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In Vitro Antibacterial Efficacy of Aqueous Extract of Stachytarpheta indica Against Helicobacter pylori and Lactobacillus casei

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Abstract

The increasing prevalence of antibiotic-resistant pathogens has intensified the search for plant-derived antimicrobial agents. Stachytarpheta indica (L.) Vahl, a traditionally used medicinal herb, possesses bioactive phytochemicals with potential therapeutic applications; however, its antibacterial effects remain insufficiently studied. The present investigation evaluated the in vitro antibacterial efficacy of aqueous leaf extract of S. indica against two clinically relevant bacteria: Helicobacter pylori and Lactobacillus casei. Extract were prepared by infusion (aqueous) while antibacterial activity was assessed using the agar well diffusion method. Results demonstrated solvent- and organism-dependent susceptibility patterns. The aqueous extract showed stronger inhibition against L. casei, likely due to tannin- and glycoside-associated mechanisms. These findings indicate that S. indica possesses broad-spectrum antibacterial potential, with differential activity linked to solvent polarity and bacterial cell structure. Overall, the study supports the medicinal relevance of S. indica and highlights its prospective value in developing plant-based antibacterial formulations.

Keywords: Stachytarpheta Indica, Antibacterial Activity, Phytoextracts, Helicobacter Pylori, Agar Well Diffusion Assay

1. Introduction

The rise of antimicrobial resistance has renewed global interest in medicinal plants as sources of novel therapeutic compounds. According to the World Health Organization, bacterial infections remain a major cause of morbidity worldwide, and the reduced efficacy of conventional antibiotics necessitates alternative strategies for infection control (WHO, 2017). Medicinal plants are rich in secondary metabolites such as phenolics, flavonoids, alkaloids, and terpenoids, which contribute to antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities (Harborne, 1998; Mandal, DebMandal, Kumar, & Pal, 2010). These phytochemicals exert diverse antibacterial mechanisms including disruption of cell membranes, inhibition of nucleic acid and protein synthesis, and modulation of microbial enzymatic systems (Cushnie & Lamb, 2005).

Stachytarpheta indica (L.) Vahl (family Verbenaceae) is a perennial herb distributed across tropical and subtropical regions. Ethnobotanical reports document its traditional use in fever, gastrointestinal

disturbances, skin infections, and inflammatory disorders (Barrett, 1994; Nath, Dutta Choudhury, & Das, 2013). Pharmacological evidence from related Stachytarpheta species indicates antimicrobial, hepatoprotective, wound healing, and anti-inflammatory properties (Liew & Yong, 2016). However, systematic investigations on the antibacterial efficacy of S. indica against clinically relevant pathogens remain limited.

Two bacterial species of clinical interest were selected for evaluation in this study: Helicobacter pylori, a Gram-negative gastric pathogen associated with gastritis, peptic ulcer disease, and gastric carcinoma (Chey & Wong, 2007); and Lactobacillus casei, a probiotic-associated species with documented cariogenic potential under acidic oral conditions (Beighton, 2005). Investigating plant-derived antibacterial agents against these organisms is therefore relevant to both gastrointestinal and oral health contexts.

Given the documented therapeutic value of S. indica and the global demand for plant-based antimicrobial alternatives, the present study aims to evaluate the in vitro antibacterial activity of aqueous leaf extract of S. indica against H. pylori and L. casei using standardized microbiological assays.

2. Materials and Methods

Plant Collection and Identification

Fresh leaves of Stachytarpheta indica (L.) Vahl were collected from rural habitats around Bhopal, Madhya Pradesh, India. Preliminary identification was carried out based on morphological characters including phyllotaxy, leaf morphology, inflorescence, and floral structure. Taxonomic authentication was verified a botanist from the department of botany and a voucher specimen was prepared and deposited for future reference. Herbarium preparation and preservation followed the procedures outlined by Johansen (1940).

Preparation of Plant Material

Collected leaves were washed under running tap water followed by distilled water to remove surface contaminants. Clean leaves were shade-dried for 7–10 days to prevent degradation of thermo-sensitive phytoconstituents, a practice recommended for medicinal plant processing (Kothari, Seshadri, & Borde, 2012). Dried leaves were pulverized into fine powder using a mechanical grinder and stored in airtight, moisture-proof containers protected from light.

