Evaluation of *in Vitrio* Antimicrobial Potential and Phytochemical Composition of Some Medicinal Plants against Pathogenic Microbes in Kashmir, India

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Abstract

Background : With no new antibiotics in the market and the rapid emergence of multi drug resistance ,currently there is a crisis type situation in public health. The need to find some alternative sources of antimicrobials is essentially the need of the hour. The current study evaluates the antimicrobial and anti fungal activity of methanolic and aqueous extracts of some traditionally used medicinal plants in Kashmir valley ,India

Methods : Antibacterial and antifungal assays were performed by agar well diffusion methods. Bacterial strains employed were Bacillis subtilis, Staphylococcus aureus, Klebsiella Pneumonia.,Pseudomonas aeruginosa, Proteus vulgaris, and Eschechiae coli. Fungal strains employed were Pencillium chrysogenum, Aspergillus fumigates, Sacchoromyces cerevisiae and Candida Albicans. The qualitative phytochemical screening was performed by using the standard methods.

Results : Antibacterial assay: The antibacterial potential of each crude extract against six different varities with the type of bacterial strain and the solvent was used. The antibacterial potential of plant extracts was compared to standard antibiotic namely Gentamycin (10µg/disc) whose zone of inhibition was found 11-25mm against (*Klebsiella pneumonia)*, 9-14mm against (*Proteus vulgaris)*, 11-21mm against (*Pseudomonas aeruginosa)* , 9-22mm against (*Bacillus subtilis)*,11-26 mm against (*Staphylococcus aureus)* and 9-16mm against (*Escherichia coli)*. The methanolic extracts of *Pseudophegopteris levingei* and *coriandum sativum* were most effective against most of the bacterial strains. *Klebsiella pneumonia* was found most susceptible to the methanolic extract of *Pseudophegopteris levingei* (24mm). *Pseudomonas aeruginosa* was found most susceptible to the methanolic extract of *coraindum sativum*(21mm). *Staphylococcus aureus* and *proteus vulgaris* were found most susceptible to *Artemesia absinthium and Datura stromonium* with inhibition zone diameters of 22mm and 24mm,respectively at the concentration of 100mg/ml.

Similarly, Escherichia coli was found most susceptible to aqueous extract of *Artemisia absinthium* (16mm) and *solanum nigrum* (16mm) at the concentration of 100mg/ml. On the other hand, the aqueous extracts of certain plants including *Strobillanthes urticifolia*, *Urtica dioca*, *and Fragaria nubicola* showed no activity against *Klebsiella pneumoniae*. Also, the methanolic extracts of *Datura stramonium* and *Amaranthus caudatus* showed no activity against *E.coli* and *Bacillus subtilis*, respectively

Antifungal. The methanolic extracts of different plants showed the zones of inhibition ranging between 14.46-20.0mm against (*Candida albicans*), 14.0-20.0 against (*Saccharomyces cerevisiae*), 10.83-18.0 mm against (*Aspergillus fumigatus*), 12.0-

18.0mm (*Penicillium chrysogenum*) at the maximum concentration (100mg/ml). Aqueous extracts also showed considerable activity with zones of inhibition ranging between 11-20mm against (*Candida Albicans)* 14.0-19mm (*Saccharomyces Cerevisiae*) and 11.33-17.0mm (*Penicillium chrysogenum*) at the maximum concentration (100mg/ml). Methanolic extract of *Amaranthus Caudatus* showed the highest activity against *Saccharomyces cerevisiae* (19.0±1.49mm), and *Aspergillus fumigatus* (18.0±0.62mm) whereas the methanolic extract of amaranthus caudatus showed highest activity against *Penicillium chrysogenum* (18.0±0.30mm).

Conclusion: The present study deciphers the antimicrobial potential of the plants which can be harvested for future antimicrobial use in the present quagmire of multi drug resistance.

KEYWORDS : Antimicrobial activity, antibacterial activity, antifungal activity, phytochemical screening, methanol and aqueous extracts.

