







Article

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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *Euphorbia helioscopia*

Atividade Antimicrobiana e Triagem Fitoquímica de Euphorbia helioscopia

ABSTRACT - Medicinal Plants have been used throughout the world by human beings as a drug and remedies for various diseases since time immemorial. A study was planned to count into the antimicrobial activity and phytochemical screening of *Euphorbia helioscopia*. The plants were gathered and tested against some standard strains and some human pathogenic microorganisms i.e *Escherichia coli*, *Bacillus Subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and three fungal strain *Trichoderma*, *R hizopus nigricans*, *Aspergillus niger*. The concentrations of extracting samples (500 and 1,000 mg mL⁻¹) were used against pathogens. Ciprofloxacin was used as positive control in case of bacterial strains and Colfrimazol was used against the fungal strain while dimethyl sulfoxide as negative control. The outcomes indicated that the positive wells potency of Water extract had a 36 mm diameter of zone of inhibition against *Escherichia coli* and ethanol extract at 1,000 mg mL⁻¹ had maximum (34 mm) zone of inhibition against *Bacillus subtilis* (36 mm) zone of inhibition against *Klebsiella pneumonia* and 33 mm of zone of inhibition against *Trichoderma harzianum*. Likewise, water extract at a concentration of 1,000 mg mL⁻¹ resulted highest value of zone of inhibition (36 mm) against *Staphylococcus aureus*, a zone of inhibition (mm) against *Salmonella typhi*, 36 mm zone of inhibition against *Pseudomonas aeruginosa*, (32 mm) zone of inhibition against *Rhizopus nigricans*, a 34 mm zone of inhibition against *Acremonium* and (34 mm) zone of inhibition against *Aspergillus niger*. The most susceptible bacteria were *K. pneumonia* and *Bacillus subtilis*, while *E. coli* was the most resistant bacteria and showed zone of inhibition. The ethanolic extract had tannins, lipid, total proteins, carbohydrates, flavonoids, Alkaloid and polyphenolics.

Keywords: ayurvedic, Euphorbiaceae, Labeena, secondary metabolites, indigenous.

RESUMO - Plantas medicinais têm sido utilizadas em todo o mundo por seres humanos como droga e remédio para várias doenças desde tempos imemoriais. Um estudo foi planejado para contar com a atividade antimicrobiana e triagem fitoquímica de *Euphorbia helioscopia*. As plantas foram recolhidas e testadas contra algumas estirpes padrão e alguns microrganismos patogênicos humanos (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi* e *Pseudomonas aeruginosa*) e três estirpes fúngicas (*Trichoderma*, *Rhizopus nigricans* e *Aspergillus niger*). As concentrações de amostras de extração (500 e 1.000 mg mL⁻¹) foram utilizadas contra patógenos. A ciprofloxacina foi usada como controle positivo no caso de cepas bacterianas, e o colfrimazol foi utilizado contra a cepa fúngica, enquanto o dimetilsulfóxido foi usado como controle negativo. Os resultados indicaram que a potência positiva dos poços do extrato aquoso apresentou diâmetro de 36 mm de inibição contra *Escherichia coli* e o extrato etanólico a 1.000 mg mL⁻¹ apresentou zona de inibição máxima (34 mm)

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contra a zona de inibição de *Bacillus subtilis* (36 mm) contra *Klebsiella pneumoniae* e 33 mm de zona de inibição contra *Trichoderma harzianum*. Da mesma forma, extrato de água na concentração de 1.000 mg mL⁻¹ resultou em maior valor de zona de inibição (36 mm) contra *Staphylococcus aureus*, uma zona de 30 mm de inibição contra *Salmonella typhi*, zona de 36 mm de inibição contra *Pseudomonas aeruginosa*, zona de inibição de 32 mm contra *Rhizopus nigricans*, zona de 34 mm de inibição contra *Acremonium* e zona de inibição de 34 mm contra *Aspergillus niger*. As bactérias mais suscetíveis foram *K. pneumoniae* e *Bacillus subtilis*, enquanto *E. coli* foi a bactéria mais resistente e mostrou zona de inibição. O extrato etanólico continha taninos, lipídios, proteínas totais, carboidratos, flavonoides, alcaloides e polifenóis.

Palavras-chave: ayurvédica, Euphorbiaceae, metabólitos secundários, indígenas.

