Pharmacogn J. 2020; 12(3): 619-623
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Fumaria indica (L), a Famous Medicinal Herb of Tribal Regions of Jabalpur, Madhya Pradesh: Broad Spectrum Antibacterial and Phytochemical Profilng Against Some Pathogenic Microorganisms

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ABSTRACT

This work describes the broad spectrum antibacterial properties of methanolic and chloroform extracts of *Fumaria indica* herb in different concentrations (50 mg/ml, 100 mg/ml and 150 mg/ml) against *Bacillus subtilis*(MTCC 10110), *Staphylococcus aureus*(MTCC96), *Escherichia coli* (MTCC 77), *Pseudomonas aeruginosa* (MTCC1688) and *Klebsiella pneumonia* (MTCC4032) using agar well diffusion method compared to standard antibiotic ciprofloxacin. Results have shown significant activities against the tested microorganisms *viz., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* than other strains. Minimum inhibitory as well as minimum bactericidal concentrations against *Bacillus subtilis and Klebsiella pneumonia* were evaluated. The study indicates the possible potentiality of *F. indica*to act as an active antibacterial agent in the modern drug formulations. As the target plant species serves for the tribal medicinal purpose in several tribal regions of Madhya Pradesh, hence, the aim of the present study is to link comparatively the possible traditional use of this herb with the modern antibiotic usage.

Keywords:*Fumaria indica*, Antibacterial activity, Zone of Inhibition, Tribes, Phytochemicals.

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History

- Submission Date: 28-01-2020;
- Review completed: 22-02-2020;
- Accepted Date: 16-03-2020.

DOI : 10.5530/pj.2020.12.92

Article Available online

http://www.phcogj.com/v12/i3

INTRODUCTION

Fumaria indica (L.) belongs to family Fumariaceae, and genus Fumaria commonly called shahtera "Pit papra" in the tribal vernacular. It is an annual herb and it's distributed all over Asia, Europe and Africa. It is a familiar weed found in the plains of India. *F. indica* have been reported various medicinal purposes to acquire pharmacological activities like antipyretic[1] hepatoprotective[2] hypoglycemic[3] antidiarrheal[4] antispasmodic[5] antihelmintic[6] antieczema[7] antiperiodic compound[8] liver complaints[9] and scrofulous skin affections.[10] Infectious diseases have threatened the continued existence of humans since very early civilizations.[11,12] The folk remedies, are still as an important part of traditional medicine[13] presently many human diseases and infections are cured by a diversity of plants[14] or plant derivative products.[15] The main cause for the continuing attention and broad research on plants for antibacterial properties is the manifestation of challenging strains of bacteria.[16,17] These strains are competent to survive with the same pace as their genetic evolution requires continuous development of new drugs against them.[18] Therefore, bacteria in fastidious are imposing require for new drugs.[19]Infectious diseases are persistent and are major explanation of premature death all over world.[20,21]The prevalence of severe infections in human beings has significantly increased all over the world and it has become the leading cause of mortality in developing countries.[22]

About 80% population of the world relies on plants as a natural source of medicine.[23] They are used medicinally in different countries and are a source of many potent and powerful drugs.[24]This study was aimed on validating the traditional use of selected medicinal plants against common bacteria, causing several human infections including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, andKlebsiella pneumonia*[25-27]by evaluating their *in vitro* antibacterial activity. The plants investigated in this study commonly used to treat the infectious diseases and the associated symptoms are listed in (Table 1).

MATERIALS AND METHODS

Plant materials and extraction

The plant was collected from Bagrachi village of Jabalpur district. The collection was done in March, 2017. To confirm and authenticate the identified plant taxonomically, the samples were examined at State Forest Research institute (SFRI) under voucher no. 586. The plant was selected based on reports of its widespread use among the tribal communities. The collected plant materials were air-dried and finely powdered using a blender. To prepare methanol and chloroform extracts of the plant materials, 20 g of each powdered plant material was extracted with 200 ml of methanol and chloroform for 48 h at room temperature. The extracted suspensions were filtered through Whatman No. 1 filter paper (Himedia) and the filtrates were concentrated to dryness using a rotary evaporator, to yield the crude extract (Table.2)

Cite this article: Ali S, Bansal S, Mishra RP. *Fumaria indica* (L), a Famous Medicinal Herb of Tribal Regions of Jabalpur, Madhya Pradesh: Broad Spectrum Antibacterial and Phytochemical Profilng Against Some Pathogenic Microorganisms. Pharmacogn J. 2020;12(3):619-23.

