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Original Article

Study of Antibacterial Activity and Phytochemical Screening of Two Selected Ethnobotanical Plants of Asteraceae from West Bengal, India

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ABSTRACT

The objectives of the present study were to evaluate the quantitative and qualitative analysis of some phytochemicals and antibacterial properties of two medicinal plants *Glossocardia bidens* (Retz.) Veldkamp and *Grangea maderaspaterna* (L.) L poir under Asteraceae family from Purulia, West Bengal, India. Preliminary phytochemical analysis (alkaloids, carbohydrates, flavonoids, phenol, phlobatannins, saponin, terpenoids, and tannin) and quantitative analysis of total phenolic content and total flavonoid content were done by using appropriate experimental protocols according to the standard methods found in literature study. The antibacterial activity was observed using agar well diffusion method. Determination of Minimum Inhibitory Concentration (MIC) was also done to detect that concentration which showed complete bacterial growth inhibition. The preliminary phytotochemical analysis of these two experimental plants revealed the presence of the secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenol, phlobatannins, saponin, terpenoids, and tannin in ethanolic extract. The estimation of total phenol and flavonoid content in the ethanolic root extract of *Glossocardia bidens* (Retz.) Veldkamp and the ethanolic leaf extract of *Grangea maderaspaterna* (L.) L poir also showed good results. The antibacterial activity test showed good results in the ethanolic root extract of *Glossocardia bidens* (Retz.) Veldkamp and ethanolic leaf extract of *Grangea maderaspaterna* (L.) L poir.

Keywords: Medicinal plants, Phytochemical Screening, Antibacterial Activity, *Glossocardia bidens* (Retz.) Veldkamp, *Grangea maderaspaterna* (L.) L poir, Pathogenic Bacteria.

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INTRODUCTION

Contagious diseases can be considered as one of the prime threats to human health all over the world. Most of them are induced by microorganisms such as bacteria, viruses, fungi and other pathogenic microbes.[1] It is stated that bacteria are responsible approximately near about 30% for all diseases, which leads to high rate of death cases every year.[2] In the current years, research on medicinal plants has generated a lot of responses globally. Huge amount of evidence has gathered to demonstrate the favourable potential of medicinal plants used in several traditional, complementary and substitute systems of medicine to cure human diseases.[3] Plants have plenty of a wide variety of secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenol, phlobatannins, saponin, tannin and terpinoids which have been obtained *in vitro* to have antibacterial properties.[4-8] There are numerous reports documenting the efficiency of plant extracts on microorganism by many researchers in different corners of the earth.[9,10] Even though various plant species have been experimented for their antimicrobial efficacy but an enormous majority has not been assessed thus far. It is crucial for the systematic evaluation and scientific authentication of plants used as ethnomedicine for several diseases. Hence, there is an urgent requirement to screen medicinal plants for discovering their promising biological activity. Herbal medicines are in huge demand in the urban as well as developing countries due to their wide range of medicinal and biological applications.[11] The healing capability of the plant play significant role in the initial health care of about 80% of the global population.[12] The practices of novel bioactive compounds as antimicrobials from plant extract have become widespread because of the effective life span of antibiotic is restricted. Also, the over prescription and application of antibiotics are triggering antimicrobial resistance.[13,14] According to WHO, an herbal plant is a bioactive plant that can be used for curative purposes or that

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is a pioneer to the production of chemical and pharmaceutical commodities.[15] In literatures like the Vedas and the holy Bible stated the importance and wide spread usage of herbal medicines obtained from common traditional plants can easily be related with the popularity of herbal medicine and their curative properties in modern time.[16] From ancient times plants were used for disease treatment without understanding the presence of compound and their approach of action. Over the epochs, societies all over the world have developed their own folk lore to make sense of medicinal herbs and their application.[17]

The usage of phytochemicals from plant extracts with identified antimicrobial properties, can be of great importance in therapeutics of human ailments.[18] Many researches have been done recently in several nations to establish effectiveness of plant derived medicines. The search of new antibiotic is the need of the time because most of the notorious disease causing bacteria has become multidrug resistant. Herbal industry has taken a boom in the last three decades because scientific research throughout the world understood the importance of secondary metabolites from different plant sources could be the way out to get new antibacterial agents. These secondary active metabolites have a significant role in the management and cure of various types of human diseases.[19] On this basis, the medicinal plants with traditionally recognised bioactive properties approach promising projections for forth coming research and drug development.[20]