Introduction : Pathogenic microbes have always posed serious threats to the health of humans and other animals. In fact infective diseases are the second leading cause of death worldwide (WHO, 2002). However with the discovery of antibiotics in 20th century, scientific community began to synthesize synthetic or semi synthetic antimicrobial drugs but ironically, the misuse of antibiotics by human, the employment of antibiotics in veterinary practices and the growing presence of antibiotics in water, soil and food have contributed to the problem of antibiotic resistance.[1] Antimicrobial resistance is a serious global challenge and could endanger the lives of future generations. After more than 50 years of widespread use of so called “miracle drugs”, synthetic antibiotics are no longer as effective as they once used to be. Virtually most of the bacterial infections throughout the world are becoming resistant to antibiotics.[2] In general, bacteria have the genetic ability to transmit and acquire resistance to drugs that are used as therapeutic agents.[3] As resistance to antibiotics becomes more common there is greater need for alternative treatments. Out Of the two million people who acquire bacterial infections in U.S. hospitals each year, 70% of cases now involve strains that are resistant to at least one drug. In U.K., Methicillin resistant *staphylococcus aureus* (MRSA), which was at low level a decade ago, has now increased to about 50% of all *Staphylococcus aureus* isolates.[4] Antimicrobial resistance occurs due to the excessive use of antimicrobials itself. Since 37 years ago, no new classes of antibiotics were discovered and all antibiotics that entered the markets during this period were modification of existing molecules.[5]

The plant kingdom is a treasure house of potential drugs. It is estimated that there are about 2.0 lake to 5.0 lakh species of plants on earth. Among them only a negligible percentage has been explored for phytochemical and medicinal properties. In fact only less than 1% of some 250,000 higher plants have been screened for their phytochemistry or pharmacology.[6] Medicinal plants have been a part of traditional healthcare on all the continents of the world for thousands of years. There is evidence that Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyhock (*Alcea rosea*) [7] for medicinal purpose.. These plants are still used widely in ethno medicine around the world. Medicinal plants are rich in a numerous variety of secondary metabolites of

antimicrobial properties such as saponins, tannins, alkaloids, alkenyl phenols, glycol alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters. [8][9] Current global drug development program may not be able to provide new effective antibiotics in 10 to 20 years. [10] However, medicinal plants are expected to be a better candidate for the development of future antimicrobials. The present study is an attempt to evaluate the phytochemical and antimicrobial potential of some traditionally used medicinal plants in the valley of Kashmir.

Materials and Methods

Collection and identification of plant material

Six 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).

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 4ºc.

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 [11] with some modifications. 100µl of standardized inoculum (0.5 Mc Farland) of

each test bacterium was inoculated on molten Mueller Hinton Agar, homogenized 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 [12] with some modification 100µl of standardized inoculum (0.5 Mc Farland) of each test fungi were inoculated on sterile molten Sabouraud Dextrose Agar homogenized 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. Flavinoids were detected by lead acetate test while the rest of phytochemicals were detected by the methods described earlier [13]

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 color 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% Fecl3 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 colored 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 (H2SO4) was added under layered

Results

The antibacterial potential of each crude extract against six different varities with the type of bacterial strain and the solvent used. The antibacterial potential of plant extracts was compared to standard antibiotic namely Gentamycin (10µg/disc) whose zone of inhibition was found (11-25mm) against *Klebsiella pneumoniae*, (9-14mm) against *Proteus vulgaris*, (11-21mm) against *Pseudomonas aeruginosa* , (9-22mm) against *Bacillus subtilis*,11-26 mm against *Staphylococcus aureus* and 9-16mm against *Escherichia coli*. The methanolic extract of *Pseudophegopteris levingei* and *coriandum sativum* were most effective against most of the bacterial strains. *Klebsiella pneumonia* was found most susceptible to the methanolic extract of *Pseudophegopteris levingei*

(24mm). *Pseudomonas aeruginosa* was found most susceptible to the methanolic extract of *coraindum sativum*(21mm). staphylococcus aureus and proteus vulgaris were found most susceptible to *artemesia absinthium and Datura stromonium* with inhibition zone diameters of 22mm and 24mm,respectively at the concentration of 100mg/ml.

