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Phytochemical analysis, stability, and antimicrobial activity of eighteen medicinal plants studied against five multi-drug resistant human pathogens

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ABSTRACT

Introduction and aim. Several medicinal plants from India have been reported to be effective against human pathogens, but comprehensive comparative studies are scarce. The aim of this study has been to evaluate and compare the antimicrobial activity, phytochemical composition, and stability at different temperatures and pH of ethanolic extracts of eighteen Indian medicinal plants which are prevalent in North-east India and thrive abundantly.

Material and methods. These plants included S*yzygium cumini, Cannabis sativa, Camellia sinensis, Murraya koenigii, Alstonia scholaris, Terminalia chebula, Flemingia strobilifera, Azadirachta indica, Prunus persica, Euphorbia thymifolia, Averrhoa carambola, Paderia foetida, Psidium guajava, Spondias pinnata, Garcinia cowa, Litsea cubeba, Micania macrantha*, and *Phlogocanthus thyrsiflorus.* Their potent medicinal properties made them the ideal choice for this study which included the use of agar well diffusion method and phytochemical analysis.

Results. Agar well diffusion has been used to test their antibacterial activity, which demonstrated higher antibacterial activity of *G. cowa* extract against *Vibrio cholerae* and *Staphylococcus aureus*, whereas *S. pinnata extract* was most effective against *V. cholerae and Enterococcus faecium*.

Conclusion. Phytochemical analysis revealed the presence of tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, and phenols. The antimicrobial activity of these plant extracts remained stable at higher temperatures and varying pH levels.

Keywords. antimicrobial activity, antibiotic resistance, extracts, medicinal plants, phytochemicals

Introduction

Infectious diseases are a major cause of increased morbidity and mortality and one of the irresistible threats to the human race. Treatments of infectious diseases are mostly carried out by antibiotics. Thus, the success of curing an infectious disease relies on the availability of effective antibiotic. The problem mounts up due to the development of antibiotic resistance by the microbial pathogens.[1-3] The use and misuse of antimicrobial drugs accelerate the emergence of drug-resistant microbes.[4,5] Poor infection control practices, inadequate sanitary conditions, and improper food-handling, etc. encourage the further spread of multidrug-resistant pathogens.[6,7] Development of plant-based antibiotic formulation is an alternative to such situations.[8]

In recent years, the growing interest in the exploration of the medicinal properties of plant extracts due to their diverse array of bioactive compounds has promoted investigations into enhanced extractions of phytochemicals and the potential use of the various plant extracts to address several health conditions including treatment against microbial infections and autoimmune diseases like Rheumatoid arthritis.[9] For this, the utilization of enzyme complexes for the recovery of plant extracts has proved to be a successful methodology. Enzymes like zympex-014 have been proven to effectively enhance phenolic recovery along with antioxidant capacity in *Pongamia pinnata* leaf extract.[10]

Azadirachta indica, a widely recognized medicinal plant has been studied extensively for its anti-inflammatory effects where it has shown to reduce inflammation in carrageenan-induced rats.[11] Plants such as *Citrus paradisi*, *Thymus vulgari*s and *Caltha palustris var. alba* have also been reported to reduce inflammation.[12-14] In the same way, *T. vulgaris* and *C. palustris var. alba* have been revealed to showcase other health benefits too such as antioxidant, antibacterial, and antidiabetic properties thereby implying their suitability for medicinal use.[12,13]

Similarly, investigations into phytochemicals obtained from plants such as *Phyllanthus emblica* , *Teucrium stocksianum* and *Salvia hispanica* have shown significant antioxidant properties.[15-18] Furthermore, studies have indicated the ability of phytochemicals extracted from several medicinal plants such as *Melia azedarach, P. emblica, T. vulgaris,* and *C. palustris var. alba* to act as antimicrobial agents thereby displaying their capability to be used in the treatment of microbial infections.[12,13,15,19] *M. azedarach* has been identified to possess the potential to have therapeutic applications against diseases such as tuberculosis.[20] This therefore has shown the immense potential of plant extracts as valuable sources of therapeutic agents and emphasized the need for research in this field.

