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Assessment of Antibacterial and Antifungal Potential of Selected Medicinal Plants of Nandurbar District, Maharashtra

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Abstract:

In the present study, we have prepared the extracts of four selected medicinal plants from Nandurbar district of Maharashtra. The extracts were tested for potential antibacterial activity against Gram-positive (*Bacillus subtilis* and *Bacillus cereus*) and Gram-negative (*E. coli and Pseudomonas aeruginosa*) bacterial strains and showed high antibacterial potential for the tested bacterial strains. Further, extracts were also tested for their antifungal activity against common pathogenic fungi, *viz.: Fusarium solani*, *Fusarium oxysporum* and *Penicillium citrinum* and showed excellent antifungal properties. Based on the results obtained in the current study, we may treat these extracts as potential candidates for the development of pharmacological antimicrobial agents.

Keywords: antibacterial, antifungal, selected plant species and pharmacology

1. Introduction

Nature has long served as a vital source of therapeutic agents for treating and preventing various diseases. The plant kingdom, in particular, represents a vast repository of bioactive compounds widely utilised in traditional and modern medicine. These phytoconstituents continue to inspire the discovery and development of novel drugs with improved efficacy and safety for managing diverse pathological conditions (Atanasov et al., 2015; Cragg & Newman, 2013). In India and globally, herbal medicines have gained prominence as primary healthcare resources due to their perceived effectiveness, accessibility, and minimal adverse effects compared to synthetic drugs (WHO Report, 2019). Since ancient times, plants have been employed for their medicinal value in treating a broad-spectrum of ailments, attributed to their rich phytochemical diversity, cost-effectiveness, and long-standing traditional use (M. M. Pandey et al., 2013). The therapeutic potential of plants stems from their diverse parts, flowers, fruits, bark, roots, leaves, and seeds, which contain a range of active constituents. Indian forests, in particular, are renowned as repositories of medicinal and aromatic plants, forming an essential base for developing natural remedies against numerous diseases (J. Sharma et al., 2012; P. Sharma & Samant, 2014).

In the present study, we have worked on some of the medicinal plants that mostly occur in the Nandurbar district of Maharashtra, India. Nandurbar district in Maharashtra boasts a rich diversity of medicinal plants, largely attributed to its forested landscapes and the traditional knowledge of indigenous communities such as the Bhil, Pawara, Tadvi Gavit, Mavchi, Vasave, Dhanka, and Barela tribes. Ethnobotanical surveys have

recorded numerous species used to treat ailments, including cough, dysentery, jaundice, abdominal pain, malaria, and snake bites. Plant families like Menispermaceae, *Euphorbiaceae, Bombacaceae, Bignoniaceae, Myrtaceae, Moringaceae, Caricaceae, Sapotaceae, Fabaceae,* and a few more are well represented, with stem bark, leaves, and roots being the most commonly utilised parts (Dhale et al., 2018; Patil et al., 2023). These plants are not only integral to traditional healthcare but also form an essential part of daily life for tribal communities, especially in regions where modern medical facilities are limited. Many of the plant species have shown pharmacological potential through phytochemical analyses. Despite this wealth of traditional knowledge, concerns are growing about its preservation, as younger generations are increasingly less engaged with ancestral healing practices. Safeguarding and systematically documenting this ethnobotanical heritage is vital for maintaining local healthcare traditions and unlocking the therapeutic potential of these plants for modern medicine. The plant species used in the study have been discussed in the subsequent sections of this chapter.

1.1 *Ricinus communis* L:

Ricinus communis L., commonly called the castor oil plant, is a member of the *Euphorbiaceae* family and holds great economic and medicinal value worldwide. It is a fast-growing perennial shrub or small tree that can reach heights of 10–12 meters in suitable conditions. The plant has large, glossy, palm-shaped leaves, hollow stems, and spiny, three-lobed fruit capsules that contain its distinctive seeds. Thought to have originated in tropical Africa, especially Ethiopia, castor is now widely naturalised and cultivated across tropical and subtropical regions of Asia, South America, and Africa. Today, India, Brazil, and China are the leading producers, with India accounting for nearly 90% of the world's castor seed output (Anjani, 2012). The seeds of *Ricinus communis* contain a high amount of fixed oil, ~ 40–55%, known as castor oil, which is widely valued for its use in medicine, cosmetics, and various industries (Abomughaid et al., 2024). Traditionally, castor oil has been used as a purgative, anti-inflammatory agent, and skin moisturiser. In Ayurveda and other traditional medical systems, different parts of the plant are used to treat ailments such as arthritis, constipation, skin disorders, and infections. (Ch Malathi Suvarna, P Sriya, Md Arshad, 2018). Besides its medicinal importance, castor oil is also a vital industrial raw material, used in the production of lubricants, paints, varnishes, nylon, and biodiesel, owing to its high content of ricinoleic acid (K. V. et al., 2025a; Ramothloa et al., 2025a)

Phytochemical studies show that *Ricinus communis* contains a variety of biologically active compounds. The seeds possess ricin, a highly toxic protein that inhibits ribosomes, and ricinine, an alkaloid known for its insecticidal properties (Ribeiro et al., 2016). Although ricin is extremely poisonous, it has attracted scientific interest for its possible use in targeted cancer treatments when safely modified or detoxified (Jennifer Audi, Martin Belson, Manish Patel, 2005). The oil extracted from the seeds, however, does not contain ricin and is considered safe for medicinal and industrial applications, making it one of the most versatile natural oils. Additionally, flavonoids, terpenoids, and sterols found in different parts of the plant enhance its pharmacological value. Ecologically, *R. communis* is a hardy plant that thrives in semi-arid and marginal lands, making it an ideal crop for cultivation in regions with low rainfall and poor soil fertility (Anjani, 2012). The ability of Ricinus communis to thrive under a wide range of climatic conditions has contributed to its broad distribution, with both wild and cultivated forms found across Africa, Asia, and South America.(Salihu, B Z, Gana, A K, Gbadeyan, T and Alabi, 2014) In addition to its agricultural and economic value, the plant supports sustainable development, as castor oil derivatives are increasingly being used as bio-based substitutes for petroleum products, promoting environmentally friendly industrial practices (Cafaro et al., 2025). Given its ethnomedicinal relevance, phytochemical richness, and wide industrial utility, *Ricinus communis* continues to attract global research attention. The dual nature of the plant as a source of valuable oil and bioactive compounds on the one hand and a toxic species on the other makes it a fascinating subject of pharmacognostic investigation.

Pharmacognostic Importance of *Ricinus communis:* Pharmacognostic studies of *Ricinus communis* L. are vital for its proper identification, authentication, and standardisation, as adulteration and substitution are common issues in crude drug markets. Macroscopic features such as the large palmate leaves, spiny fruit capsules, and mottled seeds with a distinctive caruncle serve as primary diagnostic characteristics (S. N. Pandey et al., 2025). Microscopic examination of the leaf reveals dorsiventral structures with polygonal epidermal cells, anomocytic stomata, and multicellular covering trichomes, which are useful diagnostic markers. Powder microscopy further aids in identification by showing fragments of palisade cells, xylem vessels, and calcium oxalate crystals(P. V. Sharma, 1998).

