी रंगभूमीवर अधिराज्य गाजवणाऱ्या ‘नटसम्राट’ या नाटकातील भाषा ‘काव्यात्म’ अशीच असलेली आपल्या नाटकातून पदोपदी लक्षात येईल हे मात्र नक्की. या संदर्भात डॉ. उषा देशमुख म्हणतात की, ‘नटसम्राट’ या नाटकातील अनुभवाचे स्वरूप महाकाव्य सदृश्य असे आहे. महाकाव्यातील काव्यात्मक प्रमाने ‘नटसम्राट’ मधील संवादातून जाणवते.

‘नटसम्राट’ या नाटकाच्या अगदी सुरुवातीपासून शिरवाडकरांनी शब्दांची केलेली पखरण



यात नाटकातून प्रत्ययास येताना दिसते आहे. एकीकडे प्रेक्षकांनी भारावून जावे आणि एकीकडे टाळी द्यावी अशी वाक्य आहेत की, ती ऐकता ऐकतच स्वतःच्या नकळत प्रेक्षकांना रडवितात. उदा. – जेव्हा स्वतःची मुलगी आपल्या वडिलांवर पैसे चोरीचा आरोप करते. तेव्हा मन सुन्न होते. व अप्पासाहेब बेलवककर ‘नटसम्राट’ असणारा बाप मुलगी व आपली पत्नी कावेरी (सरकार) समोर व्यक्त होताना म्हणतात, माझ्या सरकार, तिला माहीत आहे, माझ्या क्रोधाला पाय नाहीत, हात नाहीत, लुळापांगळा आहे तो; वडान्यांनी अर्धवट चिरलेल्या डुकराचं धड नुसतं. गटारात तडफडणारं. मी पैसे चोरले? नाही. सरकार, मी रागावणार नाही.पण तुम्हांला सांगून ठेवतो, मी रडणारही नाही. डोळ्यांत आसवं जमायला लागली तरी खिळे मारून डोळ्यांच्या खाचा करीन संभाजीसारख्या, पण रडणार नाही. ‘नटसम्राट’ या नाटकामध्ये मुक्त काव्य व गद्यकाव्य यामधून भावना व्यक्त करण्याचे वि.वा.शिरवाडकर (कुसुमाग्रज) यांच्या भाषाशैलीचे एक उत्कृष्ट उदाहरण पाहावयास मिळते. कुसुमाग्रजांच्या काव्यात्म भाषाशैलीचे महत्त्व आधोरेखित झालेले दिसते.

कुणी घर देता का घर, एका तुफानाला पंख मिटून पडण्यासाठी हा अनुभव वाचकांना अंतर्मुख होण्यास भाग पाडतो हे नक्की, त्यात गूढ विचारही प्रकट झालेला दिसतो.

नटसम्राट या नाटकाला एक भाग्यवान कलाकृती आहे.असे सांगून तिच्या लोकप्रियतेची कारणे विशद करताना श्री.दिगंबर पाध्ये म्हणतात की, (आलोचन १९७३) ‘प्रेक्षकांना’ आपला वाटणारा विषय नाटकातून स्पष्ट करताना त्यातील भावनात्मक आवाहन आणि त्यासाठी समर्थ भाषा’ या घटकामुळे नाट्यकृती नाटकाला अधिक लोकप्रियता ही मिळालेली आहे हे निश्चितच.

संघर्ष—मराठी नाटकांमधून संघर्ष हा दाखवण्यात आलेला असतो. संघर्ष हा नाटकाचा आत्मा मानला जातो. रंगमंचावर होणारा वादविवाद किंवा युद्ध म्हणजे संघर्ष नव्हे तर ‘अंतरिक द्वंद्व’ म्हणजे ‘संघर्ष’ होय.नाटकातील संघर्षाचे सुरू नाट्यगत कथा नाटकाच्या स्वरूपावर अवलंबून असते.नाटक सुखांत आहे, शौकात्म आहे.की सामाजिक समस्येची निगडित आहे. इत्यादी घटकावर नाट्यगत संघर्ष हा अवलंबून असतो. याप्रमाणेच मराठी भाषेतील एक प्रसिद्ध नाटक म्हणजे ‘नटसम्राट’ हे आहेत. यामध्ये देखील सामाजिक, मानसिक,कौटुंबिक संघर्ष हे आलेले दिसून येतात. त्याचे काही उदा.पुढे दिले आहे. सुरुवातीच्या काळात ‘नट’ म्हणून करावा लागणारा संघर्ष तो असा – चाळीस वर्ष, रोज रात्री प्राण पणाला लावून जिंकलं आहे ते.गणपतराव बेलवलकर फार अहंकारी आहे असं लोक म्हणतात. म्हणू देत. वयाच्या पंधराव्या वर्षी घराच्या चारी भिंती चार बाजूंना कोसळून पडल्या. डोक्यावर आकाशाच छप्पर दिसायला लागलं. सराई



नसलेल्या वाळवंटातून वाटचाल करायला लागलो. पायाला जाळीत होती जमीन आणि मस्तकाला जाळीत होतं आकाश वडील आणि मुलगी यांच्या नात्यांतील 'बाप' म्हणून असणारा संघर्ष या ठिकाणी दाखवण्यात आलेला आहे.

नलू: हो तुम्ही चोरले! तुम्ही चोरले! तुम्ही चोरले!

कावेरी 'नले, नले' म्हणत धडपडत उठते, आणि तिच्या मागोमाग जाऊन शेवटी भिंतीचा आधार घेऊन उभी राहते. अप्पासाहेब क्षणभर निश्चल उभे राहतात, नंतर भ्रमातल्या माणसाप्रमाणे हेलकावणाऱ्या अस्थिर पावलांनी खुर्चीकडे जातात. क्षणभर पाठीच्या आधाराने उभे राहतात व नंतर खुर्चीवर बसतात.

अप्पा:मी चोर आहे (छातीवर हात आढळत) मी चोर आहे. अप्पा बेलवलकर नटसम्राट' लक्षवधीची मालमत्ता गुलाल- बुक्याप्रमाणे ज्यानं उधळून दिली. तो हा गणपतराव बेलवलकर चोर ठरला- आपल्या पोरांच्या घरांमध्ये! परमेश्वरा, मी तर सारं काही सहन केलं असतं. - पण हे

कावेरी : (भिंतीचा काँटचा आधार घेत त्यांच्याजवळ येते. आणि खांद्यावर हात ठेवते) ऐकलंत का ?

