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Research Article

EVALUATION OF PHYTO-ACTIVE COMPOUNDS, ANTIOXIDANT, AND ANTIBACTERIAL EFFICACY OF ETHNOMEDICINAL PLANTS USED BY MANIPURI TRIBE IN BANGLADESH

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Abstract

The present study aimed to investigate the medicinal properties (phytochemical screening, antioxidant potentiality, and antibacterial activity) of seven plant species used by the Manipuri tribe in Bangladesh. Most extracts showed effective antimicrobial activity against the tested pathogenic species and doses of minimum inhibitory concentration of plant extracts ranged from 12.5 µg/mL to 100 µg/mL. When compared to seven medicinal plants, the crude extract of *Melastonia malabathricum* and *Oxalis corniculata* exerted best minimum inhibitory doses against all studied bacterial (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella sp and Staphylococcus aureus*). Both *Oxalis corniculata* and *Melastoma malabathricum* showed best performance against *Salmonella* sp. *Aerva sanguinolenta* exhibited good performance to retard the growth of *Staphylococcus aureus, Pseudomonas* spp, and *Escherichia coli.* Furthermore, four medicinal plants (*Oxalis corniculata*, *Aerva sanguinolenta, Melastoma malabathricum,* and *Mikania scandens*) displayed potential activity against *E. coli* growth. The results of the phytochemical screening revealed that the extracts of plants (*Houttuynia cordata*, *Oxalis corniculata*, *Leucas aspera*, *Mikania scandens*, *Aerva sanguinolenta*, *Spilanthes acmella*, *Melastoma malabathricum)* contained alkaloids, steroids, phenols, proteins, flavonoids, saponins, amino acids, coumarins, and other compounds. The presence of phytochemicals varied from plant to plant. The methanolic extract of *Houttuynia cordata* contained the highest amount of total phenolic compounds, measuring 94.53 ± 0.45 mg/g gallic acid equivalent (GAE) of dry leaf powder. High total flavonoid content was found in the ethanolic crude extract of *Melastoma malabathricum*, with 78.76 ± 0.67 mg/g quercetin equivalent (QE) of dry leaf powder. The ethanolic and methanolic extracts of *Melastoma malabathricum* showed the highest total tannin content, 51.20 ± 0.09 and 50.50 ± 0.17 mg/g tannic acid equivalent (TAE) of dry leaf powder, respectively. *Houttuynia cordata, Melastoma malabathricum, and Oxalis corniculata* extracts exhibited potent antioxidant activity. To extend the current study, in vivo models are needed to prove the effectiveness of leaves as alternative drugs.

Introduction

Since the dawn of human civilization, traditional medicine has relied on plants to treat infections, as they have always been integral to human societies. The World Health Organization (WHO) reports that approximately 80% of people in developing nations depend on traditional medicine, primarily plant-based, for essential healthcare (Basualdo et al., 2007). Notably, around 95% of modern drugs have roots in traditional medicinal plants (Rosakutty and Roslin, 2012). However, the rise of multi-drug-resistant (MDR) bacteria poses significant global health challenges, undermining the effectiveness of antibiotics and causing severe infections and high mortality rates (Visvesvara et al., 2007; Rose et al., 2001). MDR bacteria are pathogens that are resistant to at least one antimicrobial agent in three or more categories, exacerbate disease severity, and pose significant public health risks (Sweeney et al., 2018; Aslam et al.,

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2018). The misuse of antibiotics plays a major role in resistance, with projections indicating nearly 10 million annual deaths due to antibiotic resistance by 2050 (Tagliabue and Rappuoli, 2018). To address this crisis, there is an urgent need for innovative strategies, including optimizing antibiotic use, developing alternative treatments, and exploring plant-based solutions (Khameneh et al., 2021).

Plants offer phytochemicals, which are a promising alternative to antibiotics due to their accessibility, affordability, and potent antimicrobial properties with minimal or no side effects. Their diverse chemical structures and eco-friendly synthesis of bioactive compounds enable novel antibacterial mechanisms (Gorlenko et al., 2020). Natural compounds such as flavonoids, terpenes, alkaloids, and phenolic acids not only exhibit antimicrobial activity but also possess antioxidant, anti-inflammatory, antiviral, and anticancer properties (Mandalari et al., 2007; Parham et al., 2020). Phenolic compounds are distinguished for their ability to neutralize free radicals, thereby mitigating oxidative damage linked to diseases such as cancer and cardiovascular disorders (Sahu and Saxena, 2013). Compared to synthetic antioxidants like BHA and BHT, which are associated with potential carcinogenic risks, plant-derived antioxidants are considered safer and less toxic (Zhu et al., 2011).

In Bangladesh, indigenous communities, such as those in Kamalganj Upazila of Sylhet, rely on medicinal plants for various healthcare purposes, showcasing their rich ethnomedicinal traditions. This study explores the antimicrobial potential of native plants against pathogenic bacteria, aiming to identify their therapeutic value in combating infectious diseases.

