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ANTIBACTERIAL ACTIVITIES OF MEDICINAL PLANTS AGAINST MULTIDRUG RESISTANT URINARY TRACT PATHOGENS

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Abstract

Urinary tract infections (UTI) caused by multi-drug resistant (MDR) bacterial pathogens have become a serious global health concern. Main etiological agents for UTI are *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Recently, medicinal plants have found great popularity in medical treatment for different kinds of infections including urinary tract infections. The study has been planned to evaluate the efficacy of alkaloids, flavonoids, saponins and crude extracts of medicinal plants i.e. *Syzygium aromaticum, Glycerrhiza glabra,Laurus nobilis* and *Brassica rapa* against MDR urinary tract pathogens through agar well diffusion method. To investigate the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBCs), dilution method was used. Quantitative evaluations of phytochemicals indicated the presence of alkaloids in higher concentrations. Results obtained for the antibacterial activities, the crude extracts of the four plants showed significantly higher inhibition zones as compared to other phytochemicals. The MIC values obtained for different extracts varying from 7.5-15 mg/ml. Comparig the activities of the extracts of the the four medicinal plants it was found that *Syzygium aromaticum* was the most potent plant against the tested bacterial pathogens indicating its strong candidateship for the drug development.

Key words: Medicinal plants, Urinary tract infections, MDR Bacterial strains, MICs and MBCs.

Introduction

Medicinal plants have very strong relationship with the human society since the dawn of human civilization. According to the reports of world health organization (WHO), about 80% of the third world populations rely on traditional herbal therapies (Dubey *et al*., 2012). There are several studies that have analyzed the therapeutic potential of the plants (Djeussi *et al*., 2013; Habiba *et al.,* 2016; Mustafa *et al*., 2016). Both phytochemical and photochemicals have significant therapeutic properties. Several study reports have been published to prove the efficacy of the antimicrobial potency of these plants (Prusti *et al*., 2008). Certain phytochemicals correspond to the medicinal effectiveness of these plants, which include alkaloids, flavoniods (secondary metabolites) showing significant inhibitory antimicrobial potential against microganisms (Iwu *et al*., 1999; Alanis, 2005). These phytochemicals usually show greater antibacterial potential then the original individual components (Lai & Roy, 2004). Recently different studies have been published on the antimicrobial activities of different plants extracts (Almagboul *et al*., 1988; Tetyana *et al*., 2002; Bonjar, 2004; Islam *et al*., 2008). Due to easy access and less toxicity, great trend has been observed among the local inhibitants using medicinal plants in traditional therapies (Khan *et al*., 2009).

With the emergence of multidrug resistant bacteria pathogens, a significant failure has been observed in the effectiveness of drugs in the current era (Noumedem *et al*., 2013). This resistance is mainly due to extraordinary usage of antimicrobial medicines resulting in accumulation inside the body (Hancock, 2005). Although there are several pharmaceutical companies which have developed several types of antibacterial drugs in the recent years but the resistance is still increasing and becoming a global problem (Middleton *et al*., 2005). Due to the emergence of MDR strains of pathogenic bacteria, efforts are going to check the potential of medicinal plants so as to combat with these pathogenic agents (Mahmood *et al*., 2008). The phytochemicals of these medicinal plants have the ability to stop the growth of multi drug resistant organism (Delaquis *et al*., 2002).

Urinary tract infections are the most ubiquitous extraintestinal diseases caused by pathogenic bacteria. All over the world, millions of people are diagnosed with urinary tract infections (UTI) every year (Shaikh *et al*., 2005). Urinary tract infections caused by MDR strains of pathogens have become a severe health problem all over the world. The main infective driving force that causes such kind of infections are *Escherichia coli*, *Candida albicans*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis* (Diab *et al*., 2002). Usually female are more affected by these kinds of infections but still the reason is not known. Particularly the elder women and postmenopausal women are frequently affected (Shaikh *et al*., 2005; Raz, 2001). The different phytochemicals of medicinal plants showed antibacterial activities against the pathogens causing urinary tract infections. Since 1990s, plants extracts have found great popularity in medical treatment of different

infections including UTI (Rios *et al.*, 1988). Several plants have been investigated to treat the UTI, and other types of infection caused due to the pathogenic organisms (Eisenberg *et al*., 1993).

There are several medicinal plants used in traditional medication in district Dera Ismail Khan (D. I. Khan) northwest region of Pakistan for the treatment of UTI including *Syzygium aromaticum, Glycyrrhiza glabra, Laurus nobilis* and *Brassica rapa*. Hence the study has been planned to investigate the efficacy of diffent phytochemicals of the selected medicinal plants on the multidrug resistant pathogenic bacteria through their phytochemical investigation and *in vitro* antibacterial activity. The present research will provide baseline information to further investigate these plants both *In vitro* and *In vivo* in order to investigate new biologically active compounds for the production of novel drugs. This study will provide scientific validation to the traditional knowledge of using medicinal plants against infectious human diseases.