While phytochemicals have become a prime area of investigation to develop antimicrobial agents, studies portraying microbial production of antimicrobial compounds and the development of biomaterials with integration of plant science have also become emerging topics of research. Bioactive polar and non-polar metabolites extracted from bacteria such as *Bacillus* sp. have exhibited antimicrobial properties apart from

other significant biological activities, including antidiabetic, anti-inflammatory, antioxidant, and anti-hemolytic effects. Compounds isolated from the bacteria like 1,2-benzenedicarboxylic acid have shown potential anticancer properties against epithelial glioblastoma cancer genes too.[21]

The exploration of plant extracts in modern medicine has focused on plant-derived compounds and their synthesized nanoparticles which have displayed potential as a promising alternative to traditional therapies. One significant area of research has involved the synthesis of silver nanoparticles (AgNPs) using plant extracts that show antimicrobial properties. For example , inhibition zones in agar well diffusion assays have been observed for AgNPs synthesised using fenugreek leaf extract against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa.*[22] Similarly, research on *Calliandra harrisii* leaf extract synthesized AgNPs has displayed substantial activity against multidrug-resistant *Staphylococcus* species and has even been reported to outperform conventional antibiotics such as vancomycin and cefoxitin. Docking studies conducted reveal the efficacy of these nanoparticles against virulent genes of the multi-drug resistant bacteria thereby suggesting their potential in addressing the growing issue of antibiotic resistance.[20]

Several researchers are now exploring plant-derived substances as potential alternatives or complement to antibiotics. Studies such as those on *Crocus sativus*, have demonstrated the effectiveness of their modified compounds as plant-based antimicrobials.[23]

Also since plant-based formulations contain a large number of molecules that work in synergy to cause an antimicrobial effect, development of resistance is much slower. North-eastern India is mostly inhabited by a large number of tribes, who prefer using plant-based cures rather than bottled medicines.[24,25] There have been studies on the antimicrobial activity of medicinal plants against pathogens. However, comparative analyses of different medicinal plants and their effects on various pathogens are lacking. The novelty and rationale of this study lie in comparing the antimicrobial activity of these plants and assessing their stability under different temperatures and pH.

Aim

In this study, we compared the efficacy of eighteen such medicinal plants against five human pathogens (*Vibrio cholerae, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium* and *Pseudomonas aeruginosa)*. The plants used were *S. cumini, C. sativa, C. sinensis, M. koenigii, A. scholaris, T. chebula, F. strobilifera, A. indica, P. persica, E. thymifolia, A. carambola, P. foitida, P. guajava, S. pinnata, G. cowa, L. cubeba, M. macrantha,* and *P. thyrsiflorus.*

Material and methods

Collection of plant materials

Plant materials were collected from villages named Lekai gaon, Jokai gaon near Assam Medical College and Hospital Campus of Dibrugarh District of Assam which are found to occur in abundance throughout the North-eastern region of India. The collected Plants were *S. cumini* (1), *C. sativa* (2), *C. sinensis* (3), *M. koenigii* (4), *A. scholaris* (5), *T. chebula* (6), *F. strobilifera* (7), *A. indica* (8), *P. persica* (9), *E. thymifolia* (10), *A. carambola* (11), *P. foitida* (12), *P. guajava* (13), *S. pinnata* (14), *G. cowa* (15), *L. cubeba* (16), *M. macrantha* (17), *P. thyrsiflorus* (18). It may be noted that, throughout this article, these numbers of plants were maintained for convenience in discussion and data presentation. Collected plant materials were washed with tap water twice and finally washed with distilled water. Washed materials were dried at room temperature under shade. The dried plant materials were then pounded in mortar and pestle and used for extraction.

Preparation of the plant extract

Plant extracts were prepared using the maceration technique with ethanol as the solvent.[26] Initially, 10 grams of dried plant material were weighed and soaked in 40 milliliters of ethanol, agitated at 80 rpm for 24 hours at room temperature. The resulting mixture was filtered through Whatman no. 1 filter paper, and the plant residue was re-soaked in another 40 milliliters of ethanol. This process was repeated twice to ensure thorough extraction. The combined filtrate was then concentrated using a rotary evaporator and reconstituted with ethanol. The final extracts were stored at 4ºC for subsequent use.

