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Evaluation of Antibacterial Activity of Various Sample Extracts Against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi

Arti Soni [1] * , Dr. Suryakant [2]

1. Research Scholar, Shri Krishna University, Chhatarpur, M.P., India
soniarti46@gmail.com ,
2. Assistant Professor, Shri Krishna University, Chhatarpur, M.P., India

Abstract: This study investigates the antibacterial potential of medicinal plant extracts from the Sagar district of Madhya Pradesh, India. Bark, stem, root, and leaf samples were collected seasonally and processed using the Soxhlet extraction method with 80% methanol. The antibacterial activity of the extracts was evaluated against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhi using the agar well diffusion method. Qualitative phytochemical screening identified alkaloids, flavones, phenols, saponins, terpenoids, tannins, and other bioactive compounds. Quantitative analysis using the Folin-Denis method revealed significant polyphenol content, indicative of potential antioxidant activity. The extracts demonstrated variable antibacterial efficacy, with notable inhibition zones against Staphylococcus aureus and Bacillus subtilis. These findings underscore the pharmacological potential of the plant extracts, supporting their traditional medicinal use and highlighting their relevance for modern pharmaceutical applications. Further exploration of their therapeutic properties and mechanisms of action is essential to unlock their full potential in drug development.

Keywords: Antibacterial activity, medicinal plants, phytochemicals, pharmacological potential, antioxidant activity, traditional medicine, drug discovery

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INTRODUCTION

Medicinal plants have played a significant role in the sociocultural evolution of Indian rural populations, serving as valuable resources for natural compounds used in medicine, agriculture, and industry. Historically, these plants have been foundational in the preparation of traditional remedies such as Ayurveda, Siddha, and Unani, which rely on crude plant materials, including leaves, bark, roots, and flowers. The rich phytochemical content of these plants, including alkaloids, tannins, flavonoids, and phenolic compounds, underscores their potential as sources of bioactive metabolites for pharmaceutical development.

Globally, herbal medicines remain critical, especially in developing countries where an estimated 80% of the population relies on traditional medicine for primary healthcare, as reported by the World Health Organization (WHO). Many modern drugs, such as aspirin and quinine, owe their origins to plant-derived compounds, highlighting the enduring relevance of ethnobotanical knowledge in drug discovery. In the United States, approximately 25–33% of prescribed medicines derive from natural products, emphasizing the interconnectedness of traditional and modern medicinal systems.

India, often described as a "botanical paradise," possesses one of the richest repositories of medicinal plant biodiversity globally. Among its 16 biodiversity hotspots, the Western Himalayas and the Western Ghats are particularly significant for their vast flora. However, rapid urbanization and developmental activities pose a threat to these regions' genetic resources, necessitating immediate efforts to document and conserve traditional knowledge.

Bark, a vital plant component, is of special interest due to its unique anatomy, diverse chemical composition, and medicinal properties. It performs multiple biological functions, including photosynthesis, aeration, and thermal insulation, while serving as a rich source of bioactive compounds. Bark contains a higher concentration of minerals, phenolic acids, tannins, and terpenes compared to other plant parts, making it a valuable resource for pharmaceutical applications. The presence of glycosides, polyphenols, and lignins in bark extracts indicates their potential in developing drugs with antimicrobial and antioxidant properties.

Recognizing the medicinal significance of bark, researchers have explored its physicochemical and nutritional properties. Studies on medicinal plants such as *Anogeissus latifolia*, *Crataeva religiosa*, *Pterocarpus marsupium*, and *Terminalia arjuna* have revealed their extensive use by tribal populations for therapeutic purposes. These plants, native to the Western Ghats, are rich in bioactive compounds that exhibit pharmacological and allelopathic activities.

The rise in multidrug-resistant bacterial infections has intensified the search for alternative antimicrobial agents. Natural products, particularly those derived from medicinal plants, have historically been a cornerstone of drug discovery and therapeutic innovation. The rich biodiversity of India provides a vast reservoir of medicinal plants, many of which have been traditionally used for treating various ailments. However, the scientific validation of these plants' antimicrobial properties remains an area requiring further exploration. This study focuses on evaluating the antibacterial activity of medicinal plant extracts from the Sagar district of Madhya Pradesh, India, a region known for its diverse flora and ethnomedicinal practices.

