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Antibacterial evaluation of three widespread weeds *Mazus japonicus, Fumaria indica and Vicia faba* from Pakistan

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Present study was carried out to explore the antibacterial potential of three weeds *Mazus japonicus, Fumaria indica* and *Vicia sativa* grown widely in Pakistan. Different extracts (aqueous, methanolic and petroleum ether) of the respective weeds were prepared and tested against nine bacterial strains using agar well diffusion assay. Bacterial strains included both gram positive (*Staphylococcus aureus, Bacillus anthracis, Bacillus megaterium, Enterococcus faecium, Enterrococcus faecalis* and *Enteroccocus sp*) and gram negative (*Pseudomonas putida, Escherichia coli* and *Escherichia coli top10*) bacteria. Ten different concentrations of each extracts were used. *Enterococcus faecalis* JH22 and *Bacillus megaterium* MB141 were the most resistant bacteria while *Escherichia coli top10* was found highly susceptible and inhibited by all three extracts of *Mazus japonicas* and *Fumaria indica*. *Vicia sativa* was effective only against *Staphylococcus aureus* and *Pseudomonas putida* at limited crude extract concentration while all other strains showed resistance against different extracts of the respective plant. Amongst the plant extracts screened for antibacterial activity, methanolic extracts showed best antibacterial activity whereas aqueous and petroleum ether were found least active. This study significantly supports the usage of these widespread weeds as traditional medicines for various bacterial infections.

Keywords: weeds, antibacterial, well diffusion assay, *Vicia sativa, Mazus japonicus*

Introduction

There has been a continual battle between the man and microorganisms specially bacteria. These bacterial epidemics have been the major cause of morbidity and mortality of human beings. Since then man is searching for the agents that has the potential to fight against these bacterial infections, antibiotics were the main agents utilized against pathogenic microorganisms. Different antibiotics have been discovered and invented by human beings for long time. The evidence of tetracycline has been found in human skeletal remains from ancient Sudanese Nubia dating back to 350–550 CE (Nelson *et al.,* 2010). Another source of antimicrobials in the pre-antibiotic era was through the medicinal plants compounds. The best-known example is the discovery of a potent anti-malarial drug, qinghaosu (artemisinin), which

was extracted in the 1970s from Artemisia plants, used by Chinese herbalists for thousands of years as a remedy for many illnesses (Cui and Su, 2009).

Pakistan is one of the countries blessed with extensive floral biodiversity (species, genetic and habitat) distributed in various biomes and is among major countries in the use of herbal drugs and the practice of traditional medicine is widespread here. About 6000 flowering plants have been reported out of which about 400 to 600 are considered to be medicinally important and 1000 species have been recognized to possess phytochemical properties (Shinwari and Gilani, 2003; Jabeen *et al.,* 2009). Not only the medicinal plants but weeds are also efficiently utilized in developing country like Pakistan as a source of herbal medicines. Weeds represent a valuable resource for the underdeveloped countries and therefore, their pharmacological properties should be investigated like other medicinal plants in order to meet the increasing demand of antimicrobial drugs.

The use of plant extracts against these bacterial infections is as old as existence of bacteria. Despite of remarkable development in modern medicine system, potential of plant derived bioactive compounds is considered to be the novel alternative for the physicians against these antibiotic resistant bacteria. Present study, therefore, is aimed to explore the possible antibacterial potential of three weeds *Mazus Japonicus* (thunb) kuntze, *Fumaria indica* (Hausskn.) and *Vicia sativa* L. grown widely in Pakistan. Their family, distribution and traditional uses are listed in table 1. This research explores the possible pharmacological uses of weeds in medicine and helps us to investigate whether weeds can serve as an effective agent against human pathogenic disorders or not. Furthermore, this report also examines the optimal solvent for the extraction of possible antimicrobial compounds.

Table 1.Summary of weeds, their occurrence and traditional uses.

