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FUMARIA INDICA: A VALUABLE NATURAL SOURCE OF ANTIOXIDANTS FOR PROTECTION AGAINST OXIDATIVE STRESS

Tauheeda Riaz, Muhammad Athar Abbasi*, Aziz-ur-Rehman, Tayyaba Shahzadi and Muhammad Ajaib
*Department of Chemistry; Department of Botany; Government College University, Lahore-54000, Pakistan
Email: atrabbasi@yahoo.com

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ABSTRACT
The purpose of this study was to evaluate the antioxidant potential of *Fumaria indica* Pugsley. Methanolic extract of the plant was dissolved in distilled water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol sequentially. The antioxidant potential of all these fractions and remaining aqueous fraction was evaluated by four methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, total antioxidant activity, Ferric Reducing Antioxidant Power (FRAP) assay and ferric thiocyanate assay along with determination of their total phenolics. The results revealed that ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 83.41 ± 1.23% inhibition of DPPH radical at a concentration of 250 µg/ml. The IC_{50} of this fraction was 79.15 ± 0.87 µg/ml, relative to reference standard ascorbic acid, having IC_{50} of 58.9 ± 0.97 µg/mL. It also showed highest total antioxidant activity (1.029 ± 0.08), as well as highest FRAP value (249.66 ± 1.83 µg of trolox equivalents), highest total phenolic contents (123.23 ± 0.41) and highest value of inhibition of lipid peroxidation (51.16 ± 1.48% at concentration of 500 µg/ml)
Keywords: *Fumaria indica* Pugsley, DPPH assay, total antioxidant activity, FRAP value, total phenolics, Inhibition of lipid peroxidation (%).

INTRODUCTION

Antioxidants have been used as important protective agents for human health. The crude extracts of various parts of plants contain antioxidants[1]. Currently there is much interest in the protection of low density lipoprotein and important cells and organs, as well as food systems, against oxidative damage caused by superoxide, hydroxyl and peroxyl radicals. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts or isolated products from plant origin rather than looking for synthetic ones. It is an established fact that polyphenolic compounds, such as flavonoids, anthraquinones, anthocyanidins and xanthones, possess remarkable antioxidant activities which are present quite commonly in the plant family[2]. Numerous studies have shown that 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. Thus it is important to characterize different types of medicinal plants for their antioxidant potential. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Scientific experiments since the late 19th century have documented the antioxidant properties of some spices, herbs, and their components[3].

Antioxidants are used to preserve foods by retarding discoloration, rancidity, or deterioration due to auto-oxidation. However, synthetic antioxidants have been reported to be carcinogenic. Hence, several attempts to replace synthetic antioxidants with natural anti-oxidants have been developed. Antioxidative substances obtained from natural sources, such as oilseed, grains, beans, vegetables, fruits, leaf waxes, bark, roots, spices, hulls and seaweeds, have been investigated[4]. So, in this study, the antioxidant activity of a medicinal herb *Fumaria indica* Pugsley has been investigated and reported.

Fumaria indica (Hausskn.) Pugsley, syn: *F. parviflora* Lam. (*Fumariaceae*) is a small, scandent, branched annual herb growing wild in plains and lower hills. It is locally known as "Pitpapra" or "Shahtra". The plant is considered to be diuretic, diaphoretic, anthelmintic, laxative and is used to purify blood and in liver obstruction in ethnopharmacology. Pharmacological studies show that *F. indica* possesses antipyretic, antidiarrhoeal and hypoglycemic properties. It is a smooth muscles relaxant and has hydrocholeretic, by stimulating bile excretion, and hepatoprotective effects. The plant has local reputation as antidyspeptic, blood purifier, cholagogue, diaphoretic, stomachic, sedative and tonic and is also considered useful to treat constipation, abdominal cramps, fever, jaundice, leprosy and syphilis. Phytochemical investigation revealed the presence of alkaloids, *viz.* protopine, parfumine, cryptopine, copticine, fumariline, fumaramine, fumaritine, paprafumicin, paprarine, papracinine, papraline, reddeanine, fumarophycine, narlumicine, narceimine, narlumidine; steroids, *viz.* β-sitosterol, stigmasterol, campesterol; organic acids *viz.* caffeic acid and fumaric acid[5-7]. According to our knowledge, no work has been done on the comparative antioxidant potential of various fractions of *Fumaria indica* so, it was considered worthwhile to study the antioxidant potential of different fractions of *Fumaria indica* whole plant for future investigations towards the finding of new, potent and safe antioxidant compounds.