Materials and Methods

The outline of standard method of the present study was presented in **Figure 1.**

Study Area and Collection of Sample

Medicinal plants (*Houttuynia cordata, Oxalis corniculata, Leucas aspera, Mikania scandens, Aerva sanguinolenta, Spilanthes acmella, Melastoma malabathricum*) were collected from Manipuri villages in Kamalganj, Moulvibazar. Samples were stored in clean plastic bags and transported to the central laboratory at Sylhet Agricultural University for analysis. The medicinal plants used in this study are visualized in **Figure 2.**

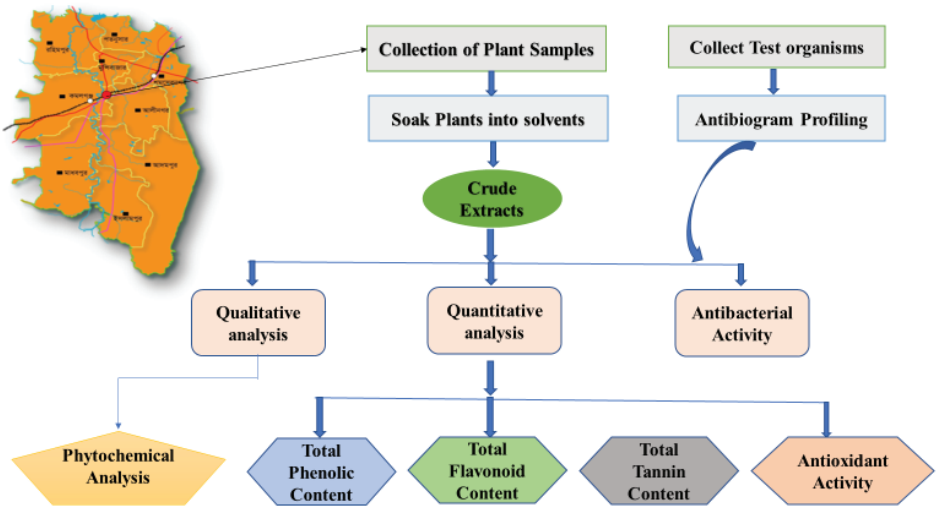
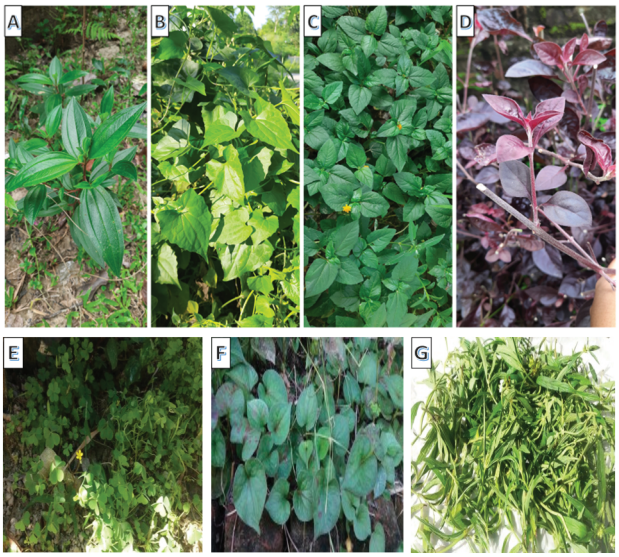


Figure 1. Study Area (Ghoramara, Adampur Bazar, Kamalganj, Moulvibazar, Sylhet) and detailed outline of Materials and Methods.



Figures 2. Plant samples. *A) Melastoma malabathricum, B) Mikania scandens, C) Spilanthes acmella, D) Aerva sanguinolenta, E) Oxalis corniculata, F) Houttuynia cordata, G) Leucas aspera*.

Solvent Extraction of Dried Leaves

Plant samples were washed, oven-dried at 45°C, and ground into powder. Five grams of each sample were soaked in 80 ml of 80% ethanol or methanol. The mixture was intermittently shaken and left for 72 hours at room temperature. Filtration was carried out using Whatman No. 1 filter paper, and the liquid was dried at 45°C to remove solvents. The dried extract was dissolved in 5 ml of 5% DMSO for further analysis.

Microbe Collection and Inoculum Preparation

Multi-drug-resistant bacterial strains (*Escherichia coli, Pseudomonas spp., Klebsiella spp., Salmonella spp.,* and *Staphylococcus aureus*) were obtained from the Department of Plant and Environmental Biotechnology, Sylhet Agricultural University. Bacteria were cultured on nutrient agar, and inoculums were prepared in nutrient broth to match 0.5 McFarland standard (1×10^8 cells/mL) for testing.

Antibiogram Profiling and Antimicrobial Activity Determination

Antibiogram tests were conducted using ten antibiotics: doxycycline, chloramphenicol, cefepime, cefuroxime, gatifloxacin, streptomycin, nalidixic acid, kanamycin, gentamicin, and vancomycin. Zones of inhibition were compared with those obtained from plant extracts.

Antimicrobial activity was tested using the disc diffusion method as described by Bauer et al., 1966 and Ahmed et al., 2019. Sterile paper discs (6 mm) saturated with plant extracts were placed on Muller-Hinton agar streaked with bacterial cultures. Before performing the antibiogram, bacteria were maintained on Muller-Hinton agar medium, Plates were incubated at 37°C for 18–24 hours, and zones of inhibition (ZI) were measured in millimeters.

