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ANTIBACTERIAL ACTIVITY OF *FUMARIA INDICA* (HAUSSKN.) PUGSLEY AGAINST SELECTED BACTERIAL STRAINS

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Abstract

Antibacterial properties of methanolic extracts of *F. indica* prepared in different doses against seven Gram-positive and Gram-negative bacterial strains i.e. *Streptococcus pyogenes, Staphylococcus aureus (1), Staphylococcus aureus (2), Shigella sonnei, Escherichia coli (1), Escherichia coli (2)* and *Neisseria gonorrhoeae* using agar well diffusion method (inhibition zone measurements) compared to gentamicin as standard antibiotic. Results showed significant activities against the test organisms with overall satisfactory statistics. *Streptococcus pyogenes, Staphylococcus aureus* strains as well as *Neisseria gonorrhoeae* showed more inhibition to methanolic extracts of *F. indica.* Minimum inhibitory as well as minimum bactericidal concentrations against all strains except *Shigella sonnei* were also recorded. Studies showed promising horizons for the use of *F. indica* as an active antibacterial component in modern drug formulations.

Key words: Antibacterial activity, Zone of Inhibition, *F. indica*, MIC, MBC.

Introduction

The acknowledgement of healing potential of plants and plant based products can be traced back to the very beginning of the history of the human race (Qasim *et al*., 2010). These "healing properties" were known before humans even had any evidence of the existence of microscopic disease causing organisms–microbes as we all know them today. Infectious diseases have threatened the survival of humans since very early civilizations and since old times (Rios & Recio, 2005; Shinwari & Qaiser, 2011). These folk remedies, though very ancient, are still an important part of traditional medicine, especially when it comes to developing countries and the exploration of plants for medicinal potential has been in focus all over again since the last few decades (Shinwari & Gilani, 2003). Plants are biochemical labs having escalated systems to orchestrate chemotherapeutically animated elements that are discovered answerable for sparing life on this planet due to their antibacterial, and antifungal which supports thorough investigation of this gift (Rates, 2001; Walter *et al*., 2011). Rich biodiversity of the planet is securing premium of new researcher to show the concealed secrets of medicinal plants from most recent few decades (Gulfraz *et al*., 2011; Shinwari *et al*., 2012).

The main cause for the ongoing attention and extensive research on plants for antibacterial properties is the appearance of resistant strains of bacteria and fungi. These strains are able to cope with the same pace as their genetic evolution requires continuous development of new drugs against them (Fazal *et al*., 2012; Shinwari *et al*., 2012). Therefore, these "superbugs" or "superstrains", bacteria in particular are imposing the need for new drugs (Zachariah *et al*., 2009).

Infectious diseases are chronic and are major cause of premature death throughout world (Parekh & Chanda, 2007; Shinwari *et al*., 2009). The incidence of severe infections in human beings has drastically increased all over the world and it has become the leading cause of mortality in developing countries (Al-Bari *et al*., 2006). The list of infectious diseases caused by bacteria as well as fungi is never ending. Highly effective drugs that are being used these days are extracted from plants. Approximately 80% population of the world relies on plants as a natural source of medicine (Swarbrick & Boylan, 2002). According to the World Health Organization, (WHO), 25% of worldwide prescribed medicines come from plants. 11% of the 252 essential drugs approved by the WHO come exclusively from plants and these figures are global which means that not only developing but even developed countries know the value of plants in medicine. These facts and figures alone are reason enough for scientists to extensively research into the antibacterial properties of plants.

Fumaria indica (Hausskn.) Pugsley (Fumariaceae), known as "Fumitory" belongs to genus *Fumaria* and family fumariaceae, is an annual herb found as a common weed all over the plains of India and Pakistan. It is a small, branched, annual herb, which grows as a weed within fields of cultivated plants, mostly crops. Commonly called "Shaterah papra" in the Pakistani vernacular, *F. indica* plants are distributed over Asia, Europe and Africa. *F. indica* is cosmopolitan in distribution all over Pakistan.