अप्पा:-(क्षीन स्वरात) काय म्हणते ?

कावेरी : दुनिया आपल्यासाठी संपली आहे आता. आपण दोघेच उरले आहोत. आपण दोघेचं मराठी रंगभूमी गाजवणाऱ्या या कलावंत बापास आपल्या स्वतःच्या मुलीने पैसे चोरल्याचा आरोप केला आहे हे निश्चितच विचार करण्यास भाग पाडणारी बाब आहे.

निष्कर्ष:

१. कविवर्य शिरवाडकर यांच्या 'नटसम्राट' या नाटकाने मराठी रंगभूमीला हा एक स्वतंत्र ओळख मिळवून दिली.
२. 'नटसम्राट' ही एक शोकात्मिका आहे, हे खरे असले तरी अनेक कुटुंबातील वास्तवता देखील आहे.
३. कविवर्य कुसुमाग्रजांचे मराठी नाटक साहित्य प्रकारातील स्थान हे मराठी पुरते मर्यादित न राहता वैश्विक पातळीवरचे आहे हे स्पष्ट होते.
४. नटसम्राट या नाटकाने मराठी नाट्य रसिकांच्या मनावर अधिराज्य निर्माण केले आहे.
५. वि.वा. शिरवाडकर यांच्या काव्यात्मक भाषा शैलीचा परिचय नाट्य रसिक वाचकाला येतो.
६. 'नटसम्राट' या नाटकातून नाटक आणि प्रेक्षक यांचे अतूट नाते निर्माण होते.
७. रसिक प्रेक्षकांना डोळ्यासमोर ठेवूनच नाटकाची रचना केली जाते.

समारोप :- नाटक हा दृकश्राव्य असा सांघिक कलाविष्कार आहे. अशा स्वरूपाचा असलेला



आपणाला ज्ञात असेलही परंतु या नाटक मराठी वाङ्मय प्रकारातून इतर साहित्य प्रमाणे समाजातील मानवी जीवनाचे दर्शन हे घडवण्याचा एक यथोचित असा प्रयत्न असतो नाटकाला विषयाचे व काळाचे देखील बंधन हे असल्याचे लक्षात घ्यावे लागते. नटसम्राट या नाटकाच्या संदर्भाने साकल्याने विचार केला तर हे आपणास समजले की, आप्पासाहेब बेलवकर 'नटसम्राट' म्हणून ज्यांनी मराठी रंगभूमी चाळीस वर्षे गाजविलेली आहे अशा महान नायकाला निवृत्तीनंतर एक सर्वसामान्य मुलाचा व मुलीचा 'बाप' म्हणून सहन करावा लागणारा अपमान व इतर समाज घटकांकडून एकावे लागणारे विविध नाटक संदर्भातील आक्षेप विचार निश्चितच एका प्रेक्षकांना वाचकांना अंतर्मुख होण्यास लावतात. अशी ही एक सुशिक्षित कौटुंबिक घराची स्थिती आहे. ही स्थिती आज एक ना अनेक कुटुंबामध्ये असल्याचे एका समाजातील वास्तवता सांगणारे चित्रच या नाटकाच्या शोकांतिकेतून निश्चितपणे स्पष्ट होते हे नक्कीच खरे आहे.

नाटक या साहित्य प्रकाराचे स्वरूप व व्याप्ती खूप मोठी अशी आहे. नाटक जेव्हा आपण फक्त प्रेक्षक या नात्याने पाहतो तेव्हा नाटका मागील यंत्रणाची जाणीव आपल्याला होतेच असे नाही आपण फक्त नाट्यप्रयोगाचा आस्वाद घेतो एक दृकश्राव्य साहित्य प्रकार म्हणून नाटक या कलेकडे आपण पाहू शकतो दिग्दर्शक नेपथ्यकार प्रकाश योजना पार्श्वसंगीत रंगभूषा अभिनय या घटकांचे देखील महत्त्व अनन्यसाधारण असल्याचे दिसून येते.

संदर्भ ग्रंथ

१. द.के.गंधारे-मराठी वाङ्मय प्रकार: स्वरूप संकल्पना व वाटचाल, शब्दालय प्रकाशन श्रीरामपूर, प्रथम आवृत्ती, जानेवारी २०१७.
२. डॉ सतीश कामत-मराठी साहित्य प्रवाह, प्रकार आणि प्रतिबिंब, शब्दालय प्रकाशन श्रीरामपूर, प्रथम आवृत्ती जानेवारी २०१२.
३. वि.वा.शिरवाडकर -नटसम्राट(नाटक) पॉप्युलर प्रकाशन मुंबई, प्रथम आवृत्ती १९७१.
४. डॉ.श्रीशैल भैरगुंडे- प्रथम वर्ष कला-<http://archive.mu.ac.in/mywebtest>.
५. म.द.हातकंगलेकर-निवडक ललित शिफारस, मॅजेस्टिक प्रकाशन मुंबई, प्रथम आवृत्ती १९९०.
६. संपा. विजया राजाध्यक्ष- मराठी वाङ्मय कोश खंड चौथा 'समीक्षा-संज्ञा' महाराष्ट्र राज्य साहित्य आणि संस्कृती मंडळ, मुंबई २००२.

नगदी पिकाच्या शेतीत फुले २६५ या वाणाचे अर्थशास्त्रीय महत्व

श्री. ज्ञानेश्वर बाळासाहेब पादर,^१ प्रो. डॉ. जगदीश छबुराव सोनवणे^२

^१संशोधक विद्यार्थी, संशोधन केंद्र, इतिहास विभाग कला, वाणिज्य व विज्ञान महाविद्यालय, सोनई,

ता. नेवासा जि. अहिल्यानगर, ४१४१०५

^२प्राध्यापक व संशोधन मार्गदर्शक इतिहास विभाग श्री ज्ञानेश्वर महाविद्यालय, नेवासा ता. नेवासा जि.

