Research Article

Antioxidant and Antimicrobial Potentiality Assessment of Bioactive Leaf Extracts of Four Traditionally Used Medicinal Plants

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Submission Date: 05-06-2024; Revision Date: 19-07-2024; Accepted Date: 22-08-2024.

ABSTRACT

Aim: The present study involves the screening and assessment of phytochemical composition of *V. negundo, A. carambola, A. indica* and *J. gendarussa* leaves extract. The extracts were further assessed for their antioxidant and antimicrobial potentialities against *Staphylococcus aureus, E. coli, Enterococcus* which have clinical importance in the disease biology. **Material and Methods:** Leaves of four plants, namely, *Vitex negundo*, *Averrhoea carambola, Azadirachta indica and Justicia gendarussa*, were extracted with hexane, chloroform, ethanol and methanol to determine phytochemical potential and their antioxidant and antimicrobial activity. Phytochemical potentiality was tested by spectrophotometric methods and the antimicrobial activities against *Staphylococcus aureus, Escherichia coli, Enterococcus* were screened using disc diffusion methods. Antimicrobial activity was calculated by measuring the diameter of the Inhibition Zone (IZ) around the discs. **Results:** The qualitative analysis in the study identified polyphenols, flavonoids, carbohydrates and tannins in the extracts and the amount of flavonoids, phenols, tannins and carbohydrates were quantitatively determined. All extracts contained alkaloids, flavonoids, cardiac glycosides and tannins, indicating that they had rich phytochemical profiles. Significantly, the chloroform extract of *Vitex negundo* contained the most carbohydrates, whereas the chloroform extract of *Justicia gendarussa* displayed the highest level of flavonoids. Whereas, the methanol extract of *Vitex negundo* displayed considerable phenolic components and the hexane extract of *Justicia gendarussa* showed the best antioxidant activity. The methanol and chloroform extracts of *V. negundo* possess antimicrobial properties in this study. **Conclusion:** The results of the present investigation suggest that studied plants are excellent sources of nutraceuticals against various ailments. The methanol extract of *Vitex negundo* displayed considerable phenolic components and the hexane extract of *Justicia gendarussa* contained the highest antioxidant activity. As methanol extract of *V. negundo* has significant antimicrobial property, therefore, the selected extract may be explored by phytomolecular analysis as an antimicrobial agent.

Keywords: Phytochemical, Antioxidant, Antimicrobial, Medicinal supplements.

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www.ajbls.com

DOI: 10.5530/ajbls.2024.13.60

INTRODUCTION

The common side effects of synthetic drugs and the evolution of drug-resistant mechanisms are demanding a sustainable alternative for treatment. Although synthetic drugs show better absorption properties in the physiological system, they exert some negative

effects on the body.[1] Herbal drugs are often free from unwanted side effects in the physiological systems and also more suitable for poor people in the society.[2] Plants are a valuable natural source of medication because they provide a wide variety of clinical benefits on human including antioxidant and antimicrobial properties.[3,4] A wide fraction (~80%) of the population worldwide are dependent on plant-based medicines for their immediate healthcare needs. This preference stems from plant-based medicine's greater compatibility and adaptability, which frequently has fewer side effects. Consequently, complementary medicinal systems offer an appealing alternative to synthetic drugs.[5] It is worth considering that throughout history, approximately 30% of viable plant species have been used in a complementary medicinal system. This is attributable to the diverse and unique composition of bioactive molecules found in plants. Notable drugs such as opium, aspirin and quinine have a long history of use as herbal medicines. Despite considerable advances in synthetic medicine, it is worth noting that traditional medicine derived from numerous natural sources remains the primary curative approach against a wide range of ailments among the majority of people in different countries, including India.[6] The plants' secondary metabolites like alkaloids, flavonoids, tannins, phenolic compounds, etc.

