Evaluation of invitro antimicrobial potential and phytochemical composition of some medicinal plants against pathogenic microbes in kashmir

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Abstract

Background: The rapid emergence of multidrug resistant microbes and decline in the synthesis of new drugs has forced the search for alternate sources of antimicrobial agents. Medicinal plants represent an excellent option for obtaining next generation antimicrobials. The current study evaluates the antibacterial and antifungal activity of methanolic and aqueous extracts of some traditionally used medicinal plants.
Methods: Antibacterial and antifungal assays were performed by agar well diffusion method. Bacterial strains employed were *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli*. The fungal strains used were *Penicillium chrysogenum*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae* and *Candida albicans.* The qualitative phytochemical screening was carried out by using the standard methods.
Results: The most susceptible microbial strains were *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis and Saccharomyces cerevisiae* while the least susceptible strains were *Klebsiella pneumoniae* and *Aspergillus fumigatus*. Highest antibacterial activity was exhibited by methanolic extract of *Pseudophegopteris levingei* with zone of inhibition 26.33±0.93 (*Staphylococcus aureus*), 24.33±1.48 (*Klebsiella pneumoniae*), 23.0±0.87 (*Proteus vulgaris*), 22.0±1.0 (*Bacillus subtilis*), 21.0± 0.52 (*Pseudomonas aeruginosa*) and 16.5±0.29 (*Escherichia coli*) at maximum concentration (100mg/ml). Highest antifungal activity was observed with the methanolic extract of *Amaranthus caudatus* with zone of inhibition 22.0± 0.62 (*Aspergillus fumigatus*), 21.0± 0.16 (*Candida albicans*) and 21.33±1.49 (*Saccharomyces cerevisiae*) at 100mg/ml. Phytochemical screening of plants revealed the presence of secondary metabolites like flavonoids, saponins, tannins, anthraquinones, and alkaloids. Maximum numbers of phytochemicals were detected in *Pseudophegopteris levingei.*
Conclusion: Present study reveals that the plants studied possess significant potential to be used as sources for future antimicrobials.
KEYWORDS: Antimicrobial activity, Antifungal activity, phytochemical screening, methanol and aqueous extracts.

Introduction

Current healthcare system is being challenged by the emerging menace of multiple drug resistant microbes. In fact, resistance to anti-microbial agents has become a big hurdle in the treatment of many infectious diseases. Out of two million people who acquire bacterial infections in U.S. hospitals annually, 70% of cases involve those strains that are resistant to at least one drug. In U.K., Methicillin-Resistant *Staphylococcus aureus*

(MRSA), which was at low levels a decade ago, has now increased to about 50% of all *Staphylococcus aureus* isolates [4]. In addition, the pace of generating antibiotics from microbial sources has drastically slowed down. There is desperate need of investment and research in the field of anti-infectives if a public health crisis is to be averted [5]. Exploration of medicinal plants represents an excellent option to obtain futuristic antimicrobial drugs. Medicinal plants have been traditionally used for multiple therapeutic purposes all over the world since antiquity to date [1, 2]. Traditionally used medicinal plants are the source of many novel compounds that are used for treating various microbial infections [39]. Plant based drugs are easily accessible, inexpensive and safe. Although a vast number of plant species have been tested for antimicrobial properties, but still majority of them have not been evaluated thoroughly [3]. The systematic screening of plant extracts is an excellent strategy to discover new compounds with antimicrobial potential. The present study is an attempt to evaluate the antimicrobial potential of some traditionally used medicinal plants of Kashmir valley

Materials and methods

Collection and identification of plant material

Ten medicinal plants were collected from higher reaches of Kashmir Valley, India and identified in the Centre of Plant Taxonomy (COPT), Department of Botany, University of Kashmir. Specimen of each plant is retained in the KASH herbarium of COPT under a specific voucher specimen number. The various plants collected include *Adiantum capillus* (2066-KASH), *Amaranthus caudatus* (2056-KASH), *Artemisia absinthium* (2059-KASH), *Pseudophegopteris levingei* (2071-KASH), *Datura stramonium* (2058-KASH), *Fragaria nubicola* (2063-KASH), *Hedera nepalensis* (2073-KASH), *Portulaca oleraceae (*2061-KASH), *Strobillanthes urticifolia* (2074-KASH) and *Urtica dioca* (2069-KASH).

Preparation of extracts

Whole plant samples were allowed to shade dry at 30±2°C. The dried plant materials were ground into coarse powder with the help of grinder and extracted using methanol and water as solvents, extractor (60-80°C). The extracts so obtained were concentrated with the help of rotary evaporator under reduced pressure and solid extracts waere stored in a refrigerator at 4^{o}c.

Test micro-organisms

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Test micro-organisms

The Bacterial and fungal strains were obtained from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India. Six bacterial strains including two Gram positive bacteria namely *Staphylococcus aureus* (MTCC-2940), *Bacillus subtilis* (MTCC-441) *and four* Gram negative bacteria namely *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-139), *Escherichia coli* (MTCC-739), and *Pseudomonas aeruginosa* (MTCC-424) were employed for antibacterial assay. Four fungal strains, *Candida albicans* (MTCC-227), *Saccharomyces cerevisiae* (MTCC-170),

Aspergillus fumigatus (MTCC-1811) and *Penicillium chrysogenum (*MTCC-947) were employed for antifungal assay. Bacterial and fungal strains were maintained by subculturing them on Mueller Hinton Agar and Sabouraud Dextrose Agar respectively after every fifteen days and then stored at 4ºC.Gentamycin discs and Nystatin powder was obtained from EOS Laboratories, India and served as positive controls for antibacterial and antifungal assays respectively. 10%Dimethylsulfoxide (DMSO) was used as negative control.

