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Phytochemical analysis and antibacterial activity of *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis* from District Lower Dir Village Maidan Banda of Khyber Pakhtunkhwa Pakistan

Shakir Ullah[1*], Inam Ullah[1], Majid Khan[1], Muhammad Zamir[1], Bakht Tarin Khan[1], Robeena Naz[2], Mohammad Sohil[2], Mohsin Ihsan[2], Fozia Abasi[1]

[1]*Department of Botany, Govt Post Graduate Collage Timargara, Lower Dir, Pakistan*

[2]*Department of Zoology, Govt Post Graduate Collage Timargara, Lower Dir, Pakistan*

[3]*Department of Botany, Abdul Wali Khan University, Garden Campus, Mardan, Pakistan*

[4]*Department of Zoology University of Buner Pakistan*

[5]*Department of Botany, Women University of Azad Jammu and Kashmir, Bagh Pakistan*

Key words: Phytochemical analysis, antibacterial activity, *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis.*

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Abstract

In the present research work, both qualitative and quantative phytochemical investigation of methanolic and ethanolic extracts of *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis* and antibacterial activity was carried out. The study was carried out due to their traditional uses because these plants are commonly used for the aliment of saverales disease. For the sub culturing the bacterial isolates Nutrient broth and Nutrient agar were used. For the sensitivity screening Mueller Hinton agar media was used. The antimicrobial assessments of both the ethanol and methanol extracts showed considerable inhibition activities on the selected isolated bacteria with the concentration of 20mg/ ml. *Ajuga bracteosa* exhibited more beneficial antibacterial activity in methanol extract of the leaves (30-37mm) in diameter. The leaves ethanol extract showed bacterial inhibition between (30-38mm) in diameter. But, *Bergenia ciliate,* and *Amaranthus viridis* leaves extracts as well revealed higher antibacterial activity than the stem bark and extracts of roots. In all the plants parts, Saponins and alkaloids were present. In the leaves extracts of *Bergenia ciliate,* highest amount of saponins constituent (5.10±0.11%) was recorded. In leaves extract alkaloids was present in highest amount 2.98±0.12 %. The values of MIC ranged between 1.25 10 mg/ml. From the present study we have conclude that the selected plants have high amount of phytochemicals due to which these can be used for further study to isolate the compounds for form the their pharmacological activates.

* **Corresponding Author:** Shakir Ullah ✉ shakirawkum321@gmail.com

Introduction

Phytochemicals are two types that may be primary and secondary. Proteins, common sugars and Chlorophyll are encompassed in primary Phytochemicals and secondary Phytochemicals have alkaloids, terpenoids and phenolic compounds ((Mahato & Sen, 1997). Terpenoids show numerous significant pharmacological activities i.e., anticancer, anti-malarial, anti-inflammatory, anti-viral and anti-bacterial activities also inhibition of cholesterol synthesis in the body (Krishnaiah *et al.,* 2007). Phytochemicals are naturally occurring chemical, biologically active compounds found in plants, which be responsible for health benefits for humans further these recognized to micronutrients and macronutrients (Hasler & Blumberg, 1999). They protect plants from damage and disease and contribute to the plant's color, flavor and aroma. In common, the plant chemicals that defend plant cells from environmental threats such as stress, drought, pollution, pathogenic attack and UV exposure are called as phytochemicals (Gibson *et al.*, 1998). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher *et al.*, 1999). *Ajuga bracteosa* (Family: Labiateae) is a perennial herb with diffused branching and aroma and is distributed from Nepal to Kashmir, sub-Himalayan tract, plains of Punjab and the upper Gangetic plains. The herb is in use since ancient times and recommended in Ayurveda for the treatment of rheumatism, palsy, amenorrhea and gout. It is also credited with astringent, tonic, stimulant, febrifugal and diuretic properties (Vohra and Kaur, 2011). *Bergenia ciliate* belongs to the family Saxifragaceae which have 30 genera and 580 species. B. ciliata commonly known as hairy Bergenia is a perennial herb found between heights of 800–3000 m throughout the temperate Himalayas from Southeast Tibet to Afghanistan. In Bhutan it is found in Deothang, Mongar, Phuntsoling and Ha districts. (Ahmad *et al.*, 2018). *Amaranthus viridis* L. a member of family (Amaranthaceae) which is commonly called Chowlai. *Amaranthus viridis* is a weed of cultivation wild and vegetable. In the warmer parts of the world Amaranthus viridis is distributed. The whole plant retains analgesic and anti-pyretic properties. These plant are used for the treatment of fever and pain in the traditional systems of medicine. A.viridis a diverse weed in the tropical and subtropical areas of the world, also universal to moderate regions of the words (Ferdous *et al.,* 2015). Its ethno medicinal uses include its use for treatment of jaundice, diabetes, eye infections, fractured bones, internal wounds, diarrhea, rheumatism, stomachache, and its use as a general body tonic (Ali *et al.*, 2015). The aims of the present work to carried out the research work on selected medicinal plants which was locally used against some diseases cure due to their phytochemical constituents and antimicrobial activities.