Purulia, the westernmost district of West Bengal, consists almost 30% forest cover is a rich repository of medicinal plants. Approximately 87% of the rural inhabitants of Purulia rely on wild plants for the treatment of different diseases.[11] In this study extracts of two plants of Asteraceae have been tested against two pathogenic bacterial strains to observe the growth inhibition of the bacteria. In this experiment five solvents were used to observe the efficiency of the plant extracts. The aim of this present study is to check selected plant extracts for the presence of secondary metabolites as well as the antibacterial activity against two bacterial stains. Estimation of total phenol and total flavonoid content are also done for qualitative analysis of the experimental plant extracts.

MATERIALS AND METHODS

Selection of plants

Glossocardia bidens (Retz.) Veldkamp was selected on the basis of use value and *Grangea maderaspaterna* (L.) L poir was selected because of its good availability as wild medicinal plant and less amount of literature reports were accessible in this area. These two plants of Asteraceae are traditionally used by the healers against various diseases found in Purulia district. The scientific names, vernacular names, parts used for the medication and use value of these plants are presented in the Table 1.

Use Value (UV)

The use value (UV)[21] determines the comparative importance of a species known in the neighbourhood was calculated by the formula: UV=(ΣU/n), here UV is the use value of species, 'U' is the sum of use accounts per species and 'n' is the sum of informants questioned for a given plant.

Collection of plant samples

Experimental plants *Glossocardia bidens* (Retz.) Veldkamp and *Grangea maderaspaterna* (L.) L poir of Asterace were collected in monsoon from Purulia district, West Bengal in India for experimental work. The plants were identified by Dr. K. Karthigeyan, from CNH, Botanical Survey of India, Howrah and they were also documented. The plant materials were washed carefully with running tap water and then shade dried for two weeks. The different dried parts of the plants were grinded to form powder and stored in clean labelled containers for further experiment.

Preparation of the Extract

For extraction using different solvents like chloroform, acetone, ethanol, methanol and water maceration technique was followed. 10 g of sample powder was soaked in 100 mL of solvents such as chloroform, acetone, ethanol, methanol and water in autoclaved conical flask for 48 hr. Then the extracts were filtered using Whatman No 1. filter paper and concentrated to dryness by the rotary evaporator. There after the powder was weighed individually and dissolved in the selected solvents separately and stored in a refrigerator at 4°C for phytochemical analysis and antibacterial activity tests.

Bacterial strain and culture

Authentic pure cultures of pathogenic bacteria *Pseudomonas aeruginosa (P. aeruginosa* MTCC87) and *Staphylococcus aureus* (*S. aureus* MTCC 741) were obtained from microbiological laboratory and clinical detection centre Paschim Medinipur, India. Bacteria were cultured in agar media (TSB or TSA) in aerobic condition at 37°C.

Assay for antibacterial activity

Antibacterial assay of plant samples was performed with the help of Agar well diffusion method. The fresh bacterial cultures were spread on sterile petri plates containing Muller Hilton Agar using cotton swab. Four wells of 7 mm diameter were made into the solidified agar media with sterilized corkborer. About 40 µL and 80 µL of the test sample extracts (chloroform, acetone, ethanol, methanol and water) were added into the separate wells accordingly from the stock solution of 10mg/mL by micropipette in aseptic condition. Gentamicin (20 µL from 2 mg/mL stock) was treated as positive control and 20% DMSO was used as negative control for alcoholic extracts and respective solvents

were used as the negative control from non-alcoholic extracts. After preparation, the petri plates were kept in a refrigerator for pre-diffusion of the extracts for 30 min. Then the plates were incubated in an incubator at 37°c for 24 hr. The antibacterial activity was determined by measuring the zone of inhibition. After three to four times repetition of this experiment the mean diameter of the inhibition zone was calculated.