Various medicinal uses of *F. indica* have been reported in old writings and ancient medical books. It is particularly recommended for treatment of fevers and blood disorders as well as chronic skin diseases, urinary diseases and cough. Synergic effects for relieving fever have been reported by the combination of *Fumaria indica* with *Tinospra cardifolia, Emblica officinalis, Santalum album* of *Zingiber officinale*. Fumitory is also prescribed as an antipyretic and as antiperiodic compound (Khare, 2004).

In the indigenous system of medicine, *F. indica* is listed as a laxative, diuretic and diaphoretic and has been found useful in the treatment of dyspepsia, liver complaints and scrofulous skin affections (Kirtikar & Basu, 1985). The decoction of the stem and leaves of *F. indica* is administered as a tonic, anthelmintic and aperients (Rastogi & Mehotra, 1970-1979). The present study, too, aims at the exploration of antibacterial capabilities of fumitory against an array of pathogenic bacteria.

Materials and Methods

Fresh samples of *Fumaria indica* were collected from fields near University of Gujrat, Hafiz Hayat Campus, Gujrat, Pakistan. Plants were identified by standard key from the Flora of Pakistan. The whole plants of *Fumaria indica* were sun dried for a couple of days in open air to ensure all the moisture was removed and dried overnight in the oven at 45°C. Dried plants were chopped into pieces and ground to fine powder. Fully processed ready samples were stored in air tight bags, moisture free at 4°C, ready for use whenever required.

Preparation of plant extract: Plant powder was taken in conical flasks and dissolved in methanol (powder: methanol = 5g:50ml) and kept in an orbital shaker incubator for 24 hours at 28°C and 220 rpm. Supernatant was decanted, filtered and centrifuged at 10,000 rpm for 10 mins at 28°C. The extract obtained was evaporated to 80% dryness at 40°C using a rotary evaporator. Final extracts were dissolved in Dimethyl Sulfoxide (DMSO) to yield different extract concentrations as per the experiment requirement in each case. DMSO was used as a negative control (Alipour & Khanmohammadi, 2011).

Determination of antibacterial activity: LB liquid medium (for one litre of LB liquid) was prepared by adding 10g peptone, 5g yeast extract and 10g NaCl to 950ml of distilled water. pH of the medium was set to 7.0 using 1N NaOH and made upto one litre mark using more distilled water. Medium was subject to autoclaving at liquid cycle for 20 min at 15psi, then allowed to cool to 55°C and then stored at 4°C under aseptic conditions for further use. LB liquid was prepared by the same method described above and 15g/L agar was added to the mixture prior to autoclaving. Media was allowed to cool likewise, poured aseptically into Petri plates, let harden and stored inverted at 4°C under sterile conditions until further use.

All bacterial strains were cultured on LB agar plates as well as in the prepared LB Broth and incubated overnight in an orbital shaker incubator at 37 °C to ensure the availability of active bacterial colonies and monitored to get McFarland standard OD of 0.5 at 600 nm.

Antibacterial susceptibility testing: Antibacterial activity of *F. indica* was tested by the agar well diffusion method of Hussain *et al*. (2010) with slight modifications.

Determination of MIC and MBC: Organic plant extracts that showed antibacterial activity were further impregnated to determine the Minimum Inhibitory Concentration (MIC), defined as the minimum concentration of antibacterial drug that have an ability to combat the test microorganism. Broth dilution method was used to determine the MIC, with LB broth as a medium and bacterial samples grown overnight at 37°C and diluted 100 folds in nutrient broth medium. Different concentrations of plant extracts (75mg/ml, 37.5mg/ml, 18.75mg/ml, 9.37mg/ml, 4.68mg/ml, 2.34mg/ml and 1.17mg/ml) were prepared to determine the MIC and these were decanted in test tubes containing the test organisms. All tubes were incubated at 37°C for 24 hours. After that all tubes were observed for visible turbidity. The lower concentration of the test extract that inhibited the growth of the test organism will be recorded as MIC (Hussain *et al*., 2010). The same concentrations were swabbed onto LB agar plates and kept overnight to observe bacterial growth in the plates. The lowest concentration that was able to inhibit the growth of test organisms on solid medium was Minimum Bactericidal Concentration (MBC).