Material and methods

Plant Collection and Botanical Identification

In the present study *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis,* was collected in October, 2018 from district Lower village Maidan Banda of Khyber Pakhtunkhwa. With the help of Flora of Pakistan Plant were taxonomically identified and placed in the Herbarium of Department of Botany, Govt Post Graduate Collage Timargara, Lower Dir, and Pakistan.

Solvent

Methanol and ethanol was used for the preparation of crude extract of the selected plants.

Crashing and filtration of the plant

The dried plant was powdered with the help of electric grinder. The powder were kept in air tight plastic bottles for further phytochemical analysis and antibacterial activities. 10 gm of plant powdered was retained in distinct conical flask and 90 ml of solvent i.e. (methanol and ethanol) was added to the powdered separately. Flask were covered with the help of aluminum foil and for 72 hours for the shaking purposes retained in shaker. Extracts were filtered with the help of Whatman filter paper after 72

hours and then through process of filtration plant extracts were obtained (Pirzada *et al.*, 2010).

Cultures of Bacterial

In the study the bacteria strains used are locally isolates from faeces, urine and sore swab in the Lebartey of District Head Quarter Hospital Timargara Lower Dir KPK Pakistan. The isolated bacterial consist of: *Shigella dysenteniae, Klebsiella pneumonia, Bacillus cereus*, *Campylobacter jejunum Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Salmonella typhii.*

Antibacterial Screening:

For the sub-culturing the bacterial isolates Nutrient broth and Nutrient agar were used. For the sensitivity screening Mueller Hinton agar media was used. At concentration of 20mg/ml the crude extracts were prepared in 5% aqueous dimethyl sulphoxide (DMSO). At 530nm the absorbance was read and with sterile distilled water to match that of 0.5Mc at was adjusted to Farland standard solution. Other dilutions were prepared to give a final concentration of 106 Colony Forming Unit per milliliter from the prepared bacterial solutions. With the help of sterile syringe 0.2ml each of the bacterial suspensions was obtained then spread on the petri plates on molten Mueller-Hinton agar. For 1.5 hours for the bacterial isolates test the plates were allowed to stand and well established in the seeded medium. With the help of sterile cork borer wells of equal depth were dug with a before sterilized cork borer. With the extracts avoiding splash and overfilling the wells were aseptically filled up. At 37°C for 24-48 hours the plates were incubated. As a negative control Sterile 5% aqueous DMSO was used while methicilin (2mg/ml) and streptomycin was used as the positive control. By agar well diffusion the antibacterial activities of the crude plant extracts were evaluated (Nair and Chando, 2005).

Minimum Inhibitory Concentration (MIC)

At the concentration of 20mg/ml and 1mL of Muller-Hinton broth and 1ml of the extract solution are consequently transferred. To the next test tube 1mL from the first test tube was transferred and still continue at to the last test tube. Then from 24 hours culture of bacteria 1ml was inoculated into each test tube and mixed carefully. The test tubes were then incubated at 37°C for 24 hours. With no detectable growth was measured as the MIC the tube which have lowest dilution. A sterility check negative control (aqueous DMSO, medium) and positive control (aqueous DMSO, medium, water soluble antibiotics and inoculum) were included. The MIC was measured by the method of (Sahm and Washington, 1990).

Phytochemical Screening

Tests for Saponins

In test tube 5 ml of extracts were shaken vigorously. The presence of saponins was indicated by the formation of froth (Rajesh *et al.,* 2016).