Minimum Inhibitory Concentrations (MIC)

MIC was determined by broth microdilution technique using the INT colorimetric method as described by Adeyemo et al., (2022). Bacterial cultures were adjusted to 0.5 McFarland Standard and 100 µL of each log phase bacteria were added to each well individually, and the mixture was then incubated for 18–24 hours at 37 °C. After that, bacteria were exposed to each extract of varying concentrations (200, 100, 50, 25, 12.5 µ

g/mL). The microtiter plates were incubated at 37 °C for 24 hours. Following the incubation period, each well received 50 µL of 0.2 mg/ml of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) (Sigma) as a growth indicator. The plates were further incubated for 30 minutes, and all determinations were carried out in triplicate. A purple-red color that emerged as a result of INT's reduction to formazan represented the growth of microorganisms. The MIC value was indicated as the lowest extract concentration to inhibit bacterial growth.

Analysis of Phyto-active compounds

Following chemical tests were performed to examine the active compounds' presence in plants as described by Ahmed et al. (2019), Trease and Evans (2002), Banu and Cathrine (2015), and Harborne (1998).

Alkaloids (Wagner's test): Reddish-brown precipitate indicates presence.
Flavonoids (NaOH test): Intense yellow color turns colorless with acid.
Coumarins (NaOH test): Yellow color indicates presence.
Saponins (Froth test): Persistent of froth confirms presence.
Tannins (Ferric chloride test): Dark green color indicates tannins.
Phenols (Ferric chloride test): Greenish color confirms phenols.
Quinones (Sulfuric acid test): Reddish color indicates quinones.
Steroids (Sulfuric acid test): Reddish-brown ring indicates presence.

Macronutrient Analysis

Macronutrient content was analyzed as described by Narasimhan, 2014, and Banu and Cathrine, 2015, and tabulated in Table 1.

Table 1. Analysis of macronutrients in medicinal plants.

Names of Test	Method of Test	Precipitation
Reducing Sugar	Benedict's test: 0.5 ml of the filtrate + Benedict reagent (0.5 ml) + heated on a boiling water bath (2 minutes)	Reddish Brown
Carbohydrate	Barfoed's test: Barfoed's reagent (1ml) + 2ml extract + boiling in a water bath (5 minutes)	Reddish Brown
Protein	Millon's Test: 2ml of extract + 40% NaOH (2ml) ---------> shaken it properly.	Yellowish
Amino acid	Ninhydrin Test: 1ml of sample + Ninhydrin Reagent (5 drops) -------> heating in a boiling water bath (2 min).	Purple color
Fats and Oils	0.1 g extract was pressed between filter paper and the paper observed. 2 drops of olive oil on filter paper were added to make a control.	Translucency on the filter paper

Quantitative Phytochemical Analysis

Total Phenol Contents (TPC) and Total Tannin Content (TTC)

As previously described by Keskin-Sasic et al. (2012) and Ahmed et al. (2019), the TPC was recorded after preparing the reaction mixture (20% Na_2CO_3 (1ml), Folin-Ciocâlteu reagent (0.5 ml) and deionized water (4.5 ml) and adding 0.1 ml of extract or various standard concentrations to that mixture. Before calculating the TPC, the absorbance was taken at 760 nm with Shimadzu UV-Spectrophotometer 900 against the reagent blank.

The determination of TTC content was carried out by the Folin-Ciocalteu protocol with slight modification (CI KC and Indira, 2016; Haile and Kang, 2019). The reaction mixture consisted of 35% Na_2CO_3 (1 ml), Folin-Ciocâlteu reagent (0.5 ml), and distilled water (5.5 ml). After that, 0.1 ml of extract or various standard solutions of standard tannic acid was added and kept for at least 30 minutes to complete the reaction. Finally, the absorbance was calculated and recorded at 760nm with Shimadzu UV-Spectrophotometer 900.

Total Flavonoid Contents (TFC)

The TFC was determined using a popular protocol of aluminum chloride (Csepregi et al., 2013; Ahmed et al., 2019). However, the 0.1 ml of extract or various standards was slowly added to the freshly prepared reaction mixture (0.2 ml 10% aluminum chloride solution, 0.2 ml 1M potassium acetate solution). 5.0 ml of distilled water was used to adjust the absorbance. Finally, the absorbance was taken at 420 nm.

Antioxidant Activity

The DPPH assay was performed to evaluate free radical scavenging activity (Susanti et al., 2007 and Ahmed et al., 2019). For the preparedness of the reaction mixture, 1 ml of the extract was added to 3 ml of DPPH solution (0.004%) and incubated for 30 minutes in the dark. 517 nm was maintained to take the absorbance, and the calculation of antioxidant activity was done as:

Percent Inhibition= [(AD-AS)/AD] ×100
Where, AD = Absorbance (DPPH)
AS = Absorbance (extract/ascorbic acid)

Data Analysis

Three replications were conducted for each experiment, and data was analyzed by GraphPad Prism 8.4.3.

Results

Preparation of Plant Leaves and Test Organisms

Leaves of seven ethnomedicinal plants were soaked in 80% methanol and ethanol for 72 hours and filtered for extraction. Five test organisms were collected as stock cultures and prepared in the nutrient broth. McFarland solution was adjusted to 0.5, equivalent to 1×10^8 bacterial cells/mL.