Materials and Methods

Medicinal plants collection, identification and samples preparation: Data collection was done using semistructured questionnaires. Regular field visits were arranged and conducted in various localities of the in order to record and document the medicinal plants are used for the treatment of Urinary tract infections in the study area, district D. I. Khan. Among all the documented plants only four highly prioritizing plants (*B. rapa*, *G. glabra*, *L. nobilis* and *S. aromaticum*) were selected for further *In vitro* screening. The respective plants' parts were purchased in the local market of Dera Ismail Khan and were identified by the taxonomist at the department of Botany, Kohat University of science and Technology (KUST), Kohat, Pakistan. Ethnobotanical data was collected from the literature mainly on their botanical names, family names, habit, life form, used part, recipe making, methods of these medicines taken, and animal types treated (Table 1). Collected samples were washed (tape water), dried, and cut into little pieces. These sliced pieces were shade-dried and crushed into powder by using grinder. The powdered samples were preserved in dirt-free container (closed glass) and stored until the quantitative analysis of the plants phytochemicals (Alkaloids, Flavonoids and Saponins) and antibacterial activities.

Crude extract determination: Ten grams of powdered sample was dissolved in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190–220 rpm for 24 h. The supernatant was collected slowly and evaporated in wide mouthed evaporating bowls at room temperature for 2–3 days till the final volume was reduced to one fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml and stored at 4 °C in airtight (Harborne, 1973).

Phytochemical analysis: Phytochemical analysis of all the samples was determined as follows: (Lakshmi & Geetha, 2011)

Alkaloids determination: A total of 200 ml of 20% acetic acid was added to 5 g of plant sample in a separate 250 ml beaker and covered to stand for 4 hrs. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample concentrated ammonium hydroxide was added drop wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighted. The percentage of total alkaloid content was calculated according to Obadoni and Ochuko (2001) as flow:

$$\text{Percentage of total alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \text{ X } 100$$

Determination of flavonoids: The total flavonoids content was estimated using the procedure described by Zhishen *et al*. (1999). A total of 1 ml of plant extract was diluted with 2 µl of distilled water separately followed by the addition of 150 µl of sodium nitrate (5%) solution. This mixture was incubated for 5 minutes and then 150 µl aluminum chloride (10%) solution was added and allowed to stand for 6 min. the 2 ml of sodium hydroxide (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left it for 15 minutes at room temperature. The absorbance was measured at 510 nm. Appearance of pink color showed the presence of flavonoids content. The total flavonoid content was expressed as rutin equivalent mg RE / g extract on a dry weight basis using the slandered.

Determination of saponins: Twenty grams of plant sample was mixed in 200 ml of 20% ethanol. The solution was heated on water bath for four hours at 55°C. This solution was then subjected to filtration, and the same procedure was repeated with 200 ml of 20% ethanol solution. The combined extracts were subjected to condensation at 40 ml using water bath at 90°C. The concentrate was then transferred into a funnel of 250 ml and added 20 ml of diethyl ether with constant shaking. Afterwards, removed the ether layer and collected the aqueous layer, in which 60 ml of n-butanol was added. Washed two times the collective n-butanol extract by using 10 ml of 5 % aqueous sodium chloride. The residual solution was then heated on water bath. Post evaporation, the sample remaining was electric oven dried. Later, measured the saponins concentration in percentage (Boham & Kocipai-Abyazam, 1994).

Table 1. Ethnomedicinaluses of the investigated plants.

Plant/ Family/ Local name	Habit/ Life form	Part used
Brassica rapa L./Brassicaceae/ Mustard, Sarson	Herb/ Annual, biennial	Seeds
Glycyrrhiza glabra L./ Leguminosae/ Mulethi	Herb/ Annual	Roots
Laurus nobilis L./ Lauraceae / Taiz pat	Shrub/ Perennial	Leaves
Syzygium aromaticum (L.) Merr. & L. M.Perry / Myrtaceae / Laung	Tree/ Perennial	Buds

Determination of antibacterial activity: Stock solution, each of 15 mg/ml of crude extract, alkaloids, flavonoids and saponins were dissolved in Dimethyl sulfoxide (DMSO). In order to carryout antibacterial activities three MDR strains of *A. baumannii, E. coli, and P. aeruginosa* were used and obtained from the Department of Microbiology, Kohat University of Science & Technology, Pakistan. To maintain the bacterial strains, Nutrient Broth was used at 36°C and then the selected strains were cultured on Mueller Hinton Agar (MHA) plates 24 h prior to any antimicrobial test. For all bacteria strains, overnight culture grown in broth (adjusted to 0.5 turbidity i.e., McFarland standard) was adjusted to an inoculum's density of 100 µl: 0.1 A600 culture containing 3.2 $x10^8$ colony forming unit. Further, 20 µl was spread onto 20 ml of sterile agar plates by using a sterile cotton swab. Each inoculums was spread in the way that it covers the whole surface of the medium on the plate. For media well diffusion method, 5 wells were made with the help of cork-borer (6mm in diameter) in each petri plate for different extracts. Then the extracts were introduced in wells with the help of micro pipette. In each plate four extracts (alkaloid, flavonoids, saponins, crude extract) of each plant were poured in four wells and DMSO was incorporated as negative control into the fifth well. Ampicillin antibiotic disc was used as positive control and was placed in the center of the Petri plate. The measured amount of extract in each well was approximately 100 µl. Each Petri plate was labeled with the code word of spreader bacteria and extract poured in wells. Then all plates were sealed with scotch tape and incubated at 37ºC for 24 hours. After that results were obtained by measuring the zone of inhibition in millimeters around wells by using scale (Kirby *et al*., 1957).