Plant	family	Local name	Occurrence in Pakistan	Traditional use
Mazus japonicas (Thunb.) Kuntze	Scrophulariaceae.	----	Found in moist lawns, bottom lands	Used for ornamental purposes in gardens for ground cover, plant is also effective against typhoid fever (Riaz et al. 2012).
Fumaria indica (Hausskn.)	Fumariaceae	Shahtara / Papra	Plain and lower hills of Pakistan	Traditionally used for the cure of dermatological diseases, topical ailments, cardiovascular problems, fever and headache (Murad et al.2011).
Vicia sativa L.	Leguminaceae	Rewari / Matri	Rain-fed and irrigated areas	Mostly used as livestock feed and forage and its mixture with other cereals is used for haymaking (Berger et al.,2003).

Experimental Section

Plant Material Collection

Mazus japonicus, Fumaria indica and *Vicia sativa* were collected during the month of March and April from different areas of Rawalpindi and Islamabad. The plants were identified by National

Herbarium, Department of Plant Sciences, Quaid-e-Azam University, Islamabad, Pakistan. The collected plants were properly washed with tap water and dried under shade for 15-20 days. The dried plant sample was ground into fine powder with electrical grinder and stored in airtight container for further use.

Preparation of Plant Extracts

Plant extracts were prepared by maceration technique according to the standard method of Inayatullah *et al.,* 2012 with some modifications. Three different solvents of increasing polarity i.e. petroleum ether, methanol and water were used for the preparation of non-polar, semi-polar and polar extracts, respectively.

Preparation of Hot Aqueous Extract

For the preparation of hot aqueous extracts of plants, 50 g of each dried plant powder was dissolved in 800 ml of distilled water and final volume was then made upto 1000 ml. This suspension was soaked overnight. It was then simmered for 2 hours with the help of magnetic stirrer on hot plate at temperature of 45-48°C. The extract was then filtered off through four layered muslin cloth followed by filtration through Whattman filter paper no.1 to remove impurities. Filtrate was then placed on water bath at 50-60 °C until dried mass was obtained.

Preparation of Methanolic and Petroleum ether Extracts

Fifty gram of each plant powder was soaked in 800 ml of methanol and final volume was then made upto 1000 ml. This suspension was left for 7 to 10 days at 24 °C in growth chamber with successive gentle shaking. The extracts were then filtered off through four layered muslin cloth followed by Whattman filter paper no.1. Then the filtrate was evaporated in water bath at 50^{o}C until dry mass was obtained. Dry mass was then stored in refrigerator for further use. Ether extract was also prepared following the similar methanolic extraction procedure with the solvent using ether.

Bacterial strains used

Clinically isolated nine bacterial strains were used. Six of them were gram positive i.e. *Staphylococcus aureus (ATCC6538), Bacillus sp.* (MB083) (KF055341), *Enterococcus faecalis* (SF17), *Enterococcus faecium (OG1RF), Bacillus megaterium* (MB141) *(KF055342)*, *Enterococcus faecalis*(JH22) and three of them were gram negative i.e. *Escherichia coli (Top10)*, *Escherichia coli* (ATCC 15224), *Pseudomonas putida*. Bacterial strains were provided by Microbiology and Biotechnology Research Lab of Fatima Jinnah Women University Rawalpindi. Bacterial strains were verified and identified by standard gram staining procedure.

Determination of antibacterial activity

Suspension preparation

The turbidity standard was prepared by mixing 0.5 ml of $BaCl_2$ (0.048 M) in 99.5 mL 0.3 6N H_2SO_4.$BaSO_4$. The standard was taken in screw cap test tube to compare the turbidity. The bacterial culture of selected strains were grown overnight, and subsequently mixed with physiological saline. Turbidity was corrected by adding sterile saline until McFarland 0.5 $BaSO_4$ turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. These inocula were used for seeding of the nutrient agar.

Agar Well Diffusion Assay

Agar well diffusion method was also employed for the evaluation of antibacterial activity of different plant extracts as employed by Munazir *et al.* (2012). This method depends on the diffusion of extracts from the well through the solidified agar layer in such a way the growth of microorganism is prevented entirely in circular area or zone around the cavity containing the extracts. Ten µl of bacterial suspension were poured with the help of micropipette and spread on the entire plate with the help of glass spreader. The wells of 7mm in diameter were cut out in the seeded agar layer with the help of the autoclaved cork borer. Thirty µl of extract of each specific concentration was poured into the well with the help of micropipette. Cefotaxime (10 mg/ml) was selected as a positive control and DMSO was used as a negative control. The plates were incubated at 37^{o}C for 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition around the well. All the reactions were carried out in triplicates and the average results obtained were recorded.