Antibiogram Profiling

Antibiograms of isolates confirmed multidrug resistance with tested antibiotics. All bacterial isolates showed complete sensitivity to Doxycycline (D), Gatifloxacin, and Vancomycin antibiotics; On the contrary, all test organisms exhibited 100% resistance to three antibiotics, Chloramphenicol (CL), Cefepime (CPM), and Cefuroxime (CXM) (Table 2-3). Klebsiella spp. exhibited the highest resistance to chloramphenicol (CL), cefepime (CPM), cefuroxime (CXM), streptomycin (S), and kanamycin (K) while *Pseudomonas spp*. displayed 50% susceptibility and *Staphylococcus spp.* showed 70% susceptibility to doxycycline, gatifloxacin, and other antibiotics. The zones of inhibitions varied from 7.33±0.33mm to 28.33±0.88mm (Figure 3, Table 2).

Table 2. Antibiogram Profiling of Five Test Organisms

Antibiotics	Test Organisms				
	Escherichia coli	***Staphylococcus aureus***	***Salmonella spp***	***Pseudomonas spp***	***Klebsiella spp***
Doxycycline (DO)	17.67±0.33	22±0.58	17.67±0.33	24±0.58	22±0.58
Chloramphenicol (CL)	8.67±0.33	7.68±0.33	7.33±0.33	8.33±0.33	8.33±0.33
Cefepime (CPM)	NZI	NZI	NZI	NZI	NZI
Cefuroxime (CXM)	NZI	NZI	NZI	NZI	NZI
Gatifloxacin (GAT)	26±0.58	23.67±0.33	23.33±0.33	28.33±0.88	22.33±0.33
Streptomycin (S)	15±1.53	21±0.58	8.33±0.33	24.67±0.33	NZI
Nalidixic Acid (NA)	15.67±0.33	20.33±0.33	11±0.58	23.67±0.33	NZI
Kanamycin (K)	14±0.58	19±0.58	17±0.58	20±0.58	12.67±0.88
Gentamicin (CN)	10±0.58	19±0.58	10±0.57	20±0.58	NZI
Vancomycin (VA)	15.33±0.88	18.67±0.88	19±0.57	19.33±0.88	19±1.15

NZI= "No Zone of Inhibition". Zone of Inhibition measured in millimeter (mm).

Table 3. Antibiotic Resistance Profiling of Bacterial Species

Antibiotics	Test Organisms						% S	% I	% R
	Dose (µg)	EC	SA	SS	PS	KS			
Doxycycline (DO)	30	S	S	S	S	S	100 (5)	0 (0)	0 (0)
Chloramphenicol (CL)	10	R	R	R	R	R	0 (0)	0 (0)	100 (5)
Cefepime (CPM)	30	R	R	R	R	R	0 (0)	0 (0)	100 (5)
Cefuroxime (CXM)	30	R	R	R	R	R	0 (0)	0 (0)	100 (5)
Gatifloxacin (GAT)	5	S	S	S	S	S	100 (5)	0 (0)	0 (0)
Streptomycin (S)	10	S	S	R	R	R	40 (2)	0 (0)	60 (3)
Nalidixic Acid (NA)	30	I	S	R	S	R	40 (2)	20 (1)	40 (2)
Kanamycin (K)	30	I	S	I	R	R	20 (1)	40 (2)	40 (2)
Gentamicin (CN)	10	R	S	R	S	R	40 (2)	0 (0)	60 (3)
Vancomycin (VA)	30	I	S	S	S	S	80 (4)	20 (1)	0 (0)

EC= "Escherichia coli", SA= "Staphylococcus aureus", PS= "Pseudomonas spp", KS= "Klebsiella spp", SS= "Salmonella spp", S= "Susceptibility", I= "Intermediate", R= "Resistance"

Table 4. Antibiotic Resistance Percentage of Pathogens

%	***Escherichia coli***	***Staphylococcus aureus***	***Salmonella spp***	***Pseudomonas spp***	***Klebsiella spp***
%Susceptibility	30%	70%	30%	50%	30%
% Resistance	40%	30%	60%	50%	70%
% Intermediate	30%	-	10%	-	-

Antibacterial Activity and Minimum Inhibitory Activity (MIC) of Medicinal Plant Extracts

The ethanolic and methanolic extracts were tested for antibacterial activity against five pathogens using the disc diffusion method. Methanolic extracts generally outperformed ethanolic extracts. The antibacterial potentiality of the tested Manipuri plants is presented in Table 5. Both *Oxalis corniculata* and *Melastoma malabathricum* inhibited the growth of *Salmonella sp*. The current study also revealed the minimum inhibitory activity of selected plants which are presented in Table 6. MIC values, determined by the broth dilution method, ranged from 12.5 µg/mL to 100 µg/mL. Both methanolic and ethanolic extracts of *Oxalis corniculata* exhibited the highest inhibition zone against *Klebsiella spp* (Table 5). Out of seven plants, five plants were active against *Pseudomonas sp* (Figure 4, Table 5). Both methanolic and ethanolic extracts of *Aerva sanguinolenta* exhibited the most inhibitory potentiality against *Staphylococcus aureus, Pseudomonas spp,* and *Escherichia coli* among five pathogenic bacteria. The methanolic extract of *Mikania scandens* was mostly active against *Staphylococcus aureus, Pseudomonas spp,* and *Escherichia coli* when comparing disc diffusion records and MIC values. Among all plants, four plant species (*Oxalis corniculata*, *Aerva sanguinolenta, Melastoma malabathricum, and Mikania scandens*) exerted better inhibitory activity against pathogenic *E. coli* (Table 5 and Table 6).