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC): To confirm the antibacterial potential, the quantitative measurement of plant extract was calculated so for this purpose MICs were evaluated. MICs were performed in sterilized 15 ml caped test tubes each having 10 ml nutrient broth. The concentrated solution of plants extracts' were diluted to different levels. Upper level was 15 mg/ml with the subsequent levels of 7.5 mg/ml and 3.25 mg/ml. Then 100 ul of overnight bacterial broth cultures were matched to 0.5 McFarland standards and inoculated in all the tubes. Two types of controls were also run with the experiment, positive control and negative control. As a positive control, nutrient broth and bacterial culture was fixed without plants' extract while to maintain the correct MICs value, nutrient broth and plant extract was used without bacterial culture. Results were recorded after we incubate the tubes at 37 °C for 24 hours. MIC for a specific bacterium was considered as the lowest concentration, at which no growth visible (Gunasegaran *et al*., 2011; Tian *et al*., 2008).

MBC is the minimum concentration of antibacterial agent which kills the bacteria. It was processed with subsequent steps of MIC. MIC results in which no bacterial growth was observed processed for MBC. Each selected MIC's tubes content were inoculated on prepared nutrient agar plates. Results were recorded after we incubate the plates at 37°C for 24 hours. MBC of specific plant against bacteria was examined as the minimum concentration of an antibacterial agent required to kill bacteria (Sabbioni *et al*., 2005).

Data analysis: The recorded data and observation were transferred to Microsoft and Microsoft Excel 2007 in order to tabulate and organize the results. Each experiment was replicated three times, and then the mean value of the recorded inhibition zone (mm) was taken after applying the standard deviations. Data was analyzed by using ANOVA for the determination of statistical significance (P value) among the tested phytochemical classes of a single plant producing zones of inhibitions in a single bacterial strain. Univariate ANOVA was performed in SPSS (SPSS, 2007).

Results

Ethnomedicinal plants used to treat UTI: *B. rapa*,*G. glabra, L. nobilis* and *S. aromaticum* belong to four different families namely Brassicaceae, Leguminosae, Lauraceae and Myrtaceae. Different plant parts like seeds, roots, leaves and buds were used for traditional recipe formulation against urinary tract infections that mostly administered orally to patients. Additives like water, honey and sugar were used in ethnomedicinal recipe preparations for reducing astringent taste of the remedy while ensuring the intake of complete dosage of medication. All the four plants used to treat UTI were herbs, shrubs and tree with annual or perennial life form (Table 1).

Quantitative evaluation of phytochemicals extracted from selected plants: Alkaloids are present in large quantity in *S. aromaticum* and *G. glabra* while saponins are present in large quantity in *B. rapa* and *G. glabra*. *S. aromaticum* contains higher concentration of alkaloids (26.24%) followed by *G. glabra* with 26.18%, *B. rapa* with 14.56% and *L. nobilis* with 11.24%. Saponins were observed second largest in quantity among the studied plant species after alkaloids. *G. glabra* was observed with the highest amount of flavonoids (45.2%) followed by *B. rapa* (26.55%). *L. nobilis* and *S. aromaticum* were observed with 4.12% and 3.39% saponins, respectively. Flavonoids were found in less concentration as compared to rest two of the other phytochemicals like alkaloids and saponins of the three selected plants. Highest flavonoids contents were found in *S. aromaticum* (10.5%) followed by *G. glabra* (8,9%), *B. rapa* (7.05%) and *L. nobilis* (1.85%) (Fig. 1)

Antibacterial activities: Phytochemicals like alkaloids, flavonoids, saponins and crude methanolic extracts were evaluated *In vitro* against three MDR strains of *A. baumannii, E. coli* and *P. aeruginosa*. DMSO was used as negative control, which did not show any activity. On the other side, Ampicillin was a positive control showing activities against all bacterial strains. Various plants' extracts have shown different inhibition values at 15 mg/ml concentration (Table 2). Alkaloids and crude extract of studied plants showed strong bacterial

inhibition zones as compared to flavonoids and saponins against selected bacterial strains. Among the tested phytochemical classes, alkaloids of *B. rapa* produced significantly higher inhibition zones against *E. coli* (10 mm) while the crude methanolic extract showed strong bacterial inhibition zones against *A. baumannii* (11 mm) (Table 2). Taking in account the effect of the different plant extracts, the crude extracts of *G. glabra*showed more efficacy than other three extracts against tested bacterial strains. Crude extracts showed significant highest zone of inhibition of ranges from 14 mm to 14.75 mm against all bacterial strains examined. The crude extract of *L. nobilis* showed significant highest inhibition zone of (12.75 mm) against *P. aeruginosa* while significantly highest bacterial zone of inhibition for *L. nobilis* alkaloids was observed in *E. coli* (12.5 mm). Alkaloids of *S. aromaticum* showed significant zone of inhibition (16.5 mm) against *A. baumannii* and crude extract showed convincing zone of inhibition against *P. auriginosa* (15 mm). Across all the extracts of studied plant species, the values corresponding to bacterial inhibition zones produced by the alkaloids, flavonoids, saponins and crude extract of *S. aromaticum* were higher than other studied plants indicating the efficacy of natural products (Table 2).