अहिल्यानगर, ४१४६०३

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प्रस्तावना:

भारत हा कृषिप्रधान देश आहे. भारताच्या बहुतांश भागात शेती हाच प्रमुख व्यवसाय आहे. लोकांच्या आर्थिक उत्पन्नाची प्रमुख साधने शेती आणि शेतीशी निगडित असणारे व्यवसाय हे आहे. महाराष्ट्र आणि अहमदनगर जिल्ह्याचा विचार केला तर येथे देखील प्रागैतिहासिक काळापासून शेती व्यवसाय केला जात होता.^१ आजही शेती हाच प्रमुख व्यवसाय आहे. त्यामुळे ही संपूर्ण लोकसंख्या शेतीवरच अवलंबून राहते. शेतीमध्ये वेगवेगळे पिके घेऊन आपला उदरनिर्वाह करून आपली आर्थिक प्रगती कशी घडून येईल यासाठी शेतीमध्ये अन्नधान्य पिकाबरोबरच नगदी पिके घेत असत. अन्नधान्य पिके ही केवळ उदरनिर्वाह पुरतीच मर्यादित राहतात यापासून मिळणारे उत्पन्न कमी असते. याला बाजारभाव देखील कमी मिळतो. शेतकऱ्यांच्या आर्थिक प्रगतीसाठी अन्नधान्य, कडधान्य ही पिके एवढी महत्त्वाची नसतात. त्यांच्या तुलनेत नगदी पिके हे शेतकऱ्यांना नगद उत्पन्न मिळवून देणारे पिके असतात म्हणून अहमदनगर जिल्हा, महाराष्ट्र आणि भारतभर वेगवेगळ्या प्रकारची नगदी पिके ही घेतली जातात. उत्पन्न रोख स्वरूपात मिळत असल्याने अहमदनगर जिल्ह्यातच नव्हे तर संपूर्ण महाराष्ट्र आणि भारतभर नगदी पिकांना प्राधान्य देताना शेतकरी दिसून येतो.

ऊस हे महाराष्ट्रातील प्रमुख नगदी पिक आहे. महाराष्ट्राचे राजकारण, समाजकारण आणि अर्थकारण फिरविण्याचे काम ऊस या पिकाने केले आहे. ऊसामुळेच महाराष्ट्रात सहकार चळवळीचा उदय झाला आहे. आणि आशिया खंडातील पहिला सहकारी साखर कारखाना महाराष्ट्रात प्रवरानगर, लोणी याठिकाणी निर्माण झाला आहे.^२ ऊस पिकाच्या वाणामध्ये महात्मा फुले कृषी विद्यापीठ राहुरी यांनी संशोधित केलेले वाण अधिक प्रभावी ठरलेले पहावयास मिळतात. को- ७२१९, को एम- ७१२५, संपदा- ७५२७, को एम- ८८१२१, को- ८०१४, को- ८६०३२, नीरा को- ९४०१२, फुले २६५ को एम- ०२६५, को- ९२००५ यासारखे विविध वाण विद्यापीठाने संशोधित केलेले आहेत. हे निश्चितच शेतकऱ्यांच्या फायद्याचे ठरले आहेत.

उद्दिष्ट्ये :

१. ऊस शेतीची माहिती घेणे.
२. ऊस या नगदी पिकात विद्यापीठाने संशोधित केलेल्या फुले २६५ या वाणाचा आढावा घेणे.
३. फुले २६५ या वाणाचे अर्थशास्त्रीय महत्व जाणून घेणे.

संशोधन पद्धती:

- १) वर्णनात्मक संशोधन पद्धती.
- २) निवेदनात्मक संशोधन पद्धती.
- ३) विश्लेषणात्मक संशोधन पद्धती.

फुले २६५ या वाणाची निर्मिती:

महात्मा फुले कृषी विद्यापीठाने फुले २६५ या वाणाची निर्मिती केली आहे. मध्यवर्ती ऊस संशोधन केंद्र पाडेगाव यांनी इ. स. २००७ मध्ये संशोधित करून प्रसारित केला आहे.^३ हा वाण प्रामुख्याने को- ८७०४४ या जातीच्या जनरल कलेक्शन मधून निवड पद्धतीने निर्माण केला आहे. ८७०४४ या वाणावर प्रक्रिया करून फुले २६५ या वाणाची निर्मिती झाली आहे. महाराष्ट्रात असणाऱ्या चार कृषी विद्यापीठांची एक संयुक्त कृषी संशोधन आणि विस्तार परिषद भरली गेली होती. जून २००७ मध्ये भरलेल्या या परिषदेमध्ये आणि राज्य वाण प्रसारण उपसमितीच्या ११ जुलै

२००७ रोजी मुंबई या ठिकाणी झालेल्या ४४ व्या बैठकीमध्ये या वाणाची शिफारस केली गेली आहे.^४ हा वाण प्रामुख्याने सुरू हंगाम, पूर्व हंगाम आणि आडसाली अशा तीनही हंगामासाठी शिफारस केलेला आहे. काळी कसदार मध्यम ते खोल आणि पाण्याचा उत्तम निचरा होणारी जमीन असल्यास या वाणाचे उत्पादन चांगले मिळते. फुले २६५ ही जात प्रामुख्याने मध्यम पक्वता गटातील असल्याचे दिसून येते.

फुले २६५ या वाणाची गुणधर्म आणि वैशिष्ट्ये:

हा वाण इतर वाणाच्या तुलनेत निश्चितच अधिक उत्पन्न देणारा वाण आहे. या वाणाच्या उसाची जाडी ३.५ सें. मी. असून उसाचे सरासरी वजन १.९१ किलो आहे.^५ हे वजन या अगोदर निर्माण केलेल्या इतर वाणाच्या तुलनेत अधिक आहे. हा वाण ८६०३२ या वाणापेक्षा जवळजवळ १५ ते २० टक्के अधिक उत्पन्न देतो. अधिक उत्पन्न मिळाल्यामुळे साखर उत्पादन देखील अधिक होते. हा वाण आडसाली हंगाम, पूर्व हंगाम आणि सुरू हंगाम या तिन्ही हंगामांमध्ये लागवडीस योग्य असून याचे उत्पादन अनुक्रमे २०० टन १६४ टन आणि १५० टनदर हेक्टरी एवढे मिळते.^६ या वाणाची पुढची वैशिष्ट्य म्हणजे याच्या पानाच्या देठावर कूस नसल्यामुळे पाचट सहजपणे निघते त्यामुळे तोडणी साठी हा वाण अधिक सोयीस्कर असल्याचे दिसून येते. तसेच हा वाण पाण्याचा ताण सहन करणारा वाण आहे. कानी पाण्याच्या प्रदेशात लागवडी योग्य आहे. या वाणात फुटव्यांचे प्रमाण अधिक असलेले दिसून येते. त्याचबरोबर यास तुरे कमी येतात. तुरे कमी आल्यामुळे याच्या वजनात घट होत नाही. रोगप्रतिकार शक्तीच्या बाबतीत विचार केल्यास हा इ. स. २००३ ते इ. स. २००६ या काळात या वाणाच्या विविध चाचण्या घेतल्या गेल्या त्यामध्ये हा वाण कानी या रोगास मध्यम प्रतिकारक असल्याचे दिसून आले आहे.^७ गुजरात या राज्यात नवसारी या ठिकाणी या वाणाची चाचणी घेण्यात आली त्यामध्ये हा वाण लाल कुज आणि मर या रोगास मध्यम प्रतिकारक्षम असल्याचे दिसून आले आहे.^८ त्यामुळे याच्या देखभालीसाठी शेतकऱ्याला विशेष अशी काळजी घ्यावी लागत नाही. त्यामुळे त्याच्या उत्पादन खर्चात घट होते आणि नफ्यात वाढ

होते. या वाणाची सर्वात महत्त्वाचे वैशिष्ट्य म्हणजे हा वाण क्षारयुक्त जमिनीमध्ये उत्तमरीत्या वाढतो आणि उत्पन्न देखील चांगले देतो. महाराष्ट्राचा बराचसा भाग हा क्षारपड जमीन असलेला आहे तसेच काही भागात खारे पाणी शेतीला दिले जाते. त्यामुळे अशा भागात इतर पिके तसेच उसाच्या इतर जाती चांगल्या येत नाही. अशावेळी फुले २६५ हा वाण या भागात अधिक उत्पन्न देतो. म्हणून फुले २६५ याची लागवड संपूर्ण महाराष्ट्रात मोठ्या प्रमाणावर झालेली पाहायला मिळते. तसेच अहमदनगर जिल्ह्यात देखील याचे उत्पादन मोठ्या प्रमाणावर आहे. अहमदनगर जिल्ह्याच्या जवळ जवळ सर्वच तालुक्यात या उसाची लागवड केली जाते.

फुले २६५ चे आर्थिक महत्व:

महात्मा फुले कृषी विद्यापीठाने ऊस या पिकामध्ये विशेष असे संशोधन केलेले आहे. विद्यापीठाच्या स्थापनेपूर्वी देखील अगदी ब्रिटिश काळात स्थापन झालेल्या मध्यवर्ती ऊस संशोधन केंद्र पाडेगाव येथे को - ४१९ हा वाण संशोधित करण्यात आला होता. त्यानंतर विद्यापीठ स्थापनेच्या पूर्वी को- ७४० व विद्यापीठ स्थापनेनंतर को- ७२१९, को एम- ७१२५, संपदा- ७५२७, को एम- ८८१२१, को- ८०१४, को- ८६०३२, नीरा को- ९४०१२, फुले २६५ (को एम- ०२६५), को- ९२००५ यासारखे विविध वाण विद्यापीठाने संशोधित केलेले आहेत. यामधील को- ८६०३२ हा वाण शेतकऱ्यास अधिकाधिक उत्पन्न देणारा वाण ठरला आहे. या वाणाने इ. स. १९९५-९६ ते इ. स. २०२२-२३ या २८ वर्षांच्या कालखंडात १९५१७०.२२ एवढे एकूण उत्पन्न दिले आहे.^९ तर २१७२१.८५ एवढे निव्वळ उत्पन्न या वाणाने दिले आहे. तर फुले- २६५ या संकरित व सुधारित ऊस वाणाने इ. स. २००८-०९ पासून इ. स. २०२२-२३ या १५ वर्षांच्या कालखंडात ८९०५७.३२ एवढे एकूण उत्पन्न दिले आहे.^{१०} तर निव्वळ उत्पन्न १००२५ .५५ एवढे निव्वळ उत्पन्न दिलेले पाहावयास मिळते. आज विचार केल्यास उसास प्रती टन ३००० रुपये भाव जाहीर करण्यात आला

आहे. प्रती एकरी ७० ते ८० टन एवढे उत्पन्न हा वाण देतो. $३००० * ८० = २४००००$ एवढे प्रती एकरी उत्पन्न मिळते. एक शाश्वत आणि इतर पिकाच्या तुलनेत कमी कष्टात येणारे पिक हे ऊस असते. एकंदरीत इ. स. २००८-०९ ते इ. स. २०२२-२३ या १५ वर्षांच्या काळाचा विचार केल्यास ऊस उत्पादनातून शेतकऱ्यास ३१,७४७.४० करोड एवढे उत्पन्न केवळ ऊस पिकातून मिळालेले पाहायला मिळते.^{११} ऊस पिकातील को- ८६०३२ आणि फुले- २६५ हे वाण शेतकऱ्यांची आर्थिक परिस्थिती सुधारण्यास अधिकाधिक महत्त्वाचे ठरले आहे. एक प्रकारे फुले- २६५ हा वाण शेतकऱ्यासाठी वरदानच ठरला आहे. हे नगदी पीक असून शेतकऱ्यांना नगद उत्पन्न मिळते. आणि या पिकासाठी शेतकऱ्याचे विशेष असा खर्चही करावा लागत नाही. कष्टही कमी घ्यावे लागतात. त्यामुळे हे शेतकऱ्यांच्या फायद्याचे हे ठरलेले आहे. वरील आकडेवारीचा विचार केल्यास शेतकऱ्यांना अधिक उत्पन्न मिळवून देणारा हा वाण असून साखर उद्योगास देखील हा वाण महत्त्वाचा आहे. कारण या वाणाचे उत्पन्न अधिक असून कारखान्यासाठी लागणारा कच्चा माल सहज उपलब्ध होतो.

