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Total Phenolic Content, Antioxidant and Antimicrobial Activity of *Fumaria indica* (Hausskn.) Pugsley

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Abstract: The comprised investigation examines total phenolic content, antioxidant activity and antimicrobial activity in ethanolic extract fraction of *Fumaria indica*. An *in vitro* evaluation model utilizing a β-carotene bleaching assay and DPPH free radical scavenging test was employed for the assessment of antioxidant potential of the plant extract. A modified Folin-Ciocalteu method used for total phenolic content (TPC) determination in this study estimated the TPC in *F. indica* to be as high as 11.42 mg/g of extract. The antioxidant potential of the plant extract was observed 54.85 % measured as total antioxidant activity (AA %), with reference to the BHA (92.74 %) and BHT (89.34 %) used as standards. The results of free radical scavenging property depicted the DPPH radical scavenged by the extract of *F. indica* were 48.65 %. It had antimicrobial activity against eleven bacteria and nine fungi determined using disc diffusion method. The findings of this research adjudged the extract of *F. indica* a rich source of natural antioxidant and endowed with antimicrobial activity.

Key words: Natural Antioxidant, beta carotene bleaching assay, parpatak, Ethnobotany.

Introduction

Ethno-pharmacological literature and numerous investigations in medical botany pointed to that the aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Therefore it sought important to characterize medicinal plants for tapping the herbal wealth in public good. The characterization of plants in light of modern scientific principle has now become new source of inspiration in drug research and development which has been simultaneously utilized to prevent biopiracy [15]. In this connection the antioxidant and antimicrobial potential of *Fumaria indica*, an ethnomedicinal plant in Indian medicine employed as blood purifier and in liver diseases. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth -antimicrobial activity [12]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. *F. indica* (Hausskn.) Pugsley, (Fumariaceae) commonly known as Parpata in Ayurveda, is an annual herb, distributed over the

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greater part of India, Baluchistan, Afghanistan and Persia [10]. The whole plant is widely used in traditional and folkloric systems of medicine. In traditional systems of medicine the plant is well known for its diuretic, anthelmintic, diaphoretic, stomachic laxative, and is used to purify blood and in liver obstruction in ethnopharmacology [4]. The whole plant forms a constituent of many common household, Ayurvedic, Unani medicinal prepartions and marketed polyherbal liver formulations. It is also used as component of various herbal products such as Livokriti syrup, Esno capsule and Ayurveda capsule, available in Indian market [7]. Pharmacological studies reported that *F. indica* possesses antidiarrhoeal, anti-inflammatory and anti-nocciceptive, hepato-protective, anxiolytic, central nervous system depressant and chemopreventive effect [5,9,13,14,16]. Phytochemical investigation revealed the presence of protopine, cryptopine, papracinine, narceimine, narlumidine, fuyuziphine; steroids, *viz*. β-sitosterol, campesterol; organic acids *viz*. fumaric acid [6]. The plant selected for study was based on its availability and its various therapeutic activities in various ailments mentioned in Ayurveda. The present investigation reported first time, the results of the combined investigations on *in-vitro* antioxidant and antimicrobial activity of 50 % ethanolic extract of the whole plant of *F. indica*. As stated earlier, several polyphenols are known to possess excellent antioxidant effects, especially *in vitro*, and the amount of total polyphenols present in a plant has been suggested to correlate with the antioxidant activity. In this connection, the comprised study represents first report on phenolic content and related antioxidant activity of the extract of *F. indica*.

Materials and methods

Plant material and extraction

Plants of *F. indica* were collected from the rural area around Lucknow, India in the month of December. The plant material was identified, authenticated taxonomically by taxonomist of National Botanical Research Institute, Lucknow, India and a voucher specimen (NBR-21) was preserved for future reference. Air-dried powdered material of *F. indica* (100 g) was exhaustively extracted with 10 volumes of 50 % aqueous ethanol using percolation method. This process of extraction was repeated four times, the extract was filtered, concentrated on rotavapour (Buchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) under high vacuum (1.33 Pa) and at temperature of – 40 ± 2°C (yield 9.85 %).

