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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1574-1584. **OPEN ACCESS**

Impact of Geological Zones on *Euphorbia Helioscopia* Phytochemicals and Their Antimicrobial Activity

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This paper communicates results of a study designed for investigation of environmental effects on phytochemicals in *Euphorbia helioscopia* and antimicrobial activity of different solvent fractions. Plant was collected from different areas and labeled as sample-1 to 7. The alkaloids, saponins, tannins, flavonoids, phenols and steroids are tested positive. Flavonoid is high as 7.148 and 7.341 % in sample-1 and 5 respectively. Maximum phenols 1.993 % is in sample-3, while no significant difference is noticed in others. Maximum saponins 1.078 % is recorded in sample-6, whereas high value of alkaloid 0.327 % is determined in sample-1. Highest antimicrobial activity 26 mm is shown by hexane extract against *Kleibsiella pneumonia*, its second highest and equal activity 16 mm are observed against *Erwinia carotovora* and *Bacillus atrophenus.* Almost equal activities 13 mm, 13 mm, 12 mm and 12 mm by ethyl acetate fraction measured against *Bacillus subtilis*, *Bacillus atrophenus*, *Pseudomonas aeruginosa* and *Erwinia carotovora* respectively, it's maximum activity 17 mm is against *Kleibsiella pneumonia*. Equal activities 9 mm is determined for aqueous extract against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus atrophenus* and *Candida albicans*. Highest activity 23 mm by crude extract is against *Kleibsiella pneumoniae*, while lowest 9 mm is recorded against *Salmonella typhi*.

Keywords: Euphorbia helioscopia, Environmental effect, Secondary metabolites, Antimicrobial activity

INTRODUCTION

Most of the plants have natural medicinal properties. Currently extensive attention is given to medicinal plants, because they can be used safely for the benefits of human beings. Phytochemical constituents of the plants have power of the medicine, which causes certain pharmacological action (Akinmoladun et al. 2007, Aslam and Ahmad 2016). Alkaloids, flavonoids, tannins, saponins, glycosides and phenolic compounds are some of the most significant bioactive phytochemicals (Edeoga et al. 2005). Most of the modern world drugs are derived from these natural compounds. Phytochemicals derived from medicinal plants, vegetables or fruits, acts or give protection against various diseases (Asif 2015, Sharma and Gupta 2015).

In most cases infectious diseases causes death, and this accounts for almost one-half of the all death in the tropical countries and serious problem in developed countries. A calculation was made and the results showed that the main causes of death are the infectious diseases and in 8 % of the 9 deaths occurring in United States (Demisew and Dagne 2001). Problems associated with the use of antibiotics; include adverse effects on the host having hypersensitivity, immune suppressant and allergic reactions, so the drug resistances have been developed due to the indiscriminate use of these drugs (Fair and Tor 2014). These problems forced the researchers to find new antimicrobial agents. With the medicinal

importance of antibiotic resistance in bacteria, there is a great need for more effective therapeutic agents. Thus it is felt to develop alternative antimicrobial drugs from medicinal plants (Demisew and Dagne 2001). They are effective in treating infectious diseases and minimize many of the side effects which are often caused by synthetic antimicrobials drugs (Luitel et al. 2014, Majhi et al. 2015).

Euphorbia helioscopia belongs to the family Euphorbiaceae, commonly known as sun spurge. Its main phytochemical constituents are euphoheliosnoids, euphornin B, euphoscopins, euphornins, epieuphorscopins, euphohelioscopin, elioscopinolide, and two a-hydroxyhelioscopinoli; a quaiane lactone, hemistepsin; two nor-sesquiterpenoids, namely 4,5-dihydroblumenol A and aglycone of icariside B2 and six flavonoids, namely lecochalcone B, glabrone and 4,5,7-trihydroxyflavanone respectively. It is used for the treatments of various diseases such as skin diseases, gonorrhea, migraine, warts cures, intestinal parasites, and it is also antiperiodic (Duke and Ayensu 1985, Saleem and Ahmad 2016). Its leaves and stems are febrifuge and vermifuge, and root is anthelmintic. The plant is cathartic. It has anticancer properties. The milky sap is used for skin diseases. Its oil obtained from seeds has the properties of purgation (Chopra et al. 1956, Wang et al. 2012).