Determination of MIC value

Minimum Inhibitory Concentration (MIC) of the sample extracts was determined by broth dilution method[22] to measure the concentration of the extracts at which bacterial growth was completely inhibited. The concentrations of 50, 100, 200, 400, 800, 1600 mcg were prepared by diluting the sample extracts in LB broth. The standard antibiotic gentamicin was also prepared by the same procedure. The LB broth which was not treated considered as negative control. The whole process was done under aseptic conditions at 37°C. After 24 hr of incubation minimum inhibitory concentration was observed. The absorbance was measured using spectrophotometer at 600 nm (OD_{600}).

Qualitative Phytochemical Screening

Preliminary qualitative phytochemical analysis of the solvent extracts of the experimental plants were performed by following the standard methods.[23-25]

Test for Alkaloids

Wagner's reagent test was performed to observe the presence of alkaloids in the sample extracts. 2 mL of extract was treated with 4 drops of Wagner's reagent and reddish brown colour was observed.

Tests for Carbohydrates

2-3 drops of Molisch's reagent were added to 2 mL of the plant extract. Then few drops of concentrated H_2SO_4 were added along the side of the test tube containing the extract slowly and carefully. After 2 min red or dull purple ring was appeared.

Test for Flavonoids

2 mL sample extract was treated with a few drops of 20% sodium hydroxide. A deep yellow colour was visible which was slowly became colourless when diluted HCl was added drop by drop on it. It proved the presence of flavonoids in the sample.

Test for Phenols

5% aqueous ferric chloride was added to a fraction of extract. A dark blue or dark purple colouration was observed.

Test for Phlobatannins

A red precipitate was formed when 2 mL of sample extract was boiled with 1 mL of 1% aqueous hydrochloric acid and it indicated the presence of phlobatannins.

Test for Saponins

2-3 mL of extract was added to 6 mL of water in a test tube. The mixture solution was then shaken strongly. Foam was formed and that confirmed the presence of saponins.

Test for Tannins

2 mL of sample extract was treated with 10% alcoholic ferric chloride and the solution turned into blue or greenish colour due to the presence of tannins.

Test for Terpenoids

1 mL of chloroform was treated with 2 mL of extract. Then a few drops of concentrated H_2SO_4 were added in to it. Observation of immediately produced reddish brown precipitate specified the presence of terpenoids.

Determination of Total Phenolic content

The Total Phenolic Content (TPC) was estimated by Folini-Cocalteu method with slight modification using Gallic Acid (GA) as the standard.[26] Approximately 1 mL of sample extract with concentration of 1 mg/mL was taken in a test tube. Then 1 mL of 7.5 % Na_2CO_3 was added into it. After 8 min, 1.25 mL of Folin-Ciocalteu's reagent (1:1) was added and incubated for 30 min. The absorbance was taken at the wavelength of 760 nm by a UV-Vis spectrophotometer. The standard curve of gallic acid was prepared with 20, 40, 60, 80 and 100 µg/mL concentrations respectively. The total phenolic content was calculated in milligram gallic acid equivalent per gram of extract (mg/gm) using calibration curve.

Determination of Total Flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric method with slight alteration.[27] The mixture of 1 mL sample (1 mg/mL), 4 mL H_2O, 0.3 mL 5% $NaNO_3$, 0.3 mL $Alcl_3$ and 1 mL of 1M NaOH was added in a volumetric flask. Then the

Table 1: Selected medicinal plants of Asteraceae traditionally used by the healers.

Scientific Name	Vernacular Name	Parts Used	Use Value	Ailment
Glossocardia bidens	Bishainandi	Leaves, roots and flowers	0.89	Pimples and skin infection
Grangea maderaspaterna	Bhuikodom	Roots	0.13	Tumors

ultimate volume in the flask was made up to 10 mL with distilled water and incubated for 25 min in dark. Quercetin was used as standard to determine of total flavonoid. The Optical Density (O.D) was measured against the blank at 510nm wavelength using a UV-vis spectrophotometer. The total flavonoid content was calculated in milligram quercetin equivalent per gram of sample (mg/gm).