INTRODUCTION

The awareness of medicinal plants has long been traced for many centuries in different medical organizations such as Ayurveda, Unani and Siddha. In India, it is reported that conventional healers use 2,500 plant species and 100 species of plants serve as regular sources of medicine (Revathi and Parimelazhagan, 2010). In Pakistan about 6,000 species of higher plants have been reported and from these 600 to 700 species are used for medicinal uses. 50% of the Pakistani population is normally treated by some 50,000 practitioners of conventional medication. Largely due to deficiency of proper health facilities the native medicinal plants have been especially used by the village community as a medical specialty based on long experiences of elders, and this data has been handed along orally from production to output without any written documents (Nasrullah et al., 2012). Long before man discovered the existence of germs, the estimate that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles were well taken. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medications are even admitted as component of the habitual discourse of various maladies (Rios and Recio, 2005). Ethnomedicinal studies are substantial in illuminating significant indigenous plant species, especially for the breakthrough of new crude drugs. Documentation of indigenous medicinal knowledge of traditional plant species has contributed several vital modern drugs (Mahmood et al., 2013).

The family Euphorbiaceae is a big family of flowering plants with 300 genera and about 7,500 species. This family takes place principally in the tropics, with most of the species in the Indo-Malayan region and tropical America. A large variety occurs in tropical Africa, but they are not as abundant or varied as in these two other tropical regions. The leaves are alternate, rarely opposite, with stipules (Mahbubur Rahman and Akter, 2013). They are mainly simple, but where compound, are always palmate, never pinnate. Stipules may be reduced to hairs, glands, or thorns, or in succulent species are sometimes lacking. *E. helioscopia* were used to treat various human ailments (Ashfaq et al., 2019a; Bahadur et al., 2018a). Leaves and stems of *E. helioscopia* are used for vermifuge and febrifuge actions, roasted pepper mixed with seeds are used in cholera, the oil obtained from seeds used in constipation and roots are used as anthelmintic. Additionally, the plant has been employed in studies for different pharmacological activities such as insulin secretagogin and antibacterial (Saleem et al., 2014). The radially symmetrical flowers are unisexual, with the male and the female flowers usually occurring on the same plant. As can be expected from such a large family, there is a wide variety in the structure of the flowers. They can be either monoecious or dioecious. The stamens (the male organs) can number from one to 10 (or even more). The female flowers are hypogynous, that is, with superior ovaries. Species of Euphorbiaceae have been used by the local population of many countries in folk medicines as remedies against several diseases and complaints such as cancer, diabetes, diarrhea, heart diseases, hemorrhages, hepatitis, jaundice, malaria, ophthalmic diseases, rheumatism and scabies etc. The plants of Euphorbiaceae are also known to cure gonorrhoea, urino-genital infections, jaundice and are used as diuretic and astringent (Kumar and Chaturvedi, 2010).

Sun spurge, is an annual plant rising 10 to 50 cm high with erected reddish stem, oval alternate leaves and small yellow green flowers. It is an endemic flora of North Africa and most

of the Europe and Asia. *E. helioscopia* has been regarded as a medicinal plant and was employed in folk medicine in several nations round the world. In traditional medicine, sun spurge, locally named “Labeena” (referring to the latex when plant bleeds). Many secondary metabolites have been covered from sun spurge plant, including diterpenoid, triterpenoid, tannins and steroid, which offered to sun spurge herb a wide array of bioactive functions. In fact, *Euphorbia helioscopia* plant was reported to possess antibacterial and antifungal effects (Ben Mohamed Maoulainine et al., 2012).

E. helioscopia is an important Chinese medicinal herb species in the family Euphorbiaceae. Plants of *Euphorbia* have been used in the traditional medicine for treatment of cancers, tumors and warts for hundreds of years. It is well known that they contain irritant and tumor-promoting ingredients.

Rather, several species are used in folk medicine as drugs and crude materials for pharmaceutical preparations. This work was therefore planned to produce a protocol for callus induction from leaf of *E. helioscopia* and understand the origin of callus by histological analysis; to investigate the antibacterial activity of the ethanol and distilled water extracts of *Euphorbia helioscopia* against important bacterial species; To provides new scientific information about *Euphorbia helioscopia* based on the biological potential and phytochemical analysis that has never been covered before.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Laboratory of Botany Department, Islamia College (Chartered University) Peshawar, Pakistan, which is located at 33°59' N latitude, 71°28' E longitude and at an altitude of 1,550 m above sea level in Peshawar valley.

Plant material and identification

Plant of *Euphorbia helioscopia* was collected from University Campus Peshawar Khyber Pakhtunkhwa, Pakistan during April 2016 and their taxonomic identification was taken from senior botanist of the institution.