Similarly, Escherichia coli was found most susceptible to aqueous extract of *Artemisia absinthium* (16mm) and *solanum nigrum* (16mm) at the concentration of 100mg/ml. On the other hand, the aqueous extracts of certain plants including *Strobillanthes urticifolia*, *Urtica dioca*, *and Fragaria nubicola* showed no activity against *Klebsiella pneumoniae*. Also, the methanolic extracts of *Datura stramonium* and *Amaranthus caudatus* showed no activity against *E.coli* and *Bacillus subtilis*, respectively. The detailed antibacterial potential of plant extracts is shown in tables 2-7.

Antifungal activity

The methanolic extracts of different plants showed the zones of inhibition ranging between 14.4-20.0mm against (Candida albicans), 14.0-20.0 against (Saccharomyces cerevisiae), 10.0-18.0 mm against (Aspergillus fumigatus), 12.0-18.0mm (Penicillium chrysogenum) at the maximum concentration (100mg/ml). Aqueous extracts also showed considerable activity with zones of inhibition ranging between 11-20mm against candida Albicans 14.0-19mm (Saccharomyces cerevisiae) and 11.33-17.0mm (Penicillium chrysogenum) at the maximum concentration (100mg/ml). Methanolic extract of Amaranthus caudatus showed the highest activity against Saccharomyces cerevisiae (19.0±1.49mm), and Aspergillus fumigatus (18.0±0.62mm) whereas the methanolic extract of amaranthus caudatus showed highest activity against Penicillium chrysogenum (18.0±0.30mm).

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 fumigates. (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 < Penicillium chrysogenum< Candida albicans of anthraquinones and phlobtannins.

The maximum numbers of tested phytochemicals were detected in Pseudophegopteris levingei (i.e., 10/12) and least in Amaranthus caudatus (i.e., 6/12) Flavonoids and Tannins 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.[14] 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.[15] 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. [16] 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.[17] While as 1% of all vaginal yeast infections occur due to Saccharomyces cerevisiae[18]. Medicinal plants were the first weapons that the man used against pathogenic microbes. Multiple studies have reported the antimicrobial potential of plants. [19-21] 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 [22] This could be due to many factors like soil composition, climate, age and vegetation cycle stage, quality of extracted product. [23,24] 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 [14]. Moreover, the type of solvent has an important role in the process of extraction. [25,26] 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 [27]. 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.[28] Bioprospection of secondary metabolites is an important step in the development of new drugs[29,30]. 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. [31] Flavonoids are known to be synthesized

by the plants in response to microbial infection.[32] 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. [33,34] Tannins posses a wide range of anti-infective activities.[35] Tannins have the ability to complex with proteins through hydrogen bonding, hydrophobic interactions as well as covalent bond formation.[36,37] Their antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins and also to complex with polysaccharides [38]. Terpenes are effective against bacteria fungi, viruses, and protozoa [39-43]. Multiple studies have proved the antimicrobial potential of alkaloids. Their mechanism of action is attributed to their ability to intercalate with DNA.[44-47] 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.[48] 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. Acknowledgement The authors are highly thankful to the department of Clinical Biochemistry, University of Kashmir for providing all the necessary facilities to carry out this valuable research.

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Table 1. Preliminary phytochemical careening of selected medicinal plants.
Note: (-) = Absent, (+) = Present

S No.	Plant name	Solvents	Alkalods	Anthraquinones	Cardiac Glycoside	Cardenolides	Flavinoids	Phenols	Phlobtann.	Saponins	Steroids	Tannins	Terpenoids	Volatile Oils
1	Adiantum Capillus	Aqueous	+	+	-	+	+	+	+	+	+	+	-	-
		Methanol	-	-	-	-	+	+	-	+	-	+	+	-
2	Amaranthum caudatus	Aqueous	-	-	-	-	+	+	+	+	-	+	+	-
		Methanolic	-	-	-	-	+	-	-	+	-	+	+	+
3	Artemisia absinthium	Aqueous	+	+	-	+	+	+	-	+	+	+	-	-
		Methanolic	-	-	-	-	+	-	-	-	-	+	+	+
4	Pseudophegopteris Levingi	Aqueous	+	-	+	+	+	+	+	+	-	+	+	+
		Methanolic	-	-	+	-	+	+	+	+	+	+	-	+
5	Coriandrun Sativum	Aqueous	+	-	-	+	+	+	+	+	-	+	-	-
		Methanolic	-	-	+	-	+	-	-	+	+	+	+	+
6	Datura Stromonium	Aqueous	-	+	+	+	+	+	+	+	-	+	-	+
		Methanolic	-	-	+	-	+	-	+	+	-	+	+	+