Antibacterial assay

Antibacterial assay was performed by Agar well diffusion method as described by Irshad et al [45] with some modifications. 100µl of standardized inoculum (0.5 Mc Farland) of each test bacterium was inoculated on molten Mueller Hinton Agar, homogenised and then poured into sterile petri plates to yield a uniform depth of 4mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5mm in diameter were used to make uniform and equidistant wells into each petriplate. 100µl of each concentration (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) of plant extracts, prepared in 10%DMSO were loaded into different peripheral wells. Gentamycin (10µg/disc) disc was placed at the centre of each petriplate and served as positive control, while as 10%Dimethylsulfoxide served as negative control in a separate petri plate. The petri plates were then incubated at 37ºC for 18 to 24 hours in an incubator. The plates were then observed for the zones of inhibition. Antibacterial potential was evaluated by measuring the diameters of zones of inhibition in millimeters (mm) with the help of a standard measuring scale. The lowest concentration of the extract (between the range 10-100mg/ml) which does not permit the growth of test bacteria was considered as minimum inhibitory concentration (MIC).

Antifungal assay

Antifungal assay was also performed by the method of agar well diffusion as described by Ahmad et al [46]. with some modification 100µl of standardized inoculum (0.5 Mc Farland) of each test fungi were inoculated on sterile molten Sabouraud Dextrose Agar homogenised and poured into a sterile petri plate to yield a uniform depth of 4mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5mm in diameter were used to make five wells at periphery and one well at centre of each petriplate. 100µl of each concentration (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) of plant extract, prepared in 10%DMSO were loaded into five different peripheral wells. 100µl of Standard antibiotic Nystatin (0.5mg/ml) was loaded into the central well while as 10%Dimethylsulfoxide alone was used as negative control in a separate petri plate. The plates were then incubated at 32ºC for 24 to 36 hours. After incubation period, the plates were observed for the zones of inhibition. Antifungal potential was evaluated by measuring inhibition zone diameters in millimeters (mm) with the help of standard measuring scale. The lowest concentration of the extract (between the range 10-100mg/ml) that prevented visible growth of test fungi was considered as minimum inhibitory concentration (MIC).

Phytochemical screening

Qualitative phytochemical screening of both the aqueous and methanolic extracts was carried out to know the nature of phytochemicals present in them. Flavonoids were detected by lead acetate test while the rest of phytochemicals were detected by the methods described earlier [6].

Test for steroids

To 0.5 ml of solvent extract, 2ml of acetic acid was added and then 2ml of concentrated sulphuric acid was added. Appearance of Blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for tannins

To 5ml of solvent extract, two drops of 5% $Fecl_3$ were added. Production of greenish precipitate indicated the presence of tannins.

Test for terpenoids

To 5 ml of solvent extract, 2ml of chloroform was added and then 3ml of concentrated sulphuric acid was added carefully. Appearance of reddish brown colouration of the interface was regarded as positive for the presence of terpenoids.

Test for flavonoids

To 2 ml of solvent extract, a few drops of lead acetate solution were added. Formation of yellow coloured precipitate was regarded as positive for the presence of flavonoids.

Test for alkaloids

To 2ml of solvent extract, a little amount of picric acid solution was added. Formation of orange colour indicated the presence of alkaloids.

Test for saponins

About 1 ml of solvent extract was introduced into a tube containing 1ml of distilled water and the mixture was vigorously shaken for 2 minutes. Formation of froth indicated the presence of saponins.

Test for anthraquinones

2ml of solvent extract was added to 10 ml of benzene, and then 0.5ml of ammonia solution was added. The mixture was shaken well. Violet colour in the layer phase indicated the presence of anthraquinones.

Test for phenols

To 2 ml of solvent extract, 2ml of ferric chloride solution was added. Formation of deep bluish green solution indicated the presence of phenols.

Test for cardiac glycosides

To 2ml of solvent extract, 2 ml of glacial acetic acid containing 1 drop of ferric chloride was added. Then 2ml of concentrated sulphuric acid (H_2SO_4) was added under layered

Results

Antibacterial activity

The methanolic extracts of different plants showed the zones of inhibition ranging between 12.0-24.33mm against (*Klebsiella pneumoniae*), 11-16mm (*Escherichia coli*) 13-21mm (*Pseudomonas aeruginosa*), 10-22mm (*Bacillus subtilis*), 12-26.33mm (*Staphylococcus aureus*) and 10-23mm (*Proteus vulgaris*) at the maximum concentration (100mg/ml). Aqueous extracts exhibited the zones of inhibition ranging between 11-14.33mm against (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*), 11-15mm against *Bacillus subtilis*, 13-16 against *Staphylococcus aureus* and 13-14.44mm against *Escherichia coli* at the maximum concentration (100mg/ml). Methanolic extract of *Pseudophegopteris levingei* showed highest activity against all the tested bacteria with the zone of inhibition 26.33±0.93 (*Staphylococcus aureus*), 24.33±1.48 (*Klebsiella pneumoniae*), 23.0±0.87 (*Proteus vulgaris*), 22.0±1.0 (*Bacillus subtilis*), 21.0± 0.52 (*Pseudomonas aeruginosa*) and 16.5±0.09 (*Escherichia coli*) at maximum concentration (100mg/ml). Among aqueous extracts the highest activity was

exhibited by ***Pseudophegopteris levingei*** against *Staphylococcus aureus* (16.48±0.85mm) and *Bacillus subtilis* (15.75±0.61mm), *Datura stramonium* against *Proteus vulgaris* (14.0±0.09mm), *Klebsiella pneumoniae* (15.57±0.39mm), and *Pseudomonas aeruginosa* (14.0±0.57mm), *Artemisia absinthium against Escherichia coli* (15.33±0.55mm) at maximum concentration (100mg/ml). The results were compared to positive control (Gentamycin), which showed the zone of inhibition 25.82±0.95 mm against *(Klebsiella pneumoniae),* 25.45±1.56 mm (*Bacillus subtilis), 26.33*±1.93 (*Proteus vulgaris),* 25.83±1.44 (*Pseudomonas aeruginosa),* 27.42±1.75mm (*Staphylococcus aureus*) and 20.50± 1.41mm against *(Escherichia coli*) (Table2-7).