Keeping in view the importance of *Euphorbia helioscopia* the present study was aimed to investigate the geological impact on *Euphorbia helioscopia* in respect of its chemical constituents and antimicrobial activities.

MATERIALS AND METHODS

2.1. Collection and identification of plant materials

The plant specimens (whole plant) were collected from the lands located at North West Frontier parts called Khyber Pakhtunkhwa (KPK) of Pakistan, including districts of Charsadda, Mohmand Agency, Mardan, Swabi, Peshawar, Nowshehra and Malakand Agency. Plant samples were identified by authors at PCSIR laboratories complex, Ministry of Science and Technology, Peshawar, Pakistan, and the Voucher specimens were preserved for reference at PCSIR laboratories complex, Peshawar. The collected plant specimens were dried under shade and then ground into uniform powder by milling machine, and stored in air-tight glass containers away from sun light for next analysis.

2.2. Sample preparation

The methanolic extract of each sample is prepared into 1000 mL conical flask by soaking 150 g plant sample in about 500 mL commercial grade methanol (90-95 %). After extraction for overnight (22 h) at room temperature the extract is filtered by using Whatman filter paper, and this extraction and filtration is repeated for three times. To collect the extract the filtrate is concentrated to dryness through rotary evaporation at low temperature (40 °C). Then transferred the extract to a pre-weighed china dish by dissolving with little methanol and put the china dish on steam water bath (80-90 °C) to evaporate the methanol. Finally the phytochemical constituent of each sample is determined qualitatively and quantitatively following the standard procedures as discussed elsewhere (Edeoga et al. 2005, Evans 2002, Harborne 1973, Singh et al. 2018).

2.3. Qualitative analysis of phytochemicals

2.3.1. Alkaloids

A drop spotted on the filter paper from plant methanolic extract then put “Draggen droft reagent” on that spot, finally washed the spot with tape water. The Draggen droft was removed away with water and alkaloid remained on filter paper (green yellow color). This green yellow color indicated the presence of alkaloids in this plant.

2.3.2. Saponin

In a dry test tube 5-6 mL distilled water was taken and added about 0.2 g of methanolic extract to it. It was dissolved and shaken vigorously to achieve a stable persistent froth. The froth was persisted for 5 minutes or more which showing that saponin is present in the sample and test is positive.

2.3.3. Flavonoids

In a test tube 5 mL dilute ammonia solution was taken and a small amount of each plant methanolic extract was dissolved completely, subsequently followed by drop wise addition of concentrated H_2SO_4 with the help of a small pipette. A yellowish coloration is observed which is more prominent at bottom of tube, and the coloration after a while disappeared when allowed to rest which indicating the presence of flavonoids.

2.3.4. Phenols

Into a test tube 2 mL of the test solution was taken followed by addition of the ethyl alcohol and

few drops of the ferric chloride solution, and then it was observed for coloration (Evans 2002, Harborne 1973).

2.3.5. Tannins

Into 100 mL conical flask about 0.25 g of the plant methanolic extract was taken. And 20 mL distilled water was added then put in a water bath at 100 °C. At boiling, it is filtered and cooled to room temperature, then transferred to a test tube and added 1-5 drops of 0.1 % ferric chloride, the blue black color was observed which indicated the presence of tannins.

2.3.6. Steroids

Into 50 mL conical flask 0.5 g of the plant extract dissolved by a suitable amount of methanol was taken. The solution was filtered and dried by rotary evaporation. Subsequently dissolved by 3 mL chloroform since steroids is soluble in chloroform. After filtration, a small amount of the filtrate was taken in a test tube and added 1-6 drops of 1/9 ratio H_2SO_4/acetic anhydride mixture. A green ring was observed which showing the presence of steroids.

2.4. Quantitative analysis of phytochemicals

2.4.1. Alkaloids

Into 250 mL beaker about 0.50 g of plant extract dissolved in 150 mL of 5 % HCl solution was taken, while keeping the pH of filtrate in range of 1-2 pH, since salt is formed in acidic medium by alkaloids. Then solution was filtered into 500 mL conical flask and to the residue left again added 125 mL of 5 % HCl solution, this dissolution and filtration was repeated for 3 times. To wash the filtrate, it was transferred to 500 mL separating funnel added $CHCl_3$ and well shook, the aqueous layer was collected, and repeated washing for two times. Subsequently the aqueous layer was basified in separating funnel by gradually addition of NH_4OH and shaking slowly while checking the pH, the addition of NH_4OH was stopped when pH reached to 9-10, the color became light dark reddish. Finally to collect the alkaloids, the $CHCl_3$ was added to the basic solution in separating funnel and was shaken well and collected the lower $CHCl_3$ layer containing alkaloids, and this separation was repeated for 3 times. Then the alkaloids was separated by rotary evaporation, dried in vacuum desiccator at room temperature, and weighed (Edeoga et al. 2005, Evans 2002, Harborne 1973, Singh et al. 2018).