Results and Discussion

Antibacterial activity of *F. indica*: The antibacterial activity of *Fumaria indica* extracts was assessed at two different concentrations i.e. 75mg/ml and 100mg/ml after 24 and 48 hours of inoculation with test organisms. Table 1 showed the antibacterial activity of *F. indica* extracts at a concentration of 75mg/ml after 24 hours of inoculation. Results indicated that the susceptibility of all bacterial strains showed significant differences ($p<0.05$). The extract was most effective against *Streptococcus genes*, followed by *Neisseria gonorrhoeae* and *Staphylococcus aureus* (25293) with zones of inhibition of 12.000±2.000, 11.000±0.000 and 11.333±0.289 respectively. The extracts of *F. indica* at 75mg/ml, however, could not inhibit the bacterial growth of *Staphylococcus aureus* (38541), *Escherichia coli* (25922) and *Shigella sonnei*, whereas, least activity was observed against *E. coli* (35318) (Fig. 1). Comparisons of these results with the activity of standard antibiotic (Gentamicin) revealed that the highest activity in terms of Activity Index (AI) and Percent Activity (PA) of *F. indica* extracts was observed against *Streptococcus genes* (AI = 0.86, PA = 86%), followed by the other three bacterial strains with close AIs and PAs as given in Table 1. Table 2 illustrated the antibacterial activity of *F. indica* extract at concentration of 75mg/ml after 48 hours of incubation. The susceptibility of all bacterial strains to the extracts did not show significant differences ($p<0.05$) as described in terms of equal ranks assigned via DMRT. Both *Neisseria gonorrhoeae* as well as *Staphylococcus aureus* (25923) were most susceptible to the extracts. Both showed equal maximum inhibitory activity zones (10.333±0.577) followed by *Streptococcus genes* with an inhibition zone of 9.833±1.155 (Fig. 2). The extracts proved to be ineffective against *Staphylococcus aureus* (38541), *E. coli* (25922), *E. coli* (35318) and *Shigella sonnei*. Comparison of the activity of the extract with standard antibiotic gentamicin showed that the highest activity in term of AI and PA was observed against *Streptococcus genes* (AI=0.70, PA = 70%). Shinwari *et al.* (2013) conducted a study regarding the antibacterial activity of 11 medicinal plant species among which the antibacterial activity of *F. indica* extracts were also studied. Methanolic extracts *of F. indica* showed antibacterial activity of 11±0.000 against *S .aureus* at a concentration of 50 mg/ml, whereas a zone of inhibition of 13±0.000 against *S. sonnei* was recorded at the same concentration as well as ZI of 11±0.000 and 10±0.000 against *Yersinia enterocolitica* and *Listeria monocytogenes*, respectively. However, no studies regarding Percentage activity and Activity index were conducted. Table 3 showed the antibacterial activity of *F. indica* extracts at a concentration