Preliminary phytochemical screening and HPTLC analysis

Preliminary qualitative phytochemical screening of *F. indica* extract was tested for the presence of major chemical constituents. The 50 % ethanolic extract of *F. indica* was analyzed by TLC, which showed the presence of phenolics by given blue spot on spraying with Folin-Ciocaltue reagent. HPTLC profile and analysis of 50 % ethanolic extract of *F. indica* was performed on pre-activated (100°C) silica gel 60/UV_{254} HPTLC plates (Merck) separately and along with caffeic acid. The separate plate was then eluted in solvent system chloroform: methanol (80:20) and plate along with caffeic acid in toulene: ethyl acetate: formic acid (7:5:1). After elution, the plates were dried and densitometrically scanned on a TLC scanner III at 366 and 284 nm using Win Cat software (CAMAG, Switzerland); the peak area was recorded and the calibration curve was prepared by plotting the peak area against the concentration of caffeic acid applied.

Determination of the total phenolic content (TPC)

Total phenolic content in the extract was determined by the modified Folin-Ciocalteu method [17]. An aliquot of the extract (1 ml) was mixed with 0.5 ml Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate (20 %). Tubes were vortexed for 15 s and allowed to stand at 40°C for 30 min in order to develop colour. Absorbances were then measured at 765 nm using the UV-VIS spectrophotometer. Total phenolic content was expressed as mg/g Gallic acid equivalent. The result of assay was obtained from three parallel determinations.

Antioxidant activity (AA)

β-carotene bleaching assay was carried out according to the method developed by **Barriere *et al*. (2001)**. β-Carotene (0.5 mg) was dissolved

in 1 ml of chloroform, then 25 µl of linoleic acid and 200 mg tween 40 were added. After removing chloroform in the preparation under reduced pressure, 100 ml of oxygenated water was added and mixed properly to obtain a stable emulsion. 3 ml aliquot of emulsion were mixed with 300 µl methanol solution of the extract and incubated for 48 h at room temperature. The same procedure was repeated with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at the same concentration and a blank containing only 300 µl of methanol. After the incubation period the absorbance of the mixtures were measured at 490 nm. Antioxidant capacities of the sample were compared with those of BHA, BHT and the blank.

DPPH free radical scavenging activity

The test sample (3ml extract solution in water) was mixed with 1ml (0.1 mM solution of DPPH in methanol) and different concentrations (50-250 µg/ml) were prepared. After 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity. BHA and BHT were used as a standard control. Each assay was repeated three times and recorded as mean of the triplicate experiments. Capability to scavenge DPPH radical was calculated using following equation [19].

% Scavenging Effect = [1-Abs. (s)/Abs. (c)] x 100

Where, Abs. (s) = Absorbance of sample, Abs. (c) = Absorbance of control

Antimicrobial activity

Microorganisms

50 % Ethanolic extract of the sample was tested against a range of 20 microorganisms, including 11 bacteria and 9 fungi species. Antibacterial activity was evaluated against five Gram (+) and six Gram (-) bacteria. The G (+) bacteria used was: *Bacillus subtilis* 121, *Staphylococcus aureus* 96, *Micrococcus luteus* 106, *Bacillus cereus* 430 and *Streptococcus pneumoniae* 2672. The G (-) bacteria utilized in the assay were: *Escherichia coli* 443, *Proteus mirabilis* 1429, *Klebsiella pneumoniae* 109, *Pseudomonas aeruginosa* 429, *Salmonella typhimurium* 98, *Enterobacter aerogenes* 111. The antifungal activity was tested against nine organisms *Cryptococcus albiolus* 2661, *Aspergillus niger* 640, *Epidermophyton floccosum 613, Aspergillus flavus* 1973, *Candida albicans* 183, *Tricophyton rubrum* 296, *Microsporum fulvum* 2837, *Microsporum gypseum* 2829, *Microsporum canis* 2820 were used. These organisms were identified and procured from Institute of Microbial Technology (IMTECH) Chandigarh, India.