RESULTS

Qualitative phytochemical screening

The preliminary qualitative phytochemical analysis of the plant extracts indicated the presence of secondary metabolites in good range, such as carbohydrates, alkaloids, phenol, flavonoids, terpenoids, saponin, phlobatannins and tannins presented in Table 2. Both ethanolic and methanolic plant extracts gave good results in comparison to other solvent extracts.

Antibacterial activity assay

The extracts of the studied plant samples showed differing degree of inhibition activity against the pathogenic bacteria. The results of antibacterial activity were exhibited in respect to the growth inhibition zones. The diameter of the growth inhibition zones was measured. The ethanol extract of *Glossocardia bidens* (Retz.) Veldkamp root and the ethanol extract of *Grangea maderaspaterna* (L.) L poir leaf were observed to be more active against *S. aureus* and *P. aeruginosa* in respect to other solvent extracts showed in Table 3. The inhibition zone of the root extract (ethanol) of *Glossocardia bidens* (Retz.) Veldkamp against *S. aureus* and *P. aeruginosa* were 40 µL- 10.5±.5, 80 µL-12.16±76 and 40 µL-10.33±0.57, 80 µL-11.33±.57 respectively. The inhibition zone of the leaf extract (ethanol) of *Grangea maderaspaterna* (L.) L poir against these two bacteria *S. aureus* and *P. aeruginosa*were 40 µL- 11±1, 80 µL- 13±1 and 40 µL- 9.13±0.32, 80 µL- 9.86±0.23 respectively.

Determination of MIC value

The MIC value showed that the microorganisms were vulnerable to the minimum inhibitory concentration of selected plant extracts. The values are presented below.

MIC value of the standard drug (Gentamicin) for *S. aureus* was <100 mcg.

MIC value of the standard drug (Gentamicin) for *P. aeruginosa* was<100 mcg.

MIC value of ethanolic root extract of *Glossocardia bidens* (Retz.) Veldkamp for *S. aureus* and *P. aeruginosa* was<400 mcg.

MIC value of ethanolic root extract of *Glossocardia bidens* (Retz.) Veldkamp for *P. aeruginosa* was <400 mcg.

MIC value of ethanolic leaf extract of *Grangea maderaspaterna* (L.) L poir for *S. aureus* was <400 mcg.

MIC value of ethanolic leaf extract of *Grangea maderaspaterna* (L.) L poir for *P. aeruginosa* was <800 mcg.

Estimation of Total Phenol content

Gallic acid standard graph (Figure 1) was used for the quantitative estimation of total phenol content. Total Phenol content in ethanol root extract of *Glossocardia bidens* (Retz.) Veldkamp and ethanol leaf extract of *Grangea maderaspaterna* (L.) L poir were 86.14±0.438 and 64.187±0.410 mg/gm gallic acid equivalent.

Estimation of total Flavonoid Content

Quercetin standard graph (Figure 2) was used for the estimation of total flavonoid content. Total flavonoid content in ethanolic root extract of *Glossocardia bidens* (Retz.) Veldkamp and ethanolic leaf extract of *Grangea maderaspaterna* (L.) L poir were 92.44±1.756 and 95.23±1.315 mg/gm quercetin equivalent respectively.

DISCUSSION

The outstanding biological activity of medicinal plants can be determined by their properties of the phytochemicals. Most of the plant parts like root, stem, leaves and fruits even some times

Table 2: Showing preliminary phytochemical screening of selected plants.

Plant Name	Solvent	Alkaloids	Carbohydrates	Flavonoids	Phenol	Phlobatannins	Saponin	Tannin	Terpinoids
Glossocardia bidens	Chloroform	-	-	+	+	-	-	+	+
	Acetone	+	-	-	+	-	+	-	-
	Ethanol	+	+	+	+	+	+	+	+
	Methanol	+	+	+	-	+	+	+	+
	Water	-	+	-	-	+	+	+	-
Grangea maderaspaterna	Chloroform	+	-	+	-	-	-	-	+
	Acetone	+	-	-	-	-	+	-	-
	Ethanol	+	+	+	+	-	+	+	+
	Methanol	-	+	+	-	-	+	+	+
	Water	-	+	-	-	-	+	+	-

Table 3: Showing antimicrobial activity of different parts of selected plans.

