INVESTIGATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *FUMARIA PARVIFLORA* LAM.

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**ABSTRACT**

The plant species *Fumaria parviflora* Lam. is a well-known and widely used herb in Indian traditional medicinal system. The study was aimed to evaluate the antioxidant and antimicrobial activities of the hydroalcoholic extract of *Fumaria parviflora* Lam. (whole plant). Antioxidant activity was determined by DPPH (1,1 diphenyl-2-picrylhydrazyl) free radical scavenging method and IC₅₀ (Half-maximal inhibitory concentration) value of the extract was determined which was compared with the IC₅₀ value of the standard Ascorbic acid. The extract was also evaluated for its antimicrobial potential and the tests were carried out using the disc diffusion method. The extract considerably inhibited the growth of most of the tested bacterial and fungal strains. Thus, the results of the study justified the traditional use of *F. parviflora* parts as a remedy to various health disorders.

**Keywords:** Antimicrobial, Antioxidant, DPPH, *Fumaria parviflora*.

1. **INTRODUCTION**

People all over the world, being surrounded by a wide range of disease causing factors, are more or less concerned about their health. Pathogens and environmental pollution are the two such factors causing innumerable health hazards to human beings. Although, a large number of antimicrobial drugs have been formulated by pharmaceutical industries, resistance to some of these drugs by microbes has also been increased [1]. According to WHO (2018) [2, 3] antimicrobial resistance, (AMR) occur when microorganisms (such as bacteria, fungi, viruses, and parasites) change when they are exposed to antimicrobial drugs (such as antibiotics, antifungals, antivirals, antimalarials, and anthelmintics). As a result, the medicines become ineffective and infections persist in the body, increasing the risk of spread to others [1-3]. On the other hand, several environmental and biological factors cause free radicals to form and accumulate in the body which cause oxidative damage to cellular constituents leading to various kinds of chronic and degenerative diseases. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule [4, 5]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [6-8]. The search for new drugs to combat diseases and infections has become the focus of research in herbal remedies [9]. Medicinal plants are regarded as the important sources of pharmaceuticals for the formulation of herbal drugs because they cure diseases safely as compared to the allopathic medicines [1]. Extracts of different plant organs, including roots, leaves, bark, flowers, fruits and seeds, may contain distinct phytochemicals with activity against bacterial or fungal pathogens [10, 11]. The traditional use of medicinal plants as drugs is considered safe and cost effective remedy [1]. In folk medicine, a single plant species is often used to treat more than one type of disease or infection [11, 12]. Phytochemicals act through diverse mechanisms and hence much useful in the treatment of various diseases. Therefore, the formulation and utilization of more potential, plant-based drugs is an urgent need of the hour.
\textit{Fumaria parviflora} Lam. (Family: Fumariaceae), commonly known as fine-leaved fumitory (in English), \textit{Shahatra}, \textit{Pittapara} or \textit{Pittapapada} and Dhamgajra (in Hindi) \[13\]. The name of the genus is derived from the Latin \textit{fumus terrae}, which means "smoke of the earth" \[14-16\]. Plant is a native of Europe commonly found over the greater parts of India as a winter season weed, mostly in wheat field \[17, 18\]. Leaves of \textit{F. parviflora} possess anti-inflammatory activity as they inhibit various cytokines and have antioxidant effects and free radical scavenging activity \[19\]. The dried plant is regarded as efficacious in low fever, and is also used as an anthelmintic, diuretic, diaphoretic and aperients and to purify the blood in skin diseases \[17, 18\]. This plant has relaxing and styptic characteristics and it is also found to have laxative and diuretic properties \[20\]. It increases \textit{Vata}, removes indigestion, biliousness, fever, burning of the body, fatigue, urinary discharges, vomiting, thirst, enriches the blood and is useful in leprosy \[17,18\].

Keeping in view the importance of \textit{Fumaria parviflora} in traditional medicine, the present study was conducted to estimate its bioactive potential experimentally. In the present study, the plant extract was tested for its \textit{in vitro} antioxidant potential and antimicrobial efficacy against some pathogenic bacterial and fungal strains.

2. MATERIAL AND METHODS

2.1. Collection, identification and authentication of plants

The plant material was collected in the month of July, 2020 and identified taxonomically by Dr. Suman Mishra, Consultant taxonomist, Xcellventure Institute of Fundamental Research Pvt. Ltd., Bhopal (MP) \[16, 21\]. She is also a Botany scientist in MFP-PARC, Barkheda pathani, Bhopal \[16, 21\]. The plant was identified as \textit{Fumaria parviflora} Lam. belonging to family Fumariaceae by its macroscopic, microscopic and powder microscopic examination \[16, 21\].