Tests for Steroids

0.5g extract of sample with the addition of 2ml H_2SO_4 was mixed with 2ml of acetic anhydride. The presence of steroids is indicates by colour change from violet to blue or green (Kokate *et al.,* 2008).

Tests for Flavonoids

For flavonoids detection, with sodium hydroxide (NaOH) solution extracts were treated. The presence of flavonoids was indicate by Red precipitation formation (Kokate *et al.,* 2008).

Tests for Tannins

For the tannins detection Ferric chloride was used. The plants extracts was mixed with Ferric chloride ($FeCl_3$) solution. The presence of tannins was indicated by Formation of blue green coloration. (Kokate *et al.*, 2008).

Tests for Alkaloids

For of alkaloids detection, a few drops of Wagner's reagent (Potassium iodine) are add to 2 ml of extracts. By the formation of reddish brown precipitate the presence of alkaloids was confirmed (Khandewal *et al.,* 2015).

Tests for Phenols

For detection of phenol, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml of extracts in a test tube. The presence of phenol was indicated deep bluish green coloration formations (Dahiru *et al.,* 2006).

Tests for (Cardiac) Glycosides

For cardiac glycosides detection, 2 ml of extracts solution were shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid (H_2SO4) and iron tri chloride ($FeCl_3$). Brown ring formation indicated the presence of Cardiac glycosides (Soni *et al.*, 2011).

Quantative Analysis of Phytochemicals

Total Phenol

Using a soxhlet apparatus for 2 hours, 2g each extracts were mixed with 1ml of diethyl ether. For 15 minutes extracts were boiled with 50ml of ether for the extraction of the phenolic components. Into the flask, 5ml of the extracts were pipetted and then added 10ml distilled water. 5ml of concentrated amyl alcohol and 2ml of ammonium hydroxide solution was also added. To the mark the samples were made up and for 30 min left to react for colour development. At 505 nm the wave length was measured (Dahiru *et al.,* 2006).

Total Cardiac glycosides

5ml of each extracts was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layer with 1ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Rajesh *et al.*, 2016).

Total quantification of Alkaloid

Into a 250ml beaker, 5g of each sample was weighed and in ethanol was added 200ml of 10% acetic acid, which is then covered and allowed to stand for 48 hours. After the completion of filtration process, on water bath to ¼ the extracts were concentrated of to the original volume. Until the precipitation was collected concentrated ammonium hydroxide was added in drops to the extract, with dilute ammonium hydroxide washed at and then filtered. The residue which was obtained from the filtration is alkaloid which was further dried and weighed (Harborne, 1973).

Determination of total saponins constituents

Into a conical flask 20 gm of each extracts were put and 100 cm3 of 20% ethanol, aqueous were added. Over a hot water bath for 4 hours the samples were heated with continuous stirring at about 55°C. The residue re-extracted with another 200 ml 20% ethanol, and mixture was filtered. At about 90°C through water bath the combined extracts were reduced to 40 ml. Into a 250 ml separatory funnel the concentrate was transferred and 20 ml of diethyl ether was added and vigorously shaken. 60 ml of n-butanol was added. With 10 ml of 5% aqueous sodium chloride the combined n-butanol extracts were washed twice. In a water bath the remaining solution was heated. The samples were dried in the oven to a constant weight after evaporation; the saponin content was calculated (Gracelin *et al.*, 2013).

Determination of total tannins constituents

500 mg of the sample was weighed in a 50 ml plastic bottle. Added 50 ml of distilled water and shacked for 1 hours in a mechanical shaker. In a 50 ml volumetric flask was filtered and made up to the mark. Into a test tube, 5 ml of the filtered was pipetted out and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium Ferro cyanide. At 120 nm their absorbance was cheeked out (Gracelin *et al.*, 2013).

Statistical Analysis

The zones of inhibition of extracts of plants i.e. Methanolic and ethanolic extracts were showed as the Mean± Standard Deviation. The result was taken is triplicate. Analysis was done by SPS 2013.

Results

In the present study *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis* studied. The results shown the presence of phytochemicals in their parts

(stem bark, roots and leaves,) in numerous amounts. Extraction for the preparation of infusion local users of these plants employ primarily water as solvent of decoctions in diverse parts of Maidan Banda Village.