ऊस शेतीमध्ये विद्यापीठाने संशोधित केलेले वाण प्रभावी ठरलेले आपणास पहावयास मिळतात. त्यामध्ये ८६०३२ हा वाण देखील अधिक उत्पन्न देणारा होता. मात्र फुले २६५ हा वाण सर्वात प्रभावी ठरला आहे. कारण या वाणाची लागवड कोणत्याही हंगामात करता येते तसेच कोणत्याही प्रकारच्या जमिनीमध्ये लागवडीसाठी योग्य असा फुले २६५ हा वाण आहे. क्षारपड जमिनीमध्ये कोणतेही पिक चांगले येत नसते. अशा जमिनीमध्ये उगवण क्षमता चांगली आणि वाढ अधिक असलेला वाण म्हणजे फुले २६५ हा होय .



संदर्भ:

१. पाठक अ.शं.महाराष्ट्र राज्य गॅझेटियर, प्रवरा खोरे, प्राचीन कालखंड २०१३, पृष्ठ.क्र.०७
२. बालवाड आर., आपला अहमदनगर जिल्हा, विद्याभारती प्रकाशन, लातूर पृ. क्र. २१
३. शेंडे एन., ऊस शेतीचे आधुनिक तंत्र,(संपादित), सकाळ प्रकाशन, २०२२, पृ. क्र. २६
४. कित्ता पृष्ठ क्र. ३१
५. कित्ता पृष्ठ क्र. ३१
६. www.mpkv.in, varieties, sugarcane, page No. 06
७. शेंडे एन., ऊस शेतीचे आधुनिक तंत्र,(संपादित), सकाळ प्रकाशन, २०२२, पृ. क्र. ३२
८. कित्ता पृष्ठ क्र. ३२
९. कृषी दर्शनी, महात्मा फुले कृषी विद्यापीठ, राहुरी, २०२४, पृ. क्र.४२२
१०. कित्ता पृष्ठ क्र. ४२२
११. कित्ता पृष्ठ क्र. ४२२



डिजिटल माध्यमांतील मराठी भाषा संरचना: आणि प्रवृत्ती

प्रानिवृत्ती हनुमंता सोनवणे.,

सहाय्यक प्राध्यापक, मराठी विभाग

कला वाणिज्य व विज्ञान महाविद्यालय सोनई ता.अहिल्यानगर. नेवासा जि.

(सावित्रीबाई फुले पुणे विद्यापीठ संलग्नित)

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सारांश :

मराठी भाषा गेल्या काही दशकांत खूप बदलली आहेपूर्वी फक्त छापील पुस्तके ., वर्तमानपत्रे आणि रेडिओवर मराठी भाषा ऐकायला मिळायची, पण आता स्मार्टफोन, इंटरनेट आणि सोशल मीडिया यांमुळे मराठीचा वापर रोजच्या आयुष्यात वाढला आहेडिजिटल युगाने मराठीला नवं स्वरूप दिलं आहे ज्यात . मराठी टायपिंग, ऑनलाइन साहित्य आणि व्हॉइस असिस्टंट यांचा समावेश आहेयामुळे मराठी भाषा . जगभरातील मराठीभाषिकांपर्यंत पोहोचत आहेदुसरीकडे., डिजिटल युगाने मराठीला नवे आव्हानेही दिली आहेतयुनिकोड फॉन्ट्स ., मराठी कीबोर्ड आणि ऑनलाइन ट्रान्सलेशन टूल्स यांमुळे मराठी लिहिणं सोपं झालं आहे .डिजिटल प्लॅटफॉर्मवर मराठी कंटेंट क्रिएशन करणारे युवा मराठीला आधुनिक बनवत आहेत . यामुळेमराठी भाषा फक्त महाराष्ट्रापुरती मर्यादित राहिली नाही तर जागतिक स्तरावर पोहोचली आहे. डिजिटल तंत्रज्ञानाच्या झपाट्याने झालेल्या प्रसारामुळे संवादाच्या साधनांमध्ये मूलभूत बदल घडले आहेत . इंटरनेट, स्मार्टफोन आणि विविध सामाजिक माध्यमांच्या आगमनानंतर भाषिक व्यवहार अधिक वेगवान, संक्षिप्त आणि बहुआयामी झाला आहेमराठी भाषा या प्रक्रियेत सक्रियपणे सहभागी होत असून तिच्या . रचनेत, शब्दसंपदेत, लिपीवापरात आणि अभिव्यक्तीशैलीत नवनवीन प्रयोग दिसून येतातडिजिटल . माध्यमांमध्ये देवनागरीऐवजी रोमन लिपीचा वापर, इंग्रजी मराठी-कोडमिक्सिंग-, इमोजी व दृश्यचिन्हांचा व्यापक उपयोग, संक्षिप्त रूपे, आणि मीमया अभ्यासात .संस्कृती यांचा प्रभाव आढळतो-शब्दरचनात्मक) डिजिटल माध्यमांतील मराठी भाषेची संरचना, वाक्यरचनात्मक व अर्थविषयकआणि (.तिच्या सामाजिक प्रवृत्तींचा अभ्यास करण्यात आला आहे

बीज शब्द : डिजिटल माध्यमे, मराठी भाषा, समाज भाषाशास्त्र, कोडमिक्सिंग-, कोडस्विचिंग-, रोमन लिपी, इमोजी, डिजिटल रजिस्टर, भाषिक परिवर्तन

प्रस्तावना:

भाषा ही समाजाच्या सांस्कृतिक, सामाजिक आणि बौद्धिक जीवनाचे प्रतिबिंब असतेकाळानुसार समाजात . बदल घडत असतानाभाषेतही विविध स्तरांवर परिवर्तन होत असतेमाहिती व तंत्रज्ञानाच्या युगात . इंटरनेट .डिजिटल माध्यमांचा प्रसार झपाट्याने वाढला आहे, स्मार्टफोन आणि सामाजिक माध्यमांच्या वाढत्या वापरामुळे संवादाची साधने व पद्धती मोठ्या प्रमाणावर बदलल्या आहेत मराठी भाषा देखील या बदलांपासून अलिप्त राहिलेली नाहीफेसबुक ., व्हॉट्सअॅप, ट्विटर)X), यूट्यूब, ब्लॉग्स, वेबसाइट्स आणि विविध डिजिटल प्लॅटफॉर्मवर मराठी भाषेचा वापर मोठ्या प्रमाणावर होत आहेडिजिटल माध्यमांमध्ये . रोमन लिपीतील लेखन .मराठी भाषेचा वापर करताना अनेक नव्या भाषिक वैशिष्ट्यांचा उदय झाला आहे, इंग्रजीमराठी मिश्र भाषा-, संक्षिप्त शब्दप्रयोग, इमोजी आणि मीम्सयांचा वापर यामध्ये दिसून येतोया सर्व . बदलांचा मराठी भाषेच्या संरचना, शैली आणि वापरावर महत्त्वपूर्ण प्रभाव पडत आहेतयामुळे डिजिटल . माध्यमांतील मराठी भाषेची रचना आणि तिच्याप्रवृत्तींचा अभ्यास करणे आवश्यक ठरते.