MATERIALS AND METHODS

Plant Material

The plant *Fumaria indica* Pugsley, was collected from district Kotli, Azad Kashmir in October 2010, and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, Government College University, Lahore. A Voucher specimen (G.C.Bot.Herb.969) has been deposited in the Herbarium of the Botany Department of the same university.

Extraction and Fractionation of Antioxidants

The shade-dried ground whole plant (0.82 g) was exhaustively extracted with methanol on the soxhlet apparatus. The extract was evaporated in rotary evaporator (Laborta 4000-efficent Heidolph) at 45^{0}C under vaccum to yield the residue (63 g), which was dissolved in distilled water (700 mL) and partitioned with *n*-hexane (600 mL × 4), chloroform (600 mL × 4), ethyl acetate (600 mL × 4) and *n*-butanol (600 mL × 4) respectively. These organic fractions and remaining water fraction were concentrated separately on

rotary evaporator (*n*-hexane at 38 ^{o}C, chloroform at 40 ^{o}C , ethyl acetate at 47 ^{o}C , *n*-butanol 50 ^{o}C and water at 60 ^{o}C under vaccum) and the residues thus obtained were used to evaluate their Phytochemical constituents and *in vitro* antioxidant potential.

Chemicals and Standards

DPPH$^{\cdot}$ (1,1-Diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-Tripyridyl-s-triazine), Trolox, Gallic acid, Follin Ciocalteu's phenol reagent, ascorbic acid and BHT (Butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride, ferrous chloride, ceric sulphate, hydrocloric acid, copper sulphate, aluminium chloride, lead acetate, acetic acid and ammonia from Merck (Pvt.) Ltd. (Germany).

Phytochemical Screening

Phytochemical screening of all the five crude extracts i.e. *n*-hexane soluble fraction, chloroform soluble fraction, ethyl acetate soluble fraction, *n*-butanol soluble fraction and remaining aqueous fractions was carried out to identify the phytochemical constituents using the following standard procedures[8-10].

Test for Alkaloids

For the test of alkaloids the TLC (thin layer chromatography) card having spots of the studied crude extracts was sprayed with Draggendorff's reagent. Appearance of orange colour indicates the presence of alkaloids.

Test for Terpenoids

Two methods were used to test presence of terpenoids. First, Ceric sulphate solution was sprayed on TLC card having spots of each crude extracts. TLC card was heated on TLC heater. Appearance of brown colour indicates the presence of terpenoids. Second, to 0.5 g of each of the extract was added 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for Saponins

To 0.5 g of each crude extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Tannins

2 ml of each crude extract was taken in test tube and 5 ml of *n*-butanol-HCl solution was added. Mixture was warmed for 1 hour at 95^{o}C in a water bath. Appearance of red colour indicated the presence of tannins.

Test for Sugars

Solutions of the each crude extract (0.5 g in 5 ml water) were added to boiling Fehling's solution (A and B) in a test tube. Formation of red precipitates indicated the presence of sugars.

Test for Phenolics

Neutral ferric chloride was added to each crude extract. Appearance of bluish green colour indicated presence of phenolics.

Test for flavonoids

Four methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of each crude extract in water. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicated the presence of flavonoids. Second, a few drops of 1% aluminium chloride solution were added to each crude extract. A yellow colouration indicated the presence of flavonoids. Third, the TLC card having spots of each crude extract was sprayed with Benedict's reagent. Green fluorescence in UV light indicated the presence of flavonoids. Fourth, the TLC card having spots of each crude extract was sprayed with lead acetate solution. Green fluorescence in UV light indicated the presence of flavonoids.

Test for cardiac glycosides (Keller-Killiyani test)

To 0.5 g of each crude extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Antioxidant Assays

Following antioxidant assays were performed on all the studied fractions.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of each crude extract of plant were examined by comparison with that of known antioxidant, ascorbic acid using the reported method[11]. Briefly, various concentrations of the samples (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 60 µg/mL, 30 µg/mL, 15 µg/mL, 8 µg/mL) were mixed with 3 ml of methanolic solution of DPPH (0.1mM). The mixture was shaken vigorously and allowed to stand at room temperature for one an hour. Then absorbance was measured at 517 nm against methanol as a blank in the UV-visible spectrophotometer (CECIL Instruments CE 7200 Cambridge England). Lower absorbance of spectrophotometer indicated higher free radical scavenging activity.

The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Percent Scavenging activity} = \frac{100 - \text{Absorbance of Test compound}}{\text{Absorbance of Control}} \times 100$$

Each sample was assayed in triplicate and mean values were calculated.