Plant Name	Solvent	Plant Parts	Zone of Inhibiton	
Glossocardia bidens			*S. aureus*	*P. aeruginosa*
	Chloroform	Root	40 µL-3.66±.57 80 µL-5.33±.28	40 µL-3.73±.46 80 µL-4.1±.36
		Stem	40 µL- - 80 µL- -	40 µL-3.66±.28 80 µL-4.66±.57
		Leaf	40 µL-4.83±1.25 80 µL-6±.5	40 µL-NM 80 µL-NM
		Flower	40 µL-NM 80 µL-NM	40 µL-3.23±.25 80 µL-3.93±.11
	Acetone	Root	40 µL- - 80 µL- -	40 µL-NM 80 µL-NM
		Stem	40 µL-7.33±.57 80 µL-9.16±.76	40 µL-NM 80 µL-NM
		Leaf	40 µL-.35±.5 80 µL-7.1±.17	40 µL-4.06±1 80 µL-5.33±.57
		Flower	40 µL-4.83±.28 80 µL-5.16±.28	40 µL-5.83±.76 80 µL-7.33±.57
	Ethanol	Root	40 µL- 10.5±.5 80 µL-12.16±76	40 µL- 10.33±.57 80 µL-11.33±.57
		Stem	40 µL-7±1 80 µL-7.66±.57	40 µL-9.16±.76 80 µL-9.5±.5
		Leaf	40 µL-6.6±.55 80 µL-7.16±.57	40 µL-8±.5 80 µL-8.16±.28
		Flower	40 µL-6.23±.46 80 µL-6.93±.11	40 µL-7.96±.05 80 µL-8.86±0.32
	Methanol	Root	40 µL-8.56±.57 80 µL-8.43±.49	40 µL-8±.5 80 µL-8.36±.32
		Stem	40 µL-6.06±0.49 80 µL-6.85±.36	40 µL-7.23±.25 80 µL-7.86±.23
		Leaf	40 µL-6.7±.26 80 µL-7.53±.45	40 µL-6.33±.57 80 µL-7.23±.25
		Flower	40 µL-5.66±.28 80 µL-6.33±.28	40 µL-6.83±.28 80 µL-7.4±.52
	Water	Root	40 µL-4.66±.28 80 µL-5.9±.36	40 µL-4.66±.57 80 µL-5.66±.28
		Stem	40 µL- - 80 µL- -	40 µL- - 80 µL- -
		Leaf	40 µL-4.76±.4 80 µL-5.7±.26	40 µL-5.66±.57 80 µL-5.9±.26
		Flower	40 µL-NM 80 µL-NM	40 µL- - 80 µL- -

Plant Name	Solvent	Plant Parts	Zone of Inhibiton	
Grangea maderaspaterna			*S. aureus*	*P. aeruginosa*
	Chloroform	Root	40 µL- 6.23±.25 80 µL- 6.96±.05	40 µL- NM 80 µL- NM
		Stem	40 µL- NM 80 µL- NM	40 µL- 5.76±.49 80 µL- 7.23±.49
		Leaf	40 µL- 4.1±.85 80 µL- 5.2±.72	40 µL- 7.16±1 80 µL- 8.16±.30
		Flower	40 µL- 6.2±.72 80 µL- 7.23±.25	40 µL- 7.4±.4 80 µL- 8.16±.28
	Acetone	Root	40 µL- 7.46±.55 80 µL- 8.2±.3	40 µL- 7.23±.25 80 µL- 8.03±.15
		Stem	40 µL- 4.5±.5 80 µL- 5.8±.28	40 µL- NM 80 µL- NM
		Leaf	40 µL- NM 80 µL- NM	40 µL- 4.06±1 80 µL- 5.56±.4
		Flower	40 µL- 6.23±.25 80 µL- 7.23±.49	40 µL- 6.5±.5 80 µL- 7.4±.52
	Ethanol	Root	40 µL- 7.5±.5 80 µL- 8.66±.76	40 µL- - 80 µL- -
		Stem	40 µL- 7±1 80 µL- 8±.5	40 µL- - 80 µL- -
		Leaf	40 µL- 11±1 80 µL- 13±1	40 µL- 9.13±.32 80 µL- 9.86±.23
		Flower	40 µL- NM 80 µL- NM	40 µL- 7.73±.64 80 µL- 8.8±.26
	Methanol	Root	40 µL- 0.22±.28 80 µL- 8.23±.25	40 µL- 5.83±0.28 80 µL- 7.73±.64
		Stem	40 µL- 5.83±.28 80 µL- 6.83±.28	40 µL- 5.66±.57 80 µL- 6.93±.4
		Leaf	40 µL- 8.66±1.15 80 µL- 9.33±1.04	40 µL- 7.83±0.28 80 µL- 8.46±.55
		Flower	40 µL- - 80 µL- -	40 µL- 7.23±.25 80 µL- 8±.2
	Water	Root	40 µL- NM 80 µL- NM	40 µL- - 80 µL- -
		Stem	40 µL- 6.5±.86 80 µL- 7.5±.45	40 µL- - 80 µL- -
		Leaf	40 µL- NM 80 µL- NM	40 µL- 4.3±.85 80 µL- 6±.1
		Flower	40 µL- 7.16±.76 80 µL- 7.6±.6	40 µL- 5.13±.9 80 µL- 5.8±.26