Minimum inhibitory inhibition (mm)

More antimicrobial activities was shown by *Bergenia ciliate* among the three plants. The *Bergenia ciliate* showed maximum inhibition against *S. dysenteriae* (33.18±1.6) in stem barks extracts.

Fooled by leaves extracts (32.14±0.7) against *P. Aeruginosa* and roots showed (27.63±0.6) inhibition against *K.pneumoniae*. The other two plants showed less antibacterial activity compared to the *Bergenia ciliate* (Tables- 1, 2, 3).

Table 1. Minimum inhibitory inhibition (mm) as showed in Methanol's and Ethanol extracts of the plants at 20mg/ml.

Name of Bacterial	*Bergenia ciliate*						References	
	Leaves		Bark		Root		Strept.	Methy.
	ME	EE	ME	EE	ME	EE		
S. dysenteriae	29.16±1.5	35.23±1.7	23.03±0.5	33.18±1.6	18.0±0.7	23.05±1.8	39.13±1.6	23.00±1.9
P. aeruginosa	32.14±0.7	34.55±0.7	22.14±0.7	19.22±1.8	16.17±0.3	21.41±1.2	37.17±1.6	16.0±1.05
E. coli	32.04±1.7	34.25±1.7	22.08±0.6	22.14±0.7	17.34±1.7	18.56±1.7	0.00±1.0	0.00±1.0
S. typhi	32.13±1.7	34.23±0.6	24.54±0.6	19.16±0.7	13.13±1.5	19.37±1.4	0.00 ±0.0	13.11±0.0
B. cereus	32.13±1.4	33.75±0.8	18.17±0.8	28.34±1.3	14.82±1.5	17.36±1.4	40.00±0.0	18.6±1.0
S.aureus	38.17±1.9	36.36±1.7	37.44±0.7	36.43±1.3	13.34±0.8	17.25±1.5	45.16±1.0	0±00.0
C. jujenum	32.14±1.5	35.63±1.5	25.16±0.7	27.04±1.7	12.13±0.4	18.22±1.5	0±00.0	0.00±0.0
K.pneumoniae	33.21±0.4	33.50±1.8	32.24±0.6	31.45±1.7	26.24±0.7	27.63±0.6	0.00±0.0	0.00±0.0

ME: methanol extracts, EE: Ethanol extracts.

Methycilin and Streptomycin ere used as the positive reference drugs at concentration of 2mg/ml.

Table 2. Mean inhibitory inhibition (mm) as showed by the crude Methanol's and Ethanol extracts of the plants at 20mg/ml.

Name of Bacterial	*Ajuga bracteosa*						References	
	Leaves		Bark		Root		Strept.	Methy.
	ME	EE	ME	EE	ME	EE		
S. dysenteriae	16.19±1.5	24.76±1.8	12.24±0.7	29.14±0.8	8.64±1.7	16.18±0.5	39.13±1.5	22.0.5±1.5
P. aeruginosa	30.13±1.8	34.72±1.7	18.45±0.5	24.12±1.7	25.22±0.5	30.64±1.8	40.17±1.6	17.0±1.6
E. coli	30.19±1.5	35.35±1.8	28.18±0.6	31.62±1.5	10.51±1.4	13.18±1.8	0.00±0.0	16.00±1.5
S. typhi	33.18±0.5	37.60±1.6	22.00±0.5	28.11±0.5	12.40±1.6	9.08±0.5	0.0 ±0.0	12.11±0.0
B. cereus	20.14±1.6	26.00±0.5	12.34±0.5	16.30±1.6	6.12±0.5	9.26±0.5	40.00±0.0	18.6±1.0
S.aureus	24.18±1.6	27.11±0.5	22.30±0.5	26.13±1.6	10.00±0.6	15.34±1.6	45.16±1.0	0±0.0
C. jujenum	18.12±1.6	38.74±1.6	20.14±0.5	25.16±1.6	16.13±0.5	14.70±1.6	0±0.0	0.0±0.0
K.pneumoniae	25.18±0.6	38.74±1.6	26. 35±1.5	30.46±1.6	18.30±1.6	21.18±1.6	0.0±0.0	0.0±0.0

ME: methanol extracts, EE: Ethanol extracts.

Methycilin and Streptomycin ere used as the positive reference drugs at concentration of 2mg/ml.