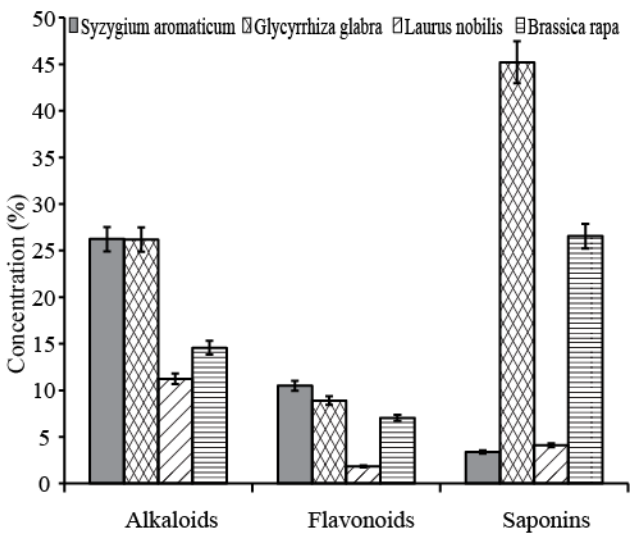

Fig. 1. Quantitative Phytochemical evaluation of the selected medicinal Plants (Concentration as %) represents the total amount of alkaloids, flavonoids and saponins in selected medicinal plants).

The MIC values of phytochemicals and crude methanolic extracts against all tested MDR bacterial strains ranged between 7.5-15 mg/ml. Mostly the MICs values of all the plants were 7.5 mg/ml for two bacterial strains *A. baumannii* and *E. coli* while there were some phytochemicals which showed MICs values of 15 mg/ml. The MBC values of phytochemicals and crude methanolic extracts against all tested MDR bacterial strains ranged between 15->15 mg/ml. The MIC and MBC values of plant's extract against MDR isolates were shown in (Table 3).

A literature survey was also conducted to confirm wether the investigated plants have been reported for the targeted strain of the bacterial pathogens. By reviewing the literature (Table), it was found that *Brassica rapa* has not been checked against the targeted pathogens in any previous studies. Similarly the other three plants have been checked partially against the three pathogens.

Discussion

Previous studies revealed that locals peoples of D. I. Khan region used various medicinal plants for the treatment of infectious diseases especially urinary tract infections. They have sound traditional knowledge regarding use of ethnomedicinal plants and preparation of recipes through their personal experience, ancestral recommendations and long effectiveness. Published literature shows that locals inhibitants of the studied region used several medicinal plants for the treatment of different ailments that indicates their higly dependency on natural products (Mussarat *et al*., 2014).

Different research studies have been conducted on antibacterial, antifungal, anti-inflammatory activities of medicinal flora (Samy & Ignacimuthu, 2000; Palombo & Semple, 2001; Kumaraswamy *et al.,* 2002). Plant phytochemicals have the capacity to combine with the extracellular and soluble proteins in order to break the membranes of microbes (Ali, 1999). Numerous studies from Pakistan and other countries have reported several phytochemicals, which were scientifically validated against a particular disease. Most of these phytochemicals have been identified from the traditional uses of medicinal plants against a particular disease (Coolborn & Bolatito, 2010).

Accessing the antibacterial potential of the plants extracts such as alkaloids, flavonoids, saponins and crude extracts of *S. aromaticum* showed convincing zone of inhibition against the three tested MDR strains of bacterial pathogens. *P. aeruginosa* appeared to be relatively more susceptible as compared to the other two bacteria. These results can be satisfied by the findings of previous research reports in which the ethanolic extracts of *S. aromaticum* showed the strongest inhibition potential towards multidrug resistant bacteria (Khan *et al*., 2009). The inhibitory action of *S. aromaticum* is referred to the presence of several compounds, mainly eugenol, eugenyl acetate, betacaryophyllene, 2-heptanone (Chaieb *et al.,* 2007) Reports have also proved that ethanolic extract of *S. aromaticum* extracts have very strong inhibitory potential against the gramnegative bacteria (Masoud & Gouda, 2012).