Physicochemical analyses, including ash content, extractive values, and fluorescence studies, are commonly used to ensure the quality of plant materials and to identify any adulteration. Preliminary phytochemical evaluation of various Ricinus communis extracts indicates the presence of alkaloids, flavonoids, terpenoids, saponins, and glycoside compounds responsible for its wide range of pharmacological effects. The seed oil, known as castor oil, contains a high proportion of ricinoleic acid (about 80–90%), which accounts for its laxative, anti-inflammatory, and wound-healing properties. These pharmacognostic characteristics serve to verify the plant's identity and establish quality benchmarks for its safe therapeutic application (Gassim et al., 2024; K. V. et al., 2025b). Phytochemical analysis of *Ricinus communis* using standard qualitative and quantitative techniques, such as Soxhlet extraction, thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and spectrophotometric assays, has confirmed the presence of various bioactive compounds across different plant parts. Roots are particularly rich in alkaloids, saponins, and tannins, whereas leaves and stems contain high levels of flavonoids and phenolic compounds. Differences in metabolite content among plant parts, extraction methods, and solvents emphasise the need for standardised procedures to ensure accurate pharmacognostic evaluation and reproducible biological activity (Abomughaid et al., 2024; S. N. Pandey et al., 2025). These phytochemical insights provide a scientific basis for the traditional medicinal applications of *R. communis*, including its antioxidant, antimicrobial, anti-inflammatory, and laxative effects. Knowledge of the chemical profile is also essential for quality control, safety assessment, and the development of herbal formulations from castor plant extracts. However, caution is necessary due to the presence of ricin, a highly toxic protein concentrated in raw seeds, which is effectively removed or inactivated during the production of castor oil (Ch Malathi Suvarna, P Sriya, Md Arshad, 2018).

The pharmacognostic importance of *Ricinus communis* stems from its unique diagnostic characteristics, physicochemical properties, and diverse phytochemical composition, which help ensure its authenticity and therapeutic consistency. In Ayurvedic medicine, the plant is highly valued for its broad therapeutic applications, particularly in the management of Vata-related disorders, highlighting its role as a classical medicinal species. These insights underscore the value of combining pharmacognostic research with traditional knowledge to promote the safe and effective use of *R. communis* in contemporary healthcare.

1.2 *Bombax ceiba* (L.)

Bombax ceiba (L.), widely known as the silk cotton tree, red cotton tree, or Semal, belongs to the family Malvaceae (previously classified under Bombacaceae). It is a tall, deciduous tree that can grow up to 25–30 meters in height. The tree is easily recognised by its straight, prickly trunk, palmately compound leaves, and large, bright crimson flowers that bloom during the dry season, often when the branches are bare of leaves (Kirtikar, K R, 1991). The species is widely distributed throughout tropical and subtropical regions, including India, Sri Lanka, Southeast Asia, southern China, and certain parts of Africa. It grows best in well-drained soils and open plains and is commonly found in mixed deciduous forests or along the outskirts of villages. (P. K. Warrier, 1994). Traditionally, *Bombax ceiba* has been highly valued in Ayurveda, Unani, and several folk medicinal practices. Its roots and bark are commonly used as demulcents and astringents to treat ailments such as dysentery, diarrhoea, and excessive menstrual bleeding. The flowers are also regarded for their cooling, diuretic, and anti-inflammatory effects (Karole et al., 2017; Khare, 2007).

In Ayurveda, *Bombax ceiba* (L.), commonly known as Semal or the silk cotton tree, is regarded as a valuable medicinal species with diverse therapeutic uses. It is characterised by *Kashaya* (astringent), *Tikta* (bitter), and *Madhura* (sweet) tastes, along with *Guru* (heavy) and *Snigdha* (unctuous) qualities. The plant exhibits a *Sheeta virya* (cooling potency) and *Madhura vipaka* (sweet post-digestive effect), making it particularly effective in pacifying *Pitta* and *Kapha doshas* (P. H. Chaudhary & Tawar, 2019; H. Kumar et al., 2025). Owing to these pharmacodynamic attributes, *B. ceiba* is extensively used in traditional medicine for its cooling, astringent, anti-inflammatory, and rejuvenating properties (P. K. Warrier, 1994; Sarkar Phyllis et al., 2022). The roots and bark of *Bombax ceiba* are highly regarded in traditional medicine for their soothing and astringent actions. They are commonly employed in conditions such as dysentery, diarrhoea, leukorrhea, and menorrhagia. Bark decoctions are used to calm irritated mucous membranes and reduce inflammation of the gastrointestinal tract. The roots are also valued as general tonics that help enhance digestion and promote overall strength and vitality (Kirtikar, K R, 1991; P. K. Warrier, 1994)

Phytochemical studies have shown that *Bombax ceiba* contains a variety of bioactive secondary metabolites, including flavonoids, alkaloids, tannins, glycosides, saponins, and phenolic compounds, which contribute to its therapeutic effects (Kamble et al., 2017; Shukla et al., 2020). Specific constituents such as shamimin, bombasin, and ceibanol have been reported to possess antioxidant, anti-inflammatory, and hepatoprotective

properties. Experimental studies using ethanolic and methanolic extracts of the bark and flowers have demonstrated significant antimicrobial and wound-healing activities. These observations provide scientific validation for its traditional medicinal applications and highlight its pharmacological relevance (Taher et al., 2024). Beyond its targeted therapeutic uses, *Bombax ceiba* is regarded as a general *rasayana* (rejuvenative) herb, promoting overall vitality and enhancing immune function. Its cooling and restorative qualities make it especially beneficial during hot seasons or in conditions associated with an excess of *Pitta dosha*. The diverse pharmacological activities of the plant correspond closely with its traditional applications, highlighting its significance in classical Ayurvedic literature as well as in contemporary ethnomedicinal research (P. K. Warrier, 1994; Shukla et al., 2020).

From a pharmacognostic point of view, a thorough examination of Bombax ceiba is crucial for ensuring the quality and standardisation of herbal preparations. Detailed observation of its macroscopic and microscopic features, such as the structure of xylem, the occurrence of mucilage cells, and the nature of trichomes, serves as a reliable basis for proper identification and authentication of the plant material (Mukherjee, 2002). Physicochemical parameters, including total ash, acid-insoluble ash, extractive values, and fluorescence properties, are important indicators for determining the purity and detecting any adulteration of the plant material. Alongside these tests, preliminary phytochemical analysis helps verify the presence of major bioactive constituents that are responsible for the plant's medicinal value (Hait, 2021; Muyumba et al., 2021).

In recent years, the increasing global demand for herbal medicines has underscored the need for reliable pharmacognostic standards. Many crude drugs are vulnerable to adulteration and substitution, leading to variations in efficacy and safety. Establishing diagnostic criteria for *B. ceiba* will therefore ensure the authenticity, consistency, and safety of herbal formulations derived from this plant. Moreover, integration of pharmacognostic data with phytochemical and pharmacological findings strengthens the scientific validation of traditional medicinal plants (K. Das, 2019).

Pharmacognostic Importance of *Bombax ceiba* (L.): *Bombax ceiba (*L.) is pharmacognostically important because multiple plant parts (bark, roots, flowers, gum and seeds) have been long used in Ayurveda and ethnomedicine and therefore require reliable identification, standardisation and quality control (P. Chaudhary & Khadabadi, 2012). Pharmacognostic investigations (macroscopy, microscopy, ash and extractive values, fluorescence analysis and chromatographic fingerprinting) have been reported to establish diagnostic characters, detect adulteration and provide baseline data for monograph development (P. H. Chaudhary et al., 2014). Phytochemical screening consistently shows flavonoids, polyphenols, steroids, glycosides and related constituents, findings that help explain the plant's reported antioxidant, anti-inflammatory, antimicrobial, aphrodisiac and antidiarrheal activities demonstrated in several *in-vitro* and *in-vivo* studies (Diab et al., 2022). Taken together, these pharmacognostic and phytochemical data justify Bombax ceiba's inclusion in traditional tonics and formulations (for instance, Semal/Musli preparations) and support its further development as a standardised herbal ingredient for further therapeutic applications.