भाषा ही मानवी समाजाची मूलभूत अभिव्यक्ती प्रणाली आहेसमाजातील राजकीय ., आर्थिक, तांत्रिक आणि सांस्कृतिक बदलांमुळे भाषेत सातत्याने परिवर्तन घडत असतेडिजिटल युगात संवादाचे प्रमुख . माध्यम म्हणून सामाजिक जाळे आणि तत्काळ संदेशवहन ॲप्स उदयास आलेफेसबुक ., व्हाट्सॲप, ट्विटर ,यूट्यूब, ब्लॉग्स, वेबसाइट्स यांसारख्या माध्यमांनी दैनंदिन संवादाची व्याप्ती प्रचंड वाढवली आहेया . माध्यमांतील संवाद अनौपचारिक, वेगवान आणि बहुविध स्वरूपाचा असतोमजकूर ., ध्वनी, चित्र, व्हिडिओ आणि इमोजी यांच्या संयोगातून अर्थनिर्मिती होतेत्यामुळे पारंपरिक लेखनशैलीतील नियमांना . .मराठी भाषेने या नव्या संदर्भात स्वतःची नवी अभिव्यक्तीशैली निर्माण केली आहे.येथे दुय्यम स्थानमिळते प्रस्तुत संशोधनात या बदलांचा संरचनात्मक आणि समाजभाषाशास्त्रीय दृष्टिकोनातून अभ्यास करण्यात येत आहे.

उद्दिष्टे :

- १.डिजिटल माध्यमांमध्ये वापरल्या जाणाऱ्या मराठी भाषेच्या स्वरूपाचा अभ्यास करणे .
- २.डिजिटल माध्यमांतील मराठी भाषेची शब्दरचना व वाक्यरचना समजून घेणे .
- ३ स्वचिंग या प्रवृत्तीचा अभ्यास-मिक्सिंग आणि कोड-डिजिटल संवादात आढळणाऱ्या कोड .करणे.
- ४रोमन लिपी ., इमोजी आणि संक्षिप्त रूपांचा मराठी भाषेवर होणारा परिणाम विश्लेषित करणे.
- ५.डिजिटल माध्यमांमुळे मराठी भाषेत होत असलेल्या सामाजिक व भाषिक परिवर्तनाचा अभ्यास करणे .

विषय विवेचन :

१. **लिपीविषयकप्रवृत्ती:** मराठी लेखन आणि लिपी विषयक प्रवृत्तींमध्ये प्रामुख्याने देवनागरी लिपी)पारंपारिक वअधिकृत (आणि रोमनलिपी) इंग्रजी/सोशलमीडियासाठी (चा वापर वाढला आहे .लिखाणात शुद्धलेखनाचे नियम, उच्चारानुसार लेखन, आणि लोकव्यवहारातील प्रचलित शब्द रूपांचा विचार केला जातो .डिजिटल युगात देवनागरी चे सुलभीकरण आणि इंग्रजी अक्षरांचा वापर ही प्रमुख प्रवृत्ती आहे.

डिजिटल माध्यमांमध्ये रोमन लिपीतील मराठी मोठ्या प्रमाणात वापरली जाते उदाहरणार्थ .- “MI ATAGHARIAHE”, “KHUP MAST”, “KAYCHALALAY?” इत्यादीही लेखनरूपे ध्वन्यात्मक . देवनागरी लिपीपेक्षा रो.पद्धतीने तयार होतातमन कीबोर्ड अधिक सुलभ असल्याने हा वापर वाढलेला दिसतोतथापिऔपचारिक पोस्ट्स ., लेख किंवा व्हिडिओ शीर्षकांमध्ये देवनागरीचा वापर टिकून आहेथोडक्यात., आजची लिपीविषयक प्रवृत्ती ही पारंपारिक देवनागरी आणि आधुनिक डिजिटल रोमन लिपी यांचा समतोल साधणारी आहे.

२.**शब्दरचनात्मक बदल:** डिजिटल माध्यमांमध्ये मराठी शब्दसंपदेत नवीन शब्दांचा समावेश मोठ्या प्रमाणावर होत आहे .इंग्रजी शब्दांचे मराठीमध्ये रूपांतर करून वापरले जात आहे .उदाहरणार्थ – पोस्ट करणे, डाउनलोड करणे, फॉरवर्ड करणे, अपडेट देणे इत्यादी.तसेच संक्षिप्त रूपांचा वापर देखील मोठ्या प्रमाणावर दिसून येतो .उदाहरणार्थ - GM (GOOD MORNING),TC (TAKE CARE) इ.शब्दरचनात्मक बदल म्हणजे एखाद्या शब्दाच्या रचनेत)STRUCTURE) बदल करून त्याचा नवीन शब्द तयार करणे .या प्रक्रियेत उपसर्ग, प्रत्यय, संधी, समास इत्यादींचा वापर करून शब्द बदलला जातो.इंग्रजी शब्दांना मराठी प्रत्यय लावण्याची प्रवृत्ती वाढलेली आहे .

उदाहरणार्थ –

- “पोस्ट केला”
- “डाउनलोड केल”
- “लाईक केलीस”
- “कमेंट केलीस”

येथे इंग्रजी मूळ शब्द मराठी क्रियापदांच्या चौकटीत सामावले जातात .