Investigating the antibacterial potential of different extracts of *G. glabra*, it was found that the crude extracts of the plant showed significant higher zone of inhibition against the three tested pathogens followed by alkaloids. *E. coli* showed relatively more sensitivity towards the different plants extracts. The other two bacterial strains showed various growth patterns against the plant phytochemicals. These findings may be in comparison with the study conducted by Chopra *et al.* (2013), who observed that the methanolic extract of root of *G. glabra* contain some special compounds having high antimicrobial activity. Our study can be supported by the work of Varsha *et al.* (2013) who suggested that the presence of secondary metabolites in hydro-methanolic extracts of root of *G. glabra*, the extract exhibits potent antibacterial activity. The observed antibacterial potential of the selected may be refer to the various plant secondary metabolites such alkaloids, tannins, saponins, terpens, and glycosides present in the *G. glabra*. The high medicinal

potency of the plant against the tested pathogens could provide the evidence of new biologically active components (Syed *et al.*, 2013). Our results correspond with the work of Murray (1995), who suggested that the antibacterial activity of the *G. glabra* plant is due to the presence of isoflavonoid-hispaglabridin and B, 4'-O-methylglabridin, glabridin, glabriol and 3-hydroxyglabrol. It has been also reported that it is the secondary metabolites such as; saponins, alkaloids, flavonoids in hydro-methanolic root extract of *G. glabra*, the extract exhibits potent antibacterial activity (Varsha *et al*., 2013).

The different extracts of the *L. nobilis* were investigated against the tested MDR pathogens. It was found that the crude extract of the plant was so much effective in comparison to the secondary metabolites against all the tested organisms. This potent activity of the plant crude extract may be due to the secondary metabolites including tannins, phenols and flavonoids as major ingrediants because plant secondary metabolites (PSMs) are particularly interesting in this sense (Croteau *et al*., 2000). In a study conducted by Santoyo *et al*. (2006) it was found that essential oil content of *L. nobilis* were active against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* showing different credible zones of inhibition.

Table 2. Bacterial zone of inhibition (mm) by phytochemicals of selected medicinal plants at 15 mg/ml concentration.

Plant name	Bacteria	Alkaloids (mm)	Flavonoids (mm)	Saponins (mm)	Crude (mm)	ANOVA	Ampicillin (mm)	DMSO (mm)
Brassica rapa	*Acinetobacterbaumannii*	9.75±0.96	8.25±0.50	9.0±0.82	11±0.82	NS	25	0
	Escherichia coli	10±0.82	9±0.82	9.5±0.58	9.75±0.50	p<0.05	25	0
	Pseudomonas aeruginosa	9±0.82	9.5±0.58	11±0.82	9±0.82	p<0.05	25	0
Glycyrrhizaglabra	*Acinetobacterbaumannii*	9.75±0.50	7.5±0.58	6.75±0.50	14±0.82	p<0.01	25	0
	Escherichia coli	9±0.82	9.75±0.50	8.75±0.50	14.75±0.50	p<0.01	25	0
	Pseudomonas aeruginosa	9±0.82	7.25±0.50	6.25±0.50	14.25±0.96	p<0.01	25	0
Laurusnobilis	*Acinetobacterbaumannii*	10.75±0.5	9.25±0.50	9.75±0.50	12.25±0.50	p<0.01	25	0
	Escherichia coli	12.5±1.29	5.75±0.50	9.25±0.50	12±0.82	p<0.01	23	0
	Pseudomonas aeruginosa	10.25±0.9	6±0.82	10±0.82	12.75±0.96	p<0.01	25	0
Syzygiumaromaticum	*Acinetobacterbaumannii*	16.5±0.58	12.75±0.50	15±0.82	13.75±0.50	p<0.01	25	0
	Escherichia coli	14.5±0.58	12.75±0.96	13.75±0.50	14.5±0.58	NS	23	0
	Pseudomonas aeruginosa	14.5±0.58	16.25±0.96	13±0.82	15±0.82	p<0.05	23	0

Table 3. Minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) of plant's phytochemicals against MDR isolates.

Bacteria	Phytochemicals	*Brassica rapa*		*Glycyrrhizaglabra*		*Laurusnobilis*		*Syzygium aromaticum*	
		MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Acinetobacterbaumannii	Alkaloids	7.5	15	15	>15	7.5	15	7.5	15
	Flavonoids	15	>15	15	>15	7.5	15	15	>15
	Saponins	7.5	15	15	>15	7.5	15	7.5	15
	Crude	15	>15	15	>15	7.5	15	15	>15
Escherichia coli	Alkaloids	7.5	15	7.5	15	7.5	15	7.5	15
	Flavonoids	7.5	15	7.5	15	15	>15	7.5	15
	Saponins	7.5	15	7.5	15	7.5	15	7.5	15
	Crude	7.5	15	7.5	15	15	>15	7.5	15
Pseudomonas aeruginosa	Alkaloids	15	>15	15	>15	15	>15	15	>15
	Flavonoids	15	>15	15	>15	15	>15	15	>15
	Saponins	15	>15	15	>15	15	>15	15	>15
	Crude	7.5	15	15	>15	7.5	15	7.5	15

Investigating the effects of different extracts of *B. rapa* against the tested bacterial strains, it was shown that all the extract showed sufficient. It means that the plant contain certain phytochemicals with efficient antibacterial potential. Polyphenolic compounds found in the different extracts of Brassica species have strong inhibitory potential against many pathogenic organisms (Shadomy *et al.*, 1985). Research has proven that phenolic acids are present in ionized form having polarity which facilitating them to inter through semi permeable bacterial membrane resulting in the reaction with the cytoplasm or cellular proteins (Tenore *et al.*, 2012). Study has proved that the seeds of *B. rapa*can exert high inhibitory pressure affecting the growth of almost all the human pathogenic organisms tested excluding *S. aureus* 3160, which was found to be resistant for all the three extracts of *B. rapa*. Overall, of considerable interest is that *B. rapa* seed demonstrated an appreciable antibacterial activity (Bai *et al*., 2014).