Antifungal activity

The methanolic extracts of different plants showed the zones of inhibition ranging between 14.46-21.0mm against (*Candida albicans),* 14.0-21.31 against (*Saccharomyces cerevisiae*), 10.83-22.0mm against (*Aspergillus fumigatus*) and 12.0-18.0mm (*Penicillium chrysogenum*) at the maximum concentration (100mg/ml). Aqueous extracts also showed considerable activity with zones of inhibition ranging between 14.75-17.64mm against (*Candida albicans*), 14.0-19.5mm (*Saccharomyces cerevisiae*) 13.0-21.0mm (*Aspergillus fumigatus*) and 11.33-17.0mm (*Penicillium chrysogenum*) at the maximum concentration (100mg/ml). Methanolic extract of *Amaranthus caudatus* showed the highest activity against *Candida albicans* (21.0±0.16mm), *Saccharomyces cerevisiae* (21.31±1.49mm), and *Aspergillus fumigatus* (22.0±0.62mm) whereas the methanolic extract of *Artemisia absinthium* showed highest activity against *Penicillium chrysogenum* (18.0±0.30mm). As far as aqueous extracts are concerned, highest activity was exhibited by *Hedera nepalensis* against *Candida albicans* (17.64±0.58mm), *Portulaca oleraceae* against *Aspergillus fumigatus* (21.0±1.75mm), and *Datura stramonium* with zone of inhibition 17.0±0.25mm and 19.5±1.58mm against *Penicillium chrysogenum* and *Saccharomyces cerevisiae* respectively at the maximum concentration (100mg/ml). The results were compared to positive control (Nystatin) which showed the zones of inhibition equal to 30.56±1.26mm against *Candida albicans* 30.57±1.68mm *against Saccharomyces cerevisiae*, 25.32±0.91mm against *Penicillium chrysogenum* and 27.21±1.35mm against *Aspergillus fumigatus*(Tables 8-11).

Minimum Inhibitory Concentration

The MIC of most of the plant extracts does not fall within the selected range (10-100mg/ml), thereby indicating their high antimicrobial potential (Table 12). A thorough analysis of MIC results reveal that certain bacterial and fungal strains are more sensitive to plant extracts than others. The increasing order of bacterial sensitivity to plant extracts follow the pattern- *Klebsiella Pneumoniae< Proteus vulgaris< Staphylococcus aureus < Bacillus subtilis< Escherichia coli <Pseudomonas aeruginosa*. Similarly, the increasing order of fungal sensitivity to plant extracts follow the pattern- *Aspergillus fumigatus< Penicillium chrysogenum< Candida albicans<Saccharomyces cerevisiae.*

Phytochemical screening.

The phytochemical analysis of medicinal plants revealed the presence of various secondary metabolites in them (Table 1). Out of the 10 selected plants, all 10 plants showed the presence of phenols, saponins, tannins and flavonoids, 9 plants showed the presence of terpenoids, 8 plants showed the presence of cardenolides and volatile oils, 7 plants showed the presence of cardiac glycosides, 6 plants showed the presence of alkaloids, 5 plants showed the presence of steroids and only 4 plants showed the presence

of anthraquinones and phlobtannins. The maximum numbers of tested phytochemicals were detected in *Pseudophegopteris levingei* (i.e., 11/12) and least in *Amaranthus caudatus* (i.e., 7/12) and *portulaca oleraceae* (i.e., 7/12). Flavonoids, tannins and phenols were detected in aqueous and methanolic extracts of all the plants studied. While the Alkaloids, anthraquinone and cardenolides were found absent in all the methanolic extracts and detected only in aqueous extracts of some plants.

Discussion

Pathogenic microorganisms have always posed a serious threat to human health by causing various dreadful diseases like syphilis, malaria, cholera, candidiasis, aspergillosis, and AIDs. The microbes used in the current study are associated with many infections. *Proteus vulgaris* is an opportunistic pathogen responsible for causing urinary tract infections and wound infections. *Escherichia coli* is responsible for causing severe cramps and diarrhea. *Escherichia coli* is also the causative agent of gastrointestinal and urinary tract infections [41] *Klebsiella pneumonia* is the causative agent of pneumonia, characterized by emission of bloody sputum. *Staphylococcus aureus* is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. *Pseudomonas aeruginosa* is a causative agent of many nosocomial infections (infections acquired in hospitals). *Pseudomonas aeruginosa* and *Staphylococcus aureus* are also associated with dental caries [44]. *Bacillus subtilis* can sometimes lead to food poisoning. *Candida albicans* is the causative agent of candidiasis. *Aspergillus fumigatus* can cause chronic pulmonary infections and allergic bronchopulmonary aspergillosis [11]. *Penicillium chrysogenum* can cause infection in people with severely suppressed immune systems, like those with human immunodeficiency virus (HIV) and characterized by pulmonary infection including pneumonia, localized granulomas, fungus balls, and systemic infection. The airborne asexual spores of *Penicillium chrysogenum* are important human allergens [12]. While as 1% of all vaginal yeast infections occur due to *Saccharomyces cerevisiae* [13].