2.4.2. Saponins

For a typical saponins analysis, into a 250 mL conical flask 2 g of plant extract was taken. Initially 100 mL of 20 % aqueous ethanol was added, stirred, and then the mixture was heated for 4 hours in a water bath at 55 °C with continuous stirring. Subsequently the mixture was filtered the residue left was re-extracted with 100 mL 20 % aqueous ethanol. The filtrates were combined and concentrated to 40 mL over a water bath at 90 °C. The concentrated extract was then transferred to 250 mL separating funnel, 20 mL diethyl ether was added and shaken vigorously. After shaking, allowed to separate into two layers, the aqueous layer (upper layer) was recovered while the organic layer (lower layer) was discarded, and this extraction was repeated three times. The aqueous layer was extracted thrice with 60 mL n-butanol, and then n-butanol extract was double washed with 10 mL 5 % NaCl solution. Finally the washed n-butanol extract was evaporated in a water bath and dried in the oven at 50 °C to a constant weight to give the saponins (Edeoga et al. 2005, Evans 2002, Harborne 1973, Singh et al. 2018).

2.4.3. Flavonoids

Into a 500 mL conical flask 2 g plant extract was taken, followed by addition of 150 mL 80 % aqueous methanol at room temperature and stirred for overnight. Then the whole solution was filtered through a Whatman filter paper No. 42 (125 mm), and this process was repeated. Finally, the filtrate was transferred into a crucible and dried via evaporation over a water bath to a constant weight (Bohm and Koupai-Abyazani 1994).

2.4.4. Phenols

Into a Soxhlet apparatus 2 g of plant extract was defatted with 100 mL diethyl ether for 2 h. The fat free sample was boiled for 15 minutes with 50 mL ether. Initially into a 50 mL flask 5 mL sample was pipetted, and then 10 mL distilled water was added. After that, 2 mL NH_4OH solution and 5 mL concentrated amyl alcohol was also added to this mixture. The sample was made up to the mark and left to react for 30 minutes for color development. Finally absorbance of resultant solution was measured with a UV-Visible spectrophotometer at 505 nm, and from calibration plot the amount of phenols was identified (Bohm and Koupai-Abyazani 1994, Edeoga et al. 2005, Evans 2002, Harborne 1973, Singh et al. 2018).

2.5. Antimicrobial activity

2.5.1. Plant extraction and fractionation

Into a flask 100 g coarsely powdered material of each plant was added, and extracted separately with methanol (90-95%) at room temperature for 72 h with occasional shaking. The methanolic extraction was filtered with Whatman No. 1 filter paper, and the filtrate was concentrated to semisolid material by using rotary evaporation under vacuum at low temperature (40 °C). The crude methanolic extract of each plant was initially dissolved in distilled water, and then fractionated with n-hexane, ethyl acetate, iso-butanal and aqueous to afford respective solvent soluble fractions. Finally the crude extract and various solvent fractions were further used for pharmacological investigations (Aiyelaagbe 2001, Ali-Shtayeh et al. 1997, Bauer 1966).

2.5.2. Test microorganisms

In this study, different solvent extracts of *Euphorbia helioscopia* were studied for antimicrobial activities against gram-negative and gram-positive bacteria, and fungi. The bacterial strains, *Escherichia coli* (ATCC25922), *Salmonella typhi* (ATCC0650), *Pseudomonas aeruginosa* (ATCC27853), *Bacillus subtilis* (NCTC8236), *Staphylococcus aureus* (NCTC25953), *Erwinia carotovora* (clinical isolate), *Kleibsiella pneumonia* (ATCC700603), *Bacillus atrophenus* (clinical isolate), and one fungal strain *Candida albicans* (ATCC2091) afforded by Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar, Pakistan, were used. The microorganisms were standardized and inoculated into a sterile nutrient broth (0.013g/mL) media, and finally tested by disc diffusion method (Aiyelaagbe 2001, Ali-Shtayeh et al. 1997, Bauer 1966).