Preparation of extractions of plant material

The collected plants were held for five weeks (35 days) in shade at room temperature. When the plant becomes dry, it was established with the help of pestle and mortar for the preparation of extraction in Ethanol and distilled water. The powder materials of plant were weighted through Electrical balance and 75 mg powder were dissolved in 1,000 mL of distilled water and 300 mL Ethanol in Borosilicate glass. Ethyl alcohol and water solution were reserved for 72 hours (3 days) in a shaker. After the shaking for 72 hours both solutions were filtered through Whitman filter paper No 1 and the filtrate collected in separate beakers. The weights of water filtrate were 75.55 g and Ethanol filtrate were 59.65 g. Ethanol and water were evaporated by the using of Rotatory Vacuum Evaporator. After the using of Rotatory Vacuum Evaporator, the water and Ethanol extract were poured into a china dish which were in crude form and place it in the water Bath on 55 °C for 2 hours. For the perfect purification of extracts from water and Ethanol present in minor amount in extracts. The sterile vials were used for to keep the extract from contamination. Two vials were weighted through Electrical balances which were 11.50 g (used for water extract) and 10.95 g (were used for Ethanol extract). When both extract completely dried, it was collected from china dish to these two separate sterile vials through stirrer. The weights of the vials were increased. The weight of water vials reaches up to 14.85 g and Ethanol vials reach up to 16.25 g. In the end, the pure weight of water extract was 2.35 g and Ethanol extract was 4.25 g. Both values were saved in the refrigerator at 40 °C for the prevention of pollution and other bacterial or fungal attacks.

Preparation of stock extractions

For the preparation of stock extractions and dilutions Water and Ethanolic extracts of 2.35 g in 3 mL and 4.25 g in 5 mL were dissolved in distilled water with the help of vortex mixer.

Preparation of serial dilution

For the preparation of serial dilution, the Eppendorf tube was applied. This tube was labeled from 1 to 4 (4 tubes were used). The 1st one was filled from 1000 μ stock extraction. From 2 to 4 Eppendorf tubes were filled from distilled water of about 500 μ through a micropipette by using of 500 μ tips. 500 μ of stock extraction from the 1st Eppendorf tube were aspirated to 2nd, from 2nd to 3rd, from 3rd to 4th, through micropipette. All the Eppendorf tubes were shaken through vortex mixer.

Sterilization of equipment's

All the equipment's i.e. Petri-dishes, beakers, borers, micropipette tips, common pins, Eppendorf tubes and Medium used during the research work were sterilized by the using of Autoclave on 121 °C at a pressure of a 15 lbs/sq inch for 15 minutes. The Laminar hood was sterilized by using of 75% Ethanol solution (25% distilled water and 75% ethyl alcohol) through tissue paper. The bacterial inoculation loops were disinfected through the fire with the help of spirit lamp. The whole work was done in the laminar hood for the prevention from microbes or contamination.

Source and maintenance of microorganisms

Two different types of microorganism strain were tested for the microbial activities.

Staphylococcus aureus was a standard strain and Nine pathogenic strains were used for microbial activities collected from main pathology Lab Khyber Teaching Hospital and North West Hospital, Peshawar (Pakistan) through sterile cotton Swabs.

Principal

The antimicrobials present within the plant extract can diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The ensuing zones of inhibition were uniformly circular as there was a confluent lawn of growth. The diameter of zone of inhibition was evaluated in millimeters (mm).

Standard control

For negative and positive controls, 0.05% concentrated of antibiotic and distilled water was applied for negative control. 0.05% Ciprofloxacin was prepared by the dissolution of 25 mL antibiotic (Ciprofloxacin) and 75% distal water. 0.05% Ciprofloxacin was used for positive control and distilled water was utilized as a negative control.

Antimicrobial activity

The method followed during this experimental work was well methodical. The antibacterial screening was conducted for Ethanolic extract and distill water extract of *E. helioscopia*. Antibiotics provide the primary basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. Still, overuse of antibiotics has become the major component in the emergence and diffusion of multi- drug resistant forms of various groups of microorganisms (Harbottle et al., 2006).

Test cultures used for antimicrobial screening were *Staphylococcus aureus* Gram positive, *Escherichia coli* Gram negative and *Pseudomonas aeruginosa* Gram negative.