Medicinal plants were the first weapons that the man used against pathogenic microbes. Multiple studies have reported the antimicrobial potential of plants [8-10]. In the current study, almost all the plants were found to possess antimicrobial activity; however the potential varied with the species of plants. Similar results were observed by [40**].** This could be due to many factors like soil composition, climate, age and vegetation cycle stage, quality of extracted product [14,15]. According to current study, the pattern of inhibition varied with the type of plant extract and the microorganism used which is in accordance to the results obtained by [41]. Moreover, the type of solvent has an important role in the process of extraction [16-18]. MIC of most of the plant extracts was not detected within the selected range of 10-100mg/ml which indicates the strong antimicrobial potential of extracts. Besides, MIC results revealed certain important facts regarding the susceptibility (sensitivity) of different microbial strains to various plant extracts. *Pseudomonas aeruginosa*, a gram –ve bacteria was found most susceptible (sensitive) among all the bacterial strains under study which is in agreement with the results obtained by Kavishankar et al, 2011 [19]. *Klebsiella pneumoniae* was found as the most resistant bacterial strain. Among fungal strains, *Saccharomyces cerevisiae* was detected as the most susceptible strain, while *Aspergillus fumigatus* the most resistant.

Medicinal plants are rich sources of therapeutically active compounds but only a small fraction of them have been isolated [20]. Bioprospection of secondary metabolites is an

important step in the development of new drugs [42.43]. Phytochemical analysis revealed the presence of various secondary metabolites like flavonoids, alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, and volatile oils in the plants under study. Many of these phytochemicals act as warriors in the plant defense mechanisms against predation by microorganisms. Phenolic compounds possess anti-microbial activity due to the presence of hydroxl (OH) group(s) in them [21]. Flavonoids are known to be synthesized by the plants in response to microbial infection [22]. Flavonoids are effective against a wide array of microorganisms. Their antimicrobial activity is probably due to their ability to complex with bacterial cell wall and they can also disrupt cell membranes [23,24]. Tannins posses a wide range of anti-infective activities [25]. Tannins have the ability to complex with proteins through hydrogen bonding, hydrophobic interactions as well as covalent bond formation [26,27]. Their antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins and also to complex with polysaccharides [28]. Terpenes are effective against bacteria fungi, viruses, and protozoa [29-33]. Multiple studies have proved the antimicrobial potential of alkaloids. Their mechanism of action is attributed to their ability to intercalate with DNA [34-37]. Saponins possess antimicrobial potential due to their ability to insert into lipid bilayer, bind to cholesterol and form cholesterol-saponin complex that can lyse the microbial cell membrane [38]. In addition, volatile oils, cardiac glycosides and various other phytochemicals have been also found to possess antimicrobial properties. The current study has revealed the presence of various phytochemicals in different plants and it is obvious that the plants may possess the antimicrobial potential due to any of these detected Phytoconstituents..

Conclusion

The current study suggests that the plant studied does contain compounds with antimicrobial properties. However there is need for isolation, purification and structure elucidation of such compounds so that they could be subjected to clinical trials and used as next generation antimicrobial agents.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Preliminary phytochemical careening of selected medicinal plants.

Note: (-) = Absent, (+) = Present

S.No.	Plant name	Solvents	Alkaloids	Anthraquinones	Cardiac glycoside	Cardenolides	Flavonoids	Phenols	Phlobtannins	Saponins	Steroids	Tannins	Terpenoids	Volatile oils
1	*Adiantum*	Aqueous	+	+	-	+	+	+	+	+	+	+	-	-
	capillus	methanol	-	-	-	-	+	+	-	+	-	+	+	-
2	*Amaranthus*	Aqueous	-	-	-	-	+	+	+	+	-	+	+	-
	caudatus	methanol	-	-	-	-	+	-	-	+	-	+	+	+
3	*Artemisia*	Aqueous	+	+	-	+	+	+	-	+	+	+	-	-
	absinthium	methanol	-	-	-	-	+	-	-	-	-	+	+	+
4	*Pseudophegopteris*	Aqueous	+	-	+	+	+	+	+	+	-	+	+	+
	levingei	methanol	-	-	+	-	+	+	+	+	+	+	-	+
5	*Datura*	Aqueous	-	+	+	+	+	+	+	+	-	+	-	+
	stramonium	methanol	-	-	+	-	+	-	+	+	-	+	+	+
6	*Fragaria*	Aqueous	-	+	+	-	+	+	-	+	-	+	+	-
	nubicola	methanol	-	-	+	-	+	-	-	+	-	+	+	+
7	*Hedera*	Aqueous	+	-	+	+	+	+	-	+	-	+	+	-
	nepalensis	methanol	-	-	+	-	+	-	-	+	+	+	-	+
8	*Portulaca*	Aqueous	-	-	-	+	+	+	-	+	-	+	-	+
	oleraceae	methanol	-	-	+	-	+	-	-	-	-	+	-	+
	Strobillanthes	Aqueous	+	-	+	+	+	+	-	+	-	+	+	-
9	*urticifolia*	methanol	-	-	-	-	+	-	-	+	-	+	-	+
10	*Urtica dioca*	Aqueous	+	-	+	+	+	+	-	+	-	+	+	-
		methanol	-	-	+	-	+	+	-	+	+	+	-	-