Here '-' indicated as no inhibition zone and 'NM' indicated that the zone of inhibition could not measure.

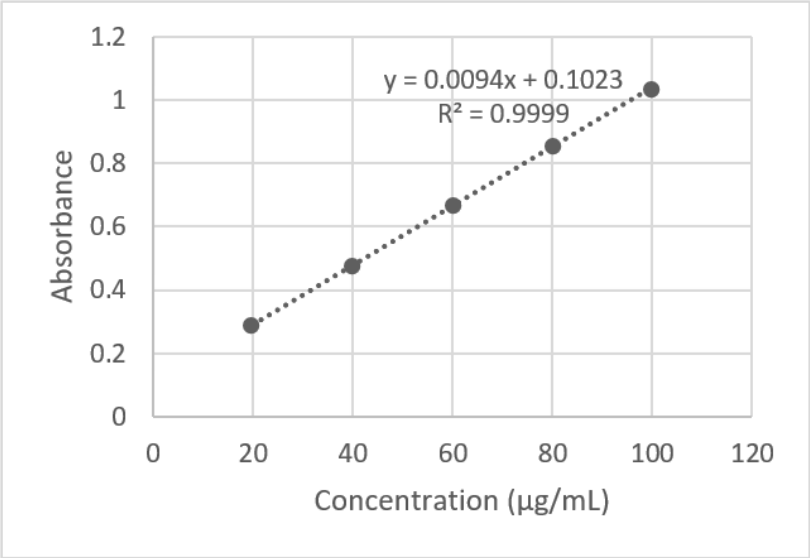

Figure 1: Gallic Acid Calibration curve.

