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Antibacterial activity of selected medicinal plants extracts against *Escherichia coli* isolates causing urinary tract infections

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Abstract

Antibiotic resistance was firstly observed soon after Alexander Fleming discovered penicillin in 1928. Resistance to commonly used antibiotics is one of the most threatening issues to public health across the globe. There has been an increasing demand to search for novel antimicrobial agents to combat the growing resistance. Medicinal plants are a rich source of many drugs especially antimicrobial drugs and these plants have been used from years to treat different types of diseases. The main goal of our study was evaluation of antimicrobial activity of the selected plant extracts against clinical isolates and then to compare resistance level of bacteria to commonly used antibiotics and medicinal plants. Methanolic extract was taken and dried in water bath; dried extracts were dissolved in Dimethyl Sulfoxide (DMSO) in different concentration (25mg/mL, 50mg/mL, and 100mg/mL). The zones of inhibition were measured by using agar well diffusion method. Methanolic Extracts of *Paganum harmala, Fogonia cretica, Ajuga bracteosa* and *Olea feroogenia showed* varying level of activities against *E. coli.* The results of the study shows that the antibacterial effect of selected plant extracts increased with increase in concentration. The extracts also showed activity against those isolates, which shows resistance to commonly used antibiotics. Therefore, it is suggested that antibacterial agents present in these plants should be isolated through advanced biochemical techniques such as HPLC for the evaluation of potency. These compounds may be evaluated as potential antimicrobial therapeutic agents against a wide range of human pathogens.

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Introduction

Scientists defined the antimicrobial resistance as ineffectiveness of drug against microbes (Coenen, Ferech *et al.,* 2007). For the first time the resistant cases were noted when new antimicrobial agents were introduced to medium (Alekshun and Levy 2007). Antimicrobial resistance is the result of evolution and is considered as the part of natural selection in the survival promotion. Certain antibiotics (Sulfamethoxazole-Trimethoprim, Ampicillin Tetracycline) were widely used before which are now no more used in many diseases (Hoge, Gambel *et al.,* 1998). Microbes can resist more antimicrobials drugs at a time, which is termed as multidrug resistance. *Staphylococcus spp* has been reported to have resistant against multiple antibiotics. Resistance mechanisms include alteration in membrane permeability, mutation in genes, which cause alteration in target site, enzymatic modification of drugs, efflux pumps that transfer the antimicrobials outside the cell. The bacteria not only resist the action of only one antibiotic while it also resists the action of structurally same antibiotics such as tetracycline resistant strains of bacteria can resist the action of oxytetracycline, chlortetracycline, doxycycline, and minocycline (Chopra and Roberts 2001). From the ancient times, medicinal plants play an important role in human health. About 80% of the word population use medicinal plants for traditional health therapies according to the World Health Organization report. Medicinal plants have been used for the derivation of more than half of the available chemotherapeutic agents (Kirbağ, Zengin *et al.,* 2009).

Antimicrobial and antioxidant activity of the phytochemicals like vitamins A, C, E and K, flavonoids, terpenoids, polyphenols, carotenoids, pigments, saponins, enzymes and minerals have been reported (Madhuri and Pandey 2009). The exact mechanisms of action of many plant extracts are not clear. The antibacterial compounds of phyto-extracts are rays of hope for the better future of medicine.

Botanists are working on medicinal plants and the horizons of knowledge about phyto-extracts are increasing with the passage of time. Such studies are very much important for making the pharmaceutical research and development strong (Shakeri, Hazeri *et al.,* 2012). Plants having compounds that helps to modulate immune responses and eliminate pathogens. Immune modulation is limited to one because modulations of specific and non-specific responses are done by phyto-extracts (Pandey and Chawdhry 2006). Phyto-extracts are very significant for treatment of wide range of infections. Recently many scientists proved the medici nal importance of plants. Plants synthesize secondary metabolites, which are important antimicrobials and extracted from plants to use in clinical setups (Nascimento, Locatelli *et al.,* 2000). Reports from Palestine shows that use of herbal medicines are on the top there. Mostly herbal medicines have very low or no side effects therefore they are used mostly. Herbal medicines are very much common as it merged in their culture. In Palestine, medicinal plants commonly found on mountains, many of them exploited in agricultural use (pesticides) (Jaradat 2005). Seeds of *Peganum harmala* reported to cause intoxication and nausea (MM 2007). They are useful for epilepsy, psychosis, loss of memory, chronic headache, kidney stone, dropsy, jaundice, colic and sciatica. These plant's seeds are used to detoxify toxins in human body. Flax seed and honey in combination are useful for the patients of dyspnea. The decoction has been used for numbness, lung and liver diseases (Mikaili, Maadirad *et al.,* 2013).