1.3 *Dolechandrone falcata* (Wall ex DC)

The *Dolichandrone falcata*, also known by its earlier names such as *Spathodea falcata* and other historical classifications under Bignonia, is a small deciduous tree belonging to the family Bignoniaceae. It is commonly found across various regions of India and is well-documented in local floras. The tree generally grows between 5 and 15 meters tall and bears compound leaves along with large, white, trumpet-shaped flowers that emit a pleasant fragrance. These flowers typically appear during the dry season. The distinctive combination of its vegetative and floral features makes this species easy to identify both in the field and in herbarium specimens (Badgujar Vishal & Surana Sanjay, 2010; Dhaswadikar et al., 2024). This species is native to India and is mainly found in seasonally dry tropical regions. It commonly grows in scrublands, dry deciduous forests, and sometimes along hedgerows or in disturbed habitats across the peninsular states, including Maharashtra, Tamil Nadu, Andhra Pradesh, and Odisha. Its tendency to thrive in dry forest patches and on hill slopes distinguishes it as an important element of the dry-deciduous flora. The tree also plays a role in supporting local biodiversity and contributes to traditional agroforestry systems in these areas (Dhaswadikar et al., 2024).

This plant has been used in traditional Indian medicine for many generations. Ethnobotanical studies and local healing practices describe the use of its bark, leaves, flowers, and fruits to treat a variety of ailments, including anaemia, menorrhagia, diarrhoea, inflammation, and wounds. The plant is also applied externally as a poultice to support bone healing after fractures. It is commonly known by vernacular names such as "Medshingi" or "Medsingi" in different regions of western and southern India. The wide range of traditional applications has

drawn scientific interest, leading to several pharmacological studies aimed at confirming its reported therapeutic properties (Badgujar Vishal & Surana Sanjay, 2010; Suhas Daswadikar, Mandar Muley, 2024).

Pharmacological studies conducted in recent years have yielded promising insights into the bioactivity of *Dolichandrone falcata*. Experimental research, both *in vitro* and *in vivo*, has demonstrated antioxidant, anti-inflammatory, antinociceptive, anxiolytic, and antimicrobial properties. Additionally, several studies have explored the plant's potential in the green synthesis of silver nanoparticles, which have shown notable antimicrobial activity. Recent preclinical work, including investigations into anti-hemorrhoidal effects and nanoparticle-mediated antimicrobial applications in recent years, has further emphasised the plant's relevance to both traditional medicine and modern biotechnology. However, most available pharmacological evidence remains preliminary, highlighting the need for standardised extract preparation, dose–response assessments, and detailed mechanistic evaluations to substantiate its therapeutic potential (Dhaswadikar et al., 2024).

Although interest in *D. falcata* has increased in recent years, several key research gaps remain. Comprehensive phytochemical studies are still needed to isolate and identify the major active compounds and to establish reliable chemical markers for quality control. Toxicological evaluations and safety assessments have been limited, and there is little clinical or translational research to substantiate its traditional medicinal claims (Sahu et al., 2025). In addition, since most of the plant material used locally is collected from the wild, conservation studies are important to ensure sustainable use. Progress in these areas will require collaboration across disciplines such as phytochemistry, pharmacology, clinical science, and conservation biology to support the responsible and evidence-based development of *D. falcata* as a medicinal resource (Kapse et al., 2024; Sabale et al., 2025).

Pharmacognostic Importance of *Dolechandrone falcata* (wall ex DC): *Dolichandrone falcata* (Wall. Ex DC.) Seem., a member of the Bignoniaceae family, is well-known in traditional medicine for its use in managing diabetes, inflammation, wounds, gastrointestinal disorders, and nervous-system conditions. Pharmacognostic investigations are essential for this species to ensure correct identification, prevent adulteration, and support the development of standardised herbal formulations. Key macroscopic traits, including leaf arrangement, flower and fruit morphology, along with microscopic tissue features, enable reliable identification. In addition, physicochemical parameters such as total ash, acid-insoluble ash, extractive values, and fluorescence characteristics provide straightforward laboratory measures to assess purity and authenticity (Badgujar Vishal & Surana Sanjay, 2010; Dhaswadikar et al., 2024; Kapse et al., 2024)

Phytochemical and chromatographic studies have helped elucidate the chemical basis of its therapeutic potential, revealing compounds such as flavonoids, phenolics, alkaloids, steroids, and glycosides. Analytical techniques, including TLC, GC–MS, and HPLC, have been employed to develop chemical fingerprints, and isolated marker compounds like dolichandroside A establish links between chemical composition and biological activity. Experimental research further supports traditional claims, demonstrating antioxidant, anti-inflammatory, antinociceptive, antimicrobial, and antidiabetic effects. Current literature highlights the importance of standardised analytical methods, consistent extraction procedures, and thorough safety and toxicological evaluations to validate *D. falcata* as a reliable and safe herbal medicine (Jang et al., 2008; Sagar Kamble, Vishal Pande, Nagesh Tour, 2024).

1.4 *Syzygium cumini* L.

Syzygium cumini (L.) Skeels, commonly known as jamun, jambolan, or black plum, is an evergreen tree belonging to the Myrtaceae family. It is widely cultivated throughout South and Southeast Asia. The tree bears shiny, opposite leaves, clusters of fragrant white flowers, and edible dark purple fruits that are rich in anthocyanins. In addition to its value as a fruit-bearing species, *S. cumini* is also planted in agroforestry systems for providing shade, improving soil fertility, and preventing erosion (Ayyanar & Subash-Babu, 2012; S. Kumar et al., 2023). Traditionally, nearly all parts of *Syzygium cumini,* including the seeds, fruits, leaves, bark, and flowers, have been used in folk medicine. The seeds and unripe fruits are commonly employed to help manage blood sugar levels, the leaves are applied for treating wounds and digestive problems, while the bark is valued for its antidiarrheal and astringent properties. These long-standing practices have encouraged extensive scientific studies aimed at confirming these traditional uses and identifying the bioactive compounds responsible for the plant's therapeutic effects (Uddin et al., 2022).

Phytochemical studies have revealed that *Syzygium cumini* contains a high concentration of bioactive compounds such as polyphenols (including ellagic and gallic acids), flavonoids, tannins, and anthocyanins. Advanced analytical techniques like GC–MS, LC–MS, and other chromatographic methods have been used

to identify both volatile and non-volatile components, creating detailed chemical profiles that aid in standardisation and in linking chemical composition with biological effects. These constituents are believed to contribute to the plant's antioxidant, anti-inflammatory, and enzyme-regulating properties, which have been demonstrated in several laboratory and animal studies (Qamar, M., Akhtar, S., Ismail, T., Wahid, M., Abbas, M.W., Mubarak, M.S.; Yuan, Y.; Barnard, R.T., Ziora, Z.M., Esatbeyoglu, 2022).

From a pharmacological standpoint, the most well-documented activity of *Syzygium cumini* is its antidiabetic potential. Experimental studies in animals, along with a few human trials, have shown that extracts from the seeds or leaves can help lower blood glucose levels and improve insulin function. In addition to its hypoglycemic effects, preclinical studies have also reported antioxidant, antimicrobial, and potential anticancer activities. Despite these promising results, researchers consistently emphasise the need for standardised extract formulations, detailed dose–response evaluations, long-term safety assessments, and rigorously designed clinical trials to establish *S. cumini* as a scientifically validated therapeutic agent (Raza et al., 2017).

Since *Syzygium cumini* serves as both a food and a medicinal plant, recent studies have highlighted its strong nutraceutical potential and the scope for developing value-added products such as functional foods and standardised herbal extracts (Ayyanar et al., 2013). At the same time, researchers stress the importance of maintaining strict quality control, establishing validated analytical markers, and thoroughly evaluating its safety and effectiveness in humans. In recent years, continued phytochemical investigations and growing clinical interest have kept jamun at the forefront of traditional medicinal plants with genuine prospects for translation into modern therapeutic and nutritional applications (Baliga et al., 2011).