Qualitative phytochemical detection in the plants extracts

Ajuga bracteosa palnts extracts Saponins and Alkaloids are present in plant parts. And tannins was absent in the leaf extracts. *Bergenia ciliate* extracts showed all the phytochemicals which mean these are higher source of plants metabolites. *Amaranthus viridis* leaf, stem barks and roots extracts showed the presence of all detected phytochemicals but steroids are absent in leaf extracts and alkaloids was absent in the roots extracts of the plants. The data are shown in the tables (Table-4).

Table 3. Mean inhibitory inhibition (mm) as shown by the crude Methanol's and Ethanol of the plants at 20mg/ml.

Name of Bacterial	*Amaranthus viridis*						References	
	Leaves		Bark		Root		Strept.	Methy.
	ME	EE	ME	EE	ME	EE		
S. dysenteriae	26.22±0.6	36.58±1.7	19.06±0.5	24.43±1.7	15.14±0.5	16.36±0.5	40.23±1.0	20.00±1.0
P. aeruginosa	35.20±1.6	38.18±0.6	32.00±0.6	34.52±1.5	30.08±0.6	36.14±0.6	39.18±1.0	15.0±1.0
E. coli	32.19±0.5	36.28±0.6	28.17±1.6	31.56±1.5	12.15±0.6	34.66±1.6	0.0±1.0	0.00±1.0
S. typhi	31.39±1.5	37.62±1.6	23.13±0.5	30.10±0.5	20.54±1.6	18.60±1.6	0.0 ±0.0	12.11±0.0
B. cereus	28.35±0.5	36.55±0.5	31.44±1.6	37.66±1.6	17.14±1.6	21.24±0.5	40.00±0.0	18.6±1.0
S.aureus	30.33±1.6	33.71±1.6	16.34±1.6	14.18±0.6	18.19±0.6	24.61±0.6	45.16±1.0	0±0.0
C. jujenum	32.14±0.5	36.47±1.6	30.11±1.6	36.65±1.6	14.16±1.6	29.34±1.6	0±0.0	0.0±0.0
K.pneumoniae	35.52±0.5	38.71±1.6	31.26±0.6	34.16±0.6	23.30±0.6	28.72±0.6	0.0±0.0	0.0±0.0

ME: methanol extracts, EE: Ethanol extracts.

Table 4. Qualitative Phytochemicals detection from the plants parts.

Phytochemicals names	*Ajuga bracteosa,*			*Bergenia ciliate,*			*Amaranthus viridis*		
	SB	LF	RT	SB	LF	RT	SB	LF	RT
Alkaloid	+++	++	++	++	++	++	++	++	--
Phenols	++	++	++	++	++	++	++	++	++
Glycosides	++	++	++	++	++	++	--	++	++
Flavonoids	++	++	++	++	++	++	++	++	++
Tannins	++	--	++	++	++	++	++	++	++
Steroids	++	++	++	--	++	++	++	--	++
Saponins	++	++	++	++	++	++	++	++	++

LF = Leave, SB = Stem bark, RT = Root.

Quantitative phytochemical detection in the plants extracts

For the quantative phytochemical detection in the plants extracts the methanolic extracts of the all three selected medicinal plants was used. In the *Ajuga bracteosa* the all phytochemicals were found in highest amounts and then followed by Amaranthus *viridis* and *Bergenia ciliate*. Phenols and flavonoids was present in highest amount which is followed by Tannins, Saponins and alkaloids. The data are shown in the (table 5).

Table 5. Quantitative phytochemicals detection from the plant parts.

	Percentage of Phytochemicals in plants (%)								
Phytochemicals name	*Ajuga bracteosa,*			*Bergenia ciliate,*			*Amaranthus viridis*		
	SB	RT	LF	SB	RT	LF	SB	RT	LF
Saponins	3.5±0.3	2.7±0.5	3.1.54±0.6	2.42±0.8	0.35±0.6	0.23±0.5	4.13±0.3	3.46±0.5	1.34±0.7
Flavonoid	0.28±0.3	0.77±0.4	0.33±0.6	0.45±0.6	0.74±0.2	0.34±0.3	0.79±0.4	0.97±0.3	0.84±0.3
Tannins	21.14±041	22.10±0.3	19.11±0.3	17.13±0.5	14.16±0.5	16.17±0.7	22.75±0.2	19.45±0.2	22.78±0.1
Alkaloid	1.60±1.3	1.72±0.4	1.81±0.4	1.36±0.4	1.38±0.1	1.23±0.2	2.38±0.2	1.34±0.4	2.09±0.3
Phenols	0.09±0.4	0.15±0.6	0.30±0.3	0.10±0.4	0.09±0.4	0.11 ±0.3	0.13±0.3	0.54±0.3	0.74±0.3

ME: methanol extracts, EE: Ethanol extracts.