2.5.3. Antimicrobial activity determination

The crude extract and subsequent various solvent-soluble fractions of *Euphorbia helioscopia* were investigated against different human and plant pathogenic strains, using the filter disc diffusion method as described in the literature previously(Aiyelaagbe 2001, Ali-Shtayeh et al. 1997, Bauer 1966, Portillo et al. 2001). Test material of each extract (1mg / 6µL) was prepared by dissolving in dimethyl sulfoxide (DMSO) or hexane. In vitro antimicrobial activity was carried out via Nutrient Agar (NA). A suitable amount (about 12-15 mL) of molten NA (0.028 g/mL) was poured into sterilized petri dishes inside a sterile environment of Laminar flow hood, and was allowed to solidify. The standard 0.5 McFarland and 10^6 CFU/mL of 50 µL from standardized microbial cultures are spread over sterile NA plates through a sterile glass spreader. A similar practice was also done for the case of fungi. Concentrations from different extracts of 1 and 2 mg $disc^{-1}$ in the form of 6 and 12 µL volume are loaded by micropipette aseptically on sterile individual filter paper discs (Whatman-I: diameter 6 mm for bacteria and fungi), and subsequently petri dishes are placed in incubator at 37 °C for 24 h. Depending on the solubility of extracts the DMSO or hexane was used as negative control. Simultaneously, the antibiotics Ciprofloxacin, Azithromycin and Clotrimazole, separately in the volume of 6 and 12 µL were also loaded on individual discs, as positive control for gram-negative bacteria, gram-positive bacteria and *Candida albicans,* respectively. After 24 h incubation, the inhibition zones were measured (in mm) and the mean value was calculated. To clear any uncertainty for each extract two duplicate trials were performed against each microorganism.

RESULTS AND DISCUSSION

Plant of *Euphorbia helioscopia* was collected from seven districts of Khyber Pakhtunkhwa, namely Charsadda, Mohmand Agency, Mardan, Swabi, Peshawar, Nowshehra and Malakand Agency, and were named as sample-1, 2, 3, 4, 5, 6 and sample-7 respectively, as represented in Table 1. And this designation was used throughout the paper.

Table 1: The plants collected from the different areas of Khyber Pakhtunkhwa, Pakistan.

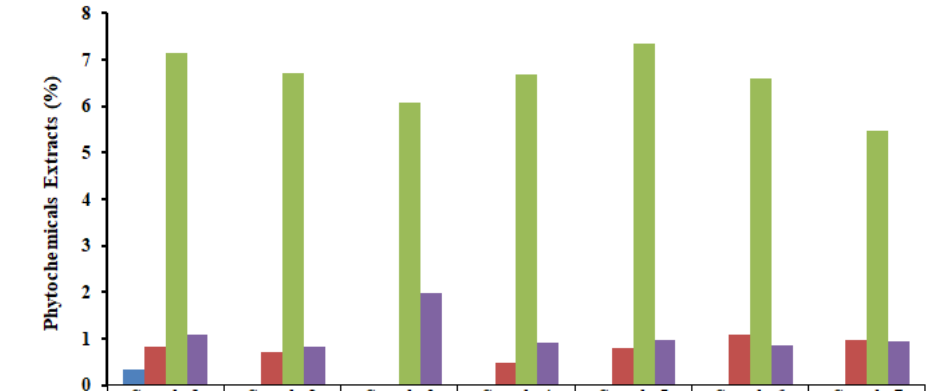
Entry	Areas	Plants
A	Charsadda	Sample-1
B	Mohmand Agency	Sample-2
C	Mardan	Sample-3
D	Swabi	Sample-4
E	Peshawar	Sample-5
F	Nowshehra	Sample-6
G	Malakand Agency	Sample-7

3.1. Qualitative analysis of phytochemicals

It is cleared through qualitative analysis as described in Table 2, all the phytochemical constituents including, alkaloids, saponins, flavonoids, phenols, tannins and steroids were present in the *Euphorbia helioscopia* plants collected from the seven various districts, and therefore the quantitative analysis was performed.

