Fumaria indica (L), a Famous Medicinal Herb of Tribal Regions of Jabalpur, Madhya Pradesh: Broad Spectrum Antibacterial and Phytochemical Profiling 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 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.

INTRODUCTION

Fumaria indica (L.) belongs to family Fumariaceae, and genus Fumaria commonly called shahetra “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 antipyretic¹ hepatoprotective¹ hypoglycemic¹ anti diarrheal⁷ antispasmodic⁵ antihelmintic⁶ antieczema⁷ antiperiodic⁸ of many potent and powerful drugs.¹⁰ 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, and Klebsiella pneumoniae²⁰⁻²¹ 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).

About 80% population of the world relies on plants as a natural source of medicine.¹¹ They are used medicinally in different countries and are a source of many potent and powerful drugs.²² 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, and Klebsiella pneumoniae 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).
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°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 Fumaria indica extracts was carried out by the standard methods. 30, 31

Test for Steroids (Liebermann-Burchard 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 H₂SO₄. 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. 36

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. 37

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. 38

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 (Figure 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 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 | fumariaceae | 586 | shahtera | pitpapa | 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 powdered sample (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% |
**Table 3: Preliminary Phytochemical analysis of *Funmaria indica* (L).**

| Extract       | Alkaloid | Terpenoids | Steroids | Flavanoids | Phenols | Glycosides | Saponins |
|---------------|----------|------------|----------|------------|---------|------------|----------|
| Aqueous       | +        | -          | -        | +          | +       | -          | -        |
| Methanol      | +        | +          | +        | -          | -       | -          | -        |
| Chloroform    | +        | +          | -        | +          | -       | -          | -        |

+Presence, -Absent.

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

| Bacterial strains | 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 | 6.33 ± 1.52 | 11.00 ± 1.00 | 1.33 ± 2.30 | 5.00 ± 1.00 | 8.00 ± 1.00 | 25.33 ± 0.57 |
| *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.](image)

**DISCUSSION**

Several studies have been confirmed for the extracts of plant species possessed activity with regard to antimicrobial properties analysed that methanolic extracts of *F. indica* was very much active against *Staphylococcus aureus* and *B. subtilis*. 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, diarrhoea, 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.

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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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