Extraction Procedure

Extraction method adopted to obtain aqueous extract:

Aqueous Extract (Infusion Method): Twenty grams (20 g) of powdered material were infused in 200 mL of freshly boiled distilled water for 30 minutes, then filtered through muslin cloth followed by Whatman No.1 filter paper.

Filtrates were concentrated using a water bath and rotary vacuum evaporation. Extraction yield (%) was calculated based on the ratio of dried extract to initial powder weight. For further studies, a stock of 100 mg/ml in sterile distilled water of extract were prepared which were serially diluted to prepare 5 different concentrations for MIC test.

Microbial Strains and Culture Conditions

Bacterial cultures of Helicobacter pylori (ATCC-43504) and Lactobacillus casei (ATCC-393) were utilized as test organisms. Selection of these species was based on their clinical relevance to gastrointestinal infections, as reported in contemporary research (Chey & Wong, 2007; Koo, Falsetta, & Klein, 2013; Beattie, 2024). Cultures were maintained on appropriate selective media: H. pylori on Columbia blood agar under microaerophilic conditions and L. casei on de Man, Rogosa, and Sharpe (MRS) agar, following standard microbiological guidelines (CLSI, 2020).

Antibacterial Assay

Antibacterial activity was assessed using the well-diffusion assay as described by Nascimento et al. (2000) with slight modifications. Mueller-Hinton agar plates were seeded with standardized bacterial suspensions adjusted to 0.5 McFarland density. Wells of 6 mm diameter were punched aseptically and filled with 20 µL of each dilution of extract solutions. Plates were incubated at 37 °C for 24–48 hours under respective atmospheric conditions, and antibacterial efficacy was quantified by measuring the diameter of inhibition zones in millimetres. All experiments were performed in triplicates and results recorded as mean ± standard deviation.

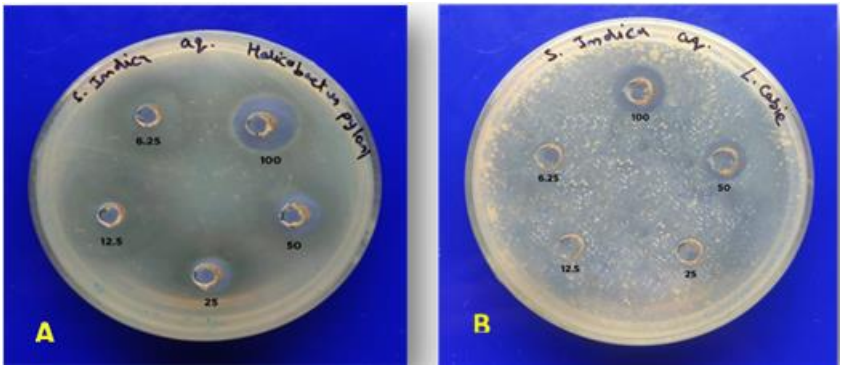

Figure: Petriplates showing antimicrobial activity (MIC) of Stachyterpheta indica leaf extract at different dilution against the test Helicobactor pylori (ATCC-43504) strains and Lactobacillus casie (ATCC-393)

3. Result and Discussion

Extraction Yield and Organoleptic Properties

Extraction yield provides preliminary insight into solvent extractability and the polarity of phytochemical constituents. As shown in Table 1, the aqueous extract produced a higher yield (44.5%). Higher aqueous yield suggests greater solubility of hydrophilic compounds such as tannins, glycosides, saponins, and carbohydrates, a trend consistent with studies on polar extractants (Sasidharan et al., 2011; Kumar et al., 2021).

Table 1: Extraction yield and organoleptic characteristics of S. indica leaf extract

Extract Type	Yield (%)	Colour	Texture	Odour
Aqueous	44.5	Dark brown	Crystalline	Organic, burnt

Antibacterial Activity

The antibacterial activity of aqueous extract of Stachytarpheta indica leaves was evaluated against Helicobacter pylori (ATCC 43504) and Lactobacillus casei (ATCC 393) using the agar well diffusion method. Zones of inhibition were recorded at serial concentrations to determine relative sensitivity and minimum inhibitory concentration (MIC) profiles.