Procedure

Antibacterial activities of the plant were held out by agar well diffusion method as described by Carron et al. (1987). Bacteria strains were first cultured on nutrient broth and incubated for 24 hours prior to experiments. Nutrient agar was melted, cooled to 40 °C and poured into sterilized Petri dishes. Wells were then immersed in media using 6 mm diameter with the avail of the sterile metal cork borer and keeping 24 mm between two adjacent wells. 4-8 hour old bacterial culture was open along the surface of nutrient agar with the help of sterilized cotton swab. These procedures were repeated thrice turned the plate 600 between each streaking. About 100 µL of 3 mg mL⁻¹ of respective extract, dissolved in DMSO was then added to the wellsprings. Other wells were supplemented with DMSO and 10 µg Ciprofloxacin disc served as positive and negative commands. The shells were then incubated for 24 hours at 370 °C the plates were then observed for Zones of Inhibition. All the experiments were conducted in triplicate.

Antifungal activity

The resistance of the pathogenic fungi to certain drugs is the major problem nowadays. Fungi are mutating to allow a broad reach of antibiotics. Test species used for this activity were *Aspergillus niger*, *Trichodeema harzianum*, *Rhizopus nigricans*, *Acremonium*.

Procedure

For performing this activity, the procedure as described by Humeera, (2013) was followed. SDA media were prepared autoclaved and poured into Petri dishes. For check in sterility, plates were incubated for 24 hours at 28 °C. On the next day-old culture of test fungi was inoculated into Petri dishes and incubated for 7 days at 28 °C, as a week-old culture is required. Slants were prepared by pouring 5 mL autoclaved SDA media to the test tubes. The test tubes were then kept in an incline position to solidify and make slants. They were incubated for 24 hours and sterility was checked. On the next day inoculums were taken from the 7 days old culture and applied on their respective slants. After 7 days results were studied by measuring the linear development on the test tube.

RESULTS AND DISCUSSION

Antimicrobial activity of extracts against (*E. coli*)

The analysis of results indicated that the highest zone of inhibition (ZOI) was recorded by water extract in positive well potency which is 36 mm against *E. coli* followed by (ZOI) against *E. coli* (34 mm) in well potency of 1,000 mg mL⁻¹ which was similar with a zone of inhibition (ZOI) by ethanol solvent extract (34 mm) in positive wells potency (Table 1).

Antimicrobial activity of extracts against (*Bacillus subtilis*)

Analysis of results presented in Table 2 revealed that zone of inhibition was significantly affected by the solvent of plant extracts against (*Bacillus subtilis*). The highest zone of inhibition (34 mm) was recorded in ethanol solvent extract in well potency of 1,000 mg mL⁻¹ against *B. subtilis* followed by a zone of inhibition (32 mm) by the concentration

Table 1 - Zone of Inhibition of *Euphorbia helioscopia* against *E. coli* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	26	28
2	1,000 mg mL ⁻¹	30	34
3	Positive	34	36
4	Negative	-	-

of 500 mg mL⁻¹ of solvent of water extract. While the lowest value of zone of inhibition (24 mm) was observed in positive well potency of solvent of water extract against *B. subtilis* which was like the zone of inhibition of ethanol solvent extract at 500 mg mL⁻¹ against *B. subtilis*.

Antimicrobial activity of extracts against *Staphylococcus aureus*

Analysis of Table 3 revealed that the highest zone of inhibition (36 mm) was recorded in water solvent extract in well potency of 1,000 mg mL⁻¹ against *S. aureus* followed by a zone of inhibition (27 mm) by the concentration of 500 mg mL⁻¹ of solvent of water extract. While the lowest value of zone of inhibition (24 mm) was observed in positive well potency of solvent of water extract against *S. aureus* which, was like the zone of inhibition of ethanol solvent extract at 1.000 mg mL⁻¹ against *S. aureus*.

Antimicrobial Activity of Extracts against (*Klebsiella pneumonia*)

Data presented in Table 4 indicated that the zone of inhibition was significantly affected by the solvent of plants extract against *K. pneumonia*. Analysis of data revealed that the highest value of the zone of inhibition (ZOI) (36 mm) was observed in ethanol solvent extracts by the concentration of 1,000 mg mL⁻¹ followed by ZOI (34 mm) in the water solvent extract by the concentration of 1,000 mg mL⁻¹ against *K. pneumonia*. While the lowest value of ZOI (24 mm) was recorded in in positive wells potency against *K. pneumonia*.

Antimicrobial activity of extracts against (*Salmonella typhi*)

It is obvious from Table 5 indicated that the application of different solvent of plant extract significantly affected zone of inhibition (ZOI) against *Salmonella typhi*. The highest zone of inhibition (30 mm) was recorded in solvent of water extract against *S. typhi* by concentration of 1,000 mg mL⁻¹ followed by ZOI (28 mm) in solvent of ethanol extract against *S. typhi*. While the lowest value of ZOI (16 mm) was observed in solvent of ethanol extract in positive wells potency against *S. typhi*.