Table 5. Antibacterial Zone of inhibition (mm ± SEM)

Plant Samples	Zone of inhibition (mm ± SEM)				
	Salmonella spp	***Staphylococcus aureus***	***Pseudomonas spp***	***Klebsiella spp***	***Escherichia coli***
HC (M)	NZI	NZI	8.33±0.33	8±0.57	10±0.58
HC (E)	NZI	NZI	8±0.58	7.67±0.33	9±0.57
OC (M)	13.33±0.88	13±1.15	12.33±0.88	15±0.57	13.33±0.88
OC (E)	15.0±0.57	14.33±0.33	15±0.58	14.33±0.33	14±0.58
AS (M)	NZI	17.33 ±0.88	12.67±0.88	NZI	16.33±0.88
AS (E)	NZI	15.33±1.86	13.67±0.88	7.67±0.67	13.33±1.20
SA (M)	9.67±0.66	17±0.57	NZI	9.33±1.20	11.33±0.88
SA (E)	10.33±0.88	14±0.57	NZI	NZI	NZI
MM (M)	17±0.58	12.33±0.67	11.33±0.88	11.33±0.88	16±1.16
MM (E)	17.67±0.67	14.33±0.88	13.32±0.88	10±0.58	13.67±0.88
MS (M)	NZI	15.67±0.88	12.33±0.87	9.67±1.20	11.33±0.88
MS (E)	NZI	12.33±0.88	12.67±0.33	NZI	14±0.57
LA (M)	8.33±0.88	NZI	NZI	NZI	10.33±0.67
LA (E)	NZI	NZI	NZI	NZI	8±0.58

Values represent mean (zone of inhibition) of four replicates ± SEM, NZI=No zone of inhibition. M=methanol, E= Ethanol, Houttuynia cordata (HC), *Oxalis corniculata* (OC), *Leucas aspera* (LA), *Mikania scandens* (MS), *Aerva sanguinolenta (*AS), *Spilanthes acmella* (SA), *Melastoma malabathricum* (MM*).*

Table 6. Determination of Minimum Inhibitory Concentrations (MIC) value using INT

Plant Samples (µg/ml)	Salmonella *spp*	Staphylococcus *aureus*	Pseudomonas *spp*	Klebsiella *spp*	Escherichia *coli*
HC (M)	-	-	100	25	50
HC (E)	-	-	100	100	25
OC (M)	50	50	50	25	25
OC (E)	12.5	25	50	50	50
AS (M)	-	50	50	-	100
AS (E)	-	50	50	100	50
SA (M)	100	50	-	-	50
SA (E)	100	50	-	-	-
MM (M)	25	25	50	50	12.5
MM (E)	12.5	25	25	50	25
MS (M)	-	25	25	100	12.5
MS (E)	-	50	50	25	25
LA (M)	50	25	-	-	100
LA (E)	-	100	-	-	50

M=methanol, E= Ethanol, Houttuynia cordata (HC), Oxalis corniculata (OC), Leucas aspera (LA), Mikania scandens (MS), Aerva sanguinolenta (AS), Spilanthes acmella (SA), Melastoma malabathricum (MM). Values represents in µg/mL.

Table 7. Preliminary phytochemical analysis of screened medicinal plant species (methanolic extract and ethanolic extract)

Plant extracts	Alkaloids	Phenol	Flavonoid	Coumarin	Steroids	Quinones	Saponin	Tannin
Test name	Wagner's Test	Ferric	2% NaOH Chloride Test	10%	H_2SO_4 NaOH	H_2SO_4 conc.	Froth conc.	Ferric Test chloride test
HC (M)	++	+	+	+	+++	++	-	+++
HC (E)	++	+	+	+	+	+	-	+++
OC (M)	+++	+	+++	++	++	+	-	+
OC (E)	+++	+	+++	++	+++	+	-	+
AS (M)	-	+	+++	+	-	+	-	++
AS (E)	-	+	+++	+++	-	+	-	++
SA (M)	-	+	+	-	-	+	-	++
SA (E)	+	+	+	-	-	+	-	++
MM (M)	+++	++	++	+	+++	++	++	+++
MM (E)	++	++	+	+	+	+	-	+++
MS (M)	-	++	++	+	-	+	-	+++
MS (E)	-	++	+++	+	-	+	-	++
LA (M)	+	+	+	+	-	+	-	++
LA (E)	+	+	+	+	+	+	-	++

'+' = indicates low concentration of phytochemicals, '++' = shows moderate concentration, '+++ '= shows high concentration, '-'= indicates absence of phytochemicals. M=methanol, E= Ethanol, Houttuynia cordata (HC), Oxalis corniculata (OC), Leucas aspera (LA), Mikania scandens (MS), Aerva sanguinolenta (AS), Spilanthes acmella (SA), Melastoma malabathricum (MM).