P. harmala is mainly used for disinfection purposes (Almasirad, Hosseini *et al.,* 2006). *A. bracteosa* is used in medicine since from very old time and has various utilizations. In ethno medicine, its use is reported as anthelmintic, astringent, antibacterial, anti-fungal, anti-inflammatory, and hypoglycemic and it remediates intestinal ailments (Israili and Lyoussi 2009).

Chinese use the above-mentioned plant in treatment of phlegm and pyrexia, as it is part of their tradition (Shen, Isogai *et al.,* 1993). It is recommended in Ayurveda to treat rheumatism, amenorrhea, and gout and palsy (Kaithwas and Majumdar 2012). It is known that most of the plant-derived compounds have substantial analgesic properties. Oil of *Olive ferrugenia royle* is the main fatty component of natural olive and mediates positive effects on cardiovascular, metabolic, inflammatory and autoimmune diseases (La Lastra, Barranco *et al.,* 2001). Extracts from olive have antioxidants properties, for this purpose, we can use it in pharmaceuticals as well as food industry (Savarese, De Marco *et al.,* 2007). Recently rate of olive usage increased many folds. Mostly people use their leaves as antidote in the recurring malarial fever. Hypertension could be prevented with use of leaves extract of olive (Ranalli, Contento *et al.,* 2006). The fresh fruits of *O. ferrugenia royale* are collected in summer and then dehydrated which is useful in lowering of glucose levels in diabetes (Ahmed *et al.,* 2015). *Fagonia indica* is useful in blood purification. Fruits of given plant are rich in ascorbic acid (Shinwari *et al.,* 2010). Extracts from given plants were tested for anticancer properties and were reported positive for anticancer properties (Graham, Quinn *et al.,* 2000). For that reason, the present study was conducted to determine the antibacterial activity of selected medicinal plants extracts against *Escherichia coli* isolates causing urinary tract infections

Material and methods

The present study was conducted in the Laboratory of Department of Microbiology, Abdul Wali Khan University Mardan, Pakistan. The plants used in this study were *Paganum harmala (seeds), Fogonia cretica (whole plant), Ajuga bracteosa (whole plant) and Olea feroogenia royle (Leaf).*

Collection of plants

The powder form of these plants were collected from local market in Mardan city.

Identification

These plants were transferred to Microbiology Laboratory where a Botanist from the Department of Botany Abdul Wali Khan University Mardan Pakistan identified it.

Preparation of the extracts

Eight Grams of air-dried powder of different parts of the selected plants were placed in 70 mL of pure methanol (100%) in a conical flask, plugged with cotton. The material was then kept at room temperature for one week. After one week, it was filtered with the Whatman No.1 filter paper and the filtrate was evaporated to dryness in water bath at 65℃ as reported (Setif 2011). The dried extract was then dissolved in Dimethyl sulfoxide (DMSO) as solvent at different concentration (25mg/mL, 50mg/mL, and 100mg/mL) and stored at -4 ℃ in the refrigerator for further use.

Test Microorganisms

The bacterial strains were obtained from Hayatabad Medical Complex Peshawar. Different strains of *E. coli* were tested in this study.

Maintenance of Bacterial Isolates

The bacterial strains were isolated by providing standard temperature and pressure using CLED media for Urine sample, MacConkey Agar and Blood Agar for all other samples. Colony morphology and different chemical biochemical testing were used to confirm the species identification. The isolated strains were preserved in LB broth with Glycerol in Eppendorf tubes and stored at -20℃ in the refrigerator for further use.

Agar Well Diffusion Method

Suspension of inoculum was swabbed uniformly on solidified plates having 25 mL Mueller-Hinton Agar (MHA). Six mm in diameter holes were made in the agar by using glass Pasteur pipettes. Forty microliter from each plant crude extract of different concentration (25 mg/mL, 50mg/mL and 100mg/mL) were added into each well made on the medium. For proper diffusion, it was allowed to stand on the bench.

Finally, for 24 hour it was incubated at 37℃. Zone of inhibition were then measured. This experiment was carried out in duplicate.