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

	Plant name	**Plant extract**	**10mg/ml**	**30mg/ml**	**50mg/ml**	**80mg/ml**	**100mg/ml**
1	**Adiantum *capillus***	Aqueous	-	-	-	10	12
		Methanolic	-	-	-	-	10
2	**Amaranthus *caudatus***	Aqueous	-	-	9	10	11
		Methanolic	-	10	11	11	12
3	**Artemesia *absinthium***	Aqueous	-	10	10	11	12
		Methanolic	-	10	12	13	15
4	**Pseudophegopteris levingei**	Aqueous	-	-	10	11	13
		Methanolic	10	11	11	12	12
5	**Coriandrum *sativum***	Aqueous	-	-	11	12	13
		Methanolic	10	11	12	13	14
6	**Datura *stramonium***	Aqueous	10	10	11	12	14
		Methanolic	**16**	**18**	**20**	**22**	**24**
		Methanolic	10	11	13	14	15
	Positive control: Gentamycin (10µg/disc)	25mm					

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

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	-	-	-	-	11
		Methanolic	-	-	10	11	12
2	**Amaranthus *caudatus***	Aqueous	-	10	10	10	11
		Methanolic	-	10	12	14	15
3	**Artemesia *absinthium***	Aqueous	10	11	11	12	13
		Methanolic	-	11	12	13	14
4	**Pseudophegopteris levingei**	Aqueous	10	11	12	13	14
		Methanolic	**14**	**18**	**19**	**22**	**24**
5	**Coriandrum *sativum***	Aqueous	-	-	-	-	11
		Methanolic	10	12	12	13	14
6	**Datura *stramonium***	Aqueous	14	14	15	15	15
		Methanolic	10	11	12	13	14

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

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	11	12	13	13	13
		Methanolic	-	-	-	8	10
2	**Amaranthus *caudatus***	Aqueous	10	11	11	11	11
		Methanolic	-	-	-	-	-
3	**Artemesia *absinthium***	Aqueous	10	11	12	13	14
		Methanolic	10	11	14	15	16
4	**Peudophegopteris levingei**	Aqueous	10	11	13	14	15
		Methanolic	**12**	**15**	**18**	**20**	**22**
5	**Coriandrum *sativum***	Aqueous	-	10	11	12	13
		Methanolic	8	10	14	15	15
6	**Datura *stramonium***	Aqueous	11	12	13	14	15
		Methanolic	7	9	10	12	13
22	**Positive control: Gentamycin (10µg/disc)**	**25mm**					

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

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	12	13	13	13	13
		Methanolic	9	9	10	10	12
2	**Amaranthus *caudatus***	Aqueous	11	12	13	14	14
		Methanolic	9	11	11	11	11
3	**Artemesia *absinthium***	Aqueous	**13**	**14**	**14**	**15**	**16**
		Methanolic	-	11	12	13	13
4	**Asplenium *species***	Aqueous	12	12	13	13	14
		Methanolic	**12**	**13**	**14**	**15**	**15**
5	**Coriandrum *sativum***	Aqueous	13	13	13	13	13
		Methanolic	12	13	14	14	14
6	**Datura *stramonium***	Aqueous	12	12	12	13	13
		Methanolic	-	-	-	-	-
	Positive control: Gentamycin (10µg/disc)	20mm					

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

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	11	11	11	11	12
		Methanolic	10	11	11	12	13
2	**Amaranthus *caudatus***	Aqueous	11	12	13	13	14
		Methanolic	10	11	12	13	14
3	**Artemesia *absinthium***	Aqueous	9	10	11	12	13
		Methanolic	11	13	14	15	17
4	**Asplenium *species***	Aqueous	-	9	11	12	13
		Methanolic	**12**	**13**	**15**	**17**	**19**
5	**Coriandrum *sativum***	Aqueous	12	12	13	13	14
		Methanolic	**14**	**16**	**18**	**19**	**21**
6	**Datura *stramonium***	Aqueous	9	10	11	12	14
		Methanolic	10	12	13	13	14
	Positive control: Gentamycin (10µg/disc)	25mm					

Table 7. Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Staphylococcus aureus*.