Table 1: Medicinal plant tested for their antibacterial activity in the study.

Scientific name	Family	Voucher number	Common name	Local name	Parts used	Traditional use
Fumaria indica (L)	fumariaceae	586	shahtera	pitpapra	Whole plant	digestion, chronic dysentery, diarrhea, Intoxication, fever, inflammations, liver complaints, skin infection, vomiting and antihelminthic,

Table 2: Yield percentage of methanol, chloroform and aqueous extract of *Fumaria indica*.

Plant	Solvent used	Weight of powderedsample (g)	Weight of Extract (g)	Extract yield (%)
Fumaria indica	Methanol	20	1.57	7.85%
Fumaria indica	Chloroform	20	1.98	9.9 %
Fumaria indica	Aqueous	20	2.32	11.6%

and then stored at −20 °C until further use. For the antibacterial activity assays, the extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50,100 and 150 mg/ml and stored at 4 °C as stock solutions.[28]

$$\text{Percentage Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}}$$

Procurement and maintenance of microbial culture

Five species of bacteria *viz., Escherichia coli* (MTCC 77), *Bacillus subtilis* (MTCC 10110) *Staphylococcus aureus* (MTCC 96) *Pseudomonas aeruginosa* (MTCC 1688) and *Klebsiella pneumonia* (MTCC 4032) were obtained from the Microbial Type Culture Collection (MTCC) Institute of Microbial Technology, The bacterial procured cultures were sub-cultured in nutrient agar medium composition of (beef extract - 3 g, NaCl - 5 g, peptone - 5 g, agar - 15 g, distilled water - 1000 ml) andincubated at 37°C. All the selected test strains were maintained and were sub-cultured after 20 days. The stock cultures of bacteria were maintained on nutrient agar slant at 4°C respectively.

Determination of antibacterial activity

Antibacterial activity was determined using well diffusion method.[29] Petri plates were prepared with 20 ml of sterile Muller Hinton agar media (HiMedia). Wells (6 mm diameter) were punched in the Muller Hinton agar and filled with plant extracts. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min for compound diffusion. The tests were conducted at three different concentrations 50mg/ml, 100mg/ml and 150mg/ml of the crude extracts. Ciprofloxacin (25µl) was used as positive control. The plates were incubated for 18-24 hours at 37±1^{0}C. Zone of inhibition was recorded in millimeters using transparent (HiMedia) antibiotic zone scale and the experiment was repeated by triplicates.

Phytochemical screening

The Preliminary qualitative phytochemical screening of *Funmaria indica* extracts was carried out by the standard methods.[30,31]

Test for Steriods (*LibermannBurchard Test*)

1 ml of the crude extract was taken and dissolved in 10 ml of chloroform and an equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and the sulphuric acid layer showed yellow with green fluorescence. These indicate the presence of steroids.[32]

Test for Terpenoids (*Salkowski test*)

2 ml of crude extract was taken and added to 2 ml of acetic anhydride and absorption of H_2SO_4. Formations of blue, green rings indicate the presence of terpenoids.[33]

Test for Saponins (*Foam test*)

5 ml of crude extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of bubbles indicates the presence of saponins.[34]

Test for Flavonoids(*Alkaline reagent test*)

2 ml of crude extract was treated with a few drops of 1N sodium hydroxide solution and observed the formation of strong yellow color. This yellow color becomes colorless on the addition of dilute hydrochloric acid, indicating the presence of flavonoids.[35]

Test for Phenolic Compounds (Ferric chloride test)

Few drops of the extract were treated with 5% aqueous ferric chloride. The formation of deepblue or black color indicates the presence of phenolic compounds.[35]

Test for Alkaloids (*Mayer's Test*)

2 ml of crude extract was treated with 2 drops of Mayer's reagent. The presence of white creamy precipitate indicates positive test.[35]

Test for glycosides (*Fehling's test*)

An equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of this solution was added to the crude extract and gently boiled. A brick-red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.[35]

Data analysis

The experiments were performed in triplicate and data were expressed as mean ± standard deviation (SD) .The data obtained was analysed using statistical package for social sciences (SPSS- Version 20).