Phytoactive chemicals and macronutrient screening of medicinal plants

Phytochemical analysis of alcoholic (methanolic and ethanolic) extract revealed secondary metabolites such as alkaloids, tannins, flavonoids, phenols, and coumarins in ethanol and methanol extracts (Table 7). Notably, saponins were detected only in the methanolic extract of *M. malabathricum*. Furthermore, the results of biochemical tests for macronutrient analysis are illustrated in Table 8.

Table 8. Preliminary macronutrient analysis of medicinal plants

Plant Samples	Carbohydrate Barfoed's Test	Reducing sugar Benedit's Test	Protein Test Millon's Test	Amino Acid Ninhydrin Test	Fats and oils Translucency
HC (M)	+	++	++	++	+
HC (E)	+	++	++	++	-
OC (M)	-	-	+++	++	+
OC (E)	-	-	+++	++	+
AS (M)	-	-	-	-	-
AS (E)	-	-	-	-	-
SA (M)	-	++	-	-	-
SA (E)	-	++	-	-	-
MM (M)	++	+++	++	++	+
MM (E)	++	+++	++	++	-
MS (M)	-	++	-	-	+
MS (E)	-	++	-	-	+
LA (M)	-	++	++	++	-
LA (E)	-	++	+	++	-

'+' = indicates low concentration of phytochemicals, '++' = shows moderate concentration, '+++ '= shows high concentration, '-'= indicates absence of phytochemicals. M=methanol, E= Ethanol, Houttuynia cordata (HC), Oxalis corniculata (OC), Leucas aspera (LA), Mikania scandens (MS), Aerva sanguinolenta (AS), Spilanthes acmella (SA), Melastoma malabathricum (MM).

Determination of TPC, TFC and TTC

Folin-Ciocalteu based method revealed that total phenolic content was highest in the methanolic extract of *Houttuynia cordata* (94.53±0.45 mg GAE/g DLP) and ethanolic extract of O. corniculata (90.49±0.38 mg GAE/g DLP) **(Table 9**). On the other hand, quercetin-based assays displayed the maximum flavonoid content in the ethanolic extract of *M. malabathricum* (78.76±0.67 mg QE/g DLP). Other notable samples included methanolic extracts of Leucas aspera (73.97±0.64 mg QE/g DLP). In addition, Tannin content was highest in ethanolic and methanolic extracts of M. malabathricum (51.20±0.09 mg and 50.50±0.17 mg TAE/g, respectively), highlighting its significant antibacterial potential. The result of the quantitative analysis of tested Manipuri plants are summarized in **Table 9**.

Table 9. Determination of Total Phenolic Content, Total Flavonoid Content, Total Tannin Content for Ethanolic and Methanolic Extracts of Selected Plants

	TPC	**TFC**	**TTC**
Plant Samples	**mg GAE/ g DLP** (Mean ± SEM)	**mg QE/ g DLP** (Mean ± SEM)	**mg QE/ g DLP** (Mean ± SEM)
HC (M)	94.53± 0.45	64.62 ± 0.56	28.85 ± 0.19
HC (E)	84.73± 0.38	44.21 ± 0.43	42.95± 0.10
OC (M)	54.57± 0.32	20.23 ± 0.57	23.66± 0.18
OC (E)	90.49± 0.38	35.19 ± 0.63	21.79 ± 0.17
AS (M)	30.22± 0.34	41.85± 0.65	18.63± 0.19
AS (E)	46.46± 3.55	38.20± 0.88	16.55± 0.24
SA (M)	44.96± 0.31	47.38± 0.49	20.73± 0.13
SA (E)	36.73± 0.31	52.99± 0.63	20.08 ± 0.17
MM (M)	87.47± 0.17	59.25± 0.78	50.50 ± 0.17
MM (E)	78.93± 0.42	78.76± 0.67	51.20 ± 0.09
MS (M)	64.93±0.31	71.36± 0.51	33.34± 0.17
MS (E)	62.81± 0.30	56.40± 0.49	26.31± 0.17
LA (M)	69.98± 0.48	73.97± 0.64	30.53 ± 0.20
LA (E)	48.38 ± 0.38	68.44 ± 0.56	36.60± 0.22

[Each value represents mean ± SEM of three replicates DLP= Dry Leaves Powder, GAE= gallic acid equivalent, QE= quercetin equivalent, TAE= Tannic Acid Equivalent]

Antioxidant Analysis

Antioxidant properties, assessed via DPPH-scavenging assays, revealed strong activity in methanolic extracts of *Cordata*, *M. malabathricum* and *O. corniculata* (Table 10). As depicted by Table 10. This result supports their potential as natural antioxidants.

Table 10. % of SCV (free radical scavenging activity) of Plant Extracts

Plant Samples	**%SCV**	**Plant Samples**	**%SCV**
HC (M)	84.22 ± 0.23	HC (E)	83.31± 0.23
OC (M)	82.32± 0.30	OC (E)	82.79± 0.38
AS (M)	77.17± 0.27	AS (E)	79.72± 0.38
SA (M)	80.11± 0.30	SA (E)	77.82± 0.10
MM (M)	83.79± 0.27	MM (E)	83.23± 0.16
MS (M)	81.15± 0.35	MS (E)	80.42± 0.20
LA (M)	81.89± 0.34	LA (E)	80.29± 0.27

[Each value represents mean ± standard deviation of three independent experiments. M=methanol, E= Ethanol, Houttuynia cordata (HC), Oxalis corniculata (OC), Leucas aspera (LA), Mikania scandens (MS), Aerva sanguinolenta (AS), Spilanthes acmella (SA), Melastoma malabathricum (MM).]