Pharmacognostic Importance of *Syzygium cumini* L.: *Syzygium cumini* (L.) Skeels, commonly known as jamun or jambolan, holds significant pharmacognostic value since almost every part of the plant, the seed, fruit, leaf, bark, and flower, is used in traditional medicine (More et al., 2024). This wide range of uses highlights the need for accurate identification, standardisation, and quality control of both raw materials and commercial preparations. Distinctive macroscopic features such as leaf arrangement, fruit, and seed shape, along with microscopic traits like epidermal cell structure, stomatal pattern, distribution of stone cells in the seed, and fibre arrangement in the leaf and bark, serve as key diagnostic tools for recognising genuine *S. cumini* and detecting adulteration (Chitnis et al., 2012). Standard physicochemical parameters, including total ash, acid-insoluble ash, loss on drying, and solvent extractive values, together with the fluorescence characteristics of the powdered drug, are routinely used in pharmacognostic evaluation. These simple laboratory tests provide effective and low-cost methods for checking the purity, quality, and proper handling of jamun seed and leaf powders or extracts (Bigoniya et al., 2012; Shanthi et al., 2014).

Chemical analysis and chromatographic fingerprinting form a key component of the pharmacognostic evaluation of *Syzygium cumini*. Various studies using HPTLC, GC–MS, and LC–MS have revealed a complex phytochemical profile, including polyphenols, ellagic and gallic acids, flavonoids, tannins, and anthocyanins (Vivek et al., 2021). These compounds provide a biochemical explanation for the plant's reported bioactivities, such as antioxidant, antidiabetic, anti-inflammatory, and antimicrobial effects, and also serve as marker compounds for standardising extracts. For example, seed polyphenols and leaf anthocyanin profiles are commonly used as chemical markers. Research on stability and shelf-life, along with validated HPTLC and HPLC methods for seed and leaf preparations, is increasingly available and plays a crucial role in transforming jamun from a traditional food-medicine into reliable nutraceutical or phytopharmaceutical products (Kaur & Bansal, 2020; Rizvi et al., 2022; Shanthi et al., 2014).

2. Materials and Methods:

2.1 Chemicals and instruments

Regular chemicals, including methanol and ethanol (Sigma-Aldrich), and microbiological culture media-Mueller-Hinton Agar (MHA), bacterial agar and Whatman filter paper number 1 were purchased from Himedia, India. The bacterial and fungal cultures were purchased from NCIM-NCL, India.

2.2 Description of the study area

The research was carried out in the region of Nandurbar district of Maharashtra, India. Geographically, this district falls under the mountainous Satpuda range. Roughly centered around 21.228° N latitude and 74.1422° E longitude in the northwest corner of Maharashtra, India. (Chaudhari et al., 2022) The samples were collected from different parts of the Nandurbar district. Fresh leaves of all the plants were cleaned using distilled water

to remove dust particles and dried in the shade. The dried leaves were powdered using a blender and further used for the preparation of an aqueous extract.

2.3 Sample Preparation:

A total of 4 plant extracts were prepared by the maceration method. One gram of sample powder was suspended in 10 mL of methanol in a 50 mL Erlenmeyer flask. The extraction process was carried out by continuously stirring on a magnetic stirrer for 24 to 48 hours to ensure maximum solute dissolution. The extracted solution was then filtered through Whatman No.1 filter paper to remove solid residues, centrifuged at 8000 rpm for 5 min, and the supernatant was collected. Different concentrations of extract samples were prepared, *viz.* 25%, 50%, and 100%, for the antibacterial activity assay and antifungal activity testing.

2.4 Antibacterial Activity of Plant Extracts:

The antibacterial assay of plant extracts was performed by the agar well diffusion method in Mueller-Hinton Agar (MHA) plates. MHA plates were prepared by adding MHA (38 gm. in 1000 mL) to distilled water and sterilized by autoclaving at 15 psi for 20 min. The media were poured into plates and kept at room temperature in a laminar hood for solidification. The test organisms (*Bacillus subtilis, Bacillus cereus, E. coli, Pseudomonas aeruginosa*) were inoculated in nutrient broth and incubated overnight at 37 °C to adjust the turbidity to 0.5 McFarland standards, giving a final inoculum of 1.5×10^8 CFU/mL. The MHA plates were lawn-cultured with standardized bacterial culture broth. The plates were pre-incubated at 4 °C for 2 h to ensure uniform diffusion of the extracts into the agar, followed by incubation at 37 °C for 24 h. Plant extracts of different concentrations, including 25 %, 50 %, and 100 %, were prepared in methanol. Wells of 6 mm were bored in the inoculated medium using a sterile Cork borer (6 mm). Each well was filled with 50 µl of extracts from different plants. The samples were allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 °C. Antibacterial activity was determined by the diffusion of the antibacterial agents (extracts) in the agar medium, which inhibited the growth of bacterial strains. After incubation, plates were observed for the formation of a clear zone around the well, which corresponds to the antimicrobial activity of the tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Clavam (100 mg/mL), an antibacterial drug, was used as a positive control, and methanol was used as a negative control in this study. All experiments were performed at least in triplicate, and the mean ± standard deviation was presented.

2.5 Antifungal Activity of Plant Extracts:

Antifungal assay of different plant extracts was performed. Potato Dextrose Agar (PDA) medium was prepared and autoclaved at 15 psi for 20 min. The media were poured into plates with different concentrations (25%, 50% and 100%) of plant extract and mixed well. (Media and plant extracts should be mixed properly). Then the plates were kept for solidification in a laminar hood. After the solidification of the plates, with the help of a sterile Cork borer, make a 6 mm diameter well at the centre of each plate. Discs of pre-grown mycelial culture were kept inverted on all PDA agar plates. All the plates were kept for incubation at RT for another 7 days. After incubation, the zone of growth was measured. Fluconazole (100 mg/mL) and methanol were used as positive and negative controls, respectively. The extracts were tested for their antifungal potential against the fungal strains, *viz.: Fusarium solani*, *Fusarium oxysporum* and *Penicillium citrinum*

3. Results and Discussion

3.1 Antibacterial studies of leaf extract of selected medicinal plants

In vitro antibacterial assay with well diffusion method

In vitro antibacterial studies of different plant extracts were performed using the well diffusion method. Both Gram-positive and Gram-negative bacterial species commonly interfering with infections, including burn and wound healing, were tested in the present studies. About four different bacterial strains, *viz. E. coli*, *P. aeruginosa* (Gram-negative bacterial species) and *B. subtilis* and *B. cereus* (Gram-positive bacterial species) were used to test the antibacterial potential of the leaf extract of 8 different medicinal plant species observed in the Nandurbar region of Maharashtra state. *Figure 1* shows representative digital images of the nutrient agar plates, where the test compounds (leaf extracts) were placed in the centre of wells in plates already seeded

with a specific bacterial strain. The negative control, methanol, showed no bacterial growth inhibition (i.e., no zone of inhibition), whereas a clear zone of inhibition was observed. The tested leaf extract of the plant species Ricinus communis did not show any antibacterial activity at the given concentrations. For the leaf extract of *Ricinus cummunis*, we used 25%, 50% and 100% concentrations. The literature shows the antibacterial potential of the aqueous leaf extract of *Ricinus cummunis* in methanol, ethanol and water too. The probable reason for the lack of antibacterial activity is that the concentration was below the required level. (Alvarez-Martinez et al., 2021) *Figure 2* shows the antibacterial potential of *Bombax ceiba* leaf extract against both Gram-positive and Gram-negative bacterial strains. But it didn't show detectable antibacterial activity. The minimum inhibitory concentration required to have antibacterial activity must be higher than the one which was used in the study.