३ .वाक्यरचना आणि शैली:वाक्यातील शब्दांची योग्य मांडणी आणि त्यांचा परस्पर संबंध याला “वाक्यरचना” म्हणतात .योग्य वाक्यरचना असल्यास वाक्याचा अर्थ स्पष्ट आणि समजण्यास सोपा होतो . विचार मांडण्याची पद्धत, शब्दांची निवड आणि भाषेची मांडणी याला “शैली”म्हणतात .चांगली शैली लेखन अधिक सुंदर, प्रभावी आणि आकर्षक बनवते .त्यामुळे वाक्यरचना आणि शैली या दोन्ही गोष्टी भाषेत महत्त्वाच्या आहेत..डिजिटल संवादात पूर्ण वाक्यांपेक्षा लघुरूपे वापरली जातात. उदा .“ओके”, “हो येतो”, “बघू”, “थँक्स”, “ग्रेट!”विरामचिन्हांचा मर्यादित वापर आणि अक्षरांची पुनरावृत्ती (“खूपच छानnnn”) ही वैशिष्ट्ये दिसतात.

४ .कोड-मिक्सिंग आणि कोड-स्विचिंग : कोड-मिक्सिंग आणि कोड-स्विचिंग या बहुभाषिक समाजात वापरल्या जाणाऱ्या भाषिक प्रक्रिया आहेत .एकाच वाक्यात दोन भाषांचे शब्द मिसळणे म्हणजे कोड-मिक्सिंग तर संभाषणाच्या संदर्भात)उदा .एका वाक्यातून दुसऱ्या वाक्यात (पूर्णपणे भाषा बदलणे म्हणजे कोड-स्विचिंग होय.मराठी आणि इंग्रजी भाषांचा एकत्रित वापर ही डिजिटल भाषेची प्रमुख वैशिष्ट्ये आहेत.

उदा .

१ (“आज Meeting आहे”

२ "(मी आता निघतो .See you tomorrow!"

कोड-स्विचिंगमध्ये पूर्ण वाक्य इंग्रजीत किंवा मराठीत बदलते, तर कोड-मिक्सिंगमध्ये शब्दस्तरावर मिश्रण होते.

५ .इमोजी आणि दृश्य संवाद:इमोजीम्हणजेभावना, प्रतिक्रिया किंवा मन:स्थिती लहान चित्रे व चिन्हांच्या मदतीने व्यक्त करण्याची पद्धत होय .आजच्याडिजिटलयुगातमोबाईल, सोशल मीडिया आणि चॅटमध्ये इमोजीं चामोठ्या प्रमाणात वापर केला जातो .😊, 😞, 👍, ❤️ यां सारखी इमोजी आनंद, दुःख, मान्यताआणिप्रेमव्यक्त करतात .त्यामुळेसंदेशअधिकस्पष्टआणिआकर्षकबनतो.दृश्यसंवादम्हणजेचित्र, चिन्ह, नकाशे, तक्ते किंवा आकृती यांच्या माध्यमातून माहिती व्यक्त करणे .वाहतूक चिन्हे, जाहिरातीतीलचित्रे, नकाशे आणि माहितीपटही दृश्य संवादाची उदाहरणे आहेत .दृश्यसंवादामुळे माहिती लवकर आणि सहज समजते.इमोजीहे भाषेचे पूरक साधन बनले आहे. काही प्रसंगी इमोजी स्वतंत्र अर्थ वाहक घटक ठरतात . त्यामुळे भाषेचीदृश्य- आधारित रचना बळकट होते.

६ .मीम संस्कृती:मीम संस्कृती ही इंटरनेटवर वेगाने पसरणारी, विनोदी प्रतिमा, व्हिडिओ किंवा मजकुराद्वारे)मीम्स (सांस्कृतिक विचार आणि भावना व्यक्त करण्याची आधुनिक डिजिटल पद्धत आहे . रिचर्ड डॉकिन्स यांनी या संकल्पनेला जन्म दिला, जीआता सामाजिक-राजकीय टीका, सहयोगात्मक हास्य आणि डिजिटलसंवादाचे प्रमुख साधन बनली आहे. हे सहसा व्यंग्यात्मक असतात आणि लोकांच्या दैनंदिन अनुभवांना प्रतिबिंबित करतात. मीम्सच्या माध्यमातून सामाजिक घटना, राजकीय प्रसंग किंवा सांस्कृतिक



संदर्भ विनोदी आणि उपरोधिक शैलीत व्यक्त केले जातात .भाषेतील लघुरूप, उपरोध आणि प्रतिमांचा वापर यात दिसून येतो.मीम संस्कृती ही डिजिटल संवादाचा महत्त्वाचा भाग बनली आहे .मीमच्या माध्यमातून लोक समाजातील घटना, राजकारण, शिक्षण किंवा दैनंदिन जीवनातील गोष्टींवर हलक्या-फुलक्या पद्धतीने प्रतिक्रिया देतात.मीममुळे संदेश लवकर समजतो, मनोरंजन होते आणि लोकांपर्यंत विचार सहज पोहोचतो .त्यामुळे आजच्या इंटरनेट युगात मीम संस्कृतीला मोठे महत्त्व प्राप्त झाले आहे.

७ .सामाजिक घटकांचा प्रभाव : डिजिटल माध्यमांच्या वाढत्या वापरामुळे मराठी भाषेच्या संरचनेत व वापराच्या प्रवृत्तींमध्ये मोठे बदल दिसून येतातसमाज .ातील विविध घटक जसे की वय, शिक्षण, प्रदेश, तंत्रज्ञानाचा वापर, आणि सामाजिकहे .सांस्कृतिक वातावरण यांचा मराठी भाषेवर प्रभाव पडतो-शिक्षणाचा स्तर भाषेच्या .सामाजिक घटक डिजिटल माध्यमांतील भाषिक अभिव्यक्तीचे स्वरूप ठरवतात उच्च शिक्षित .शुद्धतेवर परिणाम करतोवापरकर्ते डिजिटल माध्यमांवर तुलनेने शुद्ध आणि व्याकरणदृष्ट्या योग्य मराठी वापरण्याचा प्रयत्न करताततर सामान्य वापरकर्त्यांमध्ये बोली भाषेचा आणि संक्षिप्त रूपांचा . त्याचप्रमाणे महाराष्ट्रातील विविध भागांतील बोली भाषांचा प्रभाव डिजिटल .वापर अधिक आढळतो माध्यमांमध्येही दिसून येतोउदाहरणार्थ ., मराठवाडा, विदर्भ किंवा कोकणातील बोली भाषेतील शब्द सोशल मीडियावर सहजपणे वापरले जातातत्यामुळे मराठी भाषेची विविधता डिजिटल जगात अधिक . मोबाईल फोन .स्पष्ट होते, कीबोर्ड, ॲप्स आणि ऑटोकरेक्ट प्रणालीमुळे भाषेच्या लेखन पद्धतीत बदल झाले आहेतमराठी टायपिंगपेक्षा रोमन लिपीचा वापर अधिक सोपा वाटत असल्याने अनेक वापरकर्ते . डिजिटल माध्यमांमुळे मराठी संस्कृती .मराठी रोमन लिपीत लिहितात, परंपरा, सणउत्सव यांचे वर्णन - त्यामुळे भाषेच्या वापरात पारंपरिक शब्दस .आणि प्रसार मोठ्या प्रमाणावर होत आहेंपदा आणि आधुनिक शब्द यांचे मिश्रण दिसून येतेडिजिटल माध्यमांमुळे मराठी भाषेची रचना ., शब्दसंपदा आणि अभिव्यक्तीची शैली बदलत आहेत्यामुळे डिजिटल युगात .सामाजिक घटक या बदलांना दिशा देतात . मराठी भाषा अधिक गतिमान, मिश्रित आणि संवादक्षम बनत आहे.