Vitex negundo (family Verbenaceae) is an erect small shrub, traditionally considered to possess the medicinal properties of all its parts, exerting its effects on treating inflammation, eye disease, toothache, cancers, rheumatoid arthritis and sinuses.[7] *V. negundo* is native to India along with a few Eastern and Southern Asian and African countries. In the Indian medicinal system, *V. negundo* has been used to treat different gastrointestinal disorders like diarrhea, indigestion etc., cough, sore throat and several skin-related ailments. Decoction of mixture of *V. negundo* L. leaf and *Piper nigrum* is active in curing mucus inflammation and troubled hearing, while a mixture of *V. negundo* L. and sugarcane can reduce swelling. *Averrhoea carambola* (family-Oxalidaceae) is an evergreen tree or shrub having medicinal properties of antioxidant, hypoglycemic, hypotensive, hypocholesterolemic, anti-inflammatory, anti-infective, antitumor, immunomodulatory, antipyretic and antihelmintic activity.[8] *A. carambola* is native to India along with a few tropical countries like Malaysia, Indonesia and the Philippines. In the Ayurvedic medicinal system, it has been used as a digestive tonic, mood stabilizing agent. In addition, it is also used against fever, piles (specifically for inner piles), diarrhea, vomiting, hyperdipsea, haemorrhoids, intermittent fever and scrabies to cure bleeding. It has prominent antiscorbutic properties, antioxidant activity and anti-astringent potentiality. The ripe fruit is regarded for curing bleeding. *Azadirachta indica* (family Meliaceae) is a fast-growing tree native to India, Pakistan, Bangladesh and Nepal and is used as an antioxidant agent, anticancer agent, anti-inflammatory agent, hepatoprotective agent, wound healer, anti-diabetic agent, antimicrobial agent etc.[9] All parts of *A. indica* are used for medicinal purposes. *Justicia gendarussa* (family Acanthaceae) is an erect shrub used to treat ailments like fever, hemiplegia, rheumatism, arthritis, headache, muscle pain, and earache in the traditional system of disease management.[10]

Thus, all the plants have a significant role in the traditional and alternative medicine management system of Indian culture as a whole. Therefore, in the present study (Figure 1), leaves of *V. negundo, A. carambola, A. indica* and *J. gendarussa* have been selected as the source of bioactive molecules for their medicinal value analysis. Further, to extract the maximum content of the phytochemicals in our study we have used hexane, chloroform, ethanol and methanol as the solvent system for the study and thus, we have four plants leave extracts for our study to analyse its different phytochemicals content. Further, the extracts were broadcasted for their antioxidant and antimicrobial potentialities against *Staphylococcus aureus, E. coli, Enterococcus* which have clinical importance in the disease biology.

MATERIALS AND METHODS

Acquisition of plant materials

The fresh and healthy leaves were collected from mature plants of *V. negundo, A. carambola, A. indica and J. gendarussa* growing at the local area of the laboratory. The fresh leaves were separated and instantly packed in a polythene bag. The leaves were washed thoroughly and rinsed with distilled water, keeping at room temperature to dry in a shaded environment.[11]

Extract Preparation

The dried leaves were grinded and subsequently extracted by different solvents at increasing polarities in the sequence of hexane, chloroform, ethanol and methanol in a Soxhlet's extractor for 15 min. Whatman No.1 filter papers were used to filter the liquid extracts. The filtrate extracts were dried under a rotary evaporator to get a solid residue and preserved in the refrigerator for the subsequent experiment.[12]

Qualitative phytochemical screening of the plant extract

The crude extracts of hexane, chloroform, methanol and ethanol were each dissolved in their respective