Table 2 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Klebsiella pneumoniae.*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum***	Aqueous	-	-	-	-	11±0.39
	capillus	Methanolic	-	-	10±0.49	11±0.25	12±0.28
2	***Amaranthus***	Aqueous	-	10±0.37	10±0.36	10±0.52	11±0.31
	caudatus	Methanolic	-	10±0.10	12±0.63	14±0.66	15±0.25
3	***Artemisia***	Aqueous	10±0.33	11±0.20	11±0.37	12±0.25	13±0.98
	absinthium	Methanolic	-	11±0.28	12±0.29	13±0.21	14±0.57
4	***Pseudophegopteris***	Aqueous	10±0.28	11±0.30	12±0.55	13±0.68	14±0.19
	levingei	Methanolic	**14**±0.27	**18**±0.36	**19**±0.39	**22**±0.34	**24**±0.48
5	***Datura***	Aqueous	14±0.26	14±0.24	15±0.31	15±0.33	15±0.39
	stramonium	Methanolic	10±0.28	11±0.27	12±0.39	13±0.37	14±0.17
6	***Fragaria***	Aqueous	-	-	-	-	-
	nubicola	Methanolic	11±0.34	12±0.28	13±0.62	14±0.27	16±0.35
7	***Hedera***	Aqueous	-	-	-	-	13±0.38
	nepalensis	Methanolic	8±0.31	10±0.28	13±0.28	14±0.20	15±0.11
8	***Portulaca***	Aqueous	-	-	-	-	12±0.84
	oleraceae	Methanolic	9±0.25	10±0.87	11±0.85	12±0.22	13±0.47
9	***Strobillanthes***	Aqueous	-	-	-	-	-
	urticifolia	Methanolic	-	11±0.22	12±0.32	13±0.41	14±0.52
10	***Urtica dioca***	Aqueous	-	-	-	-	-
		Methanolic	9±0.36	10±0.13	10±0.39	11±0.98	12±0.35
11	**Gentamycin (10µg/disc)**	25 ±0.69mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 3 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Escherichia coli.*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum***	Aqueous	12±0.15	13±0.35	13±0.28	13±0.32	13±0.29
	capillus	Methanolic	9±0.22	9±0.33	10±48	10±0.23	12±0.31
2	***Amaranthus***	Aqueous	11±0.20	12±0.36	13±0.33	14±0.39	14±0.22
	caudatus	Methanolic	9±0.29	11±0.38	11±0.58	11±0.37	11±0.22
3	***Artemisia***	Aqueous	-	11±0.89	12±0.64	13±0.13	13±0.33
	absinthium	Methanolic	13±0.11	14±0.46	14±0.57	15±0.19	16±0.51
4	***Pseudophegopteris***	Aqueous	12±0.36	12±0.22	13±0.41	13±0.95	14±0.37
	levingei	Methanolic	12±0.82	13±0.15	14±0.31	15±0.38	16±0.09
5	***Datura***	Aqueous	12±0.73	12±0.39	12±0.29	13±0.66	13±0.12
	stramonium	Methanolic	-	-	-	-	-
6	***Fragaria***	Aqueous	11±0.64	12±0.07	13±0.88	14±0.67	14±0.13
	nubicola	Methanolic	13±0.36	13±0.85	14±0.74	14±0.69	15±0.28
7	***Hedera***	Aqueous	12±0.27	12±0.36	12±0.22	14±0.34	14±0.52
	nepalensis	Methanolic	-	-	-	-	9±0.22
8	***Portulaca***	Aqueous	11±0.55	12±0.33	13±0.85	13±0.23	14±0.49
	oleraceae	Methanolic	11±0.34	12±0.27	12±0.33	13±0.26	13±0.65
9	***Strobillanthes***	Aqueous	12±0.22	13±0.39	13±0.29	13±0.34	13±0.16
	urticifolia	Methanolic	-	-	-	-	11±0.17
10	***Urtica dioca***	Aqueous	11±0.11	12±0.19	12±0.23	13±0.38	13±0.38
		Methanolic	-	10±0.31	11±0.61	12±0.61	12±0.28
11	**Gentamycin (10µg/disc)**	20± 0.88mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 4 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Pseudomonas aeruginosa.*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum capillus***	Aqueous	11±0.28	11±0.56	11±0.25	11±0.36	12±0.07
		Methanolic	10±0.33	11±0.78	11±0.38	12±0.29	13±0.15
2	***Amaranthus caudatus***	Aqueous	11±0.59	12±0.37	13±0.55	13±0.27	14±0.30
		Methanolic	10±0.33	11±0.28	12±0.10	13±0.35	14±0.22
3	***Artemisia absinthium***	Aqueous	9±0.27	10±0.39	11±0.39	12±0.45	13±0.12
		Methanolic	11±0.25	13±0.39	14±0.34	15±0.40	17±0.18
4	***Pseudophegopteris levingei***	Aqueous	-	9±0.33	11±0.67	12±0.23	13±0.49
		Methanolic	12±0.42	13±0.35	15±0.31	17±0.58	21±0.52
5	***Datura stramonium***	Aqueous	9±0.32	10±0.38	11±0.46	12±0.15	14±0.01
		Methanolic	10±0.26	12±0.36	13±0.39	13±0.30	14±0.16
6	***Fragaria nubicola***	Aqueous	9±0.36	10±0.85	11±0.34	12±0.64	13±0.32
		Methanolic	13±0.54	15±0.49	16±0.86	17±0.44	19±0.40
7	***Hedera nepalensis***	Aqueous	13±0.39	13±0.39	13±0.66	13±0.34	13±0.39
		Methanolic	-	11±0.34	12±0.66	13±0.34	13±0.37
8	***Portulaca oleraceae***	Aqueous	12±0.69	12±0.89	12±0.64	12±0.64	12±0.18
		Methanolic	12±0.28	14±0.64	15±0.59	16±0.47	17±0.64
9	***Strobillanthes urticifolia***	Aqueous	8±0.84	8±0.57	9±0.38	10±0.33	11±0.12
		Methanolic	12±0.39	13±0.94	14±0.31	15±0.38	15±0.05
10	***Urtica dioca***	Aqueous	9±0.34	9±0.39	10±0.52	10±0.38	11±0.11
		Methanolic	14±0.05	15±0.26	16±0.52	17±0.26	18±0.01
11	**Gentamycin (10µg/disc)**	25±1.23 mm					
12	**DMSO**	0 mm					

(-) = No Activity.