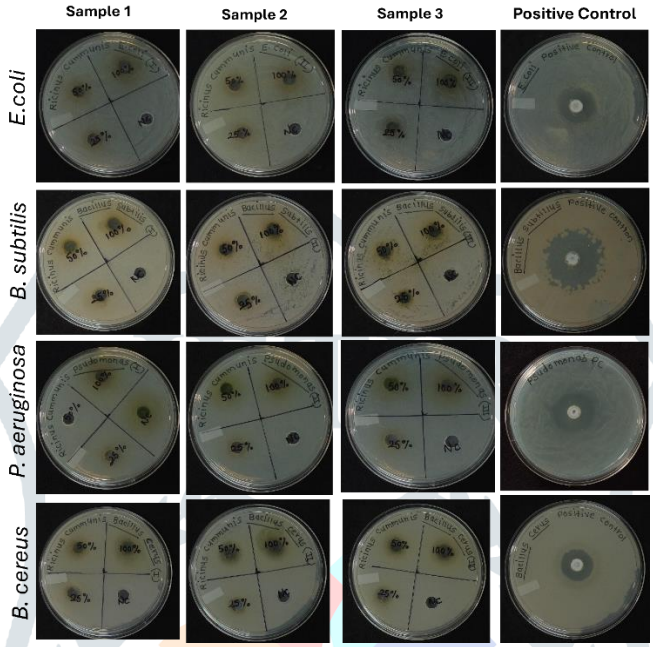

Figure 1: Antibacterial potential of *Ricinus cummunis* leaf extract. The figure shows representative digital images of the agar plates. Samples 1, 2 and 3 are the triplicate experiments. The last column of the figure shows the control sample- Clavam (100 mg/mL), an antibacterial drug.

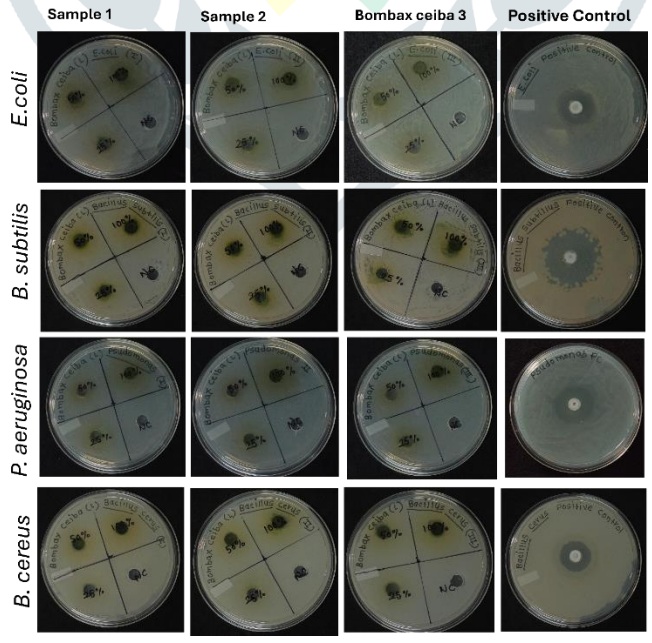

Figure 2: Antibacterial potential of *Bombax ceiba* leaf extract. The figure presents representative digital images of the agar plates showing the antibacterial activity of the *Bombax ceiba* leaf extracts. Samples 1, 2, and 3 represent the three independent experimental replicates, demonstrating the consistency and reproducibility of the observed antibacterial activity. The last column represents the positive control, Clavam (100 mg/mL), an antibacterial drug used as a reference.

Dolechandrone falcata is known for its antimicrobial potential. In the present study, it showed antibacterial activity against Gram-positive bacterial species, *viz. Bacillus subtilis* and *Bacillus cereus,* and did not show any detectable activity against the Gram-negative bacterial strains. *Figure 3* represents representative digital images of agar plates showing antibacterial activity against different bacterial strains. This selective inhibition is due to cell wall composition, which is a barrier for the entry of the extract phytochemical constituents into the bacterial cell. The activity shown by *D. falcata* is concentration-dependent. Antibacterial activity was found at its maximum for the samples having the highest concentration of the leaf extracts, as shown in *Figure 4*(a).

Bhakare et al (Bhakare et al., 2026) have shown that silver (Ag) nanoparticles loaded with *Dolechandrone falcata* extracts have antimicrobial activity against *Escherichia coli, Candida albicans, Aspergillus niger, and Trichoderma viride*. The leaves were sourced from the rocky terrain of Dahiwadi, Maharashtra (India). The phytochemical constituents of *Dolechandrone falcata* extracts are known to act on bacterial double-stranded DNA (dicarboxylic acid) and disrupt it into single-stranded DNA by generation of reactive oxygen species (ROS). Similarly, the *D. falcata* extract loaded with copper oxide nanoparticles showed antibacterial activity against Gram-positive bacterial strains such as *Staphylococcus aureus* and *B. subtilis* and Gram-negative bacterial strains such as *P. aeruginosa* and *K. pneumoniae.(Aysha et al., 2025)*

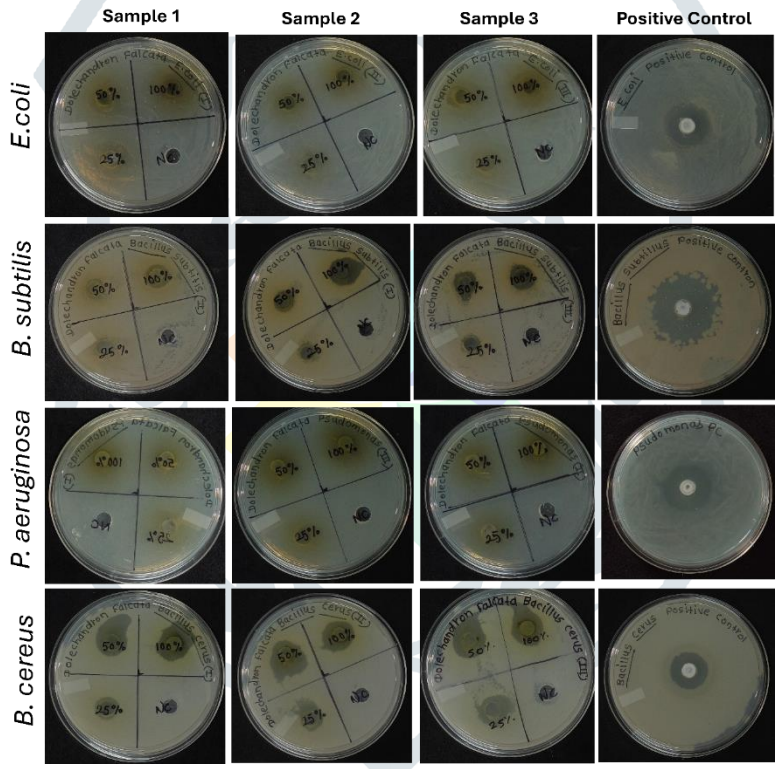

Figure 3: Digital images of antibacterial activity of *Dolechandrone falcata* leaf extract. The figure presents representative digital images of the agar plates showing the antibacterial activity of the *D. falcata* leaf extracts. Samples 1, 2, and 3 represent the three independent experimental replicates, demonstrating the consistency and reproducibility of the observed antibacterial activity. The last column represents the positive control, Clavam (100 mg/mL), an antibacterial drug used as a reference.