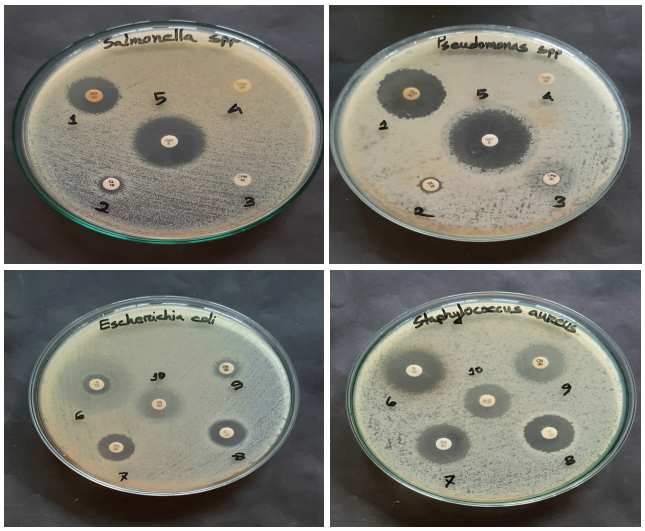


Figure 3. Antibiogram Profiling of tested organisms. Figures showing zone of inhibition of standard antibiotics including Doxycycline (DO), Chloramphenicol (CL), Cefepime (CPM), Cefuroxime (CXM), Gatifloxacin (GAT), Streptomycin (S), Nalidixic Acid (NA), Kanamycin (K), Gentamicin (CN), and Vancomycin (VA) against some pathogenic bacteria.

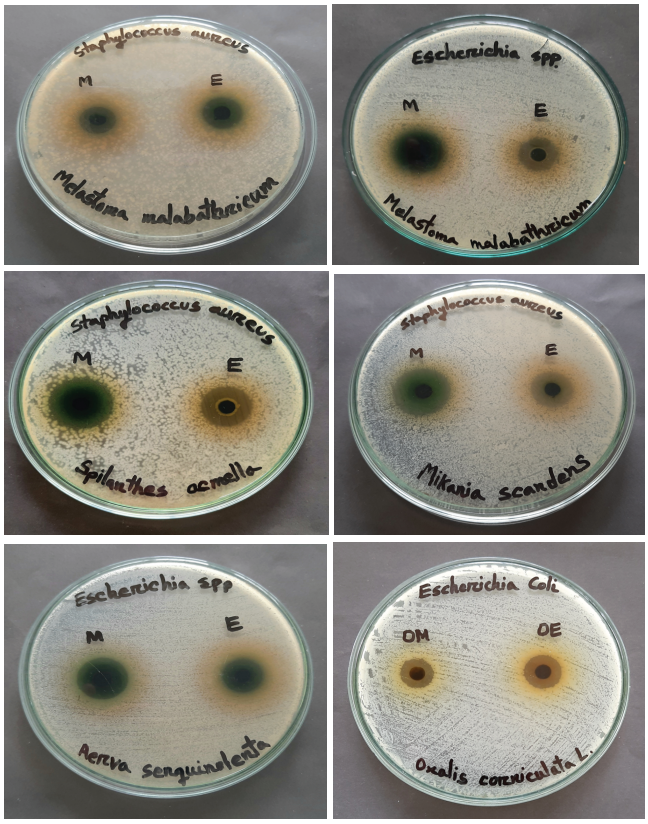


Figure 4. Antibacterial activity profiling of selected plant extract. Figures showing zone of inhibitions (mm) with methanolic and ethanolic crude extracts of plants against pathogenic bacteria. M= methanolic extract E= ethanolic extract.

Discussion

Phytochemical analysis

The current study unveils the presence of diverse phytoactive metabolites in the water and ethanol extract of the tested samples (Table 7). However, alkaloids were found in higher concentrations; phenolic compounds, flavonoids, and steroids were found in moderate concentrations. Among all plants, extracts of *Spilanthes acmella* showed poor results in phytochemical tests; they showed the presence of phenol, quinone, and tannin. *Aerva sanguinolenta* also showed the presence of tannin, quinones, coumarin, and flavonoids. *Melastoma malabathricum* exhibited the highest result of the presence of all secondary metabolites. The bioactive compounds,specifically, phenolic compounds and flavonoids disrupt microbial membranes, inactivate enzymes, and bind to bacterial cell walls, effectively inhibiting bacterial growth (Tako et al., 2020; Isnaini et al., 2019). According to Santhi et al. (2011), these substances acted as natural antibiotics, helping the body fight off microbial invasion.