Microorganisms

Gram-negative and gram-positive bacteria were used in the study. Five clinical strains were obtained from the Department of Microbiology, Assam Medical College, and Hospital of Assam. The isolates were *V. cholerae , S. aureus, E. faecalis, E. faecium* and *P. aeruginosa*. The isolates were maintained using nutrient agar media by streaking technique.[27]

Determination of antibacterial activity

The antibacterial assay was conducted with modifications to previously described methods.[28-30] Bacterial broth cultures were grown for 18 hours, and the turbidity was adjusted to 0.5 McFarland standard ($1x10^8$ CFU/mL). Then, 100 µL of each bacterial culture was spread onto sterile agar media plates. For the agar well diffusion assay, wells were punched into the agar plates using a sterile 5 mm cork borer. Each well was loaded with 100 µL of plant extract (100 mg/mL) and allowed to diffuse into the media at room temperature for 30 minutes. The plates were then incubated overnight at 37°C. The following day, the zones of inhibition (ZOI) were measured and recorded. This assay was repeated three times to ensure consistency.

Determination of minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was determined using the micro-dilution method.[31] The procedure was conducted in 96-well U-bottom plates. Plant extracts were diluted with sterile peptone water, creating a series of concentrations: 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 1.56 mg/mL, 0.78 mg/mL, and 0.39 mg/mL. For each assay, 100 µl of bacterial culture at 0.5 McFarland standard (1x10^8 CFU/mL) was used. The plates were incubated at 37°C, and the MIC was determined by visually assessing the turbidity in each well. The lowest concentration at which no bacterial growth (i.e., no turbidity) was observed was recorded as the MIC value for that extract.

Effect of temperature and pH

To assess temperature tolerance, plant extracts were heated at 4ºC, 60ºC, and 100ºC for one hour before performing the antibiotic activity assay.[32] For pH tolerance, each plant extract was exposed to acidic (pH 2) and basic (pH 10) conditions for one hour. The basic pH was achieved by adding 1M NaOH dropwise until the pH reached 10, and the acidic pH was achieved using 1N HCl.[6] After treatment, both acid- and base-treated extracts were neutralized before being used in the bioassay.

Phytochemical screening of plant materials

Phytochemicals were detected as described earlier[33] and outlined below

Tannins: 200 mg of plant material was soaked in 10 mL of distilled water and filtered. 2 mL of the filtrate was added to 2 mL of dilute $FeCl_3$. The appearance of a blue-black or violet precipitate indicated the presence of tannins and phenols.

Alkaloids: 2 mL of ethanolic extract (10 gms/40 mL) was mixed with a few drops of diluted HCl and filtered. 2 mL of the filtrate was treated with Mayer's reagent and Wagner's reagent. A creamy white precipitate with Mayer's reagent and a reddish-brown precipitate with Wagner's reagent indicated the presence of alkaloids.

Saponins: 2 mL of ethanolic extract was diluted with 20 mL of distilled water and shaken vigorously. The presence of a stable, persistent froth indicated the presence of saponins.

Glycosides: 1 g of dried leaf powder was extracted with 10 mL of 70% ethanol for a few minutes and filtered. 5 mL of the filtrate, 10 mL of water, and 0.5 mL of a strong lead acetate solution were added and filtered again. The filtrate was shaken with 5 mL of chloroform, and the chloroform layer was separated. This was dissolved in glacial acetic acid, and a drop of ferric chloride solution was added, followed by sulfuric acid, which formed the lower layer. A reddish-brown color between the two layers and a bluish-green upper layer indicated the presence of glycosides.

Flavonoids: The extracts were treated with 80% sulfuric acid. A yellowish-orange color indicated the presence of flavonoids.

Steroids and Terpenoids: The Liebermann-Burchard reaction was used. In a test tube, 2 mL of chloroform extract prepared from the leaves of the eighteen different plants selected for the study was mixed with 2 mL of acetic anhydride and a few drops of concentrated sulfuric acid. A blue-green ring between the layers indicated the presence of steroids, while a pink-purple ring indicated the presence of terpenoids.