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	Adiantum *capillus*	Aqueous	11	11	13	14	15
		Methanolic	-	10	11	11	12
2	Amaranthus *caudatus*	Aqueous	11	12	13	13	14
		Methanolic	-	11	12	14	14
3	Artemesia *absinthium*	Aqueous	13	13	14	14	16
		Methanolic	13	16	18	20	22
4	Pseudophegopteris levingi	Aqueous	12	13	15	16	16
		Methanolic	10	11	12	12	13
5	Coriandrum *sativum*	Aqueous	-	11	14	15	17
		Methanolic	-	12	13	15	16
6	Datura *stramonium*	Aqueous	11	12	14	15	16
		Methanolic	13	16	17	18	19
	Positive control: Gentamycin (10µg/disc)	27mm					

Table 8. Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Aspergillus fumigatus*.

	Plant name	**Plant extract**	**10mg/ml**	**30mg/ml**	**50mg/ml**	**80mg/ml**	**100mg/ml**
1	**Adiantum *capillus***	Aqueous	-	-	-	-	-
		Methanolic	-	8	9	10	11
2	**Amaranthus *caudatus***	Aqueous	-	-	-	-	-
		Methanolic	**14**	**15**	**16**	**17**	**18**
3	**Artemesia *absinthium***	Aqueous	-	-	-	-	-
		Methanolic	-	8	9	9	10
4	**Asplenium *species***	Aqueous	-	-	-	-	-
		Methanolic	10	11	12	13	14
5	**Coriandrum *sativum***	Aqueous	8	8	11	12	14
		Methanolic	9	10	12	13	14
6	**Datura *stramonium***	Aqueous	-	-	-	-	15
		Methanolic	-	10	11	12	13
	Positive control: nystatin (0.5mg/ml)	27mm					

Table 9. Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Penicillium chrysogenum*

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	-	-	-	-	-
		Methanolic	10	11	13	14	15
2	**Amaranthus *caudatus***	Aqueous	**-**	**-**	**-**	**-**	**-**
		Methanolic	**8**	**10**	**12**	**14**	**18**
3	**Artemesia *absinthium***	Aqueous	-	-	-	-	-
		Methanolic	-	10	11	11	12
4	**Asplenium *species***	Aqueous	-	-	-	-	-
		Methanolic	12	13	14	15	17
5	**Coriandrum *sativum***	Aqueous	-	12	13	14	15
		Methanolic	-	10	13	15	18
6	**Datura *stramonium***	Aqueous	-	14	15	16	17
		Methanolic	11	12	13	15	17

Table 10. Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Saccharomyces cerevissae.*

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	10	11	11	12	14
		Methanolic	8	12	12	13	14
2	**Amaranthus *caudatus***	Aqueous	14	15	16	17	18
		Methanolic	**15**	**17**	**18**	**18**	**20**
3	**Artemesia *absinthium***	Aqueous	14	15	16	17	18
		Methanolic	-	12	13	13	16
4	**Asplenium *species***	Aqueous	11	13	14	15	16
		Methanolic	12	12	15	16	16
5	**Coriandrum *sativum***	Aqueous	10	10	11	13	14
		Methanolic	14	16	16	17	18
6	**Datura *stramonium***	Aqueous	8	13	14	15	19
		Methanolic	-	8	13	14	14
Positive control: nystatin (0.5mg/ml)		30mm					

Table 11. Zones of inhibition in millimeter (mm) at five different concentrations of plant extracts against *Candida albicans*