SB, stem bark, RT, Roots, LF, Leaves.

Plants Extracts Minimal inhibition concentrations (MIC) Activity

Minimum inhibitory activity was showed by all the selected plants extracts against the selected bacterial strains *E.coli, S.typhi, C. jejumum* and *K. pneumoniae*. Than the reference drugs these selected bacterial were quite susceptible to the plant extracts though at higher concentrations. The plant *Ajuga bracteosa* roots showed higher activity (10nm) against the all bacteria. Then followed by *Bergenia ciliate* and which showed also a higher concentration of inhibition. The leaves, stem bark and roots extracts showed higher inhibition. The Minimum inhibitory activity of the extracts against the tested bacterial was showed in tables 6 and 7.

The reference antibiotics (methicilin and streptomycin) as the positive control in concentration of 2mg/ml as used in this study though higher in inhibitory affinity (39-45mm) (streptomycin).

Table 6. Minimal inhibition concentrations (MIC) of the plants extracts.

Bacterial organisms	*Ajuga bracteosa*						*Bergenia ciliate*					
	Bark		Root		Leave		Bark		Root		Leave	
	EE	ME	EE	ME	EE	ME	EE	ME	EE	ME	EE	ME
S. dysenteriae	10	1.2	1.2	10	10	10	10	10	10	10	10	10
P. aeruginosa	5	1.2	1.2	10	10	10	1.2	2.5	5	5	5	10
E. coli	5	1.2	1.2	10	10	10	2.5	2.5	5	5	2.5	2.5
S. typhi	5	1.2	1.2	10	10	10	5	5	2.5	5	10	10
B. cereus	5	1.2	1.2	10	10	10	5	5	5	5	10	10
S .aureus	5	1.2	1.2	10	10	10	5	5	10	10	10	10
C. jejunum	10	1.2	1.2	10	10	10	5	5	10	10	10	10
K. pneumoniae	5	1.2	1.2	5	2.5	5	5	5	5	5	10	

ME: methanol extracts, EE: Ethanol extracts.

Discussion

In the present research work the both qualitative and quantative phytochemical investigation of methanolic and ethanolic extracts of *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis* and anti- bacterial activity was carried out. The bioactive compounds on the medicinal plants employed contain various secondary metabolites such as phenols, tannins, alkaloids, flavonoids, steroids and glycosides in appreciable quantities. More antimicrobial activities was shown by *Bergenia ciliate* among the three plants. The *Bergenia ciliate* showed maximum inhibition against *S. dysenteriae* (33.18±1.6) in stem barks extracts. Fooled by leaves extracts (32.14±0.7) against *P. Aeruginosa* and roots showed (27.63±0.6) inhibition against *K.pneumoniae. Ajuga bracteosa* palnts extracts Saponins and Alkaloids are present in plant parts. . The plant *Ajuga bracteosa* roots showed higher activity (10nm) against the all bacteria. Then followed by *Bergenia ciliate* and which showed also a higher concentration of inhibition. The leaves, stem bark and roots extracts showed higher inhibition. And tannins was absent in the leaf extracts. *Bergenia ciliate* extracts showed all the phytochemicals which mean these are higher source of plants metabolites. *Amaranthus viridis* leaf, stem barks and roots extracts showed the presence of all detected phytochemicals but steroids are absent in leaf extracts and alkaloids was absent in the roots extracts of the plants. Based on the present study, we can consider the plants understudied of the *Ajuga bracteosa, Bergenia ciliate,* and *Amaranthus viridis* leaves, stem bark and roots to be good sources of antimicrobial property. The effective inhibitory potency observed with the plants parts; proof it that the inhibitory compounds were extractable by the employed solvents against the tested pathogenic bacterial isolates. This observation as reported correlates with (De and James 2002) who emphasized that these compounds are known to show medicinal activity as

well as exhibiting physiological activity. This concentration was visibly active on the tested bacterial isolates due to the combinative therapeutic actions of the various secondary metabolites contained in the plants. Some of the tested bacterial isolates such as *S.dysentariae, P aeruginosa*, *E. coli* and *K. pneumoniae* reported to be associated with nosocomial and community acquired infections (Indrayan *et al.*, 2002) were found susceptible to the plants crude extracts used in this study.