Investigating the Minimum Inhibitory Concentration (MBC) and Minimum Bactericidal Concentration (MIC) of the two medicinal plants i.e., *G. glabra* and *S. aromaticum* it was found that most of the plants' extracts showed minimum inhibition concentration at 15 mg/ml indicating that the MBCs will be greater then 15 mg/ml. There were very few extracts which showed the minimum inhibition effect at 7.5 mg/ml. In a study done by Ababutain (Ababutain, 2011), it was found that extracts of clove showed notable inhibitory effect on *B. subtilis* and *C. albicans* and their inhibition zones ranges 3.125 µg/ml-6.25 µg/ml. It was also reported that ethanolic extract of *S. aromaticum* against *E. coli* isolates showed lower MIC values ranged from 0.5 to 5.5 mg /l (Al-Jiffri *et al*., 2011). The MIC results for *G. glabra* were also the same as that of *S. aromaticum* i.e., mostly the MICs values were recorded at 15 mg/ml. In a study it was found that the MIC of *G. glabra* roots extracts were 3 µg/ml and 1 µg/ml for *E. coli* and *P. aeruginosa*, respectively (Aggarwal *et al*., 2015). From the literature survey it was found that the it was the Brassiarapawhih has been not checked in any previous studies against the tested pathogens. Furthermore, the *Glycyrrhizaglabra* has been not checked against the *Acinetobacterbaumannii*. With respect to its novelty the current study is of great importance.

Traditional herbal medicinal products used against Urinary tract infectious pathogens are therapeutically active. The crude extracts of the investigated four medicinal plants are more active against the tested urinary tract infecting MDR isolates. Higher concentration of secondary metabolites was found in *S. aromaticum* then other three medicinal plants. *S. aromaticum* is the most potent plant against the tested pathogens as compared to the other medicinal plants. The use of a particular medicinal plant to treat a particular disease in the traditional system is justified. There are certain recommendations listed below:

- Traditional knowledge on the studied medicinal plants should be documented from other regions of world in order to draw a broader conclusion on its efficacy and safety
- The studied medicinal plants should be tested against other microorganisms
- All these medicinal plants should be subjected to toxicological studies
- It is equally important to check and investigate the biological potential of the respective extracts against the mechanism of action of a particular bacterial stain.
- Scarce studies can be found on *Brassica rapa*, therefore, this species has the potential to be tested against a wide range of microorganisms and phytochemical studies.
- *S. aromaticum* contain antimicrobial agents, and therefore should further be evaluated trough other biological activities.
- Spectroscopic studies of the isolated compounds including column chromatography, crystallography, hetronuclear multiple bond correlation, hetronuclear single quantum coherence, and x-ray should be carried out for the compounds' isolation and structures' confirmation.

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References

Ababutain, I.M. 2011. Antimicrobial activity of ethanolic extracts from some medicinal Plant. *Aust. J. Basic Appl. Sci*., 5(11): 678-683.

Aggarwal, H., J. Ghosh, A. Rao and V. Chhokar. 2015. Evaluation of root and leaf extracts of *Glycrriza glabra* for antimicrobial activity. *J. Med. Bioeng*., 4(1): 81-85.

Alanis, A.J. 2005. Resistance to antibiotics: Are we in the post-antibiotic era. *Arch. Med. Res.;* 36(6): 697-705.

Ali, A.A. 1999. Studies on some medicinal plants as a source of antifungal substances in North Africa," M.Sc. Thesis, Institution of African Research and Studies, Cairo Univ, Egypt.

Al-Jiffri, O., Z.M.F., El-Sayed and F.M. Al-Sharif. 2001. Urinary tract infection with *Esherichia coli* and antibacterial activity of some plants extracts. *Int. J. Microbiol. Res*., 2(1): 1-7.

Almagboul, A.Z., A.K. Bashir, A.K.M. Salih, A. Farouk and SA. Khalid. 1988. Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity (V). *Fitoterapia*, 59: 57-62.

Bai, S., A. Malik, L. Seasotiya, P. Bharti and S. Dalal. 2104. *In vitro* antioxidant activity, total phenolic content and therapeutic potential of *Brassica campestris* (brassicaceae) seed in inhibiting human pathogens. *Int. J. Recent. Adv. Pharm. Res*., 4(2): 15-24.

Boham, B.A. and, R.Kocipai-Abyazam. 1994. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and *V. calycinum* (Ericaceae). *Pac. Sci. J*., 48(4): 458-463.

Bonjar, S. 2004. Evaluation of antibacterial properties of some medicinal plants used in Iran. *J. Ethnopharmacol*., 94(2): 301-305.