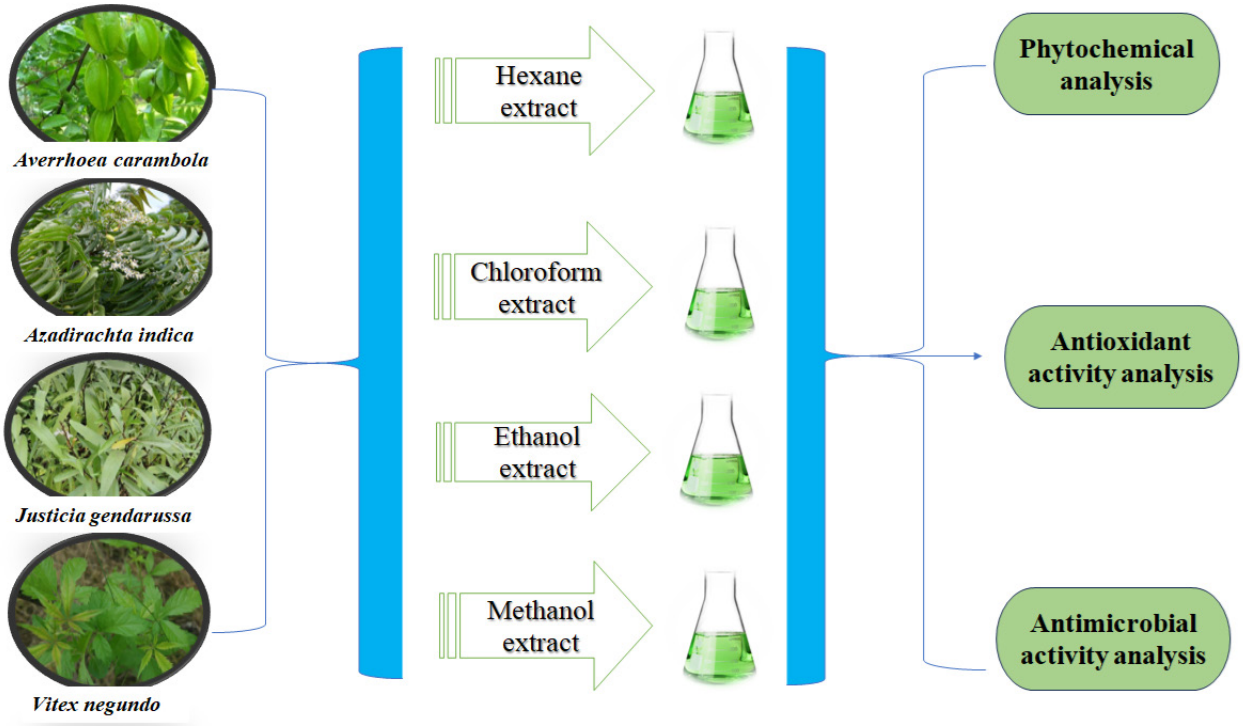

Figure 1: Flowchart showing the present study.

solvents. These solutions were used for qualitative phytochemical screening, which included the evaluation of alkaloids, flavonoids, tannins, terpenoids, cardiac glycosides, phenolic compounds, carbohydrates, amino acids, anthraquinones and phlorotannins.[13] The screening procedures were carried out in accordance with Harbourne's method.[14]

Determination of total phenol content

The total phenol content of the crude leaf extracts was evaluated by spectrophotometric analysis, which used an oxidation/reduction reaction initiated by the Folin-Ciocalteu reagent.[15] A mixture of Folin reagent (1.0 mL), sodium carbonate (0.8 mL of 1 M working solution) and a small volume (0.1 mL) of each sample were incubated at 50ºC for 5 min. The absorbance of the samples was measured using a spectrophotometer at 765 nm considering distilled water as the negative control for this experiment.

Determination of total flavonoid content

The flavonoid content of the extracts was estimated by the aluminium chloride spectrophotometric method.[16] A mixture of 1 mL extract and 0.5 mL of 10% aluminium chloride was incubated for 5 min at 50ºC. The incubated mixture was mixed with 0.05 mL of 1 M potassium acetate, followed by (after 6 min) adding 1.4 mL of distilled water. The reaction volume was stored for 30 min at room temperature. Absorbance of the solution was measured at 415 nm against quercetin as a standard solution and distilled water negative control.

Determination of carbohydrate content

The carbohydrate content in the extracts was determined by mixing 0.5 mL extract with 4.0 mL of anthrone reagent. The reaction volume was made up to 5.0 mL by adding distilled water and stored at room temperature for 30 min. Carbohydrate content was calculated by determining the absorbance of the reaction mixture at 620 nm.[17]

Determination of tannin content

The process[18] of total tannin content determination involves mixing of 0.5 mL of the extract in a test tube and addition of with 100 mg of polyvinyl polypyrrolidone. The volume is made upto 1.0 mL by adding of distilled water. The solution was incubated for 4 hr at 4ºC which is followed by centrifugation of the reaction mixture at 5000 rpm for 5 min. Absorbance was measured at 725 nm for the calculation of the tannin content in the extracts.

Antioxidant Activity determined by DPPH free radical scavenging activity

The antioxidant activity of the extracts was assessed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay for all the crude extracts.[19] Following the method, 100 µL of each extract was added to 1.4 mL

methanol solution of a 10^{-4} M DPPH. The reaction mixture was allowed to stand for 30 min, after which the absorbance was measured at 517 nm against a blank solution consisting of 100 µL methanol in 1.4 mL of DPPH radical solution. The results were quantified as a Radical Scavenging Activity (RSA) using the following equation:

$$RSA\ (\%) = [(Ao - As) / Ao] \times 100$$

Where A_o is the absorbance of the blank and as is the absorbance of the sample.