These plants, most especially their leaves and stem bark in dry powder form could be used for direct consumption as various kinds of beverages, decoctions and infusions. Healthy fresh leaves of these plants could as well be prepared as soup.

Table 7. Minim inhibition concentrations (MIC) Amaranthus viridis extracts.

Name of Bacterial	*Amaranthus viridis*						References	
	Leaves		Stem Bark		Root		Strept.	Methy.
	ME	EE	ME	EE	ME	EE		
S. dysenteriae	10.00	10.00	2.5	5.0	10.0	10.0	0.125	1.0
P. aeruginosa	1.25	1.25	2.5	1.25	1.25	1.25	0.125	1.0
E. coli	1.25	1.25	2.5	1.25	10.0	1.25	-	-
S. typhi	1.25	1.25	1.25	1.25	10.0	5.0	-	-
B. cereus	1.25	1.25	5.0	1.25	10.0	5.0	0.125	1.0
S.aureus	1.25	1.25	2.5	2.5	10.0	5.0	0.125	1.0
C. jujenum	1.25	1.25	2.5	2.5	5.0	5.0	-	-
K. pneumoniae	1.25	1.25	2.5	2.5	5.0	5.0	-	-

Streptomycin and Methycilin at concentration of 2mg/ml were used as the positive reference drugs.
ME: methanol extracts, EE: Ethanol extracts.

The percentage MIC values of the plants were based where both the ethanol and aqueous extracts have the same MIC values on the tested bacterial isolates. This perhaps helps to interpret that differences in inhibitory diameters (mm) could result in the same therapeutic potency when varied in concentrations, depending on the organism's susceptibility to the antibacterial components present in the extracts. The presence and the phytochemical components of the studied plants, the inhibitory zones and the MIC concentrations at which values were effective on the tested organisms, highlights that there were variations in the antibacterial potency of the plants extracts. The variations in the sensitivity could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum size (Gaill and Jon, 1995).
It has been reported that antibiotics are not the only antibacterial agents and this study observed the effective potency of the studied plants extracts on the selected pathogenic bacterial isolate than some highly rated antibiotics (reference drug) in disease cure and prevention ((Vohra and Kaur, 2011). Irrespective of the plants parts in this study and methods of extraction (methanol and ethanol), a dosage of between 1.2-10mg exhibited appreciable inhibitory values on the tested bacterial species. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000). The saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti as inflammatory activity and weight loss (murugan *et al.*, 2014). Saponins act as antimicrobial activity and extremely cold blooded animals, but toxicity to mammals is low (Verma *et al.*, 2013). Alkaloids have the analgesic, antispasmodic and antibacterial (Malik *et al.*, 2017) properties.

Alkaloids have been used as both antibacterial and antidiabetic properties and useful for such activities. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Akinyeye *et al.,* 2014). Glycosides are known to lower the blood pressure. Tannins are also known antimicrobial agent. Tannins (commonly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods. Tannins are water soluble plant polyphenols that precipitate proteins. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Sodipo *et al.*, 1991). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al.*, 1998).

Conclusion

The selected medicinal plants are the main basic source of the phytochemicals i.e., alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides and terpenoids. The antibacterial, plants are due to the presence of the above present phytochemicals.

The methanolic extracts of the plants displayed extensively a competitive inhibitory potency with the more effective ethanol extracts of the plants parts on the tested isolates which majority are Gram negative bacteria known for their ability to form resistance to drug. The plants parts though effective on all the bacterial isolates, there were variations in inhibitory potency resulting from variations in the secondary metabolites concentrations in the plants parts.

Recommendation

From the present research work we have recommended the selected medicinal plants for the isolation of active compounds. Also recommend at to perform other pharmacological activities in the different extracts of the plants.

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