Table 5 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Bacillus subtilis.*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum***	Aqueous	11±0.75	12±0.59	13±0.89	13±0.85	13±0.76
	capillus	Methanolic	-	-	-	8±0.59	10±0.18
2	***Amaranthus***	Aqueous	10±0.39	11±0.79	11±0.71	11±0063	11±0.51
	caudatus	Methanolic	-	-	-	-	-
3	***Artemisia***	Aqueous	10±0.76	11±0.49	12±0.83	13±0.57	14±0.13
	absinthium	Methanolic	10±0.59	11±0.96	14±0.53	15±0.42	16±0.26
4	***Pseudophegopteris***	Aqueous	10±0.81	11±0.46	13±0.47	14±0.43	15±0.61
	levingei	Methanolic	12±0.52	15±0.36	18±0.52	20±0.24	22±0.06
5	***Datura***	Aqueous	11±0.28	12±0.56	13±0.26	14±0.43	15±0.43
	stramonium	Methanolic	7±0.53	9±0.23	10±0.36	12±0.41	13±0.53
6	***Fragaria***	Aqueous	9±0.26	10±0.08	11±0.52	12±0.43	13±0.42
	nubicola	Methanolic	10±0.41	12±0.62	13±0.43	14±0.36	15±0.37
7	***Hedera***	Aqueous	11±0.26	12±0.32	12±0.31	13±0.17	13±0.06
	nepalensis	Methanolic	8±0.72	9±0.86	10±0.46	12±0.36	13±.16
8	***Portulaca***	Aqueous	10±0.86	10±0.49	11±0.87	11±0.46	12±0.53
	oleraceae	Methanolic	-	-	-	8±0.76	10±0.59
9	***Strobillanthes***	Aqueous	9±0.26	10±0.46	10±0.40	11±0.75	11±0.53
	urticifolia	Methanolic	-	-	-	10±0.46	11±0.46
10	***Urtica dioca***	Aqueous	11±0.41	11±0.30	11±0.19	12±0.23	12±0.13
		Methanolic	8±0.45	9±0.42	10±0.26	12±0.53	13±0.43
11	**Gentamycin (10µg/disc)**	25±1.89 mm					
12	**DMSO**	0 mm					

(-) = No Activity.

Table 6 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Staphylococcus aureus.*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum capillus***	Aqueous	11±0.66	11±0.56	13±0.54	14±0.47	15±0.43
		Methanolic	-	10±0.29	11±0.53	11±0.47	12±0.57
2	***Amaranthus caudatus***	Aqueous	11±0.35	12±0.35	13±0.28	13±0.39	14±0.38
		Methanolic	-	11±0.39	12±0.39	14±0.34	14±0.08
3	***Artemisia absinthium***	Aqueous	13±0.38	13±0.39	14±0.92	14±0.75	16±0.52
		Methanolic	13±0.59	16±0.95	18±0.38	20±0.28	22±0.36
4	***Pseudophegopteris levingei***	Aqueous	12±0.85	13±0.86	15±0.69	16±0.87	16±0.49
		Methanolic	14±0.68	16±0.59	22±0.52	24±0.54	26±0.23
5	***Datura stramonium***	Aqueous	11±0.81	12±0.69	14±0.51	15±0.58	16±0.50
		Methanolic	13±0.37	16±0.29	17±0.27	18±0.39	19±0.29
6	***Fragaria nubicola***	Aqueous	11±0.83	13±0.94	13±0.64	13±0.19	14±0.31
		Methanolic	-	12±0.16	13±.08	14±0.28	14±0.17
7	***Hedera nepalensis***	Aqueous	11±0.34	12±0.52	12±0.61	12±0.37	13±0.39
		Methanolic	-	13±0.64	14±0.38	15±0.39	16±0.19
8	***Portulaca oleraceae***	Aqueous	11±0.82	11±0.96	12±0.76	13±0.67	14±0.52
		Methanolic	11±0.69	12±0.61	13±0.83	15±0.62	16±0.62
9	***Strobillanthes urticifolia***	Aqueous	-	11±0.13	12±0.11	13±0.14	14±0.11
		Methanolic	-	11±0.85	13±0.86	13±0.67	13±0.92
10	***Urtica dioca***	Aqueous	11±0.34	13±0.17	13±0.20	13±0.13	15±0.10
		Methanolic	11±0.96	12±0.59	13±0.49	14±0.75	14±0.62
11	**Gentamycin (10µg/disc)**	27±1.28 mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 7 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Proteus vulgaris*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum***	Aqueous	-	-	-	10±0.45	12±0.21
	capillus	Methanolic	-	-	-	-	10±0.59
2	***Amaranthus***	Aqueous	-	-	9±0.33	10±0.32	11±0.23
	caudatus	Methanolic	-	10±0.75	11±0.34	11±0.39	12±0.65
3	***Artemisia***	Aqueous	-	10±0.39	10±0.37	11±0.33	12±0.03
	absinthium	Methanolic	-	10±0.05	12±0.28	13±0.27	15±0.58
4	***Pseudophegopteris***	Aqueous	-	-	10±0.34	11±0.39	13±0.52
	levingei	Methanolic	13±0.37	15±0.29	20±0.16	22±0.54	23±0.87
5	***Datura***	Aqueous	10±0.16	10±0.07	11±0.78	12±0.11	14±0.09
	stramonium	Methanolic	10±0.17	10±0.06	11±0.62	12±0.58	13±0.35
6	***Fragaria***	Aqueous	-	-	9±0.68	10±0.37	11±0.37
	nubicola	Methanolic	10±0.36	11±0.60	13±0.65	14±0.64	15±0.95
7	***Hedera***	Aqueous	10±0.39	11±0.38	11±0.27	12±0.58	13±0.34
	nepalensis	Methanolic	-	10±0.19	11±0.29	11±0.24	12±0.75
8	***Portulaca***	Aqueous	-	-	10±0.95	10±0.22	10±0.23
	oleraceae	Methanolic	10±0.20	11±0.17	12±0.39	13±0.32	14±0.11
9	***Strobillanthes***	Aqueous	-	-	-	10±0.52	13±0.43
	urticifolia	Methanolic	12±0.53	13±0.29	14±0.21	15±0.23	15±0.36
10	***Urtica dioca***	Aqueous	-	-	12±0.15	12±0.26	12±0.36
		Methanolic	-	-	10±0.23	11±0.16	12±0.17
11	**Gentamycin (10µg/disc)**	25 ±0.46mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 8 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Candida albicans*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum capillus***	Aqueous	-	-	-	-	-
		Methanolic	-	11±0.14	12±0.19	13±0.43	14±0.10
2	***Amaranthus caudatus***	Aqueous	-	-	11±0.28	14±0.32	17±0.36
		Methanolic	8±0.21	13±0.38	14±0.29	16±0.34	21±0.16
3	***Artemisia absinthium***	Aqueous	-	-	-	-	-
		Methanolic	-	11±0.12	12±0.31	15±0.26	16±0.22
4	***Pseudophegopteris levingei***	Aqueous	-	-	-	-	-
		Methanolic	12±0.16	13±019	14±0.54	15±0.31	16±0.02
5	***Datura stramonium***	Aqueous	-	-	-	-	-
		Methanolic	-	10±0.34	10±0.26	15±0.41	17±0.23
6	***Fragaria nubicola***	Aqueous	11±0.22	12±0.28	13±0.35	14±0.45	15±0.29
		Methanolic	12±0.35	13±0.23	14±0.42	16±0.16	18±0.25
7	***Hedera nepalensis***	Aqueous	13±0.35	14±0.17	15±0.37	16±0.39	17±0.58
		Methanolic	14±0.46	15±0.53	15±0.50	15±0.41	18±0.23
8	***Portulaca oleraceae***	Aqueous	-	14±0.83	14±0.80	14±0.63	14±0.44
		Methanolic	14±0.61	15±0.56	15±0.16	16±0.34	17±0.29
9	***Strobillanthes urticifolia***	Aqueous	-	-	-	-	10±0.12
		Methanolic	13±0.36	13±0.37	13±0.45	15±0.36	18±0.41
10	***Urtica dioca***	Aqueous	-	-	-	-	-
		Methanolic	11±0.31	12±0.35	13±0.52	14±0.89	15±0.44
11	**Nystatin (0.5mg/ml)**	30±1.93 mm					
12	**DMSO**	0 mm					