Results and discussion

Medicinal plants with antibacterial activities have been well recognized and used as traditional medicines since ancient times for the treatment of various diseases. Weeds have also been proved to contain various bioactive constituents that helped in curing various ailments and are thereby used in conventional medications in many parts of the world (Demmaa *et al.*, 2007, Stough et al., 2001, Immanuel and Elizabeth, 2009). Antibacterial, antifungal, Anticancerous, antifeedant, antiviral and antioxidant potential of weeds have been tested by many researchers and found significant results (Sanguri *et al.,* 2012, Narintom *et al.*, 2014, Huang *et al.*, 2014, Adebayo *et al.,* 2010). In our study, three weeds *Mazus japonicus, Fumaria indica and Vicia sativa* grown wildly and commonly in Pakistan were selected and evaluated for antibacterial potential against nine bacterial strains including gram positive and gram negative bacteria.

In the present study, *S. aureus* and *E. coli top 10* showed sensitivity to all the three extracts of *Mazus japonicus.* Maximum inhibitory response was found with ether extract giving inhibition zone of 11.75 mm against *S. aureus* and in case of *E. coli top 10*, aqueous extract revealed distinct zones of 15.5 mm as maximum zone of inhibition (Fig. 1). Moderate antibacterial response was observed by the aqueous and methanolic extracts of *Mazus japonicus* against *Pseudomonas* and *Bacillus* species respectively. Among three *Enterococci* species investigated, only *E. faecalis* (JH22) was found resistant to all three extracts of *Mazus japonicus*. *Enterococcus sp* SF17 and *E. faecium* (OG1RF) showed little sensitivity against aqueous and methanolic extracts respectively (Table 2).

Tiwari *et al.* (2011) reported that plant extracts from organic solvent found to be more consistent for antimicrobial activity as compared to aqueous extract. Methanol easily penetrates into plant material to extract bioactive substances as compared to aqueous and it is considered more efficient in cell wall and seed degradation. Moreover, alcoholic extracts contain higher amount of polyphenols as antimicrobial components. These compounds make complex or intercalate into cell wall resulting in the inhibition of bacterial growth. Polyphenols are well documented to have bactericide activities against a number of pathogenic bacteria resulting in inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, cell envelope transport proteins, non specific interactions with carbohydrates (Cowan, 1999). There are number of studies which reported methanolic plant extracts as best solvent compared to aqueous and petroleum ether extract for the extraction of antimicrobial substances (El-kamali and El-Karim, 2009; Sen and Batra, 2012; Ayo *et al.,* 2013).

Aqueous extract of whole plant of *Fumaria indica* did not show any promising antibacterial activity against the tested bacterial strains except against *E. coli* top10 (table 3). Natarajan *et al.* (2005) studied antibacterial activity of *Euphorbia fusiformis* against pathogenic gram positive and gram negative bacteria and its aqueous extract was found active against *E. coli* only. Methanolic plant extract of *Fumaria indica* showed higher antibacterial activity compared to aqueous and petroleum ether plant extracts. Seven out of nine bacterial strains were sensitive towards whole plant methanolic extract of *Fumaria indica. E. coli* top10 was highly susceptible and inhibited by all extracts. *Bacillus megaterium* (MB141) and *Enterococcus faecalis* (JH22) were most resistant to these extracts. Out of three strains of *Enterococcus species,* two strains (SF17 and OG1RF) were sensitive against whole plant methanolic extract of *Fumaria indica* while *Enterococcus faecalis* JH22 was resistant. *Bacillus anthracis* MB083 showed sensitivity to all concentrations (except 1mg/ml) and zone of inhibition was 11.25 mm in size (fig. 2). Petroleum ether extract of *Fumaria indica* was inactive against all the tested bacterial strains except *E. coli* and *E. coli* top10. Large and clear zones were formed in well diffusion method with maximum inhibition zone of 14.62 mm.