Chaieb, K., T. Zmantar, R. Ksouri, H. Hajlaoui, K. Mahdouani, C. Abdelly and A. Bakhrouf. 2007. Antioxidant properties of essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. *Mycosis*., 50(5): 403-406.

Chopra, P.G., D. Binda, F. Saraf, S. Inam and D. Sujata. 2013. Antimicrobial and antioxidant activities of methanol extract roots of *Glycyrrhiza glabra* and HPLC analysis. *Int. J. Pharm. Pharm. Sci*., 5(2): 157-160.

Coolborn, A.F. and B. Bolatito. 2010. Antibacterial and phytochemical evaluation of three medicinal plants. *J. Nat. Prod*., 3: 27-34.

Croteau, R., T.M. Kutchan and N.G. Lewis. 2000. Biochemistry & Molecular Biology of Plants. (Eds.): Buchanan, B., W. Gruissem & R. Jones. *Amer. Soc. of Plant Physiologists*, pp. 1250-1318.

Delaquis, P.J., K. Stanich, , B. Girard and G. Mazza. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol*., 74(1): 101-109.

Diab, A.M., M.A.A. Aziz and S.A. Salim. 2002. Plasmid encoded transferable antibiotic resistance in Gram-negative bacteria isolated from drinking water in Ismailia city. *Pak. J. Biol. Sci*., 5(7): 774-779.

Djeussi, D.E., J.A. Noumedem, J.A. Seukep, A.G. Fankam, I.K. Voukeng, S.B. Tankeo, A.H. Nkuete and V. Kuete. 2013. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complement. Altern. Med*., 13(1): 164.

Dubey,. D., M.C. Sahu, S. Rath, B.P. Paty, N.K. Debata and R.N. Padhy. 2012. Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria. *Asian Pac. J. Trop. Biomed*., 2(2): 846-854.

Eisenberg, D.M., R.C. Kessler, C. Foster, F.E. Norlock, D.R. Calkins and T.L. Delbanco. 1993. Unconventional medicine in the United States. *N. Engl. J. Med*., 328(4): 246-252.

Gunasegaran, T., X. Rathinam, M. Kasi, K. Sathasivam, S. Sreenivasan and S. Subramaniam. 2011. Isolation and identification of Salmonella from curry samples and its sensitivity to commercial antibiotics and aqueous extracts of *Camelia sinensis* (L.) and *Trachyspermum ammi* (L.). *Asian Pac. J. Trop. Biomed*., 1(4): 266-69.

Habiba, U., M. Ahmad, S. Shinwari, S. Sultana, Z.K. Shinwari and M. Zafar. 2016. Antibacterial and antifungal potential of Himalayan medicinal plants for treating wound infections. *Pak. J. Bot*., 48(1): 371-375.

Hancock, R.E.W. 2005. Mechanisms of action of newer antibiotics for Gram-positive pathogens. *Lancet. Infect. Dis*., 5(4): 209-218.

Harborne, J.B. 1973. Phytochemical Methods. Chapman and Hill, London.

Islam, B., S.N. Khan, I. Haque, , M. Alam, M. Mushfiq and A.U. Khan. 2008. Novel anti-adherence activity of mulberry leaves: inhibition of*Streptococcus mutans* biofilm by 1-deoxynojirimycin isolated from *Morus alba. J. Antimicrob. Chemother*., 62(4): 751-757.

Iwu, M.W., A.R. Duncan and C.O. Okunji. 1999. New antimicrobials of plant origin. *News antimicrobials* of *plant origin*. *In*: *Perspectives* on *New Crops* and *New Uses*, *J. Janick*, *Ed*., pp. 457-462, ASHS Press, Alexandria, Va, USA.

Khan, R., B. Islam, M. Akram, S. Shakil, A. Ahmad, S.M. Ali, M. Siddiqui and A.U. Khan. 2009. Antimicrobial activity of five herbal extracts against Multi Drug Resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*, 14(2): 586-597.

Kirby, W.M., G.M., Yoshihara, K.S, Sundsted and J.H. Warren. 1957. Clinical usefulness of a single disc method for antibiotic sensitivity testing. *Antibiot. Annu*., 892: 1956-1957.

Kumaraswamy, Y., P.J. Cox, M. Jaspars and S.D. Sarker. 2002. Screening seeds of Scottish plants for antibacterial activity. *J. Ethnopharmacol*., 83(1): 73-77.

Lai, P.K. and J. Roy. 2004. Antimicrobial and Chemopreventive Properties of Herbs and Spices. *Curr. Med. Chem*., 11(11): 1451-1460.

Lakshmi, T. and R.V. Geetha. 2001. G*lycyrrhiza glabra* Linn. commonly known as licorice: A therapeutic review. *Int. J. Pharm. Pharm. Sci*., 3(4): 20-25.

Mahmood, A.M., J.H. Doughari and N. Ladan. 2008. Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. *Afr. J. Pharm. Pharmacol*., 2(5): 089-094.