of 100mg/ml 24 hours after inoculation. Results showed that the susceptibility of all bacterial strains used had mostly significant differences ($p<0.05$) as the rankings via DMRT show in Table 3. The extract was most effective against *Neisseria gonorrhoeae*, followed by *Streptococcus genes* with zones of inhibition of 18.333±2.081 and 15.667±0.577 respectively. The extracts of *F. indica* at 100mg/ml, however, could not inhibit bacterial growth of *Shigella sonnei*, while least activity was observed against *E. coli* (35318). Comparisons of these results with the activity of standard antibiotic (Gentamicin) revealed that the highest activity in terms of Activity Index (AI) and Percent Activity (PA) of *F. indica* extracts was observed against *Straphylococcus aureus* (38541), (AI =0.75 , PA = 75%), followed by the other bacterial strains with AIs and PAs as given in Table 3. Khan *et al.* (2013) undertook some screening tests on *F. indica*. Crude methanolic extracts showed good percentage activities against *Pseudomonas aeruginosa* (77.8%), *Bacillus subtilis* (66.7%) but low against *E. coli* (33.4%) and *S. aureus* (38.5%), opposite to a moderately good percentage activity against one strain of *S. aureus* (63%) as well as one strain of *E .coli* (62%) in the present study after one day of incubation with the methanolic extracts. Whereas the other two strains of *E. coli* and *S. aureus* showed to be resistant to the methanolic extracts.

Table 4 showed the antibacterial activity of *F. indica* extracts of 100mg/ml concentration after 48 hours of inoculation period. The susceptibility of bacterial strains used in this study showed significant differences ($p<0.05$) as indicated in the table`s DMRT rankings. The extract was most effective against *Neisseria gonorrhoeae*, followed by *Streptococcus genes* with zones of inhibition of 17.667±1.527 and 15.000±1.000 respectively. The extracts of *F.indica* at 100mg/ml after 48 hours, however, could not inhibit the bacterial growth of *Shigella sonnei* and *Staphylococcus pyogenes*, while least activity was observed against *E. coli* (35318). Comparisons of these results with the activity of standard Gentamicin revealed that the highest activity in terms of Activity Index (AI) and Percent Activity (PA) of *F. indica* extracts was observed against *Straphylococcus aureus* (38541), (AI =0.79 , PA = 79%), followed by the other bacterial strains with AIs and PAs as given in Table 4. The antibacterial potential of hexane, ethanol and chloroform extracts of *F. indica* was studied by Fazal *et al*. (2012). Activities in all three solvent extracts were found against a range of bacteria including *S. aureus* as well as *E. coli*. Ethanolic and acetone extracts of *F. indica* were also found effective against *S. aureus* with ZI= 13.66±6.91 and ZI=8.66±1.36 respectively (Shinwari *et al.,* 2013). All these comparisons establish that the plant is effective against above mentioned bacteria but the present study shows considerable decline in efficiency of the extract after an additional day of incubation. Shaheen *et al.* (2013) analyzed that methanolic *F. indica* extracts were highly active against *B. subtilis*.

Table 1. Zone of Inhibition of *F. indica* against different bacterial strains after incubation of 24 hours at a concentration of 75mg/ml.

Bacterial strains	Zone of inhibition Z.I. (mm)	Control (mm) gentamicin	Activity index (A.I.)	Percentage activity (P.A.)
Staphylococcus aureus (1)	10.333 ± 0.577a	16	0.65	65
Streptococcus genes	9.833 ± 1.155a	14	0.70	70
Neisseria gonorrhoeae	10.333 ± 0.577a	16	0.65	65
Staphylococcus aureus (2)	0.000 ± 0.000b	13.5	0	0
Escherichia coli (1)	0.000 ± 0.000b	18	0	0
Escherichia coli (2)	0.000 ± 0.000b	14	0	0
Shigella sonnei	0.000 ± 0.000b	15.5	0	0

Table 2. Zone of Inhibition of *F. indica* against different bacterial strains after incubation of 48 hours at a concentration of 75mg/ml.

Bacterial strains	Zone of inhibition Z.I. (mm)	Control (mm) gentamicin	Activity index (A.I.)	Percentage activity (P.A.)
Staphylococcus aureus (1)	11.333 ± 0.289ab	18	0.63	63
Streptococcus genes	12.000 ± 2.000a	14	0.86	86
Neisseria gonorrhoeae	11.000 ± 0.000b	16	0.69	69
Staphylococcus aureus (2)	0.000 ± 0.000d	13.5	0	0
Escherichia coli (1)	0.000 ± 0.000d	18	0	0
Escherichia coli (2)	8.667 ± 1.154c	14	0.62	62
Shigella sonnei	0.000 ± 0.000d	15.5	0	0

Small letters represent rankings via DMRT at p= 0.05

Table 3. Zone of Inhibition of F. indica against different bacterial strains after incubation of 24 hours at a concentration of 100mg/ml.