Results

Antimicrobial activity of plant extracts

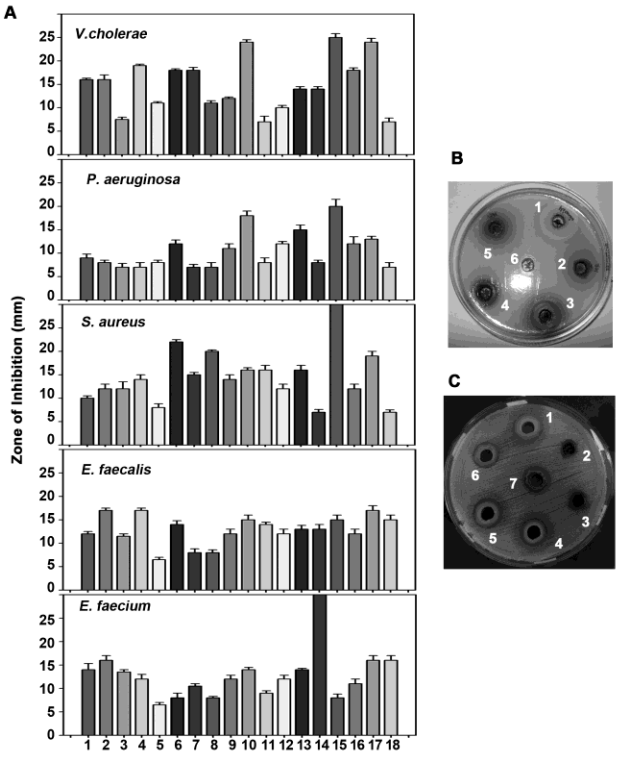

Fig. 1. Comparative study of antibacterial assay of ethanolic extract of plants (A: Zone of Inhibition. Numbers in X-axis indicate plants as mentioned in the text. Each bar indicates diameter of zone of inhibition after deducting the cup diameter ± S.D. of three replicates, B and C: Representative photos of agar well diffusion assay. B: against *V. cholerae*, with the following plant extracts. 1 – *M. macrantha,* 2 – *P.persica,* 3 – *E. thymifolia,* 4 – *A.carambola,* 5 – *P. foitida*, 6 – *P. guajava*. C: against *S. aureus* with 1 – *A. indica,* 2 – *P. persica,* 3 – *E. thymifolia*, 4 – *A. carambola*, 5– *P.foitida*, 6 – *P. guajava*, 7 – *S pinnata*)

Table 1. MIC values of ethanolic extract od plants*

Sl. No	Plant extracts	*V. cholerae*	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*E. faecium*
1.	*Syzygium cumini*	12.5	>100	50	50	50

2.	*Cannabis sativa*	6.25	50	25	25	25
3.	*Camellia sinensis*	25	50	25	100	50
4.	*Murraya koenigii,*	6.25	>100	12.5	25	100
5.	*Alstonia scholaris*	100	>100	100	100	100
6.	*Terminalia chebula*	6.25	50	6.25	25	50
7.	*Flemingia strobilifera*	12.5	>100	12.5	50	100
8.	*Azadirachta indica*	25	>100	6.25	50	50
9.	*Prunus persica*	12.5	>100	50	100	100
10.	*Euphorbia thymifolia*	3.125	25	50	50	100
11.	*Averrhoa carambola*	6.25	50	50	50	50
12.	*Paderia foetida*	25	50	100	100	100
13.	*Psidium guajava*	25	25	50	100	100
14.	*Spondias pinnata*	3.125	50	50	12.5	3.125
15.	*Garcinia cowa*	3.125	12.5	1.78	50	50
16.	*Litsea cubeba*	50	50	50	100	100
17.	*Micania macrantha*	3.125	50	25	25	50
18.	*Phlogocanthus thyrsiflorus*	>100	>100	>100	50	50

* > – indicates MIC is more than 100 mg/mL

To study the antimicrobial activity of ethanolic extract of medicinal plants, standard agar well diffusion method was used. A total of eighteen different plants were used in the study. 1-18 extracts were tested against five pathogens – *V. cholerae, S. aureus, E. faecalis, E. faecium,* and *P. aeruginosa.* The zone of inhibition for all the plant extracts against all pathogens is plotted in the same scale for comparative evaluation (Fig. 1A), and representative bioassay plates are shown in Figures 1B and 1C. Also, we have determined the minimum inhibitory concentration for all the extracts against all the pathogens (Table 1). As shown in Figure 1, *E. thymifolia* (10), G. *cowa* (15), and *M. macrantha* (17) showed higher anti-vibrio activity.

Interestingly, plants *E. thymifolia* (10) and *G. cowa* (15) also have high anti-*Pseudomonas* activity (Fig. 1 and Table 1). All the plant extracts in our study showed modest antimicrobial activity against *E. faecalis,* showing 5-15mm zone of inhibition. Almost similar results were obtained against *E. facium* except for *S. pinnata* (14). *S. pinnata* (14) extract is highly antibiotic against this pathogen (3.12 mg/mL). The results altogether suggest that though all traditionally used medicinal plants possess antimicrobial activity, they do differ in their strength and specificity.