Disc diffusion method

A disc-diffusion method was employed for the determination of the antimicrobial activity of the extract of *F. indica*. Inoculate of the bacterial and fungal strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. 100 µl of suspension containing 10^8 CFU/ml of bacteria and 10^4 CFU/ml of fungal spores spread on Muller Hington Agar medium (MHA) (Hi media, chemicals) and Potato Dextrose agar (PDA) (Oxoid, chemicals) respectively, in sterilized petri dishes (90 mm in diameter). Sterile filter paper disc (Himedia, Mumbai) with a diameter of 6 mm discs were impregnated with 10 µl volume of stock solution of extract (Conc. 50 mg/ml) and placed on the inoculated agar. Negative controls were prepared using the same solvents (DMSO) employed to dissolve the plant extracts. Streptomycin (10 µg/disc) for bacteria and Fluconazole (10 µg/disc) for fungi were used as positive reference standards to determine the sensitivity of a strain of each tested microbial species. The inoculated plates were kept at 4°C for 2 h and incubated at 37°C for 24 h for bacterial strains and at 28°C for 72 h for fungal strains. After the incubation period the diameter of the zone of inhibition in mm obtained around the well was measured [2,11]. Interpret the result of extracts sensitivity as sensitive, moderate sensitive or resistant on the basis of mean zone diameter in mm. Each assay in this experiment was repeated triplicate.

Results and discussion

Antioxidant activity and total phenolic content

The 50 % ethanolic extract of whole plant of *F. indica* was subjected to screening for their possible antioxidant activity. Three complementary test

systems, namely DPPH free radical scavenging, β-carotene/linoleic acid system, and total phenolic content have been used for the analysis. The antioxidant capacity by using β-carotene bleaching assay in plant is shown in Table 1. In antioxidant activity estimation β-carotene undergoes rapid discoloration in the absence of an antioxidant because of the coupled oxidation of β-carotene and linoleic acid. In β-carotene bleaching assay, plant showed significant antioxidant capacity. The antioxidant capacity of standard BHA and BHT was 92.74 and 89.34 % (Table 1). DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extract. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extract, measured by DPPH assay, is shown in Table 1. The plant extract at all concentration studied showed free radical scavenging activity. The phenolic content of the examined extract of *F. indica*, obtained using Folin-Ciocalteu reagent is presented in Table 1.

Preliminary qualitative phytochemical screening of *F. indica* revealed the presence of glycosides, alkaloids, flavonoids, steroids, tannins and saponins. The qualitative HPTLC profile revealed 12 spots with different R_f value (0.04, 0.08, 0.10, 0.15, 0.19, 0.27, 0.40, 0.52, 0.61, 0.69, 0.73 and 0.90) under UV light 366λ (Figure 1). The quantitative HPTLC determination shows the

Table 1. Antioxidant activity and total phenolic content of 50% ethanolic extract *F. indica*

Sample	% β-Carotene bleaching	% DPPH radical scavenging	TPC (mg/g GAE)
F. indica	54.85±1.6	48.65±0.91	11.42±0.8
BHA	92.74±1.2	83.52±1.36	-
BHT	89.34±2.3	82.63±0.85	-

BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, ± SEM

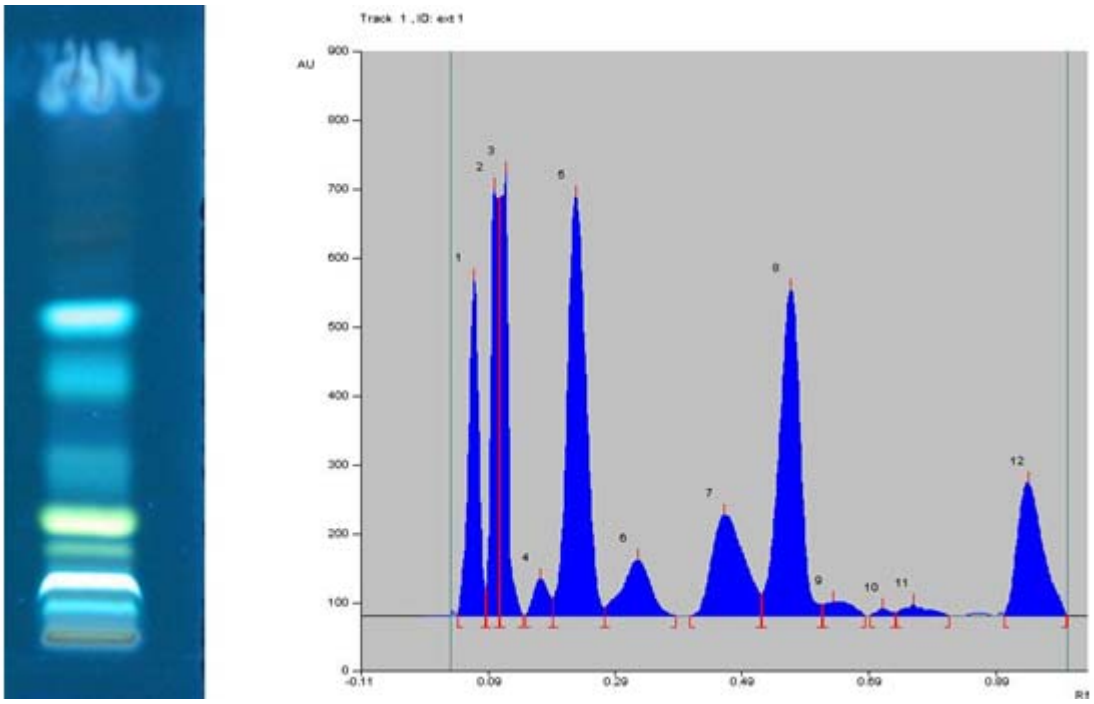

Figure 1. HPTLC profile and densitometric scanning of 50% ethanolic extract of *F. indica*

presence of caffeic acid (396 µg/g extract) in 50 % ethanolic extract of *F. indica*. Caffeic acid, a phenolic related to the derivatives of hydroxycinnamic acids, is the principal component possessing antioxidant activity [1]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, stems and bark [8].

Several studies have also reported the relationship between phenolic content and antioxidant activity. Some authors have found a correlation between the phenolic content and antioxidant activity, while others found no such relationship. Velioglu *et al.*[18] reported a strong relationship between the total phenolic content and antioxidant activity in certain plant products. The antioxidant capacity in our plant samples is possible owing to the presence of the phenolic content, but could also possibly be due to the presence of some other phytochemical such as chlorogenic acid, ascorbic acid and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant capacity. Furthermore, *in vivo* antioxidant activity of *F. indica* extract and different antioxidant mechanism is necessary in further investigations.

Antimicrobial activity

The antimicrobial effect of 50 % ethanol extracts of *F. indica* was tested against five species of G (+) bacteria, six species of G (-) bacteria and nine species of fungi. As can be seen from the results, *F. indica* showed medium antibacterial spectrum and broad antifungal spectrum against microorganisms. The test extract showed more potent activity against G (+) than G (-) bacteria. The 50 % ethanol extracts of *F. indica* showed antibacterial activity against 7 out of 11 microorganisms (Table 2) and antifungal activity against all tested microorganisms (Table 3).

Table 3. Antifungal activities of 50 % ethanolic extract *F. indica*

Test fungi	Zone of inhibition (mm)
Cryptococcus albiolus 2661	13
Aspergillus niger 640	18
Aspergillus flavus 1973	25
Candida albicans 183	18
Tricophyton rubrum 296	21
Epidermophytonfloccosum 613	24
Microsporum fulvum 2837	22
Microsporum gypseum 2829	21
Microsporum canis 2820	18

Table 2. Antibacterial activities of 50 % ethanolic extract *F. indica*

Test bacteria	Gram	Zone of inhibition (mm)
Bacillus subtilis 121	+	16
Bacillus cereus 430	+	Nil
Micrococcus luteus 106	+	9
Streptococcus pneumonia 2672	+	Nil
Staphylococcus aureus 96	+	Nil
Escherichia coli 443	-	20
Pseudomonasaeruginosa 429	-	31
Proteus mirabilis 1429	-	18
Klebsiella pneumonia 109	-	Nil
Salmonella typhimurium 98	-	15
Enterobacter aerogenes 111	-	15

Conclusions

Based on these results, it is possible to conclude that *F. indica* possesses antioxidant and antimicrobial activity. Caffeic acid might be responsible for the antioxidant activity of the plant. It is likely that the antioxidant activity of *F. indica* is a result of synergistic activities of its poly-phenolic compounds. The result of the antimicrobial study showed that *F. indica* endowed with antimicrobial activity more so against fungi. Further phytochemical studies are needed to identify active constituents responsible for the observed activity.

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