Table 2: Qualitative analysis of phytochemicals of Euphorbia helioscopia

Entry	Sample	Alkaloids	Saponins	Flavonoids	Phenols	Tannins	Steroids
A	Sample-1	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive
B	Sample-2	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive
C	Sample-3	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive
D	Sample-4	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive
E	Sample-5	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive
F	Sample-6	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive
G	Sample-7	+ ive	+ ive	+ ive	+ ive	+ ive	+ ive



	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6	Sample-7
Alkaloids	0.327	0.016	0.029	0.006	0.03	0.029	0.017
Saponins	0.823	0.725	0.022	0.479	0.79	1.078	0.974
Flavonoids	7.148	6.72	6.063	6.665	7.341	6.582	5.472
Phenols	1.085	0.839	1.993	0.925	0.972	0.85	0.946

Figure 1: Quantitative analysis of phytochemicals extracts (in %) of *Euphorbia helioscopia* from different areas.

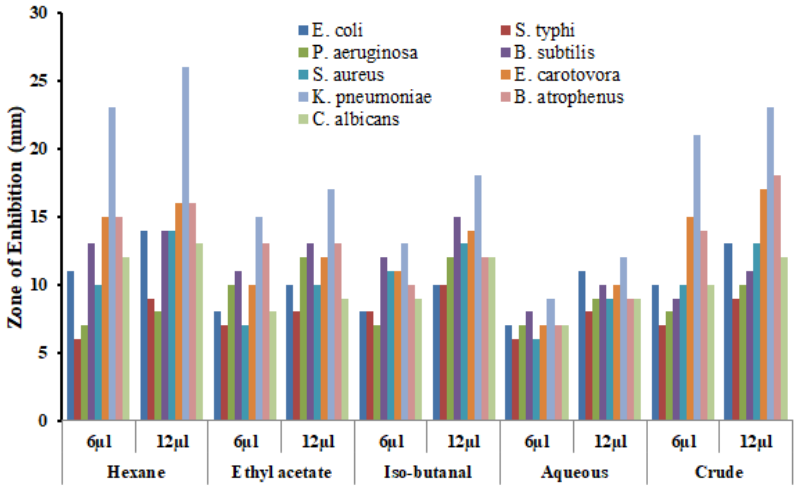


Figure 2: Antimicrobial activity of crude extract and its subsequent solvent-soluble fractions of *Euphorbia helioscopia* (zone of inhibition of growth of microbes in mm).
Gram-negative bacteria: Escherichia (E) coli, Salmonella (S) typhi, Pseudomonas (P) aeruginosa, Erwinia (E) carotovora, Kleibsiella (K) pneumoniae. Gram-positive bacteria: Bacillus (B) subtilis, Staphylococcus (S) aureus, Bacillus (B) atrophenus. Fungus: Candida (C) albicans.

3.2. Quantitative analysis of phytochemicals

The presence of phytochemical constituents in plants may be responsible for their potential use as drugs (Sofowora 1993). The medicinal plants of *Euphorbia helioscopia* from different geological areas were checked for these secondary metabolites, and the results are summarized in Table 2 and Figure 1.

3.2.1. Alkaloids

Alkaloid has a meager presence in living organism and is defined as a cyclic organic compound having nitrogen in a negative oxidation state (Van Wyk 2015). Alkaloids are divided into several subgroups on the basis of structure. These are non-heterocyclic and heterocyclic alkaloids, which are again divided into 12 major groups according to their basic ring structure. Plants having alkaloids are used since prehistory as dyes, spices and poisons(Wink and Roberts 1998). Alkaloids are famous for CNS activities (Hussain et al. 2018). As can be seen from Figure 1, the alkaloids concentration 0.327 % was highest in sample-1, followed by 0.030 % in sample-5, and a similar 0.029 % concentration was detected in sample-3 and sample-6. The lowest alkaloids contents 0.006 % was found in sample-4, while comparatively higher amounts 0.017 % and 0.016 % than lowest were reported in plants sample-7 and 2 respectively (Figure 1).