MIC was determined as the lowest concentration of extract at which growth of tested bacteria is inhibited, i.e. the concentration at which no turbidity was visible after overnight incubation (Table 1). All the

pathogens were resistant to the plant extract at a concentration below the MIC. For this study, resistant strains i.e., with higher MIC were chosen. MIC of *E. thymifolia* (10), *S. pinnata* (14), *G. cowa* (15), and *M. macrantha* (17) against *V. cholerae* was 3.125 mg/mL, and highest MIC values were obtained from *A. scholaris* (5) (100 mg/mL) and *P. thyrsiflorus* (18) (>100 mg/mL). In the case of *P. aeruginosa, the* MIC of eighteen plants ranged from 50mg/mL to greater 100 mg/mL, except *G. cowa* (15) which exhibited MIC value12.5 mg/mL. The lowest MIC value was obtained for *S. aureus* by the extract of *G. cowa* (15) (1.78 mg/mL). MIC obtained for *E. fecalis* was between 12.5 mg/mL to 100 mg/mL. For *E. faecium*, ethanolic extract of *S. pinnata* (14) showed the lowest MIC value (3.125 mg/mL).

Temperature and pH tolerance by plant extracts

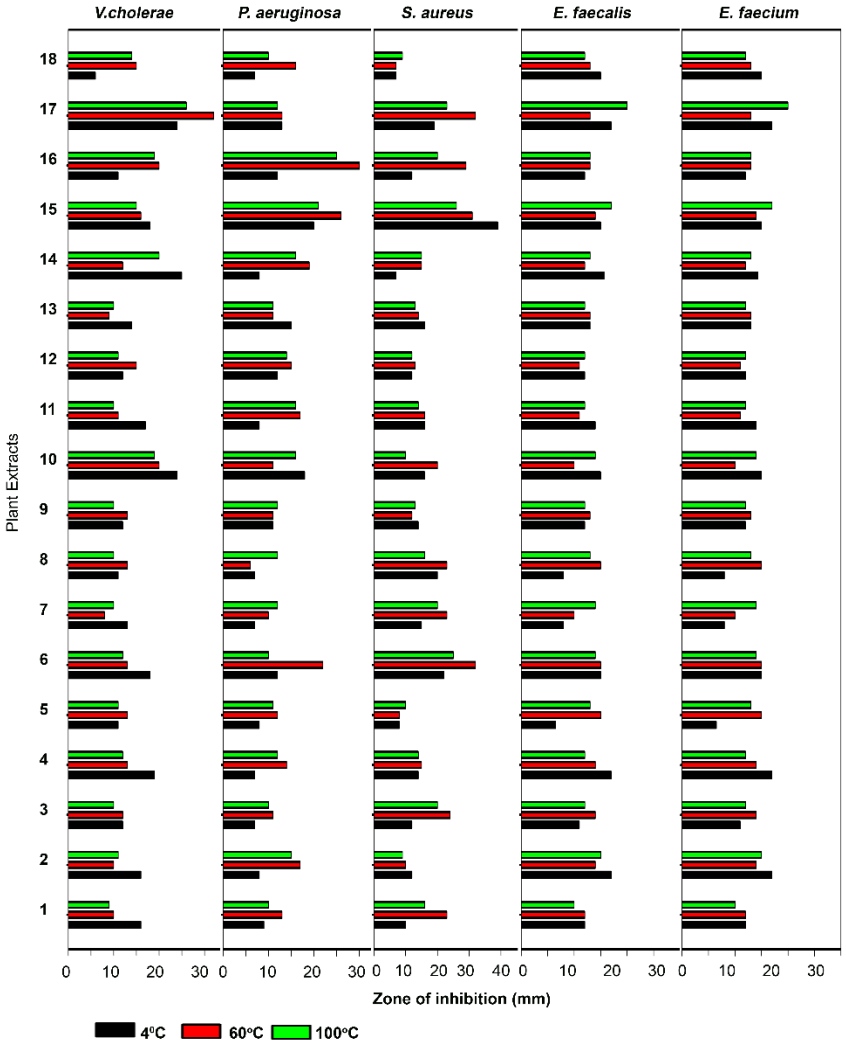

Fig. 2. Effect of different temperatures on plant extracts in terms of antibiotic activity. Plant extracts were treated at the indicated temperature for 1 hour before conducting bioassay by agar-well diffusion assay. The

zone of inhibition was measured as mentioned in Figure 1. Plant numbers are in the same order as mentioned in the text