	Plant name	Plant extract	10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml
1	**Adiantum *capillus***	Aqueous	-	-	-	-	-
		Methanolic	-	11	12	13	14
2	**Amaranthus *caudatus***	Aqueous	**-**	**-**	**11**	**16**	**20**
		Methanolic	8	13	14	15	17
3	**Artemesia *absinthium***	Aqueous	-	-	-	-	-
		Methanolic	-	11	12	15	16
4	**Asplenium *species***	Aqueous	-	-	-	-	-
		Methanolic	12	13	14	15	16
5	**Coriandrum *sativum***	Aqueous	13	14	15	16	17
		Methanolic	-	10	11	13	15
6	**Datura *stramonium***	Aqueous	-	-	-	-	-
		Methanolic	-	10	10	15	17
	Positive control: nystatin (0.5mg/ml)	30mm					

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

S. No	Plant name	Solvents	Bacterial strains						Fungal strains			
			E.C	K.P	P.A	B.S	P.V	S.A	C.A	P.C	S.C	A.F
1	*Adiantum capillus*	Aqueous	-	100	-	-	80	-	-	NA	-	NA
		methanol	-	50	-	80	100	30	30	-	-	30
2	*Amaranthus caudatus*	Aqueous	-	30	-	-	50	-	50	NA	-	NA
		methanol	-	30	-	NA	30	30	-	-	-	-
3	*Artemesia absinthium*	Aqueous	-	-	-	-	30	-	NA	NA	-	-
		methanol	30	30	-	-	30	-	30	30	30	30
4	*Asplenium species*	Aqueous	-	-	30	-	50	-	NA	NA	-	NA
		methanol	-	-	-	-	-	-	-	-	-	-
5	*Coriandrum sativum*	Aqueous	-	100	-	30	50	30	-	30	-	-
		methanol	-	-	-	-	-	30	30	30	-	-
6	*Datura stramonium*	Aqueous	-	-	-	-	-	-	NA	30	-	100
		methanol	NA	-	-	-	-	-	30	-	30	30

-= MIC value below observed range (10-100mg/ml), NA= No Activity, E.C =*Escherichia Coli*, S.A =*Staphyllococcus aureus*, K.P =*Klebsiella Pneumonia*, B.S = *Bacillus Subtillus*, P.A =*Pseudomonas aeruginosa*, P.V = *Proteus vulgaris, C.A = Candida albicans, P.C = Penicillium chrysogenum, A.F = Aspergillus fumigatus, S.C = Saccharomyces Cerevisiae*

Table 13. Percentage of relative inhibition zone diameter (%RIZD) of aqueous and methanolic extracts of plant extracts at 100mg/ml

S. No	Plant name	Solvents	**Bacterial strains**						**Fungal strains**			
			E.C	K.P	P.A	B.S	P.V	S.A	C.A	P.C	S.C	A.F
1	*Adiantum capillus*	Aqueous	65.00	44.00	48.00	52.00	48.00	55.55	0.00	0.00	46.66	0.00
		methanol	60.00	48.00	52.00	40.00	40.00	44.44	46.67	60.00	46.66	40.74
2	*Amaranthus caudatus*	Aqueous	70.00	44.00	56.00	44.00	44.00	51.85	66.67	0.00	60.00	0.00
		methanol	55.00	60.00	56.00	0.00	48.00	51.85	56.67	48.00	66.67	66.67
3	*Artemesia absinthium*	Aqueous	80.00	52.00	52.00	56.00	48.00	59.25	0.00	0.00	60.00	0.00
		methanol	65.00	56.00	68.00	64.00	60.00	81.48	53.33	72.00	53.33	37.03
4	*Asplenium species*	Aqueous	70.00	56.00	52.00	60.00	52.00	59.25	0.00	0.00	53.33	0.00
		methanol	75.00	96.00	84.00	88.00	96.00	96.29	59.25	68.00	53.33	51.85
5	*Coriandrum sativum*	Aqueous	65.00	44.00	56.00	52.00	52.00	62.96	62.96	60.00	46.67	51.85
		methanol	70.00	56.00	76.00	60.00	56.00	59.25	50.00	72.00	60.00	51.85
6	*Datura stramonium*	Aqueous	65.00	60.00	56.00	60.00	56.00	59.25	0.00	68.00	63.33	55.56
		methanol	0.00	56.00	56.00	52.00	52.00	70.37	56.67	68.00	46.67	48.14