Antimicrobial activity of extracts against (*Pseudomonas aeruginosa*)

Data in Table 6 indicated that the highest value zone of inhibition (36 mm) was observed in solvent of water extract by the concentration of 1,000 mg mL⁻¹ against *P. aeruginosa* followed by a zone of inhibition (34 mm) in solvent of ethanol extract by the concentration of 1,000 mg mL⁻¹. While the lowest value of zone of inhibition (20 mm) was recorded in ethanol solvent extracts of positive wells potency against *P. aeruginosa*.

Table 2 - Zone of Inhibition of *Euphorbia helioscopia* against *Bacillus subtilis* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	26	24
2	1,000 mg mL ⁻¹	34	32
3	Positive	24	22
4	Negative	-	-

Table 3 - Zone of Inhibition of *Euphorbia helioscopia* against *Staphylococcus aureus* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	26	27
2	1,000 mg mL ⁻¹	24	36
3	Positive	24	26
4	Negative	-	-

Table 4 - Zone of Inhibition of *Euphorbia helioscopia* against *Klebsiella pneumonia* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	28	25
2	1,000 mg mL ⁻¹	36	34
3	Positive	26	24
4	Negative	-	-

Table 5 - Zone of Inhibition of *Euphorbia helioscopia* against *Salmonella Typhi* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	20	22
2	1,000 mg mL ⁻¹	28	30
3	Positive	16	18
4	Negative	-	-

Table 6 - Zone of Inhibition of *Euphorbia helioscopia* against *Pseudomonas aeruginosa* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	26	28
2	1,000 mg mL ⁻¹	34	36
3	Positive	20	24
4	Negative	-	-

Antimicrobial activity of extracts against (*Trichoderma harzianum*)

Data presented in Table 7 showed that the highest value of zone of inhibition (33 mm) was observed in ethanol solvent extract at the concentration of 1,000 mg mL⁻¹ followed by ZOI (32 mm) in the water solvent extract by the concentration of 1000 mg mL⁻¹ against *T. harzianum*. While the lowest value for the zone of inhibition (20 mm) was recorded for water solvent against *T. harzianum* in the positive wells potency.

Antifungal activity of extracts against (*Rhizopus nigricans*)

The antifungal activity of plant extract against *Rhizopus nigricans* was found significant (Table 8). Analysis of result showed that maximum value of zone of inhibition (32 mm) was recorded in water solvent extract at the concentration of 1,000 mg mL⁻¹ against *R. nigricans* followed by a zone of inhibition (24 mm) in the water solvent extract against *R. nigricans* at the concentration of 500 mg mL⁻¹. While the minimum value for the zone of inhibition (14 mm) was observed in ethanol solvent extracts of positive wells potency against *R. nigricans*.

Antifungal activity of extracts against *Acremonium*

The antifungal activity different solvent of plant extract performed better against *Acremonium* (Table 9). The highest value of zone of inhibition (34 mm) was noted in the water solvent extract at the concentration of 1,000 mg mL⁻¹ against *Acremonium* followed by ZOI (30 mm) in water solvent by the concentration of 1,000 mg mL⁻¹ against *Acremonium*. While the lowest value for the zone of inhibition (16 mm) was recorded for water solvent against *Acremonium* in the positive wells potency.

Antifungal activity of extracts against *Aspergillus niger*

The antifungal activity result of water extract show against of *A. niger* was found excellent as compared to another antibiotic (Table 10). Analysis of the results indicated that the highest

Table 7 - Zone of Inhibition of *Euphorbia helioscopia* against *Trichoderma harzianum* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	26	24
2	1,000 mg mL ⁻¹	33	32
3	Positive	28	20
4	Negative	-	-

Table 8 - Zone of Inhibition of *Euphorbia helioscopia* against *Rhizopus nigricans* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	16	24
2	1,000 mg mL ⁻¹	22	32
3	Positive	14	20
4	Negative	-	-

Table 9 - Zone of Inhibition of *Euphorbia helioscopia* against *Acremonium* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	14	30
2	1,000 mg mL ⁻¹	20	34
3	Positive	16	20
4	Negative	-	-

Table 10 - Zone of Inhibition of *Euphorbia helioscopia* against *A. Niger* as affected by solvent of plant extracts

Wells number in plate	Wells potency	Zone of Inhibition (mm)	
		Solvent of plant extract	
		Ethanol	Water
1	500 mg mL ⁻¹	20	28
2	1,000 mg mL ⁻¹	24	34
3	Positive	16	19
4	Negative	-	-

zone of inhibition (34 mm) was observed in solvent of water extract at the concentration of 1,000 mg mL⁻¹ against *A. niger* followed by zone of inhibition (28 mm) in solvent of water extract against *A. niger* at 500 mg mL⁻¹. While the lowest value for zone of inhibition (16 mm) was recorded for ethanol solvent against *Acremonium* in the positive wells potency against *A. niger*.