Antimicrobial susceptible assays

Antibiotic sensitivity test

Antibiotic sensitivity test was conducted to test the antimicrobial efficacy of the extracts against three clinically significant bacterial strains *viz., Staphylococcus aureus* (gram +ve bacteria), *Escherichia coli* (gram -ve bacteria), *Enterococcus* (gram +ve bacteria). *E. coli;* which contributed to various diseases, including pneumonia, urinary tract infections and diarrhea. *Enterococcus* is an infectious facultative anaerobic organism that causes endocarditis, urinary tract infection, prostatitis, intraabdominal infection, cellulitis and wound infection. The sensitivities of all bacterial species were assessed by performing the disk diffusion (Kirby-Bauer) technique. [20] The turbidity of bacterial strain was standardized to ~10 CFU/mL (corresponding to 0.5 Mc Farland standards). Sterile cotton swabs were prepared, dipped into the standardized bacterial suspension and used to inoculate Muller Hinton Agar (MHA) plates. The plates were left to dry for 10 min before placing antibiotic discs on the agar surface, ensuring complete contact with the agar. The inoculated plates were then incubated at 37°C for 24-48 hr. After incubation, the zones of inhibition were measured to determine bacterial sensitivity.

Antibacterial activity of plant extracts

Leaf extracts were evaluated for their antibacterial activity by using disc diffusion methods. The stock solution of leaves extract was prepared by dissolving 0.1 g of the extract with 100 mL of their respective solvents to achieve a final concentration of 100 mg/mL. The stock solution was subsequently diluted to concentrations of 2.5, 5, 10, 20, 50 and 100 mg/mL of extract. 20 µL of each dilution was added into sterile, blank discs 6 mm in diameter. To ensure precise inoculation, 5 µL of the extract was alternately spotted on both sides of the discs, allowing each spot to dry before applying the bacterial lawn. Clindamycin, Gentamycin, and Ampicillin were used as a standard antibiotic for the present antibiotic assay positive control for bacteria *viz., Staphylococcus aureus, E. coli, Enterococcus*. Antimicrobial

Table 1: Preliminary phytochemical analysis of hexane, chloroform, ethanol and methanol extracts of four selected medicinal plants.

Sl. No.	Property	*Vitex negundo*				*Justicia gendarussa*				*Azadirachta indica*				*Averrhoea carambola*			
		Ethanol	Methanol	Hexane	Chloroform	Ethanol	Methanol	Hexane	Chloroform	Ethanol	Methanol	Hexane	Chloroform	Ethanol	Methanol	Hexane	Chloroform
1	Alkaloids	+	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
2	Flavonoids	–	–	+	+	–	–	+	–	+	–	+	+	+	–	+	–
3	Cardiac Glycosides	–	+	+	–	+	–	–	+	+	–	+	–	+	+	–	–
4	Amino acids	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
5	Tannins	–	+	–	–	+	–	–	+	+	+	+	–	–	–		+
6	Saponin	–	+	–	–	–	–	–	+	–	–	+	–	+	+		–
7	Carbohydrate	–	+	–	–	–	–	–	+	–	–	–	+	–	+	–	+
8	Anthraquinone	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
9	Phlobatannin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	Phenolic compound	–	+	–	–	–	+	–	+	+	–	+	+	–	+	+	–
11	Amino acids	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	Terpenoids	+	+	–	–	–	–	–	–	–	–	–	+	+	–	–	–

'+' indicates presence, and '-' indicates the absence of the respective phytochemicals.

activity was evaluated by measuring the diameter of the Inhibition Zone (IZ) around the discs. The assay was performed in a triplicate manner. Antibacterial activity was quantified as the mean zone of inhibition diameters (mm) produced by the leaf extract.

RESULTS

Preliminary qualitative phytochemicals evaluation

The analysis of phytochemical by hexane, chloroform, ethanol and methanol extracts of *Vitex negundo, Justicia gendarussa, Azadirachta indica, Averrhoea carambola* revealed the presence of phytocompounds in varying proportions (Table 1).

Quantitative phytochemical evaluation in the extracts

Quantitative evaluation of secondary metabolites like phenol, flavonoid, carbohydrate and tannin contents was found affective as shown in Figure 1 (A, B, C, D) respectively. The folin-ciocalteau-induced oxidation/reduction mechanism was used for spectrophotometric quantification of the phytochemicals.