Only methanolic extract of *Vicia sativa* showed zones of inhibition against *Staphylococcus aureus* and *Pseudomonas putida* at selected concentrations (fig. 3). All other strains were resistant towards *Vicia sativa* extracts (table 4). No zone of inhibition was found in aqueous and petroleum ether extracts. Valya*et al*. (2011) evaluated the antibacterial potential of *Solanum americanum* Miller against both gram positive and gram negative bacteria by agar well diffusion method. Bacterial strains they tested included *Staphylococcus aureus, Escherichia coli, Bacillus sp* and *Pseudomonas sp.* Methanolic extract of *Solanum americanum* Miller was active against *Staphylococcus aureus* and *Pseudomonas sp* only while all other strains were resistant towards methanolic extract. Their aqueous and ether extracts also showed no activity against any bacterial strains. It has been recorded that lower plants belonging to family Leguminosae are rich in phytoalexins, which include flavanone derivatives structurally analogous to the anti-MRSA flavanones and result in the inhibition of methicillin resistant *Staphylococcus aureus* (Tsuchiya *et al.,* 1996). *Vicia sativa* being member of Leguminosae family contains flavonoid phytoalexins, this might be the reason for the optimum inhibition of *Vicia sativa* against *Staphylococcus aureus.*

As the crude extracts were used, so possibly the bioactive compounds are present in insufficient quantity to be effective against bacterial strains (Taylor *et al.,* 2001). Alternatively, the bioactive compound may be present but there could be the other compounds which may have antagonistic effect or cancel out the positive effect of bioactive compound. Lack of activity can only be proved by using purified and high doses of extracts.

In our study, nine different bacterial strains were tested against the polar and non-polar extracts of three weeds used locally in Pakistan as a fodder. One can repeat the same assays by evaluating other bacterial strains in order to verify and examine the broad range antibacterial potential of this plant. As the crude extracts of the selected weeds showed bioactivity against some selected bacterial strains, these extracts can be partitioned and purified and can further be subjected to the isolation of antimicrobial constituents. This can help us to explore pharmacological significance of the investigated plants. The potential for developing antimicrobials from plants appear to be worthwhile at present as it leads to the development of new drugs which is need of hour. These weed extracts open the potential of finding new clinically effective antibacterial compounds. This study significantly supports the usage of weeds as traditional medicines for various bacterial infections.