3.2.2. Flavonoids

Flavonoids show a common benzo-pyrone structure. It is a group of polyphenolic compounds, which possess a low molecular weight. They are classified into various subclasses, like flavones, flavonols, flavonones, isoflavonones, anthocyanidins, isoflavonoids and catechines (Cook and Samman 1996, Hodnick and Mllosavljevi 1988). Flavonoids are anti-allergic, anti-inflammatory (Carvalho et al. 2006), anti-microbial, anti-cancer and antioxidant (Revathi et al. 2017). Flavonoids have no direct involvement in the growth or development of plants. If flavonoids are used as fruits and vegetables, they are beneficial to humans. More than 2000 flavonoids have been extracted from vegetables (Btissam et al. 2018). Reports show that flavonoids improve blood circulation and lower high blood pressure (Xu Yaqin et al. 2005). The flavonoids concentration 7.341 % was the highest in sample-5 and 7.148 % in sample-1 (Figure 1), while other flavonoids amounts 6.720 %, 6.665 %, 6.582 %, 6.063 % were observed in samples-2, 4, 6 and 3 respectively. The lowest content 5.472 % of the flavonoid was found in sample-5.

3.2.3. Saponins

Saponins are surface-active glycosides, which occur naturally. They form stable soap like foams in aqueous solution. This observable feature of saponins has attracted extensive attention of researchers from ancient times. Saponins are produced by plants as well as lower marine animals and bacteria (Kharkwal et al. 2012). Saponins show antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer activities (Xu Congcong et al. 2019, Zhang et al. 2001). They are also famous for activities like hypocholesterolaemic, tumourigenic, haemolytic, immunostimulatory as well as chemoprotective activities (Soetan et al. 2014). Single sugar chain saponins such as steroid and triterpenoids have strong haemolytic activity as against the two sugar chains (Soetan et al. 2014, Woldemichael and Wink 2001). Some saponins and sapogenins are effective in deactivating viruses, e.g. purified saponins mixture from Maesa lanceolata (Sindambiwe et al. 1998). Ginseng derivative shows neurotrophic and neuroprotective effects (Rudakewich et al. 2001). It is clear from Figure 1, the saponins concentration 1.078 % was the highest in sample-6, while lowest 0.022 % was in sample-3. In the rest of the samples saponins contents 0.974 %, 0.823 %, 0.790 % and 0.725 % was detected in samples-7, 1, 5 and 2 respectively, while relatively low amount 0.479 % was present in sample-4.

3.2.4. Phenols

As evident from Figure 1, the highest phenols concentration 1.993 % was found in sample-3, and second highest 1.085 % in sample-1, while the lowest amount 0.839 % was noted in sample-2. The phenols contents 0.972 %, 0.946 % and 0.925 % were present in samples-5, 7 and 4 respectively while relatively low value 0.850 % was present in sample-6. Phenols are present in abundance in nature. Their structures ranges from simple aromatic ring to a highly polymeric structure, and frequently exist in glycosidic forms (Williamson and Manach 2005). Capsaicin is present in the dried vegetables of different species of *Capsicum*. It is used for dyspepsia and flatulence, internally and externally it is used as anti-irritant (Mattila and Hellström 2007).

3.3. Antimicrobial Activity

Disc diffusion method was employed to investigate the antimicrobial activity of *Euphorbia helioscopia* crude extract and subsequent various solvent-soluble fractions against gram-negative, gram-positive bacteria and fungi. The results are represented in Figure 2, and some of the typical antimicrobial activity of fractions is photographically shown in Figure 3. Simultaneously, the standard drugs (Azithromycin, Ciprofloxacin, and Clotrimazole) as positive control and the solvents (DMSO and Hexane) as negative control were employed, and the results are summarized in Table 3 and Figure 3. Antimicrobial characteristics of substances are good tools to control the undesirable microorganisms specifically in treatment of infectious diseases and in food spoilage (Mohanta et al. 2007, Osama et al. 2017). Most of the available antibiotics are obtained from natural or semi synthetic resources (Ribeiro da Cunha et al. 2019). About 20 % are derived from plants worldwide. Chemical substances obtained from plants have great remedial power and are good in the treatment of infectious diseases. Active components in medicinal plants generally interfere in the growth and metabolism of microorganisms in a negative way (Mohanta et al. 2007, Osama et al. 2017). The reports show several plant extracts, which carry antimicrobial activities against numerous pathogenic bacteria and fungi (Bylka et al. 2004). As demonstrated in Figure 2 and Figure 3, our results clearly show the potential of the whole plant extract against a variety of microorganisms responsible for a wide range of infections, additionally the samples for the use of low dose (6µl) and high dose (12µl) were also studied and the results are summarized in Figure 2.