The methanol extract of *V. negundo* showed higher phenol content (Figure 2 (A)) than the other extracts. Chloroform extracts of *V. negundo* and *J. gendarussa* show the presence of high flavonoid content (Figure 2 (B, C)). In contrast, methanol extract (*A. carambola*) and ethanol extract (*V. negundo*) show less amount of carbohydrate and flavonoid content. Chloroform extracts of *V. negundo* and *J. gendarussa* show the presence of high flavonoid content. Whereas, methanol extract (*A. carambola*) and ethanol extract (*V. negundo*) show less amount of carbohydrate and flavonoid content. The ethanol extract of *A. indica* shows high tannin content, while the methanol extract of *V. negundo* exhibits inferior tannin content (Figure 2(D)).

Antioxidant Activity

DPPH RSA

DPPH RSA was performed to quantify the antioxidant activity of all the extracts of these four different

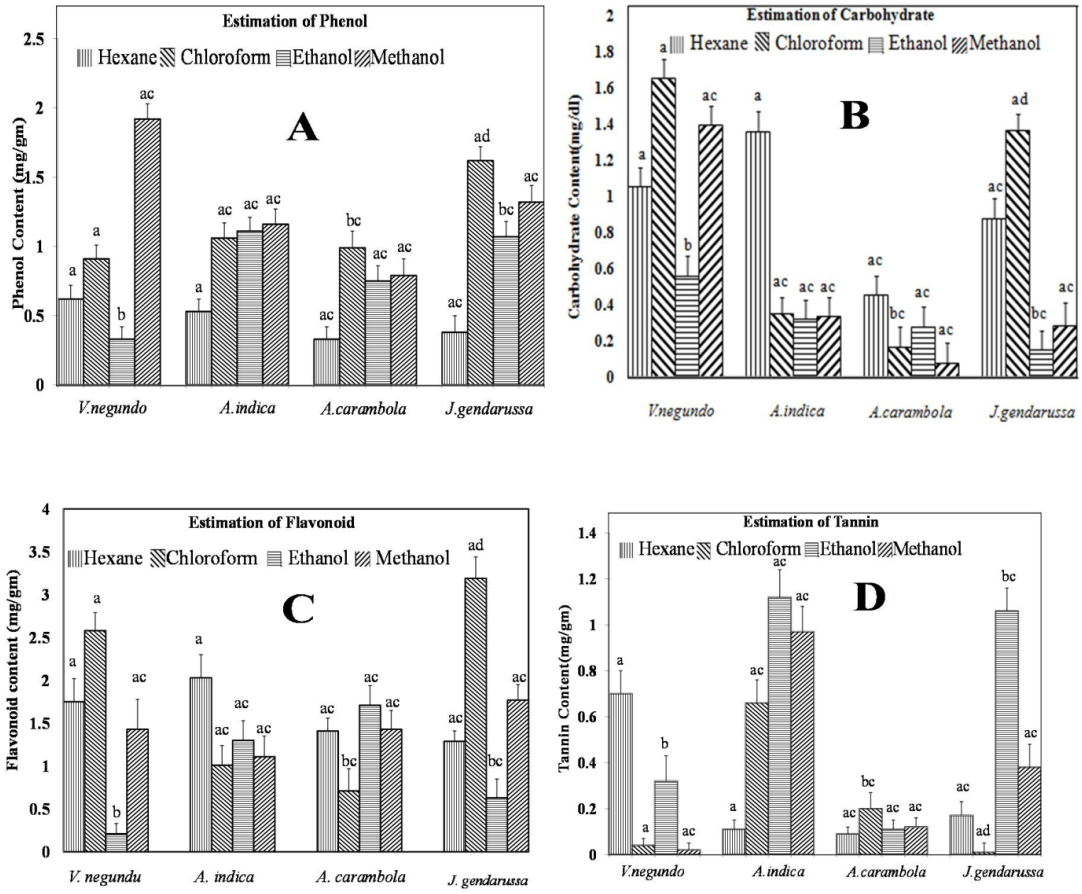

Figure 2: Determination of phenol (A), carbohydrate (B), flavonoid (C), tannin (D) content of hexane, chloroform, ethanol and methanol extracts of *V. negundo, A. indica, A. carambola, J.gendarussa*. Values are the mean of triplicates (*n* = 3) ± standard deviation.

Table 2: Percentage hatching of DPPH RSA of hexane, chloroform, ethanol and methanol extract of all the four plants *V. negundo, A. indica, A. carambola, J. gendarussa.* Values are the means of triplicate (n = 3) ± standard deviation.