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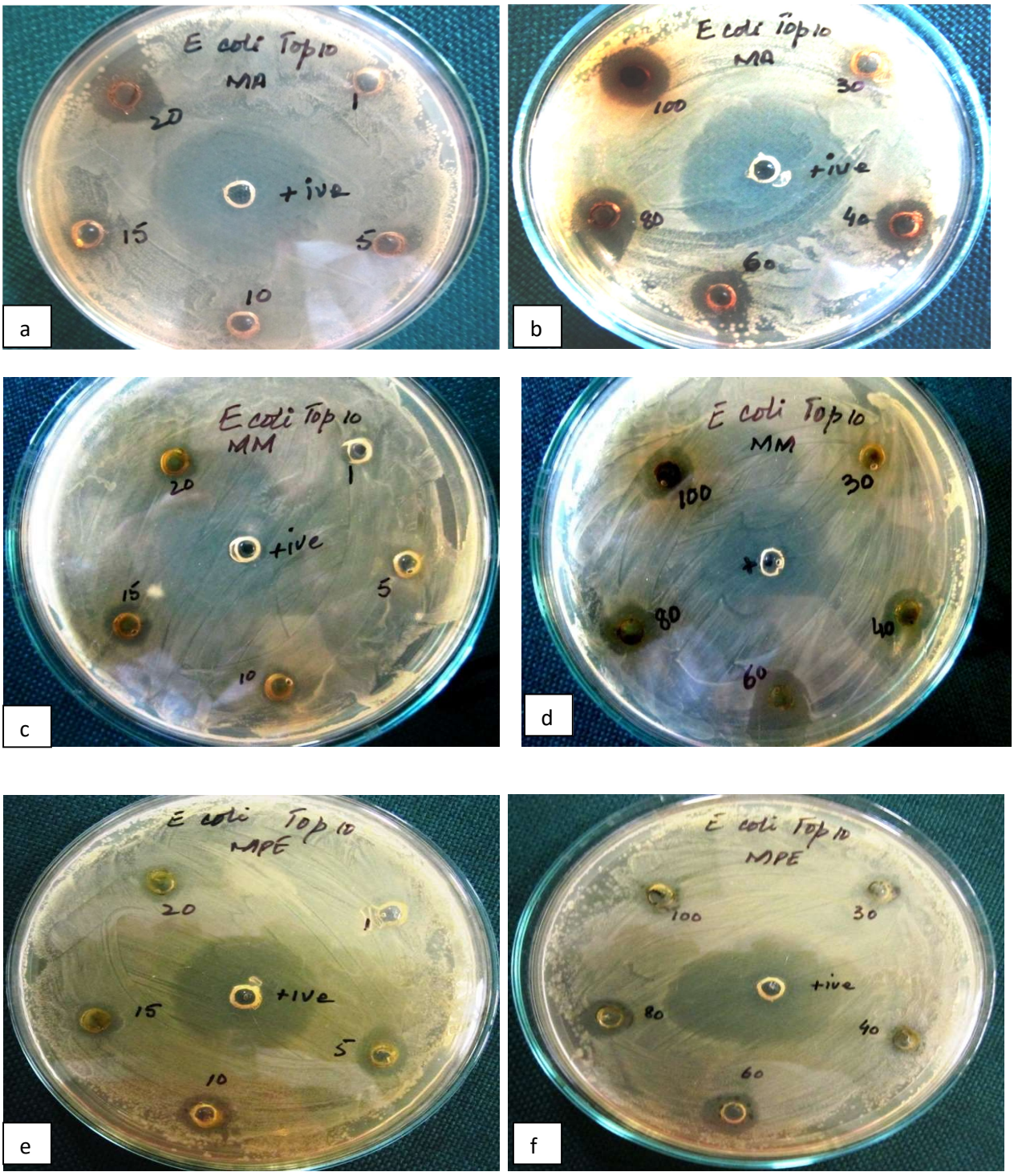

Fig. 1. Antibacterial activity of aqueous (a,b), methanolic (c,d) and petroleum ether (e,f) extracts of *Mazus japonicus* against *E. coli* top10.