Bacterial strains	Zone of inhibition Z.I. (mm)	Control (mm) gentamicin	Activity index (A.I.)	Percentage activity (P.A.)
Staphylococcus aureus (1)	0.000 ± 0.000d	17.5	0	0
Streptococcus genes	15.667 ± 0.577b	28	0.56	56
Neisseria gonorrhoeae	18.333 ± 2.081a	32	0.57	57
Staphylococcus aureus (2)	12.000 ± 0.000c	16	0.75	75
Escherichia coli (1)	10.667 ± 1.154c	16	0.67	67
Escherichia coli (2)	10.333 ± 0.577c	18	0.57	57
Shigella sonnei	0.000 ± 0.000d	26.5	0	0

Small letters represent rankings via DMRT at p= 0.05

Table 4. Zone of Inhibition of *F. indica* against different bacterial strains after incubation of 48 hours at concentration of 100mg/ml.

Bacterial strains	Zone of inhibition Z.I. (mm)	Control (mm) gentamicin	Activity index (A.I.)	Percentage activity (P.A.)
Staphylococcus aureus (1)	0.000 ± 0.000e	17.5	0	0
Streptococcus genes	15.000 ± 1.000b	26.5	0.57	57
Neisseria gonorrhoeae	17.667 ± 1.527a	32	0.55	55
Staphylococcus aureus (2)	12.667 ± 1.154c	16	0.79	79
Escherichia coli (1)	11.000 ± 1.000d	16	0.69	69
Escherichia coli (2)	9.833 ± 0.289d	17	0.58	58
Shigella sonnei	0.000 ± 0.000e	26.5	0	0

Small letters represent rankings via DMRT at p= 0.05

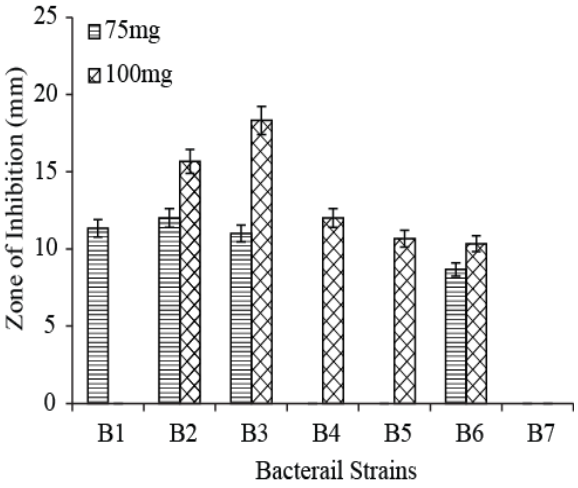

Fig. 1. Zone of inhibition of *F. indica* against different bacterial strains after incubation of 24 hours
B1= *Staphylococcus aureus* (25923), B2= *Streptococcus genes*, B3 =*Neisseria gonorrhoeae*, B4 = *Staphylococcus aureus* (38541), B5 = *Escherichia coli* (25922), B6 = *Escherichia coli* (35318), B7 = *Shigella sonnei*