Total Antioxidant Activity by Phosphomolybdenum Complex Method

The total antioxidant activities of each crude extract of plant were evaluated by phosphomolybdenum complex formation method[12]. Briefly, 500 of each crude extract was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4 mL of reagent solution. The vials were capped and incubated in water bath at 95 ^{o}C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of BHT, a reference standard. All determinations were assayed in triplicate and mean values were calculated.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was done according to Benzie and Strain[13] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa.3H_2O$ and 16 mL CH_3COOH), pH 3.6, 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution in 40 mM Hydrochloric Acid and 20 mM Ferric chloride hexahydrate solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution and then

warmed at 37 °C before using. The solutions of each crude extract and that of trolox were formed in methanol (250 . 10 L of each of crude extract solution and trolox solutions were taken in separate test tubes and 2990 L of FRAP solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FRAP solution in the dark for 30 minutes. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 593 nm by UV-visible spectrophotometer. The FRAP values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE M/mL.

Total Phenolic Contents

Total phenolics of various fractions of plant were determined reported method[14]. The 0.1 mL (0.5 mg/mL) of each crude extract was combined with 2.8 mL of 10% Sodium carbonate and 0.1 mL of 2N Folin-Ciocalteu's phenol reagent. After 40 minutes absorbance at 725 nm was checked on UV-visible spectrophotometer. Total phenolic contents were expressed as micrograms of gallic acid equivalents(GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. The curve was linear between 50 g/mL to 500 g/mL of gallic acid. Results were expressed in GAE g/mL.

Ferric Thiocyanate (FTC) Assay

The antioxidant activities of various fractions of plant on inhibition of linoleic acid peroxidation were assayed by thiocyanate method[15]. The 0.1 mL of each of crude extract solution (0.5 mg/ mL) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.02 M, pH 7.0). The linoleic emulsion was prepared by mixing 0.28 g of linoleic acid, 0.28 g of Tween-20 as emulsifier and 50.0 mL of phosphate buffer. The reaction mixture was incubated for 5 days at 40 °C. The mixture without extract was used as control. The mixture (0.1 mL) was taken and mixed with 5.0 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 minutes after addition of ferrous chloride to the reaction mixture, absorbance was recorded at 500 nm. The antioxidant activity was expressed as percentage inhibition of peroxidation (IP%) [IP% = {1-(abs. of sample) /(abs. of control)} × 100]. The antioxidant activity of BHT was assayed for comparison as reference standard.

Statistical Analysis

All the measurements were done in triplicate and statistical analysis was performed by Statistical software. All the data were expressed as ± S.E.M. Statistical analysis were determined using one way analysis of variance (ANNOVA) followed by post-hoc Tukey's test.

RESULTS

The phytochemical screening was done on all the studied fractions. Tests were performed for the detection of alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides and the results have been shown in table 1. DPPH assay was performed on all the 5 fractions and percent scavenging of the DPPH radical was calculated alongwith IC_{50} values. The values of percent scavenging of DPPH radical have been shown in Table 2 while the IC_{50} values have been given in Table 3. The total antioxidant activities of all the five studied fractions was measured spectrophotometrically by Phosphomolybednum method and compared with the standard antioxidant BHT and the results have been shown in Table 3. The FRAP values of the 5 studied fractions were calculated equivalent to trolox and results have been given in table 3. Total phenolics were determined and the table 3 shows the phenolic concentration in the five studied fractions, expressed as the µg of the gallic acid equivalents (GAEs) per g of the fraction. % inhibition of lipid peroxidation of all the fractions was determined by ferric thiocyanate assay and the results have been shown in table 3.

DISCUSSION

From the results of phytochemical screening, it was revealed that alkaloids, phenolics and flavonoids were found in chloroform fraction, ethyl acetate fraction, *n*-butanol fraction and remaining aqueous fraction, but in more concentration in ethyl acetate fraction, while *n*- hexane fraction showed absence of all these compounds. Terpenes were found to be present in all the fractions except the aqueous fraction. Tannins and sugars were present in ethyl acetate fraction, *n*-butanol fraction and aqueous fraction but not detected in *n*-hexane fraction and chloroform fraction. Saponins were present in the remaining aqueous fraction only.