Property	*V. negundo*				*A. indica*				*A. carambola*				*J. gendarussa*			
	Ethanol	Methanol	Hexane	Chloroform	Ethanol	Methanol	Hexane	Chloroform	Ethanol	Methanol	Hexane	Chloroform	Ethanol	Methanol	Hexane	Chloroform
Antioxidant activity	0.31± 0.45	0.57± 0.28	0.39± 0.38	0.51± 0.33	0.23± 0.13	0.24± 0.37	0.26± 0.40	0.59± 0.28	0.28± 0.39	0.27± 0.34	0.18± 0.21	0.13± 0.39	0.51± 0.37	0.49± 0.35	0.17± 0.44	0.19± 0.36

medicinal plants. The current study reveals moderate antioxidant activity in all the extracts (Table 2).

Hexane extract of *J. gendarussa* is found to be the most potent DPPH scavenger compared to the other extracts. Whereas chloroform extract of *A. indica shows* less potent to radical scavenging activity. DPPH radical scavenging activity of all the plant extracts in decreasing order is as follows:

Hexane extract: *J. gendarussa* >*A. indica* > *A. carambola* > *V. negundo.*

Chloroform extract: *A. carambola* > *J. gendarussa* > *V. negundo* >*A. indica.*

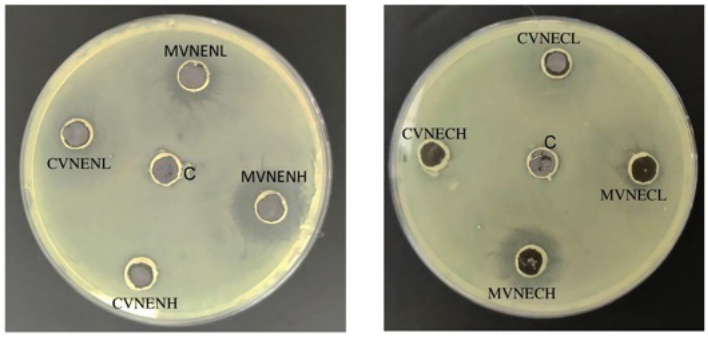

Plate 1 ***Enterococcus*** **Plate 2** ***Escherichia coli***

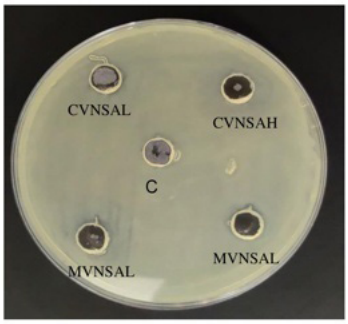

Plate 3 ***Staphylococcus aureus***

Abbreviation:
C: Control
CVNENL: Chloroform extract of *V. negundo* leave against *Enterococcus* at concentration 110 µg/ml
CVNENH: Chloroform extract of *V. negundo* leave against *Enterococcus* at concentration 120 µg/ml
MVNENL: Methanolic extract of *V. negundo* leave against *Enterococcus* at concentration 110 µg/ml
MVNENH: Methanolic extract of *V. negundo* leave against *Enterococcus* at concentration 120 µg/ml
CVNECL: Chloroform extract of *V. negundo* leave against *Escherichia coli* at concentration 110 µg/ml
CVNECH: Chloroform extract of *V. negundo* leave against *Escherichia coli* at concentration 120 µg/ml
MVNECL: Methanolic extract of *V. negundo* leave against *Escherichia coli* at concentration 110 µg/ml
MVNECH: Methanolic extract of *V. negundo* leave against *Escherichia coli* at concentration 120 µg/ml
CVNSAL: Chloroform extract of *V. negundo* leave against *Staphylococcus aureus* at concentration 110 µg/ml
CVNSAH: Chloroform extract of *V. negundo* leave against *Staphylococcus aureus* at concentration 120 µg/ml
MVNSAL: Methanolic extract of *V. negundo* leave against *Staphylococcus aureus* at concentration 110 µg/ml
MVNSAH: Methanolic extract of *V. negundo* leave against *Staphylococcus aureus* at concentration 120 µg/ml

Figure 3: Antimicrobial potentiality of selected plant crude extracts of different solvent fraction.

Ethanol extract: *A. indica* >*V. negundo* = *A. carambola* > *J. gendarussa*.
Methanol extract: *A. indica* > *A. carambola* > *J. gendarussa* > *V. negundo*.

Antimicrobial activity

The four medicinal plant extracts, hexane, chloroform, ethanol, and methanol, were investigated for antimicrobial activity using the disc diffusion method. The study targeted three bacterial strains: *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative) and *Enterococcus* (Gram-positive) (Figure 3).