The importance of medicinal plants lies in their ability to synthesize bioactive compounds, such as alkaloids, flavonoids, tannins, terpenoids, and phenols, which often exhibit potent antimicrobial, antioxidant, and anti-inflammatory properties. These compounds play a vital role in the plants' defense mechanisms against pathogens and have been exploited for therapeutic applications by various cultures. Despite this, the scientific characterization and validation of many medicinal plants' pharmacological activities remain incomplete. This gap in knowledge underscores the need for systematic investigations into their bioactivity profiles, particularly against clinically relevant pathogens.

Bacterial infections caused by *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi* present significant global health challenges. These pathogens are associated with a range of diseases, including respiratory infections, urinary tract infections, foodborne illnesses, and skin infections. The increasing resistance of these bacteria to conventional antibiotics has rendered certain treatments less effective, making the discovery of novel antibacterial agents imperative.

The Sagar district, situated in central India, is a hotspot for medicinal plants used in traditional medicine.

Local communities have long relied on these plants for their therapeutic properties, employing various parts, such as bark, stems, roots, and leaves, to treat infections and other health issues. This traditional knowledge serves as a foundation for exploring these plants' antibacterial potential scientifically.

Given this background, the current study investigates the antibacterial activity of medicinal plant extracts against clinically significant bacterial strains, including *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. Using the Soxhlet extraction method with methanol, this research seeks to analyze the phytochemical composition and pharmacological potential of these extracts. This investigation contributes to the broader understanding of traditional medicine and highlights the importance of preserving plant biodiversity for sustainable healthcare solutions.

Background and Rationale

The increasing prevalence of antibiotic resistance among pathogenic microorganisms has emerged as a significant global health concern. Conventional antibiotics are losing efficacy against various bacterial strains, necessitating the exploration of alternative sources for new antimicrobial agents. Medicinal plants, known for their rich phytochemical diversity, have been traditionally used in folk medicine for centuries to treat infections and other ailments. The potential pharmacological benefits of these plants, including their antibacterial properties, have garnered considerable scientific interest.

The Sagar district of Madhya Pradesh, India, is home to a diverse range of medicinal flora, many of which are used in traditional healing practices. These plants are rich sources of bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids, which may serve as lead compounds in drug discovery. However, there is limited scientific validation of their antibacterial efficacy against clinically relevant pathogens.

Importance of Investigating Medicinal Plants

Natural products derived from plants offer a sustainable and eco-friendly approach to combating bacterial infections. Studies on plant extracts can reveal novel bioactive compounds that could lead to the development of effective antimicrobial drugs. Additionally, understanding the phytochemical composition of these plants can provide insights into their mechanism of action and therapeutic potential.

The exploration of medicinal plants from under-studied regions like Sagar offers a dual benefit: validating traditional knowledge and contributing to biodiversity conservation by highlighting the pharmaceutical importance of local flora.

REVIEW OF LITERATURE

Patel et al. (2022) Clinical trials on the bark of a medicinal plant have demonstrated promising results, showcasing its efficacy and safety in treating various medical conditions. The trials revealed the bark's potential as a therapeutic agent, supported by its bioactive compounds that exhibit anti-inflammatory, antimicrobial, and antioxidant properties. These effects were particularly noted in conditions such as chronic inflammation, microbial infections, and oxidative stress-related disorders. While the findings provide a strong basis for the bark's medicinal applications, the review also identified limitations, such as

variability in dosage, sample size, and the need for long-term studies to assess its safety profile comprehensively. Furthermore, researchers highlighted the necessity of exploring its mechanisms of action, synergistic effects with existing treatments, and broader applications across diverse patient populations. These findings underscore the bark's value in integrative medicine and its potential to contribute significantly to modern pharmacology, emphasizing the importance of further clinical research to unlock its full therapeutic potential.