RESULTS

The preliminary qualitative phytochemical screening of different extract showed maximum number of phytoconstituents along with alkaloids, terpenoids, steroids, flavonoids, phenols (Table 3). The zone of inhibition of methanol and chloroform extracts of *Fumaria indica* on gram positive and negative bacteria at different concentrations, by using agar well diffusion method, was determined to access their antibacterial effect. Both extracts of *Fumaria indica* exhibited sensible antibacterial activity against five tested bacterial strains as compared to the standard antibiotic ciprofloxacin (Table 4). The highest zones of growth inhibition were exhibited by methanol and chloroform extract against all the microorganisms compared to aqueous extract are shown in figure 4.2 (a), (b) and (c). The methanol and chloroform extract produced a highest mean zone diameter of 13.00±1.00 mm, 11.00±1.00 mm and 10.66±0.57 mm at a dose of 150 mg/ml on *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. Lowest zone

Table 3: Preliminary Phytochemical analysis of *Funmaria indica* (L).

Extract	Alkaloid	Terpenoids	Steroids	Flavanoid	Phenols	Glycosides	Saponin
Aqueous	+	-	-	+	+	-	+
Methanol	+	+	+	+	+	-	-
Chloroform	+	+	+	+	+	-	-

+Presence, -Absent,

Table 4: Inhibition Zone of methanolic and chloroform extract of *F. indica* against different bacterial strains.

Bacterial strains	Zone of inhibition in mm						
	Methanol extract			Chloroform extract			Ciprofloxacin
	50mg/ml	100mg/ml	150mg/ml	50mg/ml	100mg/ml	150mg/ml	25µg/ml
Staphylococcus aureus	8.00 ± 1.00	12.00 ± 1.00	13.00 ± 1.00	6.00 ± 1.00	7.00 ± 1.00	10.33 ± 0.57	25.00 ± 1.00
Escherichia coli	6.33 ± 0.57	9.33 ± 0.57	10.66 ± 0.57	1.33 ± 1.88	4.33 ± 0.57	7.00 ± 1.00	24.33 ± 0.57
Pseudomonas aeruginosa	5.33 ± 0.57	7.00 ± 1.00	8.00 ± 1.00	7.00 ± 1.00	8.00 ± 1.00	11.00 ± 1.00	23.33 ± 0.57
Klebsiella pneumonia	1.33 ± 2.30	5.00 ± 1.00	6.33 ± 1.52	4.66 ± 0.57	5.66 ± 0.57	8.00 ± 1.00	25.00 ± 1.00
Bacillus subtilis	1.33 ± 2.30	4.33 ± 0.57	5.66 ± 0.57	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	25.33 ± 0.57

Values are expressed as means ± SD.