Among leaf extracts, only methanol and chloroform extract of *Vitex negundo* showed significant Zone of Inhibition (ZOI) at concentration of 110 µg/mL and 120 µg/mL. On the contrary, other study plant extracts do not exhibit significant antimicrobial activity against *Staphylococcus aureus, E. coli, Enterococcus* (Table 3). Thus, from the disc diffusion method, it can be concluded that both methanol and chloroform extract of *V. negundo* possess antimicrobial properties against Gram-positive and Gram-negative bacteria.

DISCUSSION

A considerable amount of flavonoids, cardiac glycoside, tannin, terpenoids, saponin, carbohydrate and phenolic compounds are found in the ethanol and methanol extract of these plants, while a very less amount of phytocompounds are found to be present in hexane extracts. The hexane is non-polar in nature and able to extract a lesser amount of the compound namely alkaloid, flavonoid and a few phenolic compounds. Flavonoids are known as natural biological modifiers that present in hexane, ethanol, chloroform extracts but showed no traces in the methanol extracts. Carbohydrate which constitutes the major edible part of the plant is present in the methanol extract of *V. negundo* and chloroform extract of *J. gendarussa* and *A. indica*.

Cardiac glycoside has shown strong positive result in ethanol extracts but weak positive results in chloroform extracts. In contrast phenolic compound has shown weak positive results in ethanol extracts and strong positive results in methanol extracts of *V. negundo, J, gendarussa, A. carambola*. Tannin test showed weak positive result in the hexane extract of *V. negundo, J, gendarussa, A. carambola* whereas showed a positive result in methanol extract of *V. negundo*, ethanol and chloroform extract of *J. gendarussa*, ethanol, methanol, hexane extract of *A. indica* and chloroform extract of *A. carambola*. Among the four plants screened, the most potent RSA was reported to be the hexane extract of *J. gendarussa*. The chloroform extract of *A. carambola* also showed a positive result. In the antimicrobial property investigation, phyto-extracts were analysed against gram-positive (*Staphylococus*

Table 3: Minimum Inhibitory Concentrations (MIC) (µg/mL) of leaf extracts and their corresponding inhibition zones.

Solvent used	Plant name	*Zone of inhibition (mm)		
		Staphylococcus aureus	*Escherichia coli*	*Enterococcus*
Chloroform	*J. gendarussa*	ND	ND	ND
	A. indica	ND	ND	ND
	A. carambola	ND	ND	ND
	V. negundo	4±0.6	11±0.5	14±0.5
Ethanol	*J. gendarussa*	ND	ND	ND
	A. indica	ND	ND	ND
	A. carambola	ND	ND	ND
	V. negundo	ND	ND	ND
Hexane	*J. gendarussa*	ND	ND	ND
	A. indica	ND	ND	ND
	A. carambola	ND	ND	ND
	V. negundo	ND	ND	ND
Methanol	*J. gendarussa*	ND	ND	ND
	A. indica	ND	ND	ND
	A. carambola	ND	ND	ND
	V. negundo	6±0.8	11±1.2	18±0.7

*ND stands for not detected.

aureus, Enterococcus) and gram-negative (*E. coli*) bacteria. The highest inhibitory zone was recorded with *S. aureus* when exposed to methanol and chloroform extracts of *V. negundo*, highlighting the highest plant's antibacterial efficacy, combating the organism. The effectiveness of plant extracts may indicate the presence of broad-spectrum antibiotic compounds or more generally, metabolic toxins present in the plant. The antimicrobial characteristics of *V. negundo*, *J. gendarussa*, *A. carambola* and *A. indica* suggest the potential for these plants in the formulation of novel antimicrobial therapies. The active principles responsible for these bioactivities are numerous and diverse, most of which fall under the category of secondary metabolites. Among the bacteria and fungi tested *V. negundo*, *A. indica* display noteworthy antimicrobial *J. gendarussa* and *A. carambola* also have shown considerable antimicrobial effect against the bacterial pathogens.