Analysis of TPC and TFC

The presence of flavonoids and phenolic compounds facilitates the plant's ability to be used in herbal medicines (Tungmunnithum *et al*., 2018). *M. malabathricum* methanolic extract was found to have the highest number of total flavonoids (78.76± 0. mg QE/g DLP), while *A. sanguinolent*a methanol extract had the lowest amount (20.23 ± 0.57 mg QE/g DLP) (Table 9, Figure 18). *M. malabathricum*'s ethanolic and methanolic extracts had maximum total tannin contents of 51.20 ± 0.09 mg TAE/g DLP and 50.50 ± 0.17 mg TAE/g DLP, respectively. Tuyen et al. (2018), found a higher level of tannin in the *H. cordata* extract. The ethanolic extract of *A. sanguinolenta* had the lowest tannin content (16.55 ± 0.24 mg TAE/g DLP). There could be environmental factors causing the variation in the total phenolic and flavonoid contents.

Antioxidant Properties

Plant extracts exhibited antioxidant properties, with *H. cordata* showing the highest scavenging activity, which was 84.22 ± 0.23. As per Tuyen *et al.*'s (2018) study, methanol extract exhibited the highest level of antioxidant activity in DPPH, while ethanol extract followed suit. *M. malabathricum* and *O. corniculata* demonstrated significant free radical scavenging activity, attributed to their flavonoids and phenolics. *L. aspera* leaves had moderate antioxidant activity compared to roots. Correlation analysis showed a strong positive relationship between phenolic content and antioxidant capacity. A positive correlation (*p-value <0*.0001) was observed between phenolic content and free radical ($DPPH^{\cdot}$) scavenging activity.

Antibacterial and MIC activity

Various degrees of antibacterial activity had been found with plants against tested bacteria. In this study, *M. malabathricum* and *Oxalis corniculata* extracts showed relatively higher levels of antibacterial potential, on the contrary, *Leucas aspera* extracts showed less antibacterial activity. The methanolic extract of *M. malabathricum* leaves showed maximum zones of 17±0.58 mm, 12.33±0.88 mm, 11.33 mm, 11.33mm, and 16±0.88 mm, respectively, for the *Salmonella spp., Staphylococcus aureus, Pseudomonas spp., Klebsiella spp*., and *Escherichia spp*. Ahmed et al. reported the zone of inhibition of *M. malabathricum* against *Staphylococcus aureus* (13.75±0.96) *Salmonella sp.* (16.75±0.5), Pseudomonas aeruginosa (14.5±0.58), *Klebsiella pneumoniaea* (12.25±0.96), and E. coli (11±0.82). The ethanolic extracts also showed a maximum zone against those five organisms. Besides this, the highest inhibition zones (17.33 ±0.88mm and 17±0.57 mm)

were shown by *Aerva sanguinolenta* and *Spilanthes acmella* against *Staphylococcus spp*. The plant extract from *Aerva sanguinolenta* showed very good control over *Escherichia spp., Staphylococcus spp*., and *Pseudomonas spp*. in the antibacterial activity analysis. The variability in antibacterial potency may be attributed to the presence of medicinally active compounds in the tested crude extracts. The crude extract of plants generally contains polyphenol that exerts antibacterial potency (Bouarab Chibane et al., 2019).

The minimal inhibitory concentration (MIC) obtained with all plants' ethanol and methanol extracts ranged from 12.5 µg/mL to 100 µg/mL. Similarly, *O. corniculata* and *Mikania scandens* showed strong efficacy with comparable MIC values (Table 6). However, the *M. malabathricum* and *Oxalis corniculata* methanolic and ethanolic extracts had the highest activity towards all organisms after incubation. *M. malabathricum* demonstrated the lowest MIC (12.5 µg/mL), indicating potent antibacterial activity. For example, *Escherichia spp*., one of the multi-drug resistant bacteria, was highly susceptible to the methanolic extract of *Mikania scandens* and *Melastoma malabathricum*, showing a MIC value of 12.5 µg/mL (Table 6). Similarly, O. corniculata and Mikania scandens showed strong efficacy with comparable MIC values (Table 6). Likewise, *Oxalis corniculata*'s methanolic and ethanolic extracts demonstrated bacteriostatic activity against every test organism at a MIC value less than 100 µg/mL. The methanolic extract of *H. cordata* was the most effective in inhibiting the growth of *Klebsiella,* with an MIC value of 12.5 µg/mL. *Klebsiella species* and *Pseudomonas species* growth was not inhibited by *Leucas aspera* and *Spilanthus aspera* extract. Ethanolic extracts of *O. corniculate* and *M. malabathricum* showed the highest inhibitory activity (MIC 12.5 µg/mL) against *Salmonella spp*. In the case of *Staphylococcus spp*., all plant extracts displayed a moderate to minimum result except *H. cordata*.

Conclusion

The study demonstrates that the selected ethnomedicinal plants possess significant antibacterial and antioxidant activities, attributed to their rich phytochemical profiles. Among them, *M. malabathricum* and *O. corniculata* emerged as the most promising candidates for further investigation as natural antimicrobial and antioxidant agents.

Declarations

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Availability of Data: All datasets generated or analyzed during this study are available from the corresponding author upon reasonable request.

Authors' Contributions: SS and SRA contributed equally to conducting the study. FMAH and KZ designed the study, while KZ, SS, and SRA analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript.

Competing Interests: The authors declare that they have no competing interests.

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