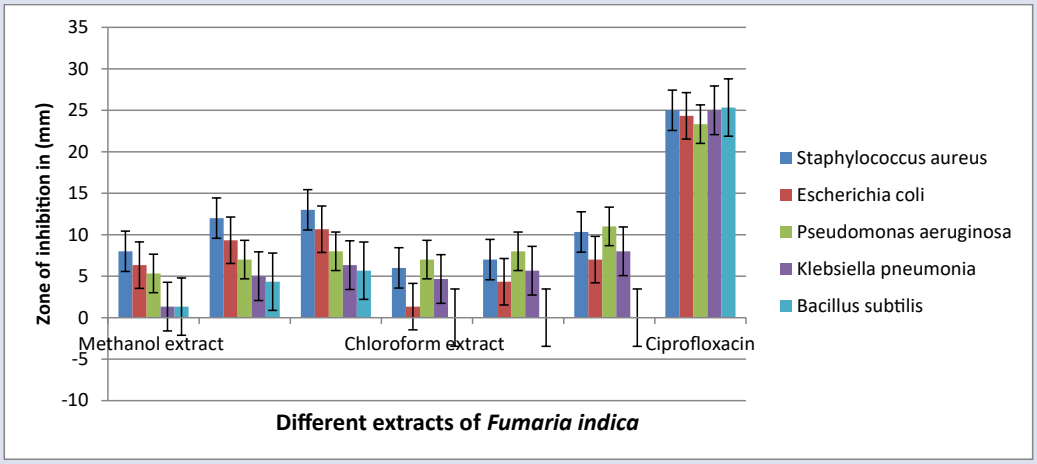
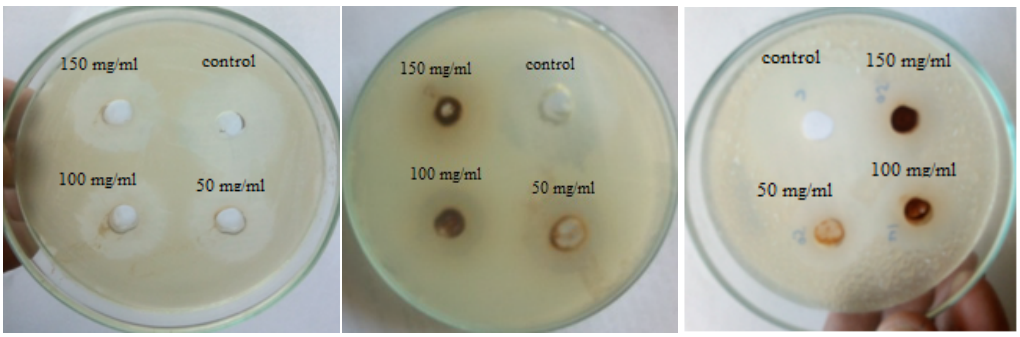


Figure 1: Inhibition Zone of methanolic and chloroform extract of *F. indica* against different bacterial strains.



(a) *Staphylococcus aureus* (b) *Pseudomonas aeruginosa* (c) *Klebsiella pneumonia*

Figure 2: Antibacterial activity *Fumaria indica*.

of growth inhibition was observed on both extracts against *Bacillus subtilis* which gave a zone of inhibition measuring 1.33±2.30 and 0.00± 0.00 respectively at a dose of 50 mg/ml shown in (Table 4). The aqueous extract does not show any zone of inhibition.

DISCUSSION

Several studieshave been confirmed for the extracts of plant species possessed activity with regard to antimicrobial properties[36,37] analysed that methanolic extracts of *F. indica* was very much active against *Staphylococcus aureus and B. subtilis*[38]. The study revealed that methanol and chloroform extract of the crude drug was very much effective at *E. coli, S. aureus and P. aeruginosa* and moderately effective at *B. subtilis* and *Klebsiella pneumonia*. The aqueous extract of the crude drug was moderately effective against all these test bacteria. The result of this work may add to overall value of the medicinal potential of *Funmaria indica*. Further chromatographic studies are required to determine

the purified bio-active compounds responsible for the antibacterial activities which could serve as useful sources for new anti microbial agents. This experimental activity has confirmed the tribal use of the plant in the treatment of infectious diseases.

CONCLUSION

The results of the present study reveal that the antibacterial activity of traditional medicinal herb *fumaria indica* extracts against all the selected pathogenic bacterial strains is remarkably fascinating. This herb thus justifies its use by tribal to treat various diseases like digestion, diarrohea, fever, inflammations, liver complaints, skin infection and vomiting. This success in the beneficial role of native tribal communities hence strongly stems the fact that this plant bears the capability of possessing most important bio-conjugated constituents that could serve as a source of novel drug design formulation.

ACKNOWLEDGEMENT

Author is grateful to the University Grant Commission, New Delhi for providing (RGNF) Rajeev Gandhi National Fellowship for this study. We are thankful to the Head, Department of Post-Graduate Studies and Research in Biological Science, Rani Durgavati University, Jabalpur for providing laboratory facilities.