Results

Well diffusion method was used to determine the antibiogram assay of selected medicinal plants. According to our results, *P.harmala* extracts showed high inhibitory activity against all tested strains of *E. coli*. (Table 1)

Zone of inhibition were increased as increased occur in concentration. (Fig. 1) Synthetic antibiotics such as CAZ-30, SXT-25, CN-10, TE-30 and E-15 were also checked comparatively with the plants extract of *P. harmala* and most of the tested strain *E. coli* shows resistance to antibiotic except some strain, which were sensitive to SXT-25, CN-10. (Table 1)

Methanolic extract of *Fogonia cretica* also shows significant inhibition against all strain of *E. coli.* Some strains were less sensitive while some are more sensitive to plants extract. (Table 2) Zone of inhibition were increased as increased occur in concentration. (Fig. 2) Synthetic antibiotics (CAZ-30, SXT-25, CN-10, TE-30 and E-15) were also checked comparatively with the methanolic extract of *Fogonia cretica.*

Most of the strains were resistant to antibiotic except some strain, which were sensitive to SXT-25, CN-10. (Table 2) Methanolic extract of *Ajuga bracteosa* also showed inhibitory activity against all strains of *E. coli*. (Table 3) Zone of inhibition were increased as increased occur in concentration. (Fig. 3)

Synthetic antibiotics (CAZ-30, SXT-25, CN-10, TE-30 and E-15) were also checked comparatively with the plants extract, in which all strains show resistant to all antibiotics other than SXT-25 and CN-10, which inhibit the growth some strains. (Table 3)

Olea feroogenia royle also shows inhibitory activity against all strain of *E. coli.* (Table 4) Zone of inhibition were increased as increased occur in concentration. (Fig. 4) Synthetic antibiotics (CAZ-30, SXT-25, CN-10, TE-30 and E-15) were also checked comparatively with the methanolic extract of *Olea feroogenia royle*. Maximum strain of *E. coli* shows resistance to synthetic antibiotics accept SXT25 and CN-10, which effectively inhibit some strains of *E. coli.* (Table 4)

Table 1. Antibacterial activity of *Paganum harmala* methanolic extracts against different isolates of *E. coli.*

Isolates	Inhibitory zone of plant extract (mm)			Synthetic Antibiotics Inhibitory zone (mm)				
	25 mg/mL	50 mg/mL	100 mg/mL	CAZ	E	SXT	TE	CN
E1	15	16	18	0	0	20	15	15
E2	18	20	25	0	0	0	20	0
E3	19	22	25	20	0	0	0	10
E4	22	25	29	17	0	0	0	25
E5	16	13	11	0	0	0	0	0
E6	15	12	10	0	0	0	0	0
E7	14	11	10	16	0	17	17	16
E8	14	12	11	15	0	18	15	17

CAZ- trimethoprim, SXT- Ceftazidime, TE-Tetracycline, CN-Gentamycin, E- Erythromycin

Table 2. Antibacterial activity of *Fagonia cretica* methanolic extracts against different isolates of *E. coli* by.

Isolates	Inhibitory zone of plant extract (mm)			Synthetic Antibiotics Inhibitory zone (mm)				
	25 mg/mL	50 mg/mL	100 mg/mL	CAZ	E	SXT	TE	CN
E1	8	10	14	0	0	19	17	13
E2	15	18	20	0	0	0	19	0
E3	13	15	18	21	0	0	0	11
E4	12	13	15	16	0	0	0	24
E5	15	17	20	0	0	0	0	0
E6	12	14	15	0	0	0	0	0
E7	10	11	13	17	0	15	16	15
E8	13	14	16	14	0	19	17	15

CAZ- trimethoprim, SXT- Ceftazidime, TE-Tetracycline, CN-Gentamycin, E- Erythromycin

Table 3. Antibacterial activity of *Ajuga Bracteosa* methanolic extracts against different isolates of *E. coli.*

Isolates	Inhibitory zone of plant extract (mm)			Synthetic Antibiotics Inhibitory zone (mm)				
	25 mg/mL	50 mg/mL	100 mg/mL	CAZ	E	SXT	TE	CN
E1	14	17	18	0	0	21	17	13
E2	12	14	16	0	0	0	19	0
E3	10	12	15	19	0	0	0	11
E4	10	12	14	18	0	0	0	26
E5	10	13	15	0	0	0	0	0
E6	12	15	17	0	0	0	0	0
E7	9	10	12	17	0	16	15	15
E8	11	12	14	13	0	20	15	18

CAZ- trimethoprim, SXT- Ceftazidime, TE-Tetracycline, CN-Gentamycin, E- Erythromycin

Table 4. Antibacterial activity of *Olea ferrugenia Royale* methanolic extracts against different isolates of *E. coli*.