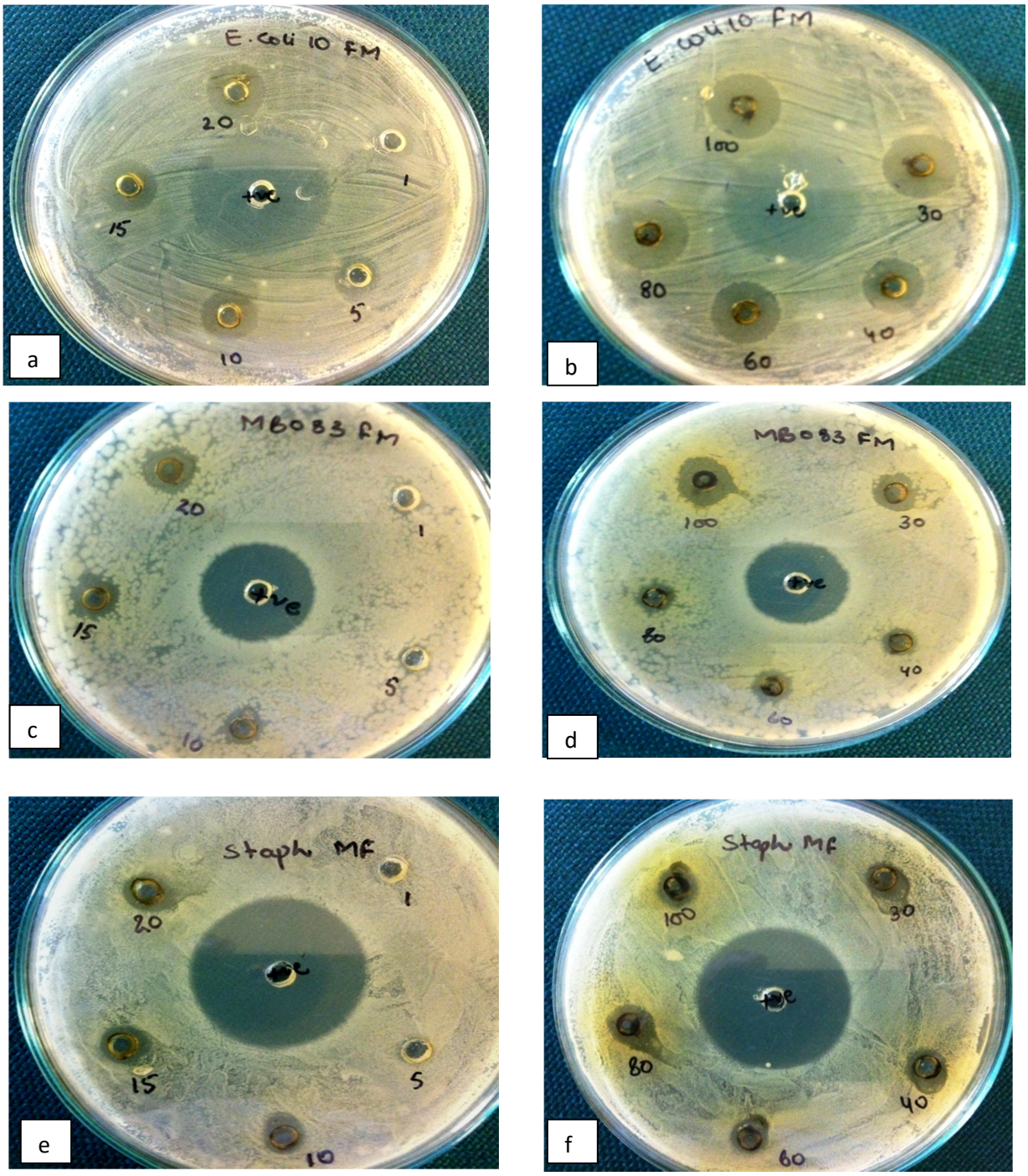

Fig. 2. Antibacterial activity of *Fumaria indica* (methanolic extract) found against *E. coli* top10 (a,b), *Bacillus sp.* MB083 (c,d),*S. aureus* (e,f).

Table 2. Antibacterial activity of *Mazus japonicus*extracts using agar well diffusion assay.

Mean diameter (± SE) of inhibitory zones (mm)