(-) = No Activity.

Table 9 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Saccharomyces cerevisiae*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum capillus***	Aqueous	10±0.03	11±0.52	11±0.40	12±0.01	14±0.16
		Methanolic	8±0.22	12±0.55	12±0.28	13±0.35	14±0.25
2	***Amaranthus caudatus***	Aqueous	14±0.22	15±0.16	16±0.42	17±0.14	18±0.43
		Methanolic	15±0.06	17±0.49	18±0.38	18±0.35	21±0.01
3	***Artemisia absinthium***	Aqueous	14±0.92	15±0.48	16±0.20	17±0.56	18±0.06
		Methanolic	-	12±0.31	13±086	13±0.02	16±0.32
4	***Pseudophegopteris levingei***	Aqueous	11±0.38	13±0.68	14±0.31	15±0.38	16±0..35
		Methanolic	12±0.35	12±0.52	15±0.09	16±0.38	16±0.35
5	***Datura stramonium***	Aqueous	8±0.02	13±0.25	14±0.54	15±0.25	19±0.38
		Methanolic	-	8±0.31	13±0.33	14±0.55	14±0.31
6	***Fragaria nubicola***	Aqueous	11±0.02	12±0.28	13±0.34	15±0.23	16±0.34
		Methanolic	14±0.55	15±0.54	15±0.21	16±0.34	17±0.51
7	***Hedera nepalensis***	Aqueous	8±0.27	15±0.33	16±0.36	17±0.28	18±0.10
		Methanolic	13±0.41	13±0.22	14±0.57	14±0.27	15±0.39
8	***Portulaca oleraceae***	Aqueous	13±0.25	17±0.02	18±0.80	18±0.27	18±0.20
		Methanolic	-	15±0.14	16±0.11	18±0.31	19±0.25
9	***Strobillanthes urticifolia***	Aqueous	11±0.10	12±0.44	16±0.28	17±0.33	18±0.36
		Methanolic	9±0.58	14±0.41	17±0.36	18±0.85	18±0.21
10	***Urtica dioca***	Aqueous	13±0.25	14±0.02	15±0.48	15±0.36	17±0.30
		Methanolic	11±0.29	13±0.12	14±0.50	14±0.16	15±0.29
11	**Nystatin (0.5mg/ml)**	30 ±1.80mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 10 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Aspergillus fumigatus*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum capillus***	Aqueous	-	-	-	-	-
		Methanolic	-	8±0.28	9±0.16	10±0.23	11±0.53
2	***Amaranthus caudatus***	Aqueous	-	-	-	-	-
		Methanolic	15±0.24	17±0.56	18±0.22	20±0.39	22±0.62
3	***Artemisia absinthium***	Aqueous	-	-	-	-	-
		Methanolic	-	8±0.83	9±0.65	9±0.17	10±0.06
4	***Pseudophegopteris levingei***	Aqueous	-	-	-	-	-
		Methanolic	10±0.97	11±0.22	12±0.71	13±0.32	14±0.36
5	***Datura stramonium***	Aqueous	-	-	-	-	15±0.32
		Methanolic	-	10±0.80	11±0.32	12±0.14	13±0.10
6	***Fragaria nubicola***	Aqueous	-	11±0.49	12±0.64	13±0.73	14±0.19
		Methanolic	10±0.93	12±0.77	12±0.19	13±0.14	13±0.31
7	***Hedera nepalensis***	Aqueous	-	-	-	-	13±0.33
		Methanolic	8±0.96	8±0.86	8±0.75	8±0.60	8±0.44
8	***Portulaca oleraceae***	Aqueous	-	12±0.33	17±0.35	19±0.10	21±0.15
		Methanolic	8±0.16	9±0.32	10±0.20	11±0.31	11±0.22
9	***Strobillanthes urticifolia***	Aqueous	-	-	-	-	13±0.02
		Methanolic	-	-	-	-	-
10	***Urtica dioca***	Aqueous	8±0.86	9±0.75	11±0.66	12±0.58	13±0.57
		Methanolic	-	11±0.70	12±0.83	13±0.38	14±0.46
11	**Nystatin (0.5mg/ml)**	27±1.16 mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 11 Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Penicillium chrysogenum*