Isolates	Inhibitory zone of plant extract (mm)			Synthetic Antibiotics Inhibitory zone (mm)				
	25 mg/mL	50 mg/mL	100 mg/mL	CAZ	E	SXT	TE	CN
E1	15	16	18	0	0	18	14	16
E2	15	18	20	0	0	0	22	0
E3	15	18	20	23	0	0	0	12
E4	14	17	21	19	0	0	0	22
E5	13	11	9	0	0	0	0	0
E6	17	15	13	0	0	0	0	0
E7	13	10	8	15	0	18	18	17
E8	18	15	13	15	0	20	14	19

CAZ- trimethoprim, SXT- Ceftazidime, TE-Tetracycline, CN-Gentamycin, E- Erythromycin

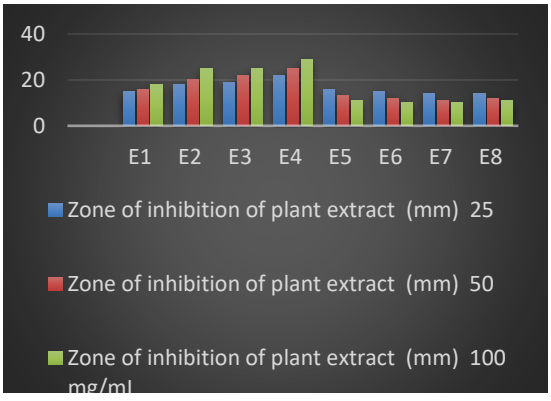

Fig. 1. Relative Zone of inhibition of different concentration of *Paganum harmala* methanolic extracts.

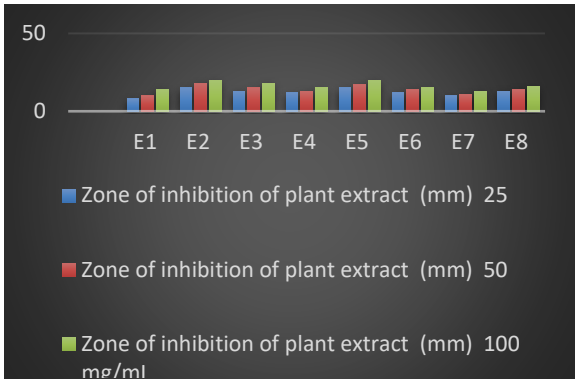

Fig. 2. Relative Zone of inhibition of different concentration of *Fagonia cretica* methanolic extracts.

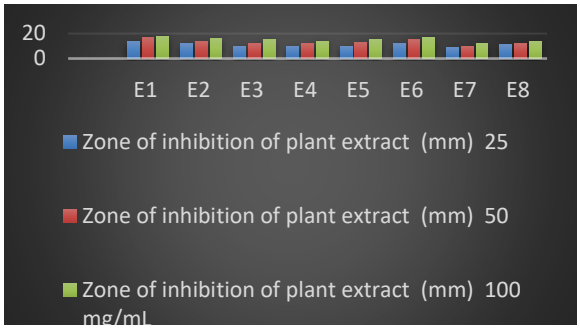

Fig. 3. Relative Zone of inhibition of different concentration of *Ajuga Bracteosa* methanolic extracts.

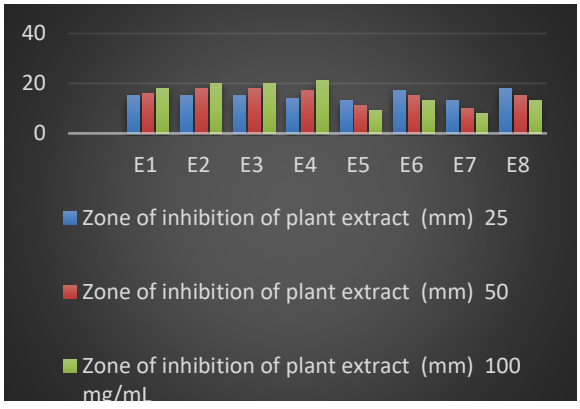

Fig. 4. Relative Zone of inhibition of different concentration of *Olea ferrugenia royale* methanolic extracts.