3.3.1. Hexane Fraction

As can be seen from Figure 2, the highest activity 26 mm was found in *Euphorbia helioscopia's* hexane extract against *Kleibsiella pneumonia* while its second highest and equal activity 16 mm was observed against both *Erwinia carotovora* and *Bacillus atrophenus*. Lowest activity 8 mm was noted against *Pseudomonas aeruginosa*. The medium and equal activity 14 mm was recorded in hexane fraction against strains of *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The activity 13 mm was detected against *Candida albicans* while relatively low zone of inhibition 9 mm was measured against *Salmonella typhi*.

3.3.2. Ethyl acetate fraction

By the *Euphorbia helioscopia* ethyl acetate fraction (Figure 2), the highest antimicrobial activity 17 mm was noted against *Kleibsiella pneumoniae* while lowest activity 8 mm was recorded against *Salmonella typhi*. The almost equal activities 13 mm, 13 mm, 12 mm and 12 mm by this fraction were indicated against *Bacillus subtilis*, *Bacillus atrophenus*, *Pseudomonas aeruginosa* and *Erwinia carotovora* respectively. While antimicrobial activity 9 mm was present low as compared to the activity 10 mm and 10 mm against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* respectively.

Table 3: Antimicrobial activity for positive control (antibiotics: Ciprofloxacin Azithromycin and Clotrimazole) and Negative Control (solvents: DMSO and Hexane) with inhibition zones in mm.

S. No.	Species	Positive Control		Negative Control	
		Ciprofloxacin		DMSO	Hexane
		6 µl	12 µl	6 µl	6 µl
1.	*E. coli*	35	36	00	00
2.	*S. typhi*	43	41	00	00
3.	*P. aeruginosa*	30	28	00	00
4.	*E. carotovora*	31	33	00	00
5.	*K. pneumoniae*	33	35	00	00
		Azithromycin			
6.	*B. subtilis*	33	35	00	00
7.	*S. aureus*	30	33	00	00
8.	*B. atrophenus*	30	34	00	00
		Clotrimazol			
9.	*C. albicans*	25	28	00	00