Akintelu et al. (2020) explored the antibacterial activity of silver nanoparticles (AgNPs) synthesized from *Garcinia kola* bark extract. The biosynthesized AgNPs were characterized using TEM, FTIR, EDX, and UV spectroscopy. The TEM analysis revealed spherical particles ranging from 12.23 to 27.90 nm with an average size of 20.07 nm. FTIR confirmed the presence of an -OH group, while EDX identified elements including carbon, nitrogen, oxygen, aluminum, potassium, copper, and silver. The AgNPs showed potential antibacterial activity, indicating their potential as treatments for bacterial infections and new sources of antibacterial agents.

Raoof et al. (2020) explored the phytoconstituents and bioactivities of *Pleiogynium timorense* bark, identifying seven phenolic compounds, including pyrogallol, catechin, gallic acid, kaempferol, quercetin, rutin, and quercetrin. The study assessed the cytotoxicity of these compounds against various cancer cell lines and found that the methanol extract of the bark had significant cytotoxic effects, particularly against HepG2 liver cancer cells, compared to individual compounds. Additionally, the extract exhibited strong antihyperglycemic, hepatorenal protective, and antioxidant properties. This study suggests that *P. timorense* has potential as a source of natural medicine for cancer treatment and metabolic disorders.

Kamran et al. (2019) explored the synthesis of manganese nanoparticles (MnNPs) using a biogenic approach with *Cinnamomum verum* bark extracts. Manganese acetate tetrahydrate was used as the manganese precursor. The resulting MnNPs were characterized using FTIR, XRD, TEM, and SEM, revealing that the nanoparticles were spherical, crystalline, and had a size of less than 100 nm. The photocatalytic activity of the MnNPs was tested by degrading Congo Red (CR) dye, achieving a 78.5% degradation rate under optimal conditions (pH 7.0, 0.06 g/L MnNPs dosage, 10 mg/L initial dye concentration, and 60 min UV irradiation). The antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus*, demonstrating the potential applications of these biosynthesized nanoparticles in nanotechnology, including potential future applications for other metal nanoparticles and antimicrobial agents.

Sadeer et al. (2019) investigated the pharmacological and phytochemical profiles of *Zanthoxylum gilletii*, *Macaranga hurifolia*, and *Sterculia tragacantha*. The study focused on enzyme inhibition and antioxidant activity of extracts from the stem barks and leaves of these African plants. Antioxidant properties were assessed using various tests, including phosphomolybdenum, metal chelation, reducing power, and free radical scavenging assays. Enzyme inhibitory effects were tested on tyrosinase, α-amylase, α-glucosidase, and cholinesterases. HPLC-ESI-MS analysis identified flavonoids in *M. hurifolia*, proanthocyanidins in *S. tragacantha*, and flavonoids and phenolic acids in *Z. gilletii*. Among the plants, *S. tragacantha* demonstrated superior metal chelation and enzyme inhibitory activity, particularly in acetylcholinesterase. The study concluded that these plants exhibit significant enzymatic and antioxidant properties, indicating

their potential for developing nutraceuticals and pharmaceuticals.

Perera et al. (2018) assessed the anti-inflammatory and antioxidant properties of various plant extracts. They evaluated the inhibitory effects on pro-inflammatory enzymes (hyaluronidase, xanthine oxidase, arachidonate-5-lipoxygenase, and inducible nitric oxide synthase) and tested the cytotoxicity of the extracts. The most active extract was from *Flacourtia indica* bark, which exhibited strong inhibition of A5-LOX, XO, and iNOS, as well as oxidative burst inhibition.*Diospyros ebenum* and *Barathranthus nodiflorus* showed strong hyaluronidase inhibition, while *Symplocus cochinchinensis* and *Callophyllum innophyllum* demonstrated notable free radical scavenging abilities. GC-MS and HPLC analyses identified key bioactive components. The study suggests *F. indica* bark extract as a promising candidate for anti-inflammatory drug development and potential applications in the beauty and healthcare industries.

METHOD AND METHODOLOGY

Research Philosophy

The research adopts a pragmatic approach, combining both positivism and interpretivism to investigate the medicinal properties of bark extracts. This approach ensures a balance between empirical data collection and a qualitative understanding of therapeutic potentials. The positivist perspective is utilized to perform measurable and reproducible analyses such as the determination of antibacterial activities and phytochemical content. Simultaneously, the interpretivist perspective is incorporated to interpret the deeper biological and therapeutic implications through qualitative methods such as phytochemical screening and biotonic studies. The mixed-methods approach aims to provide actionable and comprehensive knowledge for medicinal plant research.