Phytochemical studies of *Euphorbia helioscopia*

Chemical screening was carried out on the *n*-hexane, chloroform and methanol extracts by using standard procedure to detect the constituents as described by (Qaisar et al., 2012).

Alkaloids

About 0.2 g of each extract was warmed with 2% H₂SO₄ for 24 minutes. It was filtered and a few drops of Dragendrof's reagent were added. Orange red precipitate indicated the presence of alkaloids (Qaisar et al., 2012).

Tannins

A small quantity of each extract was mixed with water, heated on water bath and filtered. A few drops of ferric chloride solution were added to the filtrate. A dark green coloration indicated the presence of tannins.

Flavonoids

0.2 g extract was dissolved in diluted 10% NaOH and 2M HCl was added. A yellow solution that turns colorless indicated the presence of flavonoids.

Steroids

2 mL of acetic anhydride was added to 0.5 g of each extract and then added 2 mL of H₂SO₄. The color changed from violet to blue or green or red which indicated the presence of steroids (Qaisar et al., 2012). *E. helioscopia* is a common herbaceous weed found in the different parts of the world and Europe. It has been responsible for poisoning of livestock resulting a severe inflammation, particularly of mucous membranes and the eyes (Schmidt and Evans, 2004).

Plant are an important source of potentially useful structures for the evolution of novel chemotherapeutic agents. The foremost measure towards this goal is the in vitro antibacterial activity assay (Govindarajan et al., 2006). In the present work, plant body of *Euphorbia helioscopia* was selected for determining antimicrobial activity. The plant extract of *E. helioscopia* showed a good potential against tested microbes. Aerial parts of *Euphorbia hirta* show a wide spectrum of antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus* (Sudhakar et al., 2006). The bacteria selected for this study were (size gram negative i.e. *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. typhi* and two gram positive i.e. *S. aureus*, *B. subtilis*). The fungus used in thus the study was *candida albicans*. As a rule, plant is considered

active against both fungi and bacteria when the zone of inhibition is greater than 6 mm (Prabuseenivasan et al., 2006). ZOI more than or equal to 12 mm was considered the best i.e. most active; from 9-11 mm to be better, and from 7-8 to be good. The result presented that the plant extract of *E. helioscopia* was active against all tested microbes however result also suggested that *E. coli* was the most susceptible bacteria to all extract of *E. helioscopia* while *S. typhi* was the most resistant bacteria. Our result also proved that the overall antimicrobial activity of the plant extract of *E. helioscopia* was independent of gram positive and gram-negative bacteria.

The result presented in the previous study also revealed that the crude ethanol extract was more effective against, *E. coli* and *B. subtilis*, the zone inhibition increases with increasing concentration of extract. It is resolved that the entire plant of *E. helioscopia* possesses significant antimicrobial activity (Le Minh et al., 2015).

The data regarding aqueous extract showed that it was less active against tested microbes. Sataish et al. (2008) also reported similar results. *E. coli* and *B. subtilis* were more resistant to aqueous extract. Plants remain the most common source of antimicrobial agents. Their use as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa is reported to cause minimal side effects (Maghrani et al., 2005). The utilization of plants to heal diseases, including infectious one, has been extensively used by people. Plant extracts have great potential as antimicrobial compounds against microorganisms. Rediscovery of the connection between plants and health is accountable for launching a novel generation of botanical therapeutics that include plant-derived pharmaceuticals.

Further taxonomic study of the is recommended for the correct identification. Micromorphology of plants material have been proven significant in correct identification and discrimination of the species (Ahmad et al., 2018; Ashfaq et al., 2018; Ashfaq et al., 2019b; Bahadur et al., 2018b; Bahadur et al., 2019; Gul et al., 2019a; Gul et al., 2019b; Gul et al., 2019c; Naz et al., 2019; Sufyan et al., 2018; Bano et al., 2019; Nazish et al., 2019; Qureshi et al., 2019; Arshad et al., 2019; Rashid et al., 2018).

Therefore, they can be utilized in the treatment of infectious diseases caused by resistant microbes. The demonstration of antibacterial activity against both gram positive and gram-negative bacteria may be suggestive of the presence of broad spectrum antibiotic compounds. This will be of immense advantage in fighting the threat of antibiotic refractive pathogens that are so predominant in recent times (Helma et al., 2001).