the whole plants are used for the phytochemical analysis.[28] Alkaloids are one of the largest classes of compounds synthesized by plants, primarily derived from amino acids. These metabolic byproducts play significant roles in plant defense and medicinal applications.[29] In this study, the acetone, ethanol, and methanol extracts of *Glossocardia bidens* (Retz.) Veldkamp, as well as the chloroform, acetone, and ethanol extracts of Grangea *maderaspatana* (L.) L. Poir, exhibited the presence of alkaloids. Flavonoids, which are hydroxylated phenolic compounds, are also synthesized by plants as a defense mechanism against microbial infections.[30] The phytochemical analysis revealed the presence of flavonoids in the ethanolic, chloroform, and methanolic extracts of the selected plant species. Terpenoids, known for their diverse pharmacological properties-including antiviral, anti-inflammatory, antibacterial, anticancer, and antimalarial activities-also play a role in cholesterol synthesis inhibition.[31] The experimental analysis confirmed the presence of terpenoids in the chloroform, ethanol, and methanol extracts of the studied plants. Phenolic compounds are another secondary metabolites are found in ethanolic, chloroform and methanolic extracts of studied plants in this phytochemical screening test which are produced in the plants' shikimic acid and pentose phosphate by phenylpropanoid metabolization.[32] Tannin is broadly applied to a huge complex biomolecule of polyphenol which have adequate amount of hydroxyls and other groups namely carboxyl to produce strong complexes with several macromolecules.[33] Presence of tannin is visible in the Methanol, chloroform, ethanol and water extracts of *Glossocardia bidens* (Retz.) Veldkamp and the ethanolic, methanoic and water extracts of *Grangea maderaspaterna* (L.) L poir in this preliminary phytochemical test. This present experimental study explains that the ethanolic and methanolic extracts of *Glossocardia bidens* (Retz.) Veldkamp showed a good source of secondary metabolites like carbohydrates, phenol (absent in methanolic extract),terpenoids, tannins, flavonoids, alkaloids, phlobatannins and saponin in preliminary phytochemical screening test and similarly the plant extracts of ethanol and methanol of *Grangea maderaspaterna* (L.) L poir detected the presence of carbohydrates, phenol (not present in methanol extract), flavonoids, saponin, tannin, tarpinoids and alkaloids (absent in methanol extract) than other solvents such as chloroform, acetone and water (Table 2). The fact that polar solvent extracts gave good results could imply that the bioactive target compounds are highly soluble in polar solvents, leading to effective extraction and possibly strong antioxidant, antimicrobial, or other bioactivities.[34] In this current study, the antibacterial activity test of different plant parts such as root, stem, leaves and flowers of experimental plants were performed with the help of different solvent extracts including chloroform, acetone, ethanol, methanol and water. As a result, the root and leaf extracts (ethanol) of *Glossocardia bidens* (Retz.) Veldkamp and *Grangea maderaspaterna* (L.) L poir respectively gave satisfactory results against *S. aureus* and *P. aeruginosa* strains (Table 3). That's interesting! It seems like both *Glossocardia bidens* and *Grangea maderaspatana* have shown strong antibacterial properties against some common pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, particularly in their ethanol extracts. The root extract of *Glossocardia bidens* seems to have a more potent antibacterial effect than the leaf extract of *Grangea maderaspatana*, with both showing dose-dependent inhibition. The larger inhibition zones for *S. aureus* suggest this species might be a better target for Grangea maderaspatana, while *P. aeruginosa* appears to be more resistant in general. In contrast to the gram positive bacteria, the gram negative bacteria often show higher extent of resistance.[35,36] In this experiment *P. aeruginosa* exhibited more resistance than *S. aureus*. MIC values of these two experimental plant extracts (ethanol) of asteraceae against previously mentioned two bacteria, *S. aureus* and *P. aeruginosa* were also determined where gentamycin was used as the positive

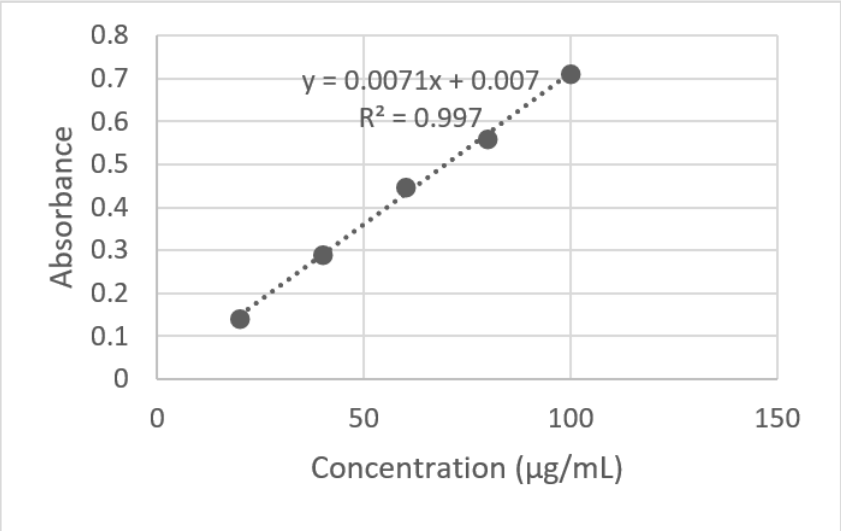

Figure 2: Quercetin Calibration curve.