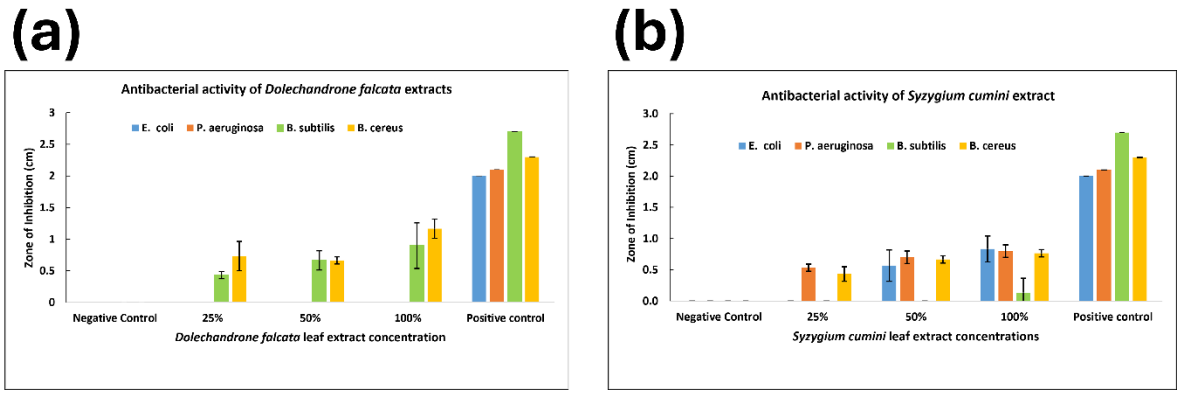

Figure 4: *In-vitro* antibacterial studies of plant extracts (a) *Dolechandrone falcata*, (b) *Syzygium cumini,* against four different bacterial strains, viz. *E. coli, P. aeruginosa* (Gram negative bacterial species) and *B. subtilis and B. cereus* (Gram-positive bacterial species). We have used Clavam (100 mg/ml) and methanol as positive and negative controls, respectively. The average zone of inhibition (ZOI) was measured on the agar plates. The error bars represent the standard deviations obtained from three sets of experiments.

***Syzygium cumini* antibacterial potential:** The results confirmed that *Syzygium cumini* leaf extract exhibited concentration-dependent antibacterial activity against the tested bacterial strains. No zone of inhibition was observed on the negative control plate, whereas clear and visible inhibition zones on positive control plates confirmed the validity of the assay. Among the tested groups, increasing concentrations resulted in progressively greater inhibition of *E. coli*, *P. aeruginosa*, and *B. cereus*, with maximum inhibition observed at 100% extract concentration. *B. subtilis*, however, exhibited comparatively weak susceptibility, showing only a small inhibition zone at the highest concentration. In a nutshell, *E. coli*, *P. aeruginosa*, and *B. cereus* appeared to be more susceptible to the *S. cumini* leaf extract than *B. subtilis*.

The *S. cumini* leaf extract is known to contain various flavonoids, terpenoids and phenolics such as sitosterol, betulinic acid, crategolic acid, quercetin, myricetin, methyl gallate, kaempferol, etc.(Srivastava & Chandra, 2013) The presence of these constituents in the extracts shows antibacterial and antifungal activity on a measurable scale. Not only does the leaf extract of S. *cumini* show this property, but also other plant parts such as seed and bark show similar properties. Recently, eco-friendly fabrication of ZnO nanoparticles from *S. cumini* seeds has been demonstrated for its potential anticancer and antimicrobial properties.(Lathika et al., 2026) Santos et al. (Santos et al., 2020) have demonstrated that the phenolic extracts were inhibitory to most common bacterial species, including *P. fluorescens, L. monocytogenes* and *S. aureus*. *Figure 5* shows the digital image of the agar plates for the antibacterial zone of inhibition and *Figure 4(b)* shows the quantitative measurements of the antibacterial potential of the *S. cumini* leaf extracts. The graph shows the minimal activity against *B. subtilis* and moderate activity against other strains used in the study. Yadav et al. (Yadav et al., 2017)confirmed that methanolic seed extracts of *S. cumini* could potentially be used to inhibit foodborne infection caused by *B. subtilis* and claimed as an alternative to prevalent antibiotics.

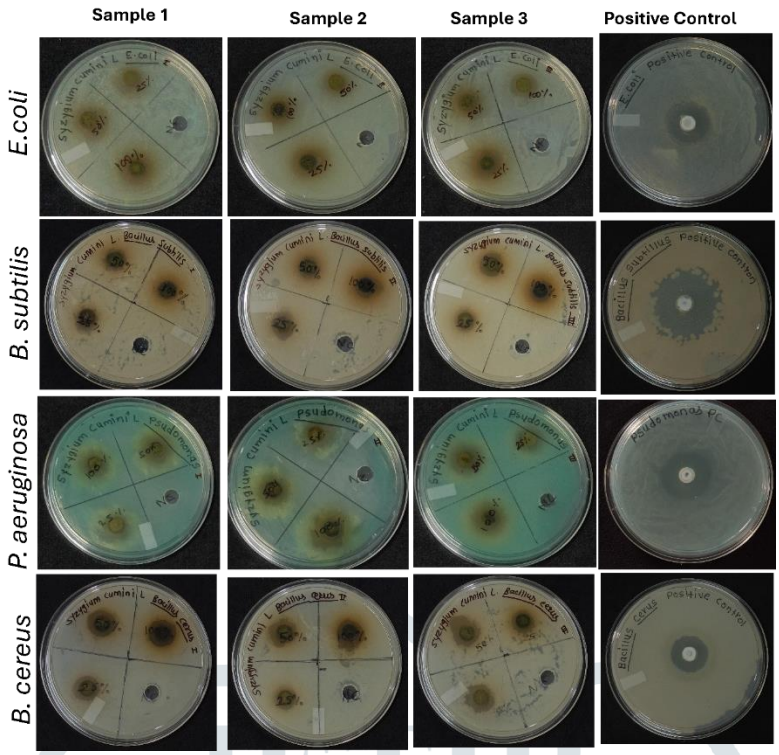

Figure 5: Antibacterial activity of *Syzygium cumini*. The figure presents representative digital images of the agar plates showing the antibacterial activity of the *S. cumini* leaf extracts. Samples 1, 2, and 3 represent the three independent experimental replicates, demonstrating the consistency and reproducibility of the observed antibacterial activity. The last column represents the positive control, Clavam (100 mg/mL), an antibacterial drug used as a reference.

3.2 Antifungal activities of selected medicinal plant extracts

3.2.1 Antifungal potential of *Ricinus cummunis* leaf extract:

Figure 6(a) and (b) demonstrate a clear concentration-dependent antifungal effect of *R. communis* leaf extract against the three tested fungal pathogens, *viz. Fusarium solani*, *Fusarium oxysporum* and *Penicillium citrinum*. In the negative control (methanol), the fungi showed maximum mycelial growth, with values of approximately 6.2–6.4 cm, confirming active fungal development in the absence of the plant extract. With increasing concentrations of *R. communis* leaf extract from 25% to 100%, a progressive reduction in the zone of mycelial growth was observed for all three fungal pathogens. The photographic observations are consistent with the graph (Figure 6 (b), showing considerably smaller fungal colonies at higher extract concentrations.

Among the tested fungi, *F. oxysporum* appeared to be the most susceptible to the *R. communis* extract. Its mycelial growth decreased from approximately 6.3 cm in the negative control to 2.7 cm at the highest extract concentrations. *F. solani* showed a similar but comparatively lower response, with growth decreasing from approximately 6.2 cm to 3.1 cm, while *P. citrinum* decreased from approximately 6.4 cm to 3.1 cm across the same concentrations. Thus, the higher concentrations of the leaf extracts indicated that the antifungal effect increased with extract concentration. The positive control showed almost no fungal growth, indicating strong antifungal activity of the reference treatment and providing a useful benchmark for comparison.