It is concluded from the results that plants possess a good impeding against pathogenic microbes. The obtained results carry the role of plants in the formal medical specialty. The potential for developing anti-microbes from higher plants appears satisfied as it heads to the maturity of new drugs, which is demanded today. These are a lot of opportunity of using plants in new drugs for the dealing of various diseases by separating the active compounds from plants. Further research is necessary to define the active compounds within these plant studies a chloroform and methanol extracted samples are needed, as they demonstrated good results compared to other extracts.

REFERENCES

- Ahmad M, Zafar M, Sultana S, Ahmad M, Abbas Q, Ayoub M. et al. Identification of green energy ranunculaceous flora of district Chitral, Northern Pakistan using pollen features through scanning electron microscopy. *Microsc Res Tech.* 2018; 81(9):1004-16.
- Arshad S, Ahmad M, Saboor A, Ibrahim FH, Mustafa MRU, Zafar M, et al. Role of trees in climate change and their authentication through scanning electron microscopy. *Microsc Res Tech.* 2019;82(2):92-100.
- Ashfaq S, Ahmad M, Zafar M, Sultana S, Bahadur S, Ullah F. et al. Foliar micromorphology of Convolvulaceous species with special emphasis on trichome diversity from the arid zone of Pakistan. *Flora.* 2019b; 255:110-24.
- Ashfaq S, Ahmad M, Zafar M, Sultana S, Bahadur S, Abbas N. Medicinal Plant Biodiversity used among the rural communities of Arid Regions of Northern Punjab, Pakistan. *Indian j. tradit. knowl.* 2019a;18(2):226-41.
- Ashfaq S, Zafar M, Ahmad M, Sultana S, Bahadur S, Khan A. et al. Microscopic investigations of palynological features of convolvulaceous species from arid zone of Pakistan. *Microsc Res Tech.* 2018;81(2):228-39.

- Bahadur S, Khan MS, Shah M, Shuaib M, Ahmad M, Zafar M. et al. Traditional usage of medicinal plants among the local communities of Peshawar valley, Pakistan. *Acta Ecologica Sinica*. 2018a;1-29.
- Bahadur S, Ahmad M, Mir S, Zafar M, Sultana S, Ashfaq S. et al. Identification of monocot flora using pollen features through scanning electron microscopy. *Microsc Res Tech*. 2018b;81(6):599-13.
- Bahadur S, Ahmad M, Zafar M, Sultana S, Begum N, Ashfaq S. et al. Palyno anatomical studies of monocot taxa and its taxonomic implications using light and scanning electron microscopy. *Microsc Res Tech*. 2019;82(4):373-93.
- Bano A, Ahmad M, Rashid S, Zafar M, Ashfaq S, Rehman S.U. et al. Microscopic investigations of some selected species of Papilionaceae through SEM and LM from Skardu valley, northern Pakistan. *Microsc Res Tech*. 2019;82(4):452-58.
- Carron EA, Maran JM, Montero L, Fernandezalga A, Dominguez A. Antimicrobial properties of some extracts obtained from some Mediterranean plants of medicinal value. *J. Pharmacognosy Phytother*. 1987;21:195-202.
- Govindarajan R, Vijayakumar M, Singh M, Rao CV, Shirwaikar A, Rawat AKS. Et al. Antiulcer and antimicrobial activity of *Anogeissus latifolia*. *J Ethnopharmacol*. 2006;106(1):57-1.
- Gul S, Ahmad M, Zafar M, Bahadur S, Sultana S, Ashfaq S. et al. Foliar epidermal anatomy of Lamiaceae with special emphasis on their trichomes diversity using scanning electron microscopy. *Microsc Res Tech*. 2019a;82(3):206-23.
- Gul S, Ahmad M, Zafar M, Bahadur S, Celep F, Sultana S. et al. Taxonomic significance of foliar epidermal morphology in Lamiaceae from Pakistan. *Microsc Res Tech*. 2019c;1-22.
- Gul S, Ahmad M, Zafar M, Bahadur S, Sultana S, Begum N. et al. Taxonomic study of subfamily Nepetoideae (Lamiaceae) by polynomorphological approach. *Microsc Res Tech*. 2019b;82(7):1021-31.
- Harbottle H, Thakur S, Zhao S, White DG. Genetics of antimicrobial resistance. *Anim. Biotechnol*. 2006;17(2):111-24.
- Helma C, King RD, Kramer S, Srinivasan A. The predictive toxicology challenge 2000-2001. *Bioinformatics*. 2001;17(1):107-8.
- Humeera N, Kamili AN, Bandh SA, Lone BA, Gousia N. Antimicrobial and antioxidant activities of alcoholic extracts of *Rumex dentatus* L. *Microbial pathogenesis*. 2013;57:17-20.
- Kumar GP, Chaturvedi A. Ethnobotanical Observations of Euphorbiaceae Species from Vidarbha region, Maharashtra, India. *Ethnobotan Leaflets*. 2010;14:674-80.
- Le Minh V, Nhu NTK, Phat VV, Thompson C, Lan NPH, Nga TVT. et al. *In vitro* activity of colistin in antimicrobial combination against carbapenem-resistant *Acinetobacter baumannii* isolated from patients with ventilator-associated pneumonia in Vietnam. *J Med Microbiol*. 2015;64(10): 11-62.
- Mahmood AAM, Malik RN, Shinwari ZK. Indigenous knowledge of medicinal plants from Gujranwala district, Pakistan. *J. Ethnopharm*. 2013;148:714-23.
- Maghrani M, Zeggwagh NA, Michel JB, Eddouks M. Antihypertensive effect of *Lepidium sativum* L. in spontaneously hypertensive rats. *J Ethnopharmacol*. 2005;100(1-2):193-97.
- Nasrullah AB, Nisar M, Suliman and Ali Z. Ethnobotanical wealth of Jandool Valley, Dir Lower, Khyber Pakhtunkhwa (Kpk), Pakistan. *Int. J. phytomedicine*. 2012;4:351-4.
- Naz S, Zafar M, Ahmad M, Memon RA, Sultana S, Bahadur S. et al. Palynological investigation of lactiferous flora (Apocynaceae) of District Rawalpindi, Pakistan, using light and scanning electron microscopy. *Microsc Res Tech*. 2019;1-9.
- Nazish M, Zafar M, Ahmad M, Sultana S, Ullah R, Alqahtani AS. et al. Palyno morphological investigations of halophytic taxa of Amaranthaceae through SEM from Salt range of Northern Punjab, Pakistan. *Microsc Res Tech*. 2019;82(3):304-16.
- Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. *BMC Complement Altern Med*. 2006;6(1):39.
- Qaisar SNA, Gilani SN, Farooq S, Rauf A, Naz R, Perveez S. Preliminary Comparative Phytochemical Screening of Euphorbia Species, *Am Eurasian J Agric Environ Sci*. 2012;12(8):1056-60.