Although the DPPH assay, used in this study for analysis of antioxidant activity has a reproducibility and applicability from the point of its simplicity and rapidity for its results but it cannot accurately measure the antioxidant activity in complex biological systems.[21]. The Disk-Diffusion Test for antimicrobial susceptibility test is a widely used age-old practice in clinical microbiology. It is a cost-effective accurate performance and it is a broad spectrum analytical antimicrobial test against different microorganisms. But it has limitations in terms of its MIC value determination ability and laziness for completion of the process.[22]

Flavonoids are primarily recognized for their antioxidant properties. Flavonoids are prominant compounds that are found in dietary plants as glycosides and contain several phenolic hydroxyl groups on their ring structures. The presence of these groups renders flavonoids as strong antioxidants and capable of effectively scavenging the reactive oxygen species.[23] The flavoinoids are a major secondary metabolite found on *J. gendarussa* and *V. negundo.* [24,25]

Saponin helps in reducing blood cholesterol by preventing its re-absorption that is useful in cardiovascular diseases.[26] It helps in preventing cancer cells from further growing and can lower the risk of the formation of cancers in humans implicating its importance in therapeutics.[27]

Terpenoids, a group of phenolic compounds are one of the most important chemical compounds and have been reported in the reduction of oxidative stress, induction of apoptosis and regulation of cell cycle. Tannin rich plants are used to treat leucorrhoea, rhinorrhoea, diarrhea. Cardiac glycosides are the compounds that help in treating congestive heart failure, cardiac arrhythmia.

In a previous study, leaves and bark of *V. negundo* were analysed against selected gram-positive bacteria namely *Bacillus subtilis, S. epidermidis, S. aureus* and Gram-negative bacteria such as *E. coli, P. aeruginosa, Salmonella typhimurium, Vibrio cholerae* and *Vibrio alginolyticus*. For this study, both polar and nonpolar extracts were prepared which showed promising antibacterial activity against *E. coli*.[28] The alcoholic (both ethanol and methanol) leaf extracts revealed significant inhibitory activity against both gram-positive and gram-negative bacteria. The results are in conformity with the previous studies to some extent and imply the effectiveness of the phyto extracts as a potent therapeutic agent for antibacterial properties.

V. negundo showed a positive inhibition in methanol and chloroform extract among all the pathogenic microbes. These activities are due to the flavonoids, glycosides, alkaloids and the essential oils found in *V. negundo*. The leaf oils have been proven to inhibit the growth of a number of pathogenic bacteria. The essential oil of *V. negundo* is also effective against two human pathogenic fungi including *Candida albicans* and *Aspergillus niger*. These bioactive compounds individually and in collaboration with each other make *V. negundo* a strong antimicrobial scaffold.[29,30] Although the hexane, alcoholic and aqueous extracts from the whole plant of *V. negundo* show no antimicrobial activity against bacteria, including *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*,[31] however, another study confirmed the antibacterial effects of ethanol extracts from *V. negundo* leaves using the agar dilution method on four bacteria: *B. subtilis*, *S. epidermidis*, *E. coli* and *P. aeruginosa*. Their research concluded with positive antibacterial activity on Gram-positive bacteria compared to Gram-negative bacteria.[32] According to another comprehensive assessment, *J. gendarussa* has a large number of important phytochemicals that are very helpful in treating a variety of illnesses. By forming an inhibitory zone, the plant's leaf extract showed strong antibacterial activity. It also showed anti-inflammatory qualities by preserving human red blood cells.[33] Present study unequivocally shows that components with antioxidant, analgesic, antibacterial, antidiarrheal and cytotoxic qualities are present in the plant extract of *A. carambola*. These results corroborate the traditional usage of this herb to treat wounds, diarrhoea and headaches.[34]

ACKNOWLEDGEMENT

The authors acknowledge the support of Principal, Eastern Karbi Anglong College for providing necessary supports to carry out the present research.

AUTHORSHIP STATEMENT

HD, CD, DD: Data collection and result compilation.
CD, MT: Protocol designing, standardization and manuscript preparation.
PK: Study designing, supervision, final manuscript compilation and editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Our study is an evaluation of four medicinal plants that have traditionally been used in the treatment of various ailments. These plants contain bioactive compounds and have potent antibacterial characteristics, which contribute to their therapeutic efficiency. The hexane extracts of *J. gendarussa* leaves expressed the highest antioxidant activity. Methanol and chloroform extract of *Vitex negundo* showed significant antimicrobial potentiality. Further research in this area could provide valuable insights into harnessing the healing potential of these plant extracts for the development of pharmaceutical drugs. However, more investigation is needed to clarify the active principles at play.

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Cite this article: Deka H, Devi C, Tabish M, Das D, Das C, Kalita P. Antioxidant and Antimicrobial Potentiality Assessment of Bioactive Leaf Extracts of Four Traditionally Used Medicinal Plants. Asian J Biol Life Sci. 2024;13(2):481-90.