Different temperature treatments of plant extracts showed that all the extracts were stable at higher temperatures i.e. retained their inhibitory property even after treating at 60ºC and 100ºC (Fig. 2). Plant extracts were not only stable at a higher temperature, for instance, exhibited improvement in their inhibition activity, which was observed with *L. cubeba* (16) against all the bacterial isolates. Similarly, *M. macrantha* (17) and *P. thyrsiflorus* (18) also showed higher ZOI at higher temperature against *V.cholerae* and *S.aureus. S. cumini* (1), *C. sativa* (2), *M. koenigii* (4), *T. chebula* (6), *A. carambola* (11) and *G. cowa* (15) showed a modest reduction in antimicrobial activity against *V.cholerae.* Similarly, *C. sativa* (2), *P. guajava* (13), *G. cowa* (15) also exhibited a decrease in inhibition activity against *S. aureus* with increase in temperature. Higher temperatures also reduced the antibacterial activity of *M. koenigii* (4) and *P. thyrsiflorus* (18) against *E. faecalis* and *E. faecium.* While the reduction in antimicrobial activity may suggest a partial loss of structural integrity of the bioactive molecules; enhancement in activity may be attributed to better extraction.

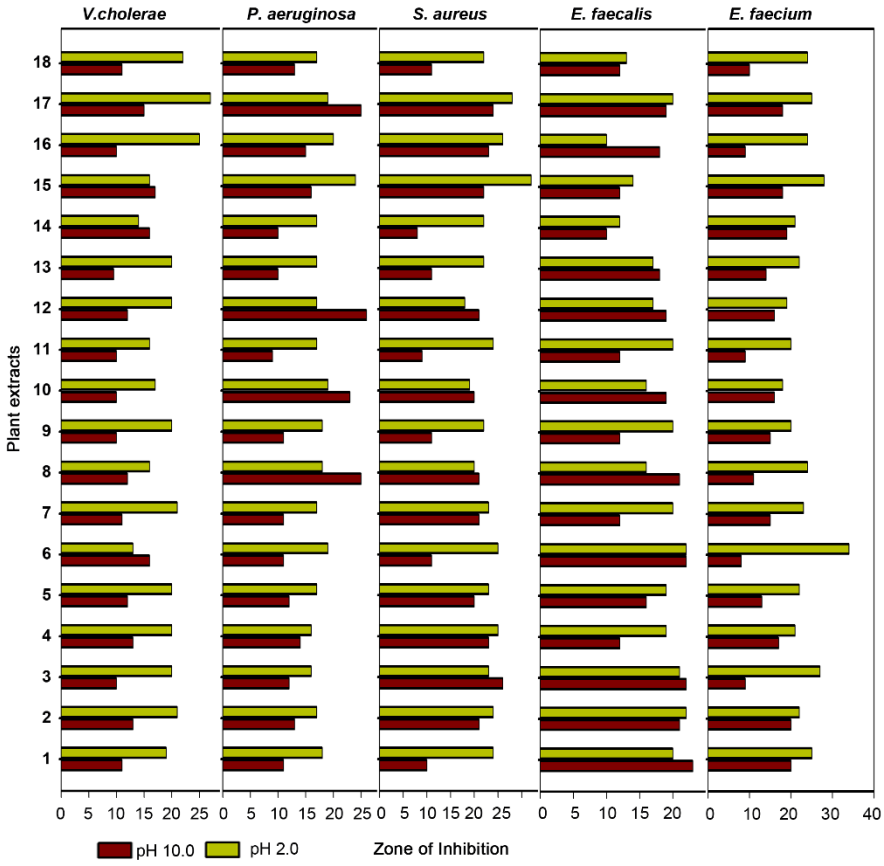

Fig. 3. Effect of different pH on plant extracts in terms of antibiotic activity. The plant extracts were treated with HCl and NAOH to adjust the pH to 2 and 10 respectively. After 30 mins pH was neutralized for

bioassay. Zone of inhibition was measured as mentioned in Figure 1. Plant numbers are in the same order as mentioned in the text

To determine the extreme pH tolerance, plant extracts were treated with acid and alkali to get a final pH of 2 and 10 respectively. The extracts were further neutralized and activity was checked against the tested pathogens. Significant antibiotic activity was retained in all the extracts against all the pathogens (Fig. 3). However, in general, we observed that treating with alkaline pH (10) was more detrimental than the acidic pH (2) with certain exceptions. For example, alkaline treated extracts of *M. macrantha* (17), *P. foitida* (12), *E. thymifolia* (10) and *A.indica* (8) showed higher activity against *P.aeruginosa* (Fig. 3). In other cases, the acid treated samples were more or equal to the alkaline treated samples against all the pathogens.