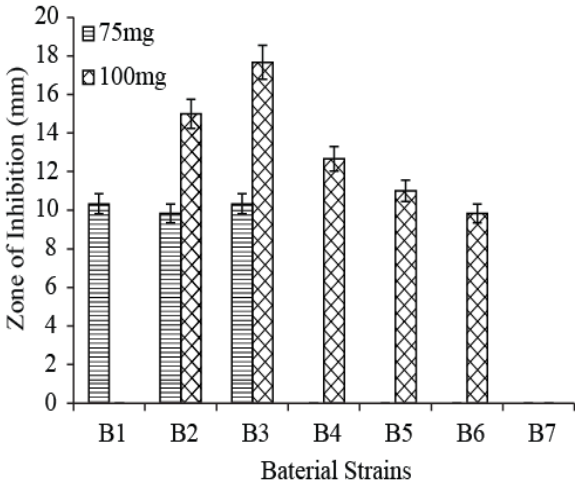

Fig. 2. Zone of inhibition of *F. indica* against different bacterial strains after incubation of 48 hours
B1= *Staphylococcus aureus* (25923), B2= *Streptococcus genes*, B3 =*Neisseria gonorrhoeae*, B4 = *Staphylococcus aureus* (38541), B5 = *Escherichia coli* (25922), B6 = *Escherichia coli* (35318), B7 = *Shigella sonnei*

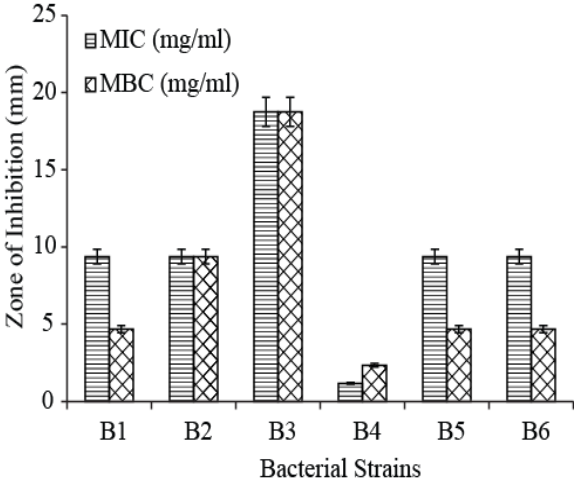

Fig. 3. MIC and MBC of *F. indica* extracts against different bacterial strains
B1= *Staphylococcus aureus* (25923), B2= *Streptococcus genes*, B3 =*Neisseria gonorrhoeae*, B4 = *Staphylococcus aureus* (38541), B5 = *Escherichia coli* (25922), B6 = *Escherichia coli* (35318), B7 = *Shigella sonnei*

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Fumaria indica* extracts.

Bacterial strains	Extract (*Fumaria indica*)	
	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus (1)	9.37	4.68
Streptococcus genes	9.37	9.37
Neisseria gonorrhoeae	18.75	18.75
Staphylococcus aureus (2)	1.17	2.34
Escherichia coli (1)	9.37	4.68
Escherichia coli (2)	9.37	4.68
Shigella sonnei	N.A.	N.A.

N.A = Not Applicable

MIC and MBC of *F. indica*: Plant extracts which showed striking activity to combat microbial population were further subjected to determine minimum concentration of antimicrobial drug. *Staphylococcus aureus* (38541) was recorded with least value 1.17mg/ml as MIC which means it was most susceptible to the methanolic extracts of *F. indica*, while its MBC (2.34mg/ml) was also recorded as least bactericidal concentration among all applied strains

(Fig. 3). *Staphylococcus aureus* (25923), *Streptococcus genes*, *Escherichia coli* (25922) and *Escherichia coli* (35318) were recorded with an MIC 9.37mg/ml each while MBC values of these bacteria were 4.68mg/ml, 9.37mg/ml, 4.68mg/ml and 4.68mg/ml respectively (Table 5). Shinwari *et al*. (2013) analyzed methanolic extracts of *F. indica* against several bacterial strains and came up with MIC and MBC ranges roughly between 10-50 mg/ml.

Conclusion

In light of the strong potential for combating pathogenic bacterial populations revealed , the results of this study strongly support the capabilities of *F. indica* as major component in possible antibiotic formulations and the plant can contribute to novel drug discoveries.

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