control. These values MIC test suggest that the standard drug (Gentamicin) is effective at lower concentrations (under 100 mcg), while the ethanolic extracts from the two plants (*Glossocardia bidens* and *Grangea maderaspatana*) have higher MIC values, which indicates that they may not be as potent against these bacterial strains, at least in comparison to Gentamicin. Srikacha *et al*., also stated similar good results of MIC of their studied plant extracts which were impressive.[37] Total phenol content and total flavonoid content of ethanolic extracts of the experimental plants [root of *Glossocardia bidens* (Retz.) Veldkamp and leaf of *Grangea maderaspaterna* (L.) L poir] were estimated and the results were found suitable. The values suggest that *Glossocardia bidens* root extract has a higher phenolic content compared to *Grangea maderaspatana* leaf extract. Phenolic compounds are often studied for their antioxidant properties and potential health benefits. The test for total phenol content of experimental plants was supported by the statement of Yuliani *et al.*,[38] They stated that phenolic compounds are present in many members of Asteraceae in a large content. When it came to the experiment for total flavonoids of studied plants, it shows that the leaf extract of *Grangea maderaspatana* has a slightly higher flavonoid content compared to the root extract of *Glossocardia bidens*, but both show relatively high levels of flavonoids. The flavonoids could contribute to a variety of bioactivities, such as reducing oxidative stress or inflammation, which might explain their use in traditional medicine. Chaudhary *et al*., presented that total flavonoid content of *Grangea maderaspaterna* (L.) L poir was notable.[39] This was also visible in this present study.

CONCLUSION

Antibiotics are one of the milestone discoveries of the 20th century. But unfortunately, antibiotic resistance has probably become the most threatening public health issue of the present century. This has happened due to extensive and careless overuse of antibiotics to treat bacterial infections. Approximately 20,000 possible resistance genes have been identified from 400 bacterial isolates.[40] This current scenario resulted in an increased interest in plant derived therapeutic agents because plants synthesize secondary metabolites that have enormous potential to act as antibacterial compounds. Using medicinal plants to treat common human ailments is an age-old practice. Plant extracts contain various phytochemicals that could be used as a potential source of novel medicines. Phytochemical screening of the plant extracts of *Glossocardia bidens* (Retz.) Veldkamp and *Grangea maderaspaterna* (L.) L poir displayed the presence of alkaloids, phenol, flavonoids, terpenoids, saponin, tannins, carbohydrates and phlobatannin. The results of this present study evidently pointed that the antibacterial activity vary with the different parts of the studied plants with different solvents. The experimental data on antibacterial potency of ethanolic extracts of *Glossocardia bidens* (Retz.) Veldkamp root and *Grangea maderaspaterna* (L.) L poir leaf come up with the source for synthesis of novel antibiotics. This investigation of screening different experimental plant extracts revealed the therapeutic effectiveness of plants utilized by the healers as traditional medicine. It recommends that these plant extracts can play an important role as antibacterial agents and can open a door to new drug discovery of different diseases caused by the harmful bacteria like *S. aureus* and *P. aeruginosa*. It can be concluded that the results from the present experimental work produce a good source for the selection of applicant plants for advance phytochemical and pharmacological investigation. The most efficient extracts can isolate as therapeutic antimicrobials and endure further pharmacological appraisal.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MIC: Minimum inhibitory concentration; **WHO:** World Health Organization; **UV:** Use Value; **CNH:** Central National Herbarium; **MTCC:** Microbial Type Culture Collection and Gene Bank; **TSB:** Tryptic Soy Broth; **TSA:** Tryptic Soy Agar; **OD:** Optical Density; **µL:** microliters; **HCL:** Hydrochloric Acid; **TPC:** Total Phenolic Content; **GA:** Gallic Acid; **Na_2CO_3:** Sodium Carbonate; **H_2O:** Water; **$NaNO_3$:** Sodium Nitrate; **$AlCl_3$:** Aluminium chloride; **1M:** 1Molar; **NaOH:** Sodium hydroxide; **UV-vis:** Ultraviolet-visible; **GAE:** Gallic Acid Equivalents; **NM:** The zone of inhibition could not measure.

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