Phytochemical analysis

Table 2. Phytochemical analysis of ethanolic extract of plants*

Plants																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Tannins	-	+	+	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+
Alkaloids	-	-	-	-	+	-	-	-	+	+	-	+	+	+	-	-	+	-
Saponins	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	-	-	+
Glycosides	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-
Steroids	-	-	-	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+
Terpenoids	+	+	-	+	-	+	-	-	-	-	-	-	+	-	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
Phenols	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	-	+

* + – presence, - – absence, Plant No. 1 – 18 as mentioned: *S. cumini* (1), *C. sativa* (2), *C. sinensis* (3), *M. koenigii* (4), *A. scholaris* (5), *T. chebula* (6), *F. strobilifera* (7), *A. indica* (8), *P. persica* (9), *E. thymifolia* (10), *A. carambola* (11), *P. foitida* (12), *P. guajava* (13), *S. pinnata* (14), *G. cowa* (15), *L. cubeba* (16), *M. macrantha* (17), *P. thyrsiflorus* (18)

The presence of phytochemicals in medicinal plants imparts medicinal values and led to the discovery of phytodrugs of immense importance. Studies of predominant phytochemicals from the ethanolic extract of eighteen plants revealed the presence of different phytochemicals like tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, and phenols (Table 2). Flavonoids were detected in the ethanolic extract of all the plants except *S. cumini* (1), *P. foitida* (12), *L. cubeba* (16) and *M. macrantha* (17). Tannins, alkaloids, and flavonoids largely contribute to medicinal properties of plants. They were detected in *A. scholars* (5), *P. persica* (9), and *E. thymifolia* (10). Besides these plants, extracts of *P. thyrsiflorus* (18), *S. pinnata* (14), *M. koenigii* (4) are also rich in various phytochemicals (Table 2).

Discussion

The evolution of antibiotic resistance in pathogenic microorganisms is a natural phenomenon. However, certain human activities, such as the misuse and overuse of antibiotics, accelerate the development and spread of multidrug resistance. The use of medicinal plant-based formulations may offer an effective approach to mitigating the emergence of multidrug-resistant pathogens. Ethnic tribes around the world extensively use plants for medicinal purposes, including those in the biodiversity-rich northeastern region of India, where local populations frequently utilize medicinal plants for common ailments.[24,34,35] Numerous research papers describe the antimicrobial properties of these medicinal plants yet given the vast diversity of both medicinal plants and microbial pathogens, the number of studies remains insufficient. Moreover, the comparison of the efficacy of various medicinal plants as antimicrobial agents against pathogens underscores the rationale and novelty of this research. This study aims to partially address this knowledge gap by providing a comprehensive and comparative evaluation of eighteen medicinal plants against five different human bacterial pathogens (Table 1).

To conduct this extensive study, we focused on ethanolic extracts of the plants. Ethanol, with a polarity index of 5.1, effectively solubilizes a wide range of biomolecules, bridging the solubility spectrum between water (9) and hexane (0).[36] Previous reports indicate that ethanol extracts of medicinal plants contain flavonoids, phenolics, alkaloids, and other substances.[28] Our analysis confirmed the presence of tannins, alkaloids, saponins, glycosides, terpenoids, flavonoids, and phenols in varying degrees across the extracts (Table 2). This table also compares the abundance of secondary metabolites among the eighteen plants; for instance, *S. cumini* (1) is rich in terpenoids and phenols, whereas these compounds are less abundant in *F. strobilifera* (7), *A. indica* (8), and *P. persica* (9). Flavonoids are crucial components of several medicinal plant extracts; the flavonoid fraction of *P. guajava* leaves, for example, inhibits the growth of *P. aeruginosa*[37-41] and may counter biofilm formation.[42] We found an abundance of flavonoids in most ethanolic extracts, supporting their role as bioactive components along with other metabolites.