2.2. Extraction

The plant material was washed and then kept for shade drying for 7 days \[16, 21\]. The dried plant sample was powdered by mechanical grinder into a fine powder \[16, 21\]. The air-dried powdered material of the whole plant of \textit{Fumaria parviflora} (100gm) was extracted with hydroalcoholic solvent [methanol and water solvent (1:1 v/v)] using Soxhletion process with the help of Soxhlet-apparatus \[16, 21\]. Excess solvent was then evaporated in a water bath at 50-100°C to obtain the crude and stored in air tight containers \[16, 21\].

2.3. Pathogenic microbe used

The standard cultures of the \textit{Escherichia coli} (MTCC443), \textit{Pseudomonas aeruginosa} (MTCC424), \textit{Staphylococcus aureus} (MTCC96), \textit{Enterococcus faecalis} (MTCC439) (bacterial strains) and \textit{Candida albicans} (MTCC227), \textit{Aspergillus niger} (MTCC282), \textit{Curvularia lunata} (MTCC283), \textit{Alternaria alternata} (MTCC2060) (fungal strains) used in the current study were obtained from CMBT Laboratory, Bhopal India.

2.4. DPPH radical scavenging activity

The DPPH assay is used to examine the antioxidant efficacy of plant extracts or compounds by determining their potential to act as free radical scavengers. \textit{IC}_{50} (the substrate concentration that causes 50% loss of DPPH) is used to interpret the assay results \[11\]. The antioxidant activity of \textit{Fumaria parviflora} Lam. (whole plant) hydroalcoholic extract was evaluated using DPPH radical scavenging assay \[22\] with some modifications. A stock solution of 0.004 g 1, 1 diphenyl-2-picrylhydrazyl (DPPH) in methanol (100 mL) was prepared, then 2 mL of this solution was mixed with 2 mL of different concentrations of \textit{Fumaria parviflora} extract. The mixture was shaken and incubated in dark at room temperature for 30 min. Absorbance was measured at 517 nm. The radical scavenging activity of the extract at various concentrations (20-100 μg/mL) was measured. Standard Ascorbic acid was used as positive control.

2.5. Assay for antimicrobial activity

Antimicrobial activity of \textit{Fumaria parviflora} hydroalcoholic extract was determined using the agar disk diffusion method \[23\] at four different concentrations of 25μg/ml, 50μg/ml, 75 μg/ml, 100 μg/ml. The stock solution was prepared by dissolving 1.0 g extract in 1000 mL methanol and then diluted to obtain different concentrations (25μg/ml, 50μg/ml, 75 μg/ml and 100 μg/mL) of the extract.

Nutrient agar media included Agar (15 gms), Yeast extract (3 gms), Peptone (5 gms) and Sodium chloride (5 gms). Distilled water was used to make the volume 1000 ml and pH of the media was maintained at 6.8±0.2

Potato dextrose agar (PDA) media was prepared by adding Potato infusion (200 gms), Dextrose (20 gms) and Agar (20 gms). Distilled water was used to make the volume 1000 ml and pH of the media was maintained at 5.6±0.2

The nutrient and potato dextrose agar media were separately dissolved in distilled water and boiled in
conical flask of sufficient capacity. Dry ingredients were transferred to the flasks containing required quantity of distilled water and heated to dissolve the media completely. The flasks containing media were cotton plugged and placed in an autoclave for sterilization at 15 lbs/inch² (121°C) for 15 minutes. After sterilization, the media in flask were immediately poured (20 ml/plate) into sterile petri plates. The poured plates were left at room temperature and allowed the medium to solidify. The microbial (bacterial and fungal) cultures were swabbed over the nutrient and potato dextrose agar plates respectively and after which the disks (6 mm in diameter) impregnated with the drug were placed on the surface of the media. The plates were incubated at 37°C for bacterial culture and 28°C for fungal culture for 24 hours. The zone of inhibition was measured for each plate by zonal scale.

2.6. Statistical method
One way ANOVA followed by Tukey’s HSD post hoc test was performed for statistical significance (α=0.05) between the Means (n=3) within a column.