A. niger growth inhibition on PDB media containing various concentrations of castor leaves methanolic extract following seven days of incubation has been shown by Carolina et al. (Carolina, 2018).Parimala et al(S. Parimala and D. Sangeetha, 2016) have studied the antifungal activity of methanolic, ethanolic and acetone extracts of *Ricinus communis* against almost 11 different fungal pathogens. Similar studies have been conducted by Rashmi et al (Rashmi et al., 2019)and discussed the effect of *R. communis* leaf extract on microorganisms with their advantages and disadvantages. The inhibitory activity of *R. communis* may be associated with its diverse phytochemical constituents, particularly phenolics, flavonoids, tannins, alkaloids and other secondary metabolites, which can interfere with fungal cell membranes, cellular metabolism and enzyme activities. (Ramothloa et al., 2025b)The stronger response of *F. oxysporum* suggests that its cellular or membrane characteristics may make it comparatively more susceptible to these bioactive compounds. Overall, the results indicate that *R. communis* leaf extract possesses promising concentration-dependent

antifungal activity, with the highest concentration showing the most pronounced suppression of all three fungal pathogens.

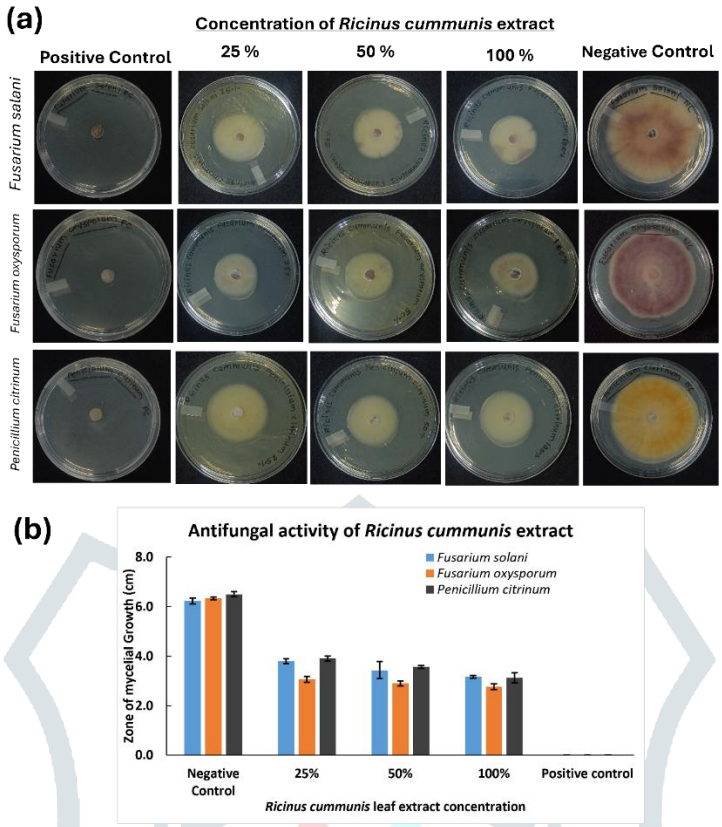

Figure 6: Antifungal potential of *Ricinus cummunis* leaf extract at various concentrations (a) qualitatively by measuring the zone of mycelial growth on PDA plates and (b) the diameter of mycelial growth (cm) plotted against the concentrations of *R. cummunis* leaf extract with Fluconazole (100 mg/mL) as positive control and methanol as negative control. The inhibition efficiency of *R. cummunis* against fungal pathogens *viz. Fusarium solani*, *Fusarium oxysporum* and *Penicillium citrinum* was tested.

3.2.2 Antifungal potential of *Bombax ceiba* leaf extract:

Figure 7 shows that *Bombax ceiba* leaf extract produced a moderate inhibitory effect on the mycelial growth of *Fusarium solani*, *Fusarium oxysporum* and *Penicillium citrinum*. In the negative control, all three fungi showed extensive growth, with mycelial growth ranging from approximately 6.2 to 6.5 cm. At 25%, the lowest extract concentration used, growth decreased to about 5.4 cm for *F. solani*, 5.2 cm for *F. oxysporum* and 5.2 cm for *P. citrinum*. At 50% concentration, the corresponding values were approximately 5.5, 5.1 and 5.1 cm, while at 100% concentration they declined slightly further to 5.4 to 5.0 cm, respectively. The reduction was also visually evident in the Petri plates, where fungal colonies appeared somewhat smaller in the extract-treated plates than in the negative controls, as can be observed in Figure 7 (a) and (b).

Overall, *P. citrinum* and *F. oxysporum* appeared to be slightly more sensitive to *B. ceiba* leaf extract than *F. solani*, particularly at the higher concentrations. However, the differences between 25%, 50% and 100% concentrations were relatively small, suggesting that the extract exhibited only a modest concentration-dependent inhibition under the conditions tested. The positive control showed almost complete suppression of fungal growth, indicating substantially stronger antifungal activity than the *B. ceiba* extract. Therefore, although *B. ceiba* leaf extracts demonstrate some antifungal potential, the relatively limited reduction in mycelial growth suggests that a higher extract potency, a different extraction solvent, or isolation of specific bioactive compounds may be required to achieve stronger inhibition. (G. Das et al., 2021)

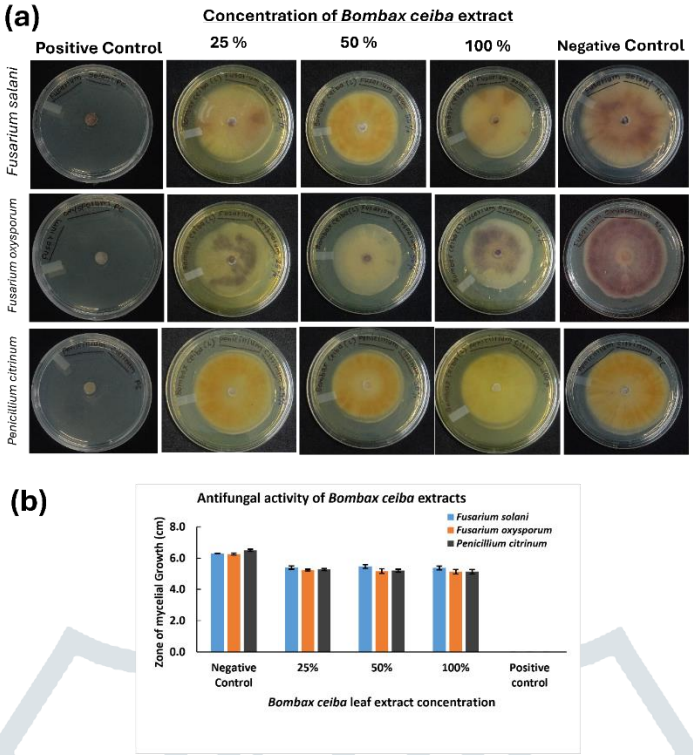

Figure 7: Antifungal potential of *Bombax ceiba* leaf extract at various concentrations (a) qualitatively by measuring the zone of mycelial growth on PDA plates and (b) the diameter of mycelial growth (cm) plotted against the concentrations of Bombax ceiba leaf extract with Fluconazole (100 mg/mL) as positive control and methanol as negative control.