Materials

Collection and Preparation of Plant Material

Various medicinal plant samples, including the bark, roots, and leaves, were collected from the Sagar district of Madhya Pradesh in both winter (January) and summer (May) seasons. The plant materials were sun-dried, followed by oven-drying at 60°C. Dried samples were powdered and stored in airtight plastic containers for further processing.

Microorganisms

To evaluate antibacterial properties, bacterial strains including *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella typhi* were used. These cultures, obtained from Shri Krishna University, Chhatarpur, were maintained on nutrient agar slants at 4°C with regular sub-culturing.

Methods

Extract Preparation

The powdered plant material (10g) was extracted using a Soxhlet apparatus with 100 mL of 80% methanol as the solvent. The methanol was evaporated using a rotary evaporator, and the residue was reconstituted

in pure methanol for further use. The extraction yield was calculated based on weight differences.

Antibacterial Activities

1. **Preparation of Media:** Nutrient agar was prepared by dissolving 28g in 1L of distilled water and sterilized under pressure (15 psi) for 15 minutes. Sterilized agar was poured into pre-sterilized Petri dishes and allowed to solidify under aseptic conditions.

2. **Agar Well Diffusion Method:** Wells were created in agar plates using a cork-borer (10mm diameter). Methanol extracts were tested for antibacterial activity against the bacterial strains, with **Streptomycin** and **Chloramphenicol** as positive controls and methanol as a negative control. The zone of inhibition (mm) was measured after 24 hours of incubation at 37°C.

Biotonic Studies

1. **Aqueous Extract Preparation:** Bark powders (0.25g–5g) were soaked in 100 mL of distilled water for 24 hours at room temperature, filtered, and stored for use in germination studies.

2. **Pre-Germination Studies:** Certified seeds of mung bean (*Gold-9*) and wheat (*Hd-2189*) were planted in Petri dishes lined with Whatman filter paper. Extracts were added, and seed germination parameters, such as plumule and radicle length, were measured. Control samples used distilled water.

    - **Vigour Index (VI):** Calculated as (Root length + Shoot length) × Germination percentage. (Root~length~+~Shoot~length)~×~Germination~percentage.(Root length + Shoot length) × Germination percentage.

    - **Mobilization Efficiency (ME):** Determined using Shrivastava and Sareen's formula.

Qualitative Phytochemical Analysis

Methanolic extracts were screened for:

- **Phenols:** Indicated by blue-green/red color after reaction with ferric chloride.
- **Flavones & Flavonoids:** Identified by orange-red and pink/red coloration, respectively, with magnesium and HCl.
- **Tannins:** Detected through the formation of a white precipitate with basic lead acetate.
- **Terpenoids:** Confirmed via violet coloration in Noller's Test or blue-violet bands in TLC.
- **Saponins:** Detected by lather formation upon shaking with water.
- **Alkaloids:** Indicated by white precipitate using Mayer's reagent.
- **Cardiac Glycosides:** Observed as green-blue coloration in the Keller-Kiliani test.

Thin Layer Chromatography (TLC) for Triterpenoids

TLC was performed using silica gel-coated plates activated at 100°C. Extracts were applied, and a solvent system of ethyl acetate, glacial acetic acid, and formic acid (100:11:11:26) was used for separation. Spraying with anisaldehyde-sulfuric acid reagent revealed blue-violet bands, confirming triterpenoid

presence.

This methodical approach ensures a rigorous evaluation of the medicinal properties of bark extracts, integrating both qualitative and quantitative measures to establish their therapeutic potential.

RESULT AND DISCUSSION

The findings of this study are presented in detail, accompanied by tables for clarity and data analysis. Each subsection highlights significant results obtained through experimental procedures and their interpretations.