Masoud, E.A. and H.A. Gouda. 2012. Effect of some natural plant extracts against gram negative bacteria in Njran Area, Saudi Arabia. *Egypt. Acad. J. Biol. Sci*., 4(1): 85-92.

Middleton, J.R., W.H. Fales and C.D. Luby. 2005. Surveillance of *Staphylococcus aureusin* veterinary teaching hospitals. *J. Clin. Microbiol*., 43(6): 2916-2919.

Murray, M.T. 1995. The healing power of herbs. Published by Prima., U.S.A., Prima Publishing, USA.

Mussarat, S., N.M., Abdel-Salam, A. Tariq, S.M. Wazir, R. Ullah and M. Adnan. 2014. Use of Ethnomedicinal Plants by the People Living around Indus River. *J. Evid. Based Complementary Altern. Med*., Article ID 212634, 14 pages.

Mustafa, G., S. Ahmed, N. Ahmed and A. Jamil. 2016. Phytochemical and antibacterial activity of some unexplored medicinal plants of Cholistan desert. *Pak. J. Bot*., 48(5): 2057-2062.

Noumedem, J.A.K., M., Mihasan, S.T., Lacmata, M. Stefan, J.R. Kuiate and V. Kuete. 2013. Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria. *BMC Complement. Altern. Med*., 13(1): 26.

Obadoni, B.O. and P.O. Ochuko. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Glob. J. Pure Appl. Sci*., 8(2): 203-208.

Palombo, E.A. and S.J. Semple. 2001. Antibacterial activity of traditional Australian medicinal plants. *J. Ethnopharmacol*., 77(2): 151-157.

Prusti, A., S.R. Mishra, S. Sahoo and S.K. Mishra. 2008. Antibacterial activity of some Indian medicinal plants. *Ethnobot. Leaflets*, 1: 227-230.

Raz, R. 2001. Hormone replacement therapy or prophylaxis in postmenopausal women with recurrent UTI. *J. Infect. Dis*., 183(1): 74-76.

Rios, J.L., M.C. Recio and A. Villar. 1988. Screening methods for natural products with antimicrobial activity: a review of the literature. *J. Ethnopharmacol*., 23(2-3): 127-149.

Sabbioni, C., R. Mandrioli, A. Ferranti, F. Bugamelli, M.A. Saracino, G.C. Forti, S. Fanali and M.A. Raggi. 2005. Separation and analysis of glycyrrhizin, 18-glycyrrhetic acid and 18-glycyrrhetic acid in liquorice roots by means of capillary zone Electrophoresis. *J. Chromatogr*., 1081(1): 65-71.

Samy, R.P. and S. Ignacimuthu. 2000. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India. *J. Ethnopharmacol*., 69(1): 63-71.

Santoyo, S., R., Lioria, L., Jaime, E. Ibanez, F.J. Senorans and G. Reglero. 2006. Supercritical fluid extraction of antioxidant and antimicrobial compounds from *Laurus nobilis* L. Chemical and functional characterization. *Eur. Food Res. Technol*., 222(5-6): 565-571.

Shadomy, S., I. Epsinel and R. Cartwright. 1985. Laboratory studies agents: Susceptibility test and bioassays. In*:*

Manual of Clinical Microbiology, 4th edition. (Eds.): Lennette, A., W. Balows, H. Hausler, S. Shadomy, Boston: Little Brown Co.

Shaikh, D., S. Ashfaq,K. Shaikh,M. Shaikh, B.S. Naqvi, Z.A. Mahmood and R. Majid. 2005. Studies on resistance/sensitivity pattern of bacteria related with Urinary tract infections. *Med. J. Islamic World Acad. Sci*., 15(1): 29-133.

SPSS Inc: 2007. SPSS Version 16.0 for Windows. SPSS, Chicago IL.

Syed, F., R. Jahan, A. Ahmed and S. Khan. 2013. *In vitro* antimicrobial activities of *Glycyrrhiza glabra* and *Fagonia arabica. J. Med. Plant Res*.,7(10): 2265-2270.

Tenore, G.C., J. Troisi, D.R. Fiore, A. Basile and E. Novellino. 2012. Chemical composition, antioxidant and antimicrobial properties of Rapa Catozza Napoletana (*Brassica rapa* L. var. *rapa* DC.) seed meal, a promising protein source of Campania region (southern Italy) horticultural germplasm. *J. Sci. Food Agric*., 92(8): 1716-1724.

Tetyana, P., E.A. Prozesky and A.K. Jager. 2002. Some medicinal properties of Cussonia and Schefflera species used in traditional medicine. *S. Afr. J. Bot*., 68(1): 51-54.

Tian, M., H. Yan and K.H. Row. 2008. Extraction of glycyrrhizic acid and glabridin from Licorice. *Int. J. Mol. Sci*., 9(4): 571-577.

Varsha, S., R.C. Agrawal and P. Sonam. 2013. Phytochemical screening and determination of anti-bacterial and antioxidant potential of *Glycyrrhiza glabra* root extracts. *J. Environ. Res. Dev*., 7(4): 1552-1558.

Zhishen, J., T. Mengcheng and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chem*., 64(4): 555-559.

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