Discussion

Both for conventional and modern medicines, medicinal plants are used as potential active source (Shariff 2001). The use of medicinal plants As an alternative of chemical antimicrobial agents

to treat bacterial infections are advantageous because many of the medicinal plants have the ability of agreeing the biological toxicity and have rarer side effects. Easy access, low price and no reported resistance of herbs to bacteria make it advantageous (Hayacibara, Koo *et al.,* 2005). In the present study the antibacterial activity of methanolic extract of *Paganum harmala, Fagonia cretica, Ajuga bracteosa* and *Olea ferrugenia royle* against different isolates of *E. coli* were studied by using well diffusion method. According to our results, *P.harmala* extracts showed high inhibitory activity against all tested strains of *E. coli*. Zone of inhibition were increased as increased occur in concentration.

Mohsenipour *et al* obtained similar results (Mohsenipour and Hassanshahian 2016) and the study reported low efficacy to prevent biofilm formation of *E. coli*. This result is also in agreement with the previous study done by Arshad *et al.,* who reported the antibacterial activity of seed extract of *P. hamala* against O_1:K_1 serotype of *E. coli* (Arshad, Neubauer *et al.,* 2008). Synthetic antibiotics such as CAZ-30, SXT-25, CN-10, TE-30 and E-15 were also checked comparatively with the plants extract of *P. harmala* and most of the tested strain *E. coli* shows resistance to antibiotic except some strain, which were sensitive to SXT-25, CN-10. Methanolic extract of *Fogonia cretica* also shows significant inhibition against all strain of *E. coli.* Some strains were less sensitive while some are more sensitive to plants extract. Zone of inhibition were increased as increased occur in concentration. Our study is in accordance with the previous study who use whole plant methanolic extract of *Fagonia cretica* in various concentrations against *Escherichia coli*.

The extract indicated more activity against all the tested bacterial strains of *E. coli* (Sajid, Alia *et al.,* 2011). Synthetic antibiotics (CAZ-30, SXT-25, CN-10, TE-30 and E-15) were also checked comparatively with the methanolic extract *Fogonia cretica.* Most of the strains were resistant to antibiotic except some strain, which were sensitive to SXT-25, CN-10. Methanolic extract of *Ajuga bracteosa* also showed inhibitory activity against all strains of *E. coli*. Zone of inhibition were increased as increased occur in concentration. Similar result are reported in another study (Khan, Prakash *et al.,* 2011) using aqueous extracts of flowers part of this plant and give significant results. Another study (Shad, Zeeshan *et al.,* 2016) concluded the highest percent zone of inhibition of hexane fraction of *A. bracteosa* against *E. coli.* Synthetic antibiotics (CAZ-30, SXT-25, CN-10, TE-30 and E-15) were also checked comparatively with the plants extract, in which all strains show resistant to all antibiotics other than SXT-25 and CN-10, which inhibit the growth some strains.

Olea feroogenia royle also shows inhibitory activity against all strain of *E. coli.* Zone of inhibition were increased as increased occur in concentration. Another study also reported appreciable bactericidal activity against *E. coli*. It is evident that the *Olea feroogenia royle* show activity against *Escherichia coli* and other clinical isolates (Mehmood and Murtaza 2018). Synthetic antibiotics (CAZ-30, SXT-25, CN-10, TE-30 and E-15) were also checked comparatively with the methanolic extract of *Olea feroogenia royle*. Maximum strain of *E. coli* shows resistance to synthetic antibiotics accept SXT25 and CN-10, which effectively inhibit some strains of *E. coli.*

Conclusion

Plants have been used as medicinal sources in the developing countries from long time. In the current study antibacterial activity of *Paganum harmala, Fogonia cretica, Ajuga bracteosa and Olea feroogenia royle* were checked against clinical isolates *E. coli*. The plant extracts showed activity against some pathogens while some pathogens are resistant to the plants extract. The results of the study shows that the antibacterial effect of plants extracts increased after increase in concentration.

Synthetic antibiotics were also used to check their antibiotic sensitivity testing in which most of the clinical isolates were resistant strains. Possibly these plants have bioactive compounds which inhibit the growth of bacteria. Therefore, it is recommended to isolate and separate these bioactive compounds responsible for this antibacterial activity using advanced scientific techniques. They might have a potential for new drug development against wide range of human pathogens.

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