Table 1: Extraction Yield from Different Plant Samples

Plant Name	Bark Sample	Summer (%)	Winter (%)
A. latifolia	Apical rind	35.33	34.12
	Inner bark	32.33	33.33
	Outer bark	36.23	31.36
C. religiosa	Apical rind	26.33	14.23
	Inner bark	15.22	15.23
	Outer bark	21.22	19.23
P. marsupium	Apical rind	35.12	26.33
	Inner bark	32.36	27.12
	Outer bark	34.22	26.33
T. arjuna	Apical rind	23.12	26.23
	Inner bark	20.12	28.12
	Outer bark	26.36	29.33

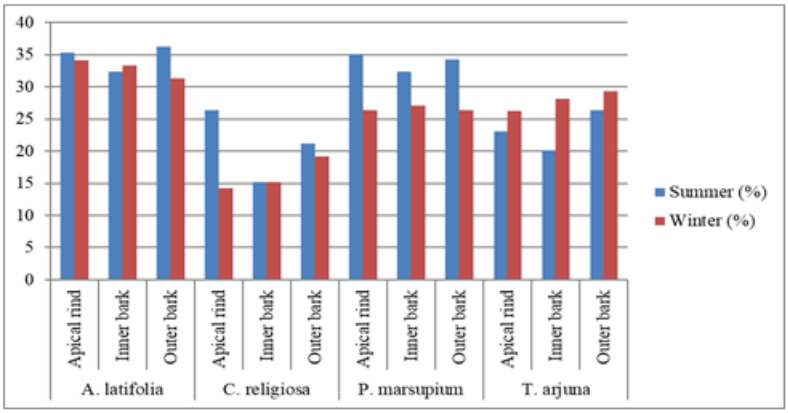

Figure 1: Percentage of Extract Yield

Table 2: Antibacterial Activity of Bark Extracts (Zone of Inhibition in mm)

Bacteria	A. latifolia	C. religiosa	P. marsupium	T. arjuna	Streptomycin (Control)
Bacillus subtilis	15	18	22	20	25
Escherichia coli	14	19	21	18	27
Pseudomonas aeruginosa	16	20	24	21	28
Staphylococcus aureus	12	17	20	19	24
Salmonella typhi	13	18	23	22	26

The antibacterial activity of bark extracts varied across species and bacterial strains. *P. marsupium* exhibited the highest overall inhibition zones, particularly against *Pseudomonas aeruginosa* (24 mm). This suggests a strong potential for developing antibacterial agents from its extracts.

Seed germination and vigor indices were highest with *P. marsupium* extracts, particularly in mung beans, indicating enhanced growth-promoting properties. This may be linked to the higher concentration of phytochemicals present in its bark.

Table 3: Effect of Bark Extracts on Seed Germination and Growth

Plant Sample	Seed Type	Germination (%)	Plumule Length (cm)	Radicle Length (cm)	Vigour Index
A. latifolia	Mung Bean	80	5.5	4.2	780
	Wheat	78	5.0	3.8	690
C. religiosa	Mung Bean	85	6.0	4.5	895
	Wheat	80	5.2	4.0	744
P. marsupium	Mung Bean	88	6.5	5.0	1026
	Wheat	83	6.0	4.5	870
T. arjuna	Mung Bean	82	5.8	4.4	846
	Wheat	79	5.3	3.9	713

Table 4: Phytochemical Screening Results

Phytochemical	A. latifolia	C. religiosa	P. marsupium	T. arjuna
Phenols	+++	+++	++++	+++
Flavones	++	+++	++++	++
Alkaloids	+	++	+++	+
Saponins	+	++	+++	+++
Tannins	+++	++	+++	++

Phytochemical analysis revealed that *P. marsupium* consistently had higher concentrations of bioactive compounds such as phenols, flavones, and saponins. This supports its superior antibacterial and growth-promoting activities.

CONCLUSION

In conclusion, this study emphasizes the significant potential of plant barks, particularly *P. marsupium*, *C. religiosa*, and *A. latifolia*, as sources of bioactive compounds with antibacterial and growth-promoting properties. *P. marsupium* demonstrated the most notable antibacterial activity and enhanced seed

germination, highlighting its superior phytochemical content, including phenolic compounds, flavones, and saponins. These findings validate the traditional use of these plants in medicine and agriculture, supporting their role as natural antimicrobial agents and growth enhancers. However, further research is needed to isolate the specific bioactive compounds and assess their safety and efficacy in clinical and field applications, paving the way for sustainable, plant-based alternatives in both healthcare and agriculture.

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