CONFLICTS OF INTEREST

No conflicts of interest.

ABBREVIATION

MTCC:Microbial Type Culture Collection, mg: milligram, ml: milliliter, µl:microliter, SD: Standard deviation, NaCl: Sodium chloride, DMSO:Dimethyl sulfoxide.

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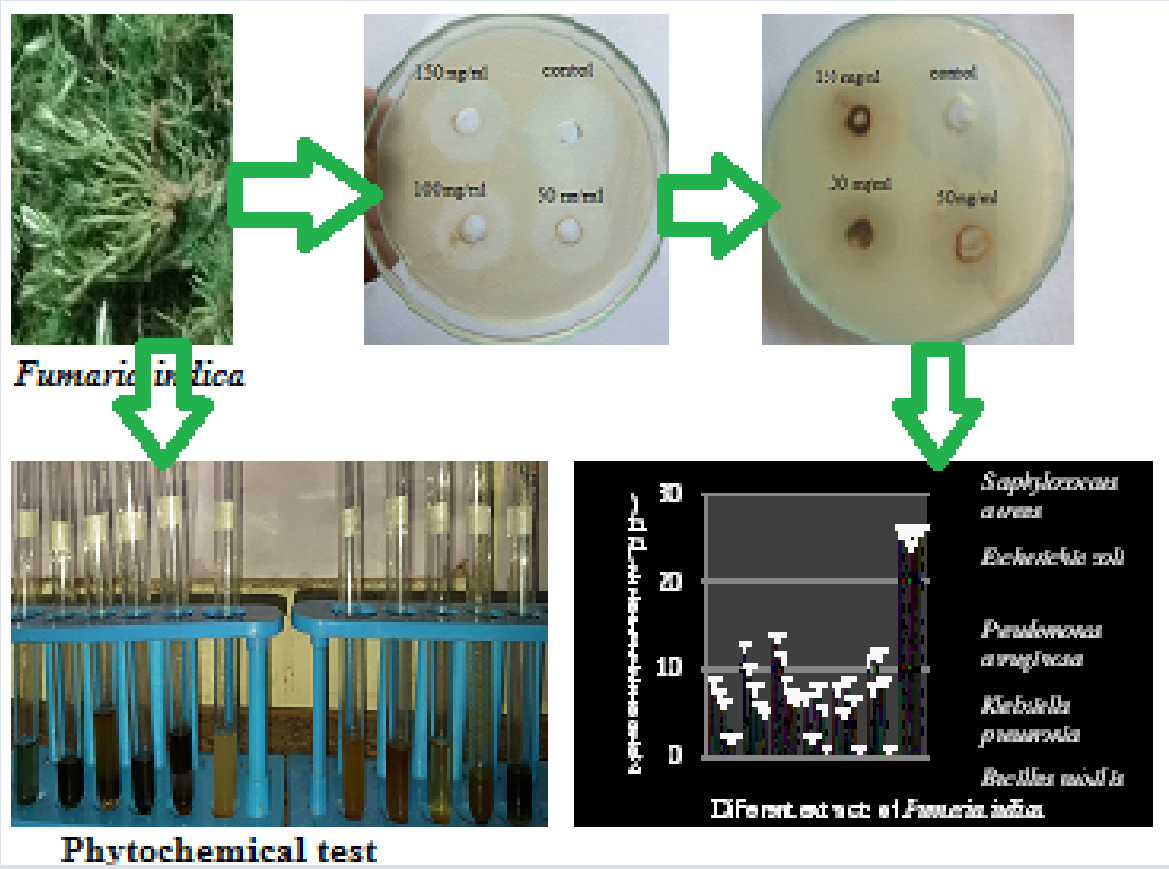
GRAPHICAL ABSTRACT



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Cite this article: Ali S, Bansal S, Mishra RP. *Fumaria indica* (L), a Famous Medicinal Herb of Tribal Regions of Jabalpur, Madhya Pradesh: Broad Spectrum Antibacterial and Phytochemical Profilng Against Some Pathogenic Microorganisms. Pharmacogn J. 2020;12(3):619-23.