- Qureshi MN, Talha N, Ahmad M, Zafar M, Ashfaq S. Morpho palynological investigations of natural resources: A case study of Surghar mountain district Mianwali Punjab, Pakistan *Microsc Res Tech.* 2019;82(7):1047-56.
- Rahman AHMM, Akter M. Taxonomy and medicinal uses of euphorbiaceae (spurge) family of rajshahi, Bangladesh. *Plant Sci.* 2013;1(3):74-80.
- Rashid S, Zafar M, Ahmad M, Lone FA, Shaheen S, Sultana S. et al. Microscopic investigations and pharmacognostic techniques used for the standardization of herbal drug *Nigella sativa* L. *Microsc Res Tech.* 2018;81(12):1443-50.
- Revathi P, Parimelazhagan T. Traditional knowledge on medicinal plants used by the Irula Tribe of Hasanur Hills, Erode District, Tamil Nadu, India. *Ethnobot Leaflets*, 2010;(14):136-60.
- Rios JL, Recio MC. Medicinal plants and antimicrobial activity. *J Ethnopharm.* 2005; 100:80-4.
- Saleem U, Hussain K, Ahmad M, Bukhari, I, Malik A, Ahmad B. Physicochemical and phytochemical analysis of *Euphorbia helioscopia* (L.). *Pak J Pharm Sci.* 2014;27(3):577-85.
- Schmidt RJ, Evans FJ. Skin irritants of *Euphorbia helioscopia* L. *Contact Dermatitis.* 2004;6:204-10.
- Sudhakar M, Rao ChV, Rao PM, Raju DB, Venkateswarlu Y. Antimicrobial activity of *Caesalpinia pulcherrima*, *Euphorbia hirta* and *Asystasia gangeticum*. *Fitoterapia.* 2006;77(5):378-80.
- Sufyan M, Badshah I, Ahmad M, Zafar M. Identification of medicinally used Flora using pollen features imaged in the scanning electron microscopy in the lower Margalla Hills Islamabad Pakistan. *Microsc Microanal.* 2018;24(3):292-9.