The most significant finding of our study is the comparison of the antimicrobial efficacy of the selected medicinal plant extracts against the different pathogens. A plant extract with high inhibitory activity against one pathogen may have little effect on another. For example, *E. thymifolia* (10) extract strongly inhibits the growth of *V. cholerae* with a MIC of 3.125 mg/mL but is less effective against *P. aeruginosa* (MIC 25 mg/mL) and nearly ineffective against *S. aureus, E. faecalis,* and *E. faecium.* Like pharmaceutical compounds that require extensive validation, the bioactivity of medicinal plant extracts should also be confirmed through multiple independent studies. For instance, our findings on the bioactivity of *S. cumini* against *V. cholerae* corroborate previous reports by Sharma et al. and Islam.[43,44] However, most of the interactions between plant extracts and pathogens reported here are novel. To our knowledge, there are no previous studies on the interactions between extracts of the selected plants and *E. faecalis* or *E. faecium.*

Our results show that these two pathogens are generally resistant to most extracts, but is susceptible to the extract of *S. pinnata* (14) (Table 1).

Additionally, there are no prior reports on the susceptibility of *V. cholerae, S. aureus, E. faecalis, E. faecium,* and *P. aeruginosa* to extracts of *F. strobilifera* (7), *E. thymifolia* (10), *A. carambola* (11), *P. foitida* (12), *S. pinnata* (14), *M. macrantha* (17), *P. thyrsiflorus* (18), and *L. cubeba* (16). Besides antimicrobial properties and phytochemical constituents, this study also evaluates and compares the efficacy of the plant extracts under extreme pH and temperature conditions. The pH analysis conducted aimed to determine the impact on the extracts upon exposure to acidic (pH=2) and basic (pH=10) conditions, to understand how the extracts react to different pH conditions of the human gut. The temperature analysis involved exposure of the extracts to various temperatures (4ºC, 60ºC, and 100ºC), which was performed to determine the adequate temperature for their transportation and storage.

Our research shows that the growth inhibition properties of the extracts are retained at both pH 2 and pH 10 (Fig. 3), with higher efficacy observed at alkaline pH. Although the reason for reduced activity at low pH is unclear, it is possible that certain components precipitate and become unavailable in the assay. This hypothesis is supported by the significant loss of antibiotic activity observed when extracts are incubated at low temperatures, such as 4°C (Table 4). The bioactive ingredients are highly thermostable, retaining activity even at 100°C, making it unlikely that their bioactivity would be lost during storage at 4°C. However, our findings indicate that storage at 4°C significantly reduces the activity of the samples.

Nonetheless, the study has limitations which gives the scope for further research. The study acknowledges the presence of different bioactive components. It does not, however, explore the synergistic or antagonistic effects of these compounds in different combinations. Furthermore, the study focuses on plants from the northeastern region of India. This region is rich in biodiversity, however, the findings may vary significantly depending on the location of the medicinal plants. Further research into these areas will help answer these questions.

Conclusion

Our work demonstrated the efficacy of eighteen medicinal plants towards five microbial pathogens. The medicinal plants and the pathogens were taken from the same geographical regions, which are from the state of Assam in India. The ethanolic extracts of the medicinal plants selected were observed to possess significant antimicrobial properties which had been tested using agar well diffusion. They also tend to possess a wide range of varying MIC values for the pathogens which range from 1.78 mg/mL to >100 mg/mL. These results suggest that the effects of compounds are variable and specific against certain pathogens. A combinatorial approach may be considered for better results and for making broad-spectrum activity. The presence of phytochemicals such as tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, and phenols in plant extracts was determined which indicated the medicinal values

of these plants and their potential to be used in the development of medications. Also, the plant extracts showed stability at extreme temperatures and pH by retaining their antimicrobial activity even though certain variations in their effectiveness were observed. While exposure to higher temperatures displayed improved inhibition activity in some plant extracts and reduced activity in some, alkaline pH showed more detrimental antimicrobial activity of the extracts compared to acidic pH.

Based on further research on these phytochemicals, the local tribals who rely on these medicines can be guided about the disease-specific use of plants.

Declarations

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Author contributions

Conceptualization, S.P.N.; Methodology, G.D.M. and J.B.; Software, G.D.M., J.B. and S.M.; Validation, G.D.M., J.B. and S.M.; Formal Analysis, G.D.M., J.B. and S.M.; Investigation, G.D.M., J.B. and S.M.; Resources, S.D., L.S. and S.P.N.; Data Curation, G.D.M. and J.B.; Writing – Original Draft Preparation, G.D.M. and J.B.; Writing – Review & Editing, G.D.M., J.B. and S.M.; Visualization, S.P.N.; Supervision, S.P.N.; Project Administration, S.P.N.; Funding Acquisition, S.P.N..

Conflicts of interest

Authors declare no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this article.

Ethics approval

Not applicable.

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