3.2.3 Antifungal potential of *Dolechandrone falcata* leaf extract:

The antifungal activity of *Dolechandrone falcata* leaf extract showed a clear inhibitory effect against all three tested fungi: *Fusarium solani*, *Fusarium oxysporum*, and *Penicillium citrinum*. In the negative control, mycelial growth was approximately 6.3–6.5 cm, whereas treatment with the leaf extract markedly reduced fungal growth at all tested concentrations. At 25%, 50%, and 100% concentrations, the mycelial growth of *F. solani* decreased to approximately 3.8, 3.4, and 3.2 cm, respectively. Similarly, *F. oxysporum* showed a stronger response, with growth decreasing from approximately 2.8 cm at 25% to 2.0 cm at 100%. *P. citrinum* exhibited comparatively lower sensitivity, with mycelial growth decreasing from about 3.9 cm at 25% to 3.5 cm at 100%, as can be clearly observed in representative digital images of PDA plates (Figure 8 (a) and the mycelial growth against the extract concentrations Figure 8 (b). Overall, the results indicate a concentration-dependent antifungal effect, with the 100% extract producing the greatest inhibition, particularly against *F. oxysporum*. Similar concentration-dependent antifungal effects of plant extracts against *Fusarium* species have been reported, with phenolic- and flavonoid-rich extracts showing substantial suppression of fungal mycelial growth. (Aboody & Mickymaray, 2020)

The differential susceptibility observed among the fungi may be associated with differences in their cell-wall composition, membrane properties, detoxification mechanisms, and susceptibility to plant-derived secondary metabolites. (Davidova et al., 2024; Seepe et al., 2021)The stronger inhibition of *F. oxysporum* suggests that constituents present in *D. falcata* leaves may interfere with fungal growth and development more effectively in this species. Plant-derived phenolics, flavonoids, tannins, terpenoids, and related compounds are known to exhibit antifungal activity through multiple mechanisms, including disruption of the fungal plasma membrane, alteration of cell-wall integrity, inhibition of essential enzymes, induction of oxidative stress, and interference with mitochondrial function.(Davidova et al., 2024) Therefore, the antifungal activity observed in the present study may be attributed to the combined action of several phytochemicals present in the *D. falcata* leaf extract rather than a single active constituent.

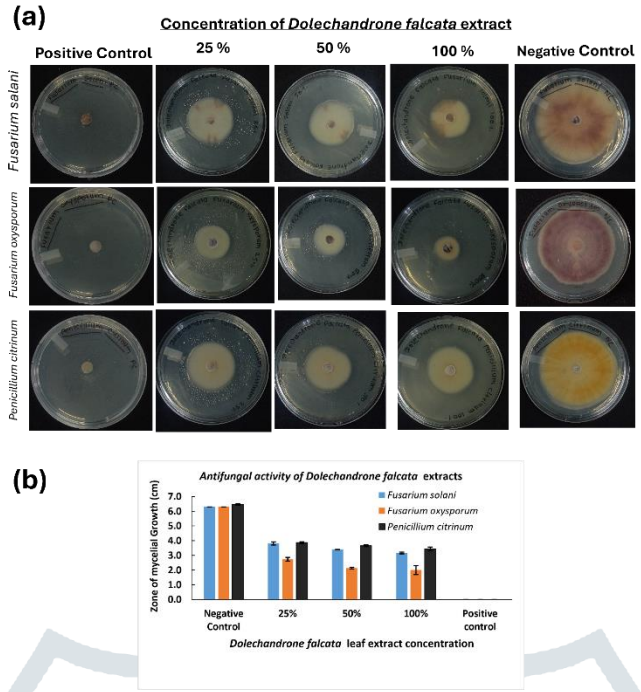

Figure 8: Antifungal potential of *Dolechandrone falcata* leaf extract at various concentrations (a) qualitatively by measuring the zone of mycelial growth on PDA plates and (b) the diameter of mycelial growth (cm) plotted against the concentrations of Dolechandrone falcata leaf extract with Fluconazole (100 mg/mL) as positive control and methanol as negative control.

3.2.4 Antifungal potential of *Syzygium cumini* leaf extract:

In the present study, results demonstrate a concentration-dependent inhibitory effect of *S. cumini* leaf extract on the mycelial growth of all three tested fungi. In the negative control, mycelial growth was approximately 6.2–6.4 cm, whereas increasing the extract concentration from 25% to 100% progressively reduced fungal growth. At 100% concentration, growth decreased to approximately 4.4 cm for *F. solani*, 3.7 cm for *F. oxysporum*, and 4.0 cm for *P. citrinum*. Based on the control values, this corresponds approximately to 29%, 40%, and 38% inhibition, respectively. Thus, *F. oxysporum* appeared to be the most susceptible organism, followed by *P. citrinum*, while *F. solani* showed comparatively greater tolerance to the extract.

A clear difference in susceptibility was also observed among the fungi at intermediate concentrations. At 50% extract concentration, *F. oxysporum* showed a marked reduction in growth (~3.8 cm), while *F. solani* remained around 5.1 cm. At 100%, however, inhibition increased further for all three organisms, indicating that the antifungal effect becomes more pronounced at higher extract concentrations. The photographic observations in Figure 9 (a) support the quantitative data in Figure 9(b), showing visibly restricted colony development at increasing extract concentrations. The absence of appreciable growth in the positive control further confirms the effectiveness of the reference antifungal treatment and provides a clear benchmark against which the plant extract can be compared.

The observed antifungal activity is consistent with previous reports on *S. cumini*. Leaf extracts of *S. cumini* have demonstrated significant antifungal activity against *Aspergillus niger*, while phytochemical investigations have reported the presence of phenolics, flavonoids, tannins, alkaloids, saponins and other bioactive metabolites that may contribute to fungal growth inhibition.(Figueiredo Junior et al., 2021) Studies using *S. cumini* extracts against *F. oxysporum* have also reported substantial suppression of mycelial growth, particularly with organic extracts, supporting the susceptibility of *Fusarium* species observed in the present experiment. In addition, leaf extracts have been shown to possess direct antifungal activity against *Candida* spp., suggesting that the antifungal potential of *S. cumini* leaves is not restricted to a single fungal group. Overall, the present findings suggest that *S. cumini* leaf extract possesses promising concentration-dependent antifungal activity, with *F. oxysporum* exhibiting the greatest sensitivity among the tested fungi.(Dhanasekaran et al., 2016; Samapti et al., 2022)

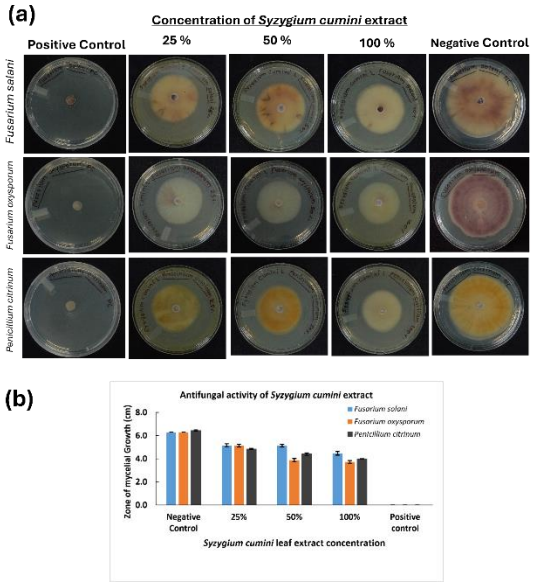

Figure 9: Antifungal potential of *Syzygium cumini* leaf extract at various concentrations (a) qualitatively by measuring the zone of mycelial growth on PDA plates and (b) the diameter of mycelial growth(cm) plotted against the concentrations of *S. cumini* leaf extract with Fluconazole (100 mg/mL) as positive control and methanol as negative control.

4. Conclusion:

The selected plant species from the Nandurbar district of Maharashtra have shown good antibacterial and excellent antifungal activity. The leaf extracts of four selected plant species have shown activity against bacterial strains including *Bacillus subtilis, Bacillus cereus, E. coli, and Pseudomonas aeruginosa.* The extracts also showed excellent antifungal activity against three different fungal species, *viz. Fusarium solani*, *Fusarium oxysporum* and *Penicillium citrinum.* This potential activity of the extract may serve as a potential source in pharmacology for the development of antibacterial and antifungal agents. A detailed biochemical analysis of these plant extracts is required to take it further for its pharmacological applications.

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