Table 2 presents the antibacterial activity of aqueous extract, demonstrating concentration-dependent inhibition against two test organisms. At 100 mg/mL, inhibition zones were highest for L. casei (19 mm), followed by H. pylori (18 mm). Aqueous extract lost activity against H. pylori and L. casei at concentrations below 12.5 mg/mL indicating relatively higher susceptibility of L. casei and H. pylori to water-soluble constituents.

Table 2: Results of antimicrobial activity (MIC) of Stachytarpheta indica aqueous extract against 2 types of ATCC bacterial strains.

S.N.	Test ATCC Bacterial Strains	Zone of inhibition (Φ in mm) against test microbes different concentration of phytochemical extract				
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
1.	H. pylori	18 mm	12 mm	11 mm	10 mm	nil
2.	L. casie	19 mm	14 mm	12 mm	11 mm	nil

Interpretation and Comparative Analysis

The results demonstrate solvent and organism-specific antibacterial effects. The aqueous extract showed the highest efficacy against L. casei, particularly at higher concentrations (19–11 mm), indicating that water-extractable constituents such as tannins, saponins, and glycosides may play a dominant role. Traditional medicinal systems often utilize water infusions for gastrointestinal disorders, including dyspepsia and diarrhoea, supporting the relevance of aqueous extracts (Barrett, 1994; Nath, Panda, & Mahapatra, 2013).

Differential susceptibility among bacterial species can additionally be explained by cell wall structural differences. H. pylori (Gram-negative) possess an outer membrane enriched with lipopolysaccharides that cannot be penetrated by hydrophilic phytochemicals. Gram-positive species such as L. casei have thicker peptidoglycan layers, facilitating interaction with tannins that form irreversible complexes with cell wall proteins (Scalbert, 1991).

Correlation with Phytochemical Composition

Correlation between observed antibacterial activity and phytochemical composition can be inferred from previously documented phytochemical investigations of Stachytarpheta indica. Whereas aqueous extracts are typically enriched with more polar constituents such as phenolics, tannins, saponins, and glycosides. Phytochemical profiles play an important role in determining antimicrobial potency. Flavonoids, for instance, are known to disrupt bacterial membrane integrity and inhibit nucleic acid synthesis, thereby exerting broad antimicrobial effects (Cushnie & Lamb, 2005). Alkaloids have been shown to interfere with peptidoglycan synthesis and protein transcription in bacteria, contributing to growth inhibition and bactericidal action (Mickymaray, 2019). Similarly, tannins exert antimicrobial effects by precipitating microbial enzymes and binding to cell wall proteins, resulting in altered membrane

permeability and enzyme deactivation (Scalbert, 1991). Terpenoids further contribute to antibacterial action through disruption of membrane lipids and inhibition of key metabolic enzymes (Huang et al., 2022).

Higher susceptibility of L. casei to the aqueous extract may be due to the abundance of tannins and other phenolic compounds that interact strongly with Gram-positive cell wall structures.

Biswas et al. (2002) showed that tannin-rich aqueous extracts inhibited oral pathogens, supporting the observed sensitivity of L. casei to aqueous fractions in this study. In addition, Nazzaro et al. (2013) highlighted the role of plant-derived phenolic compounds in modulating the growth and metabolic behaviour of probiotic bacteria, providing further context for the selective activity observed against L. casei.

Taken together, these convergent lines of evidence indicate that Stachytarpheta indica possesses broad-spectrum antibacterial potential, and that solvent polarity plays a key role in determining extract composition and corresponding antimicrobial outcomes. The differential responses observed across Gram-positive and Gram-negative bacteria further underscore the importance of phytochemical–microbial interactions and support the potential value of S. indica as a source of natural antibacterial agents.

4. Conclusion

In conclusion, the findings of this study demonstrate that Stachytarpheta indica leaf extracts possess notable antibacterial activity against H. pylori and L. casei with clear solvent-dependent differences. The aqueous extract was more effective against L. casei. These variations reflect differences in phytochemical composition and bacterial cell structure. Overall, the results support the traditional medicinal use of S. indica and highlight its potential as a source of plant-based antibacterial agents. Further studies, including compound isolation and mechanistic assays, are recommended to validate therapeutic applications.

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