Extracts	Conc. mg/ml	*S. aureus*	*P.putida*	*Bacillus sp.* MB083	*B.megaterium*MB141	*E. coli*	*E. coli* top10	*Enterococcus sp* SF17	*E. faecium*OG1RF	*E.faecalis*JH22
Water	**1**	R	R	R	R	R	11.5±0.71	7.75±0.35	R	R
	5	R	R	R	R	R	11.5±0.71	7.875±0.35	R	R
	10	R	R	R	R	R	11±0.71	8±0.71	R	R
	15	R	8.25±0.35	R	R	R	10.75±0.353	9.25±0.35	R	R
	20	R	8.5±0.71	R	R	R	12.12±0.53	8.37±0.53	R	R
	30	8±0.71	8.5±1.41	R	R	R	13±1.41	8.5±0.35	R	R
	40	9±0.35	9.25±1.06	R	R	R	13.75±1.06	8.5±0.35	R	R
	60	10±0.1	11±1.41	R	R	R	14±0.71	7.5±0.71	R	R
	80	9.75±0.36	11.25±1.06	9.75±1.76	R	R	14±0.70	9.0±0.71	R	R
	100	10±0.71	12.25±1.06	10.5±2.12	R	R	**15.5±1.41**	11.25±1.06	R	R
Methanol	**1**	R	R	R	R	R	R	R	R	R
	5	R	R	R	R	R	8.62±0.88	R	R	R
	10	R	R	R	R	R	10.8±.176*	R	R	R
	15	7.5±0.1	R	R	R	R	12.25±2.47	R	R	R
	20	8±0.1	R	R	R	R	12.5±2.12	R	R	R
	30	9.5±0.2	R	8.75±1.06	7.75±0.35	13.1±0.1	11.2±1.41	R	6.87±0.18	R
	40	9.5±0.3	R	9.25±1.1	9.0±1.41	14±0.2	12.75±0.35	R	8.3±0.35	R
	60	10.1±0.02	R	9.37±0.88	9.75±1.77	14.7±0.35	14.2±0.71	R	8.55±0.71	R
	80	10.01±0.2	R	10.5±0.71	11.1±2.83	15.75±0.35	14.25±0.35	R	8.87±0.18	R
	100	11.1±0.03	R	11.5±0.35	12±1.41	17.5±0.71	15.25±1.06	R	9.13±0.53	R
Petroleum ether	**1**	7.2±0.53	R	R	R	R	10±0.1	R	R	R
	5	7± 0.53	R	R	R	R	13.25±0.35	R	R	R
	10	8.5±0.2	R	R	R	R	13.6±0.176	R	R	R
	15	8.38±0.53	R	R	R	R	10±0.2	R	R	R
	20	9.2±0.2	R	R	R	R	10±0.1	R	R	R
	30	8.63±1.59	R	R	R	R	13±0.707	R	R	R
	40	9±1.41	R	7.5±0.35	R	R	11.75±1.06	R	R	R
	60	9.75±1.06	R	7.5±0.35	R	R	13.25±0.35	R	R	R
	80	9±1.42	R	9.75±0.35	R	R	12.5±0.707	R	R	R
	100	11.75±1.18	R	12.2±0.70	R	R	11.5±0.707	R	R	R
Cefotaxime	**100µg**	37.8±0.176a	33±0.71	24.5±0.71	33±0.71	23.5±0.75	31.5±0.70	36.5±0.2	29±0.70	21.5±1.4

R Resistant/ No inhibition zone Data represents the average of three replicates ± S.D.

The bold values represent the optimum zone of inhibition formed by the respective plant extract among the tested bacterial strains.

Table 3. Antibacterial activity of *Fumaria indica* extracts using agar well diffusion assay.

Mean diameter (± SE) of inhibitory zones (mm)

Extracts	Conc. mg/ml	*S. aureus*	*P.putida*	*Bacillus sp.* MB083	*B.megaterium*MB141	*E. coli*	*E. coli* top10	*Enterococcus sp.* SF17	*E. faecium*OG1RF	*E.faecalis*JH22
Water	**1**	R	R	R	R	R	R	R	R	R
	5	R	R	R	R	R	R	R	R	R
	10	R	R	R	R	R	R	R	R	R
	15	R	R	R	R	R	7.5±0	R	R	R
	20	R	R	R	R	R	8.5±0.70	R	R	R
	30	R	R	R	R	R	8.5±1.55	R	R	R
	40	R	R	R	R	R	11.25±1.06	R	R	R
	60	R	R	R	R	R	12.75±2.47	R	R	R
	80	R	R	R	R	R	13.16±2.08	R	R	R
	100	R	R	R	R	R	14.20±2.43	R	R	R
Methanol	**1**	R	R	R	R	R	7.62±0.17	R	R	R
	5	R	R	7.25±0.35	R	R	11.12±0.17	R	12*±0.17	R
	10	10.08±0.11	R	10.08±0.11	R	R	13.25±0.35	R	10±0.17	R
	15	10.37±0.17	R	10.75±0.35	R	R	13.87±0.17	R	8.5±0.35	R
	20	10.31±0.26	R	11.25±0.35	R	R	14.37±0.17	R	8.2±1.06	R
	30	10.62±0.17	11.3±0.17	9.83±2.58	R	8.5±0.70	18.25±0.35	8.4±0.58	7.1±0.35	R
	40	11.12±0.17	11.7±0.06	9.75±1.76	R	9.25±1.06	16.75±2.4	9.125±0.17	9.2±0.17	R
	60	10.75±1.06	12.1±0.11	10±0.70	R	10±0.70	17.25±0.35	9.87±0.17	10.1±0.17	R
	80	11.62±0.17	12.6±0.17	9.37±0.17	R	10.75±0.35	19.12±0.53	10.12±0.17	11.0±0.17	R
	100	11.25±1.76	12.4±0.17	10.75±0.35	R	11.12±0.53	**19.25±1.06**	9.75±1.06	12.0±0.17	R
Petroleum ether	**1**	R	R	R	R	R	9.75±1.76	R	R	R
	5	R	R	R	R	9.25±0.35	11.75±1.76	R	R	R
	10	R	R	R	R	10.75±0.35	12.25±0.35	R	R	R
	15	R	R	R	R	11.25±0.35	13±0.70	R	R	R
	20	R	R	R	R	12.25±0.35	14.75±0.35	R	R	R
	30	R	R	R	R	12.75±0.35	12.5±1.4	R	R	R
	40	R	R	R	R	13.75±0.35	14.2±0.2	R	R	R
	60	R	R	R	R	14.25±0.35	15.1±0.82	R	R	R
	80	R	R	R	R	14.16±1.18	15.92±0.58	R	R	R
	100	R	R	R	R	14.62±0.53	16.25±0.35	R	R	R
Cefotaxime	**100µg**	37.8±0.176a	33±0.71	24.5±0.71	33±0.71	23.5±0.75	31.5±0.70	36.5±0.2	29±0.70	21.5±1.4