DPPH radical scavenging activity is widely used to evaluate antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability[16]. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compound is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow[17]. DPPH is a preformed stable radical used to measure radical scavenging activity of antioxidant samples. This method is based on the reaction of DPPH radical that is characterized as a stable free radical with deep violet colour and any substance that can donate hydrogen atom to DPPH thus reduces it to become stable diamagnetic molecule[11]. Reduction of DPPH radical was observed by the decrease in absorbance at 517 nm where as colour changes from purple to yellow. The various fractions of *Fumaria indica* significantly reduced DPPH radicals. It was observed from the results that activity was increased by increasing the concentration of the fractions in the assay. The various concentrations of ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 83.41 ± 1.23% inhibition of DPPH radical at a concentration of 250 µg/ml. The various concentrations of the fractions which showed percent inhibition greater than 50% were found to be significant ($p < 0.05$) when compared with negative control i.e. blank. IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds[18]. IC_{50} is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process. A lower value would reflect greater antioxidant activity of the fraction. Ethyl acetate soluble fraction exhibited lowest IC_{50} value i.e. 79.15 ± 0.87µg/ml as compared to other studied fractions. Chloroform soluble fraction, *n*-butanol soluble fraction and remaining aqueous

fraction showed IC_{50} values 141.04 ± 1.78, 169.47 ± 1.39 and 217.27 ± 1.42 µg/ml respectively. The IC_{50} value was not detected in *n*-hexane soluble fraction. The results were expressed relative to ascorbic acid, a reference standard, having IC_{50} of 58.9 ± 0.97 µg/ml. The IC_{50} values of chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of remaining aqueous fraction was found to be non significant ($p > 0.05$) when compared with ascorbic acid, a reference standard.

In phosphomolybednum complex method, the reduction of Mo (VI) to Mo (V) took place by various fractions of plant which was detected at 695nm by spectrophotometer due to the formation of green phosphate Mo (V) compounds[19]. The assay was successfully used to quantify vitamin E in seeds[12] and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant polyphenols. A higher absorbance indicates a higher antioxidative activity. It was revealed from the results that ethyl acetate fraction showed highest total antioxidant activity i.e. 1.029 ± 0.08 as compared to other fractions. The total antioxidant activities of chloroform, *n*-butanol and remaining aqueous fraction were found to be 0.839 ± 0.07, 0.741 ± 0.11 and 0.523 ± 0.28 respectively. The *n*-hexane fraction showed lowest total antioxidant activity (0.316 ± 0.06). The results were compared with BHT, a reference standard having total antioxidant activity 1.22 ± 0.53. The total antioxidant activity shown by chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction was found to be significant ($p < 0.05$) while that of *n*-hexane and aqueous fraction were found to be non significant ($p > 0.05$) when compared with BHT.

FRAP assay is used for the determination of the reducing power of various samples which is shown by the colour change of the test solution from yellow to blue and green in proportionate to the reducing power of various samples. In a redox- linked colorimetric method antioxidants are used as reductants in FRAP assay and in stoichiometric excess it provides an easy reduced oxidant system[13]. Ferric form in a ferric tripyridyl triazine complex changes to ferrous form showing intense blue colour. This change was observed by measuring the absorption at 593 nm. In the reaction mixture the absorption change was linked directly with the total reducing power of electron donating antioxidants which reduced the ferric form (ferric cyanide complex) to the ferrous form. From the results it was revealed that among all the fractions the ethyl acetate fraction showed highest FRAP value (249.66 ± 1.83 TE M/mL). Chloroform fraction, and *n*-butanol fraction also showed good FRAP values i.e. 148.52 ± 1.69 TE M/mL, and 178.43 ± 1.41 TE M/mL respectively while *n*-hexane fraction and remaining aqueous fraction showed very less FRAP values i.e. 35.71 ± 0.98 TE M/mL, 32.64 ± 0.95 TE M/mL respectively. High FRAP values obtained for polar fractions may be ascribed partially to the presence of phenolic and flavonoid contents. The FRAP values of chloroform soluble fraction, ethyl acetate soluble fraction, and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with blank.

The antioxidant activity of plant materials correlated well with the phenolic content. The phenols concentration in the different extracts is expressed as microgram of gallic acid equivalents (GAEs) per gram of extract[20]. The plant fractions were evaluated by using Follin-Ciocalteu´s reagent known as FC reagent for the determination of total phenolic contents. When the phenolic compounds are reduced by different hydroxyl species then the colour of FC reagent changes from yellow to blue according to the amount of phenolic compounds present in the plant extracts. This change was observed spectrophotometrically at 725nm. Results showed that highest concentration of the total phenolics was shown by the ethyl acetate soluble fraction (123.23 ± 0.41 GAE µg/g) comparative to other five fractions. The total phenolic contents of chloroform soluble fraction, *n*-butanol soluble fraction and aqueous fraction were found to be 104.18 ± 1.94, 96.37 ± 0.83 and 46.58 ± 0.61 GAE µg/g respectively. Lowest concentration of the total phenolics was shown by the *n*-hexane soluble fraction i.e. 29.14 ± 0.68 GAE µg/g. The results for total phenolic contents of chloroform soluble fraction, ethyl acetate soluble fraction, and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and remaining aqueous fraction were found to be non significant ($p > 0.05$) when compared with blank.