	Plant name	Extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	***Adiantum capillus***	Aqueous	-	-	-	-	-
		Methanolic	10±0.57	11±0.45	13±0.41	14±0.33	15±0.23
2	***Amaranthus caudatus***	Aqueous	-	-	-	-	-
		Methanolic	8±0.46	10±0.38	11±0.42	11±0.32	12±0.12
3	***Artemisia absinthium***	Aqueous	-	-	-	-	-
		Methanolic	-	10±0.28	12±0.36	14±0.18	18±0.30
4	***Pseudophegopteris levingei***	Aqueous	-	-	-	-	-
		Methanolic	12±0.33	13±0.52	14±0.81	15±0.34	17±0.16
5	***Datura stramonium***	Aqueous	-	14±0.36	15±0.17	16±0.25	17±0.25
		Methanolic	11±0.32	12±0.08	13±0.38	15±0.13	17±0.10
6	***Fragaria nubicola***	Aqueous	8±0.36	8±0.46	10±0.42	11±0.54	11±0.18
		Methanolic	8±0.31	9±0.22	10±0.11	12±0.26	14±0.15
7	***Hedera nepalensis***	Aqueous	-	-	9±0.24	12±0.23	13±0.27
		Methanolic	-	8±0.85	9±0.57	10±0.55	12±0.43
8	***Portulaca oleraceae***	Aqueous	-	-	11±0.31	12±0.22	13±0.01
		Methanolic	8±0.80	8±0.78	8±0.73	8±0.54	8±0.49
9	***Strobillanthes urticifolia***	Aqueous	-	-	-	-	-
		Methanolic	10±0.47	11±0.64	12±0.32	13±0.47	14±0.64
10	***Urtica dioca***	Aqueous	-	-	-	-	-
		Methanolic	11±0.34	12±0.22	13±0.16	14±0.38	15±0.29
11	**Nystatin (0.5mg/ml)**	25 ±0.84mm					
12	**DMSO**	0 mm					

(-) = No Activity

Table 12 MIC of aqueous and methanolic extracts between the range (10–100) mg/ml.

S. No	Plant name	Extract	Bacterial strains						Fungal strains			
			EC	KP	PA	BS	PV	SA	CA	PC	SC	AF
1	***Adiantum capillus***	Aqueous	-	100	-	-	80	-	-	NA	-	NA
		methanolic	-	50	-	80	100	30	30	-	-	30
2	***Amaranthus caudatus***	Aqueous	-	30	-	-	50	-	50	NA	-	NA
		methanolic	-	30	-	NA	30	30	-	-	-	-
3	***Artemisia absinthium***	Aqueous	-	-	-	-	30	-	NA	NA	-	-
		methanol	-	30	-	-	30	-	30	30	30	30
4	***Pseudophegopteris levingei***	Aqueous	30	-	30	-	50	-	NA	NA	-	NA
		methanolic	-	-	-	-	-	-	-	-	-	-
5	***Datura stramonium***	Aqueous	-	-	-	-	-	-	NA	30	-	100
		methanolic	NA	-	-	-	-	-	30	-	30	30
6	***Fragaria nubicola***	Aqueous	-	NA	-	-	50	-	-	-	-	30
		methanolic	-	-	-	-	-	30	-	-	-	-
7	***Hedera nepalensis***	Aqueous	-	100	-	-	-	-	-	50	-	100
		methanolic	100	-	30	-	30	30	-	30	-	-
8	***Portulaca oleraceae***	Aqueous	-	100	-	-	50	-	30	50	-	30
		methanolic	-	-	-	80	-	-	-	-	30	-
9	***Strobillanthes urticifolia***	Aqueous	-	NA	-	-	80	30	100	NA	-	100
		methanolic	100	30	-	80	-	30	-	-	-	NA
10	***Urtica dioca***	Aqueous	-	NA	-	-	50	-	NA	NA	-	-
		methanolic	-	-	-	-	50	-	-	-	-	30

EC= *Escherichia Coli*, SA= *Staphylococcus aureus*, KP= *Klebsiella Pneumoniae*, BS= *Bacillus Subtilis*, PA= *Pseudomonas aeruginosa*, PV= *Proteus vulgaris*, CA= *Candida albicans*, PC= *Penicillium chrysogenum*, AF= *Aspergillus fumigatus*, SC = *Saccharomyces Cerevisiae*, NA= No Activity, (-)= MIC Not detected within the observed range (10-100mg/ml).