3. RESULTS

3.1. Antioxidant activity
The results of DPPH radical scavenging activity are depicted in the table-1 and graph (Fig.1) below:

Table 1: DPPH scavenging activity

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Standard Ascorbic acid</th>
<th>Fumaria parviflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg/ml</td>
<td>94.444±0.39b</td>
<td>65.30±0.25d</td>
</tr>
<tr>
<td>40 µg/ml</td>
<td>94.166±0.26b</td>
<td>90.80±0.42d</td>
</tr>
<tr>
<td>60 µg/ml</td>
<td>94.444±0.10b</td>
<td>109.90±0.48c</td>
</tr>
<tr>
<td>80 µg/ml</td>
<td>95.000±0.12b</td>
<td>158.30±1.33b</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>98.481±0.07a</td>
<td>163.30±1.40a</td>
</tr>
</tbody>
</table>

DPPH scavenging activity is expressed in percentage. Values are expressed as Mean ± SEM (n=3); values with different letters (a,b,c,d,e) in superscript are significantly different (P≤0.05) within a column.

Fig. 1: DPPH scavenging activity

3.2. Antimicrobial activity
The results of Antimicrobial activity are depicted in the table 2:

Table 2: Antimicrobial activity of F. parviflora on selected microbes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of microbes</th>
<th>Zone of inhibition (diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Escherichia coli</td>
<td>10 10 6 11</td>
</tr>
<tr>
<td>2.</td>
<td>Pseudomonas aeruginosa</td>
<td>14 12 14 15</td>
</tr>
<tr>
<td>3.</td>
<td>Staphylococcus aureus</td>
<td>11 11 12 14</td>
</tr>
<tr>
<td>4.</td>
<td>Enterococcus faecalis</td>
<td>- - - -</td>
</tr>
<tr>
<td>B.</td>
<td>Fungus</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Candida albicans</td>
<td>- - - -</td>
</tr>
<tr>
<td>2.</td>
<td>Aspergillus niger</td>
<td>13 14 14 19</td>
</tr>
<tr>
<td>3.</td>
<td>Curvularia lunata</td>
<td>10 10 12 15</td>
</tr>
<tr>
<td>4.</td>
<td>Alternaria alternata</td>
<td>9 13 16 16</td>
</tr>
</tbody>
</table>

4. DISCUSSION
Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [24, 25]. India has a rich knowledge base related to the healing properties of medicinal plants which has led the pharmaceutical companies here to take a keen interest to use this traditional knowledge in the formulation and commercialization of novel drugs [1]. The evaluation of medicinal plants for bioactive potential is an essential step in the isolation and characterization of their active
principles, important in drug development. Natural crude drug extracts isolated from plant species used in traditional medicine can be prolific resources for such new drugs [26, 27].

The results of the present study indicated that the hydroalcoholic extracts of *F. parviflora* (whole plant) exhibited considerable antimicrobial activity (table 2). The extract actively inhibited the growth of most of the tested microbes upto different extents and the maximum zone of inhibition was obtained against *Aspergillus niger* (19 mm) at the highest applied extract concentration of 100 μg/ml. The maximum zone of inhibition obtained against *Pseudomonas aeruginosa* was the highest (15 mm) among all the tested bacterial strains, at the highest applied extract concentration 100 μg/ml. A dose dependent increase in the antimicrobial activity of the extract was observed.

The results of antioxidant activity revealed that the IC₅₀ Value of the crude extract is 1196.13 μg/ml which was comparatively much higher than the IC₅₀ value of the Standard Ascorbic acid (IC₅₀ = 8.754 μg/ml). High IC₅₀ value indicates less antioxidant potential and thus the extract possesses comparatively less antioxidant potential than the Standard Ascorbic acid used in the study. The presence of important phytoconstituents like alkaloids, phenols, flavonoids, etc., revealed from the phytochemical studies performed earlier [16, 21], contributes to the bioactive potential of *F. parviflora*.

The current scenario indicates the demand for plant-based drugs across the world, due to the presence of valuable phytochemicals. The use of traditional medicine should be encouraged with an approach to identify and exploit the plant kingdom for the production of effective drugs that could be used for the treatment of microbial as well as non-microbial diseases [1, 28]. In future, more co-ordinated multidimensional research aimed at correlating botanical and phytochemical properties to specific pharmacological activities is expected [29].

5. CONCLUSION

The results revealed the promising pharmaceutical efficacy of *F. parviflora*. Thus, the data obtained from the antimicrobial and antioxidant assays may be beneficial in the formulation of cost-effective herbal drugs in future. The present study provides evidence in support of the traditional use of the plant as medicine by the indigenous communities and could be a potential source of new drug discovery. However, more in vitro and in vivo studies should be conducted to support the therapeutic use of *F. parviflora* which could draw the attention of pharmaceutical companies for the commercial production of herbal drugs. However, further studies are needed in this direction for the production of novel herbal drugs.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Source of funding

Nil

6. REFERENCES