निष्कर्ष :

या संशोधनातून पुढील निष्कर्ष समोर आले:

- १ .डिजिटल माध्यमांतील मराठी भाषा अधिक लवचिक आणि अनौपचारिक आहे.
- २ .रोमन लिपीचा वापर विशेषतः तरुण वर्गात अधिक आहे.
- ३ .इंग्रजी-मराठी कोड-मिक्सिंग सर्वसाधारण झाले आहे.
- ४ .इमोजी आणि दृश्यचिन्हे अर्थनिर्मितीची महत्त्वाची साधने ठरली आहेत.
- ५ .डिजिटल मराठी ही स्वतंत्र डिजिटल रजिस्टर म्हणून विकसित होत आहे.

समारोप :

डिजिटल युगात मराठी भाषा नव्या संवाद माध्यमांशी जुळवून घेत आहे .तिच्या संरचनेत, शैलीत आणि शब्दसंपदेत झालेले बदल समाजातील तांत्रिक आणि सांस्कृतिक परिवर्तनांचे प्रतिबिंब आहेत.भविष्यात शिक्षणव्यवस्था, भाषिक धोरणे आणि माध्यमसंस्था यांनी डिजिटल मराठीच्या स्वरूपाचा अभ्यास करून तिच्या सकारात्मक उपयोगाला प्रोत्साहन द्यावे .डिजिटल माध्यमांतील मराठी ही आधुनिक



पिढीच्या सांस्कृतिक ओळखीचे आणि अभिव्यक्तीस्वातंत्र्याचे प्रभावी साधन ठरली आहे.डिजिटल तंत्रज्ञानाच्या वेगवान प्रसारामुळे आधुनिक समाजातील संवाद प्रक्रियेत मोठे परिवर्तन झाले आहेया . इंटरनेट .परिवर्तनाचा प्रभाव मराठी भाषेवरही स्पष्टपणे दिसून येतो, स्मार्टफोन आणि विविध सामाजिक माध्यमांच्या माध्यमातून मराठी भाषेचा वापर अधिक व्यापक झाला असून तिच्या रचना, शैली आणि अभिव्यक्तीच्या पद्धतीत नवे बदल घडून आले आहेतरोमन लिपीचा वाढता वापर ., इंग्रजी-मराठी कोड-स्विचिंग-मिक्सिंग आणि कोड, संक्षिप्त शब्दप्रयोग, इमोजी व दृश्यचिन्हांचा वापर ही डिजिटल मराठीची ठळक वैशिष्ट्ये म्हणून समोर येताततसेच समा.जातील वयोगट, शिक्षणपातळी, प्रदेशीय बोलीभाषा, तंत्रज्ञानाची उपलब्धता आणि सांस्कृतिक पार्श्वभूमी यांसारख्या सामाजिक घटकांमुळे डिजिटल माध्यमांतील मराठी भाषेचे स्वरूप विविधतेने समृद्ध झाले आहे या प्रक्रियेमुळे मराठी भाषेत एक स्वतंत्र . “डिजिटल रजिस्टर” विकसित होत असल्याचे दिसून येतेत्यामुळे डिजिटल माध्यमांतील मराठी भाषेचा . .अभ्यास हा केवळ भाषावैज्ञानिक दृष्टीनेच नव्हे तर समाजभाषाशास्त्रीय दृष्टीनेही महत्त्वाचा ठरतो भविष्यात मराठी भाषेच्या विकासासाठी डिजिटल तंत्रज्ञानाचा सकारात्मक आणि सर्जनशील वापर करणे आवश्यक आहेशिक्षणसंस्था ., संशोधक आणि माध्यमसंस्था यांनी या बदलत्या भाषिक प्रवाहांचा अभ्यास करून मराठी भाषेचे जतन, संवर्धन आणि आधुनिक संदर्भात तिचा प्रभावी वापर यासाठी प्रयत्न करणे गरजेचे आहे.

संदर्भ :

१. केळकर, अशोक र.(1997) – मराठी भाषेचे स्वरूप.मौज प्रकाशन :पुणे.
२. देवी, गणेश.(2010) – भारतीय भाषांचे भविष्य.नवी दिल्ली.साहित्य अकादमी :
३. मेये, सुनील.(2018) – सामाजिक संशोधन पद्धती.पुणे.डायमंड पब्लिकेशन्स :
४. जोशी, वा) .भा .2008) शुद्धलेखन माझ्या खिशात.
५. साबनीस, शरणकुमार) .2013)मराठी भाषा आणि संस्कृती.डायमंड प्रकाशन :पुणे.
६. देशमुख, सुनील) .2019)डिजिटल युगातील मराठी भाषा.मुंबई.ग्रंथाली प्रकाशन :
७. डिजिटल भाषाविज्ञान विषयक संशोधन निबंध
८. सामाजिक माध्यमांवरील संकलित नमुना डेटा
९. मराठी भाषा आणि तंत्रज्ञान विषयक शैक्षणिक लेख