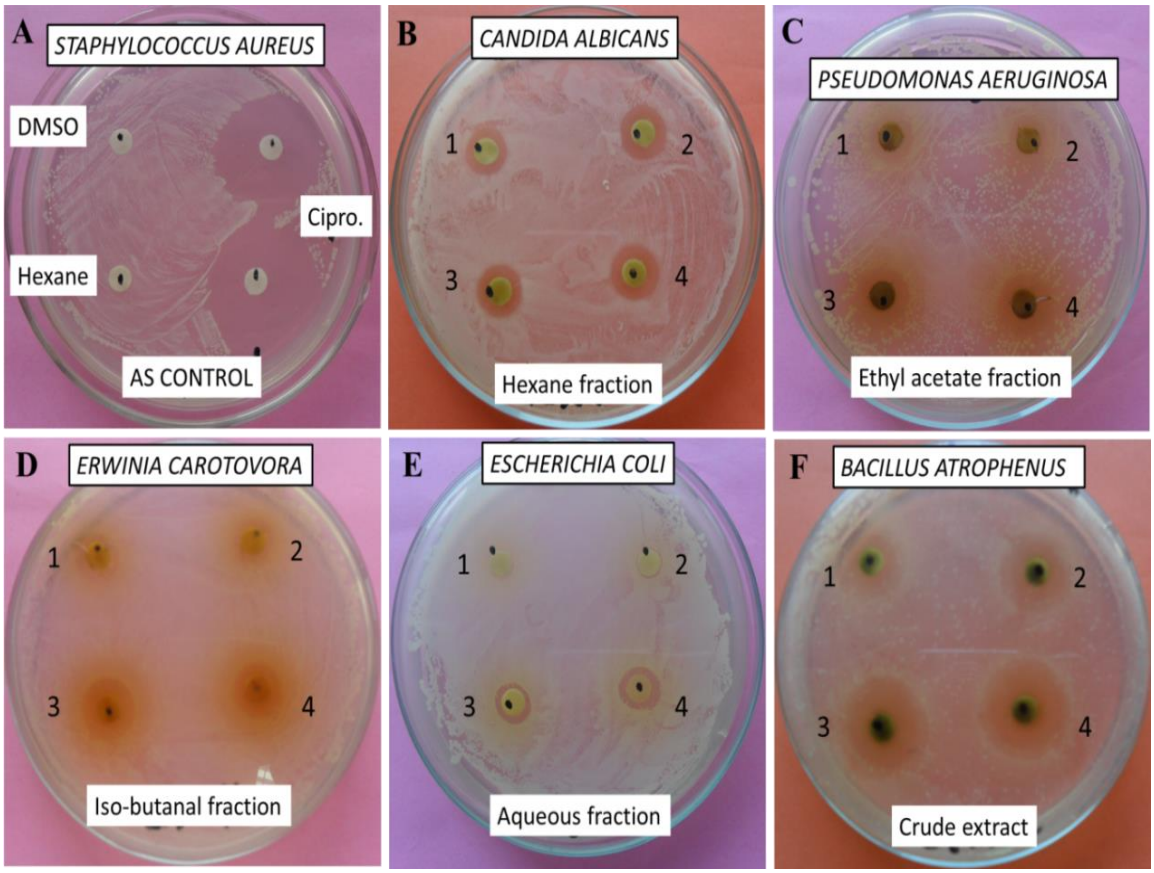


Figure 3: Photographical representation of the typical antimicrobial activities of the *Euphorbia helioscopia* crude extract and subsequent fractions against a variety of pathogenic microorganisms by disc diffusion method.

In Figure 3A, the DMSO and hexane were used as negative control, while the Ciprofloxacin (Cipro. herein) was used as a positive control. In Figure 3B-F, the digits 1 and 2 indicate 6 µl volumes, while the digits 3 and 4 indicate 12 µl volumes of the respective test materials.

3.3.3. Iso-butanal fraction

It is evident from Figure 2, that in the *Euphorbia helioscopia* iso-butanal fraction, the highest activity 18 mm was noted against *Kleibsiella pneumoniae*, second highest activity 15 mm was found against *Bacillus subtilis*, while lowest zone of inhibition 10 mm was detected against each of *Escherichia coli* and *Salmonella typhi*. The medium inhibition value 14 mm was reported against *Erwinia carotovora*, and high inhibition zone 13 mm was recorded against *Staphylococcus* by this fraction as compared to low and equal inhibition zones 12 mm against each of *Pseudomonas aeruginosa*, *Bacillus atrophenus* and *Candida albicans* species.

3.3.4. Aqueous fraction

Figure 2 shows that highest zone of inhibition 12 mm was found in aqueous fraction of *Euphorbia helioscopia* against *Kleibsiella pneumoniae,* while lowest 8 mm was present against *Salmonella typhi*. In the rest of samples, the inhibition zone 11 mm was against *Escherichia coli*, and equal zones of inhibition 10 mm was observed against both *Bacillus subtilis* and *Erwinia carotovora*. For some microbes, equal zone of inhibition, 9 mm was determined against each of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus atrophenus* and *Candida albicans*.

3.3.5. Crude extract

Figure 2 reveals that highest activity 23 mm of crude fraction was found against *Kleibsiella pneumoniae*, while lowest 9 mm was noted against *Salmonella typhi*. In this fraction the medium size zones of inhibition 18 mm and 17 mm were recorded against *Bacillus atrophenus* and *Erwinia carotovora* species respectively. The activities in descending order, 13 mm, 13 mm, 12 mm, 11 mm and 10 mm were present against

Escherichia coli, *Staphylococcus aureus*, *Candida albicans*, *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively.

CONCLUSION

Euphorbia helioscopia a well-known annual medicinal plant is abundantly available almost worldwide. Herein, the aforesaid plants were collected from different seven areas and were checked for secondary metabolites and antimicrobial spectrums against a variety of pathogens. The present study informing the common and local populaces who use this crude phytochemical constituents or whole plant for the cure of various diseases, and also provides a scientific data base, and evaluating scientifically crude phytochemical constituents and their antimicrobial activities. It is hoped that, the present study will enhance the knowledge about these plants.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This work was supported by the China Postdoc Funding for International Researcher, the research facility was provided by the PCSIR Peshawar, Pakistan.

AUTHOR CONTRIBUTIONS

HK designed, performed the experiments, and wrote the manuscript. FC reviewed the manuscript. Both authors read and approved the final version.

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