R Resistant/ No inhibition zone Data represents the average of three replicates± S.D.

The bold values represent the optimum zone of inhibition formed by the respective plant extract among the tested bacterial strains

Table 4. Antibacterial activity of *Vicia sativa* extracts using agar well diffusion assay.

		Mean diameter (± SE) of inhibitory zones (mm)								
Extracts	Conc. mg/ml	*S. aureus*	*P.putida*	*Bacillus sp.* MB083	*B.megaterium*MB141	*E. coli*	*E. coli* top10	*Enterococcus sp.* SF17	*E. faecium*OG1RF	*E.faecalis*JH22
Water	**1**	R	R	R	R	R	R	R	R	R
	5	R	R	R	R	R	R	R	R	R
	10	R	R	R	R	R	R	R	R	R
	15	R	R	R	R	R	R	R	R	R
	20	R	R	R	R	R	R	R	R	R
	30	R	R	R	R	R	R	R	R	R
	40	R	R	R	R	R	R	R	R	R
	60	R	R	R	R	R	R	R	R	R
	80	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R
Methanol	**1**	R	R	R	R	R	R	R	R	R
	5	R	R	R	R	R	R	R	R	R
	10	9.7±0.14	8.5±0.07	R	R	R	R	R	R	R
	15	**10.2±0.14**	9.7±0.14	R	R	R	R	R	R	R
	20	9.5±0.14	9.6±0.28	R	R	R	R	R	R	R
	30	R	R	R	R	R	R	R	R	R
	40	R	R	R	R	R	R	R	R	R
	60	R	R	R	R	R	R	R	R	R
	80	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R
Petroleum ether	**1**	R	R	R	R	R	R	R	R	R
	5	R	R	R	R	R	R	R	R	R
	10	R	R	R	R	R	R	R	R	R
	15	R	R	R	R	R	R	R	R	R
	20	R	R	R	R	R	R	R	R	R
	30	R	R	R	R	R	R	R	R	R
	40	R	R	R	R	R	R	R	R	R
	60	R	R	R	R	R	R	R	R	R
	80	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R
Cefotaxime	**100µg**	37.8±0.176a	33±0.71	24.5±0.71	33±0.71	23.5±0.75	31.5±0.70	36.5±0.2	29±0.70	21.5±1.4

R Resistant/ No inhibition zone Data represents the average of three replicates ± S.D.

The bold values represent the optimum zone of inhibition formed by the respective plant extract among the tested bacterial strains.

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