Oxygen reacts with unsaturated double bond on the lipid which results in generation of free radicals and lipid hydroperoxides. Peroxidation of lipids occurs both *in vivo* and *in vitro* and gives rise to cytotoxic and reactive products. These products disturb the normal functioning of the cell and can give rise to damaged or modified DNA. Hydrogen donating antioxidants can react with lipid peroxyl radicals and break the cycle of generation of new radicals. The ferric thiocyanate assay is used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate, a reddish pigment[21]. The various fractions of plant were tested by this assay and results have been shown in table 3.The fractions which showed greater values of percent inhibition of lipid peroxidation might contain primary antioxidant compounds, which are able to react aggressively with free radicals, particularly hydroxyl radicals, thereby terminating the radical-chained reaction and retarding the formation of hydroperoxide[22]. Highest percentage of inhibition of lipid peroxidation was exhibited by ethyl acetate fraction (51.16 ± 1.48%) at concentration of 500 µg/ml, while *n*-hexane soluble fraction showed lowest percentage of inhibition of lipid peroxidation (8.57 ± 0.08%). Chloroform, *n*-butanol and aqeuous fractions exhibited percent inhibition of lipid peroxidation 43.27 ± 0.75%, 36.52 ± 0.69% and 18.22 ± 0.63% respectively. The inhibition of lipid peroxidation by BHT (standard) was found to be 62.96 ± 1.18%. The results for percent inhibition of lipid peroxidation of chloroform soluble fraction, ethyl acetate soluble fraction and *n*-butanol soluble fraction were found to be significant ($p < 0.05$) while that of *n*-hexane soluble fraction and aqueous fraction were found to be non significant ($p > 0.05$) when compared with BHT.

CONCLUSION

It was observed from the results that chloroform fraction, ethyl acetate fraction, and *n*-butanol fraction showed good antioxidant activities probably due to presence of phenolics and flavonoids. Ethyl acetate soluble fraction exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 83.41 ± 1.23% inhibition of DPPH radical at a concentration of 250 µg/ml. The IC_{50} of this fraction was 79.15 ± 0.87 µg/ml, relative to ascorbic acid,

having IC_{50} of 58.9 ± 0.97 highest total antioxidant activity (1.029 ± 0.08), as well as highest FRAP value (249.66 ± 1.83 µg of trolox equivalents), highest total phenolic contents (123.23 ± 0.41) and highest value of inhibition of lipid peroxidation (51.16 ± 1.48% at concentration of 500 µg/ml) as compared to the other studied fractions. So it was concluded that chloroform fraction, ethyl acetate fraction and *n*-butanol fraction are rich in strong antioxidants. So it was concluded from the present study that these fractions are potentially valuable sources of natural antioxidants and bioactive materials and further phytochemical investigations on this plant may bring new natural antioxidants into the food industry that might provide good protection against the oxidative damage which occurs both in the body and our daily foods.

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.**Table 1**. Phytochemical constituents of various fractions of *Fumaria indica* Pugsley.

Test	*n*-hexane soluble fraction	Chloroform soluble fraction	Ethyl acetate soluble fraction	*n*-Butanol soluble fraction	Remaining Aqueous fraction
Alkaloids	−	++	+++	++	+
Terpenoids	+	++	+	_	−
Saponins	_	−	−	−	+
Tannins	−	−	+	+	+
Sugars	−	−	+	+	+
Phenolics	−	++	+++	++	+
Flavonoids	−	+	++	+	+
Cardiac Glycosides	−	-	+	+	-

Note: '+' represents presence and '–' represents absence.

Table 2. 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH) radical scavenging activity of the various fractions of *Fumaria indica* Pugsley.

Sr.No.	Sample	Concentration in assay (µg/ml)	% scavenging of DPPH ± S.E.M[a)]
1	*n*-Hexane soluble fraction	1000 500 250	29.28 ± 0.15 18.46 ± 0.09 5.30 ± 0.04
2	Chloroform soluble fraction	500 250 125 60	73.87 ± 1.71* 59.28 ± 1.23* 45.19 ± 0.91 25.85 ± 0.35
3	Ethyl acetate soluble fraction	250 125 60 30	83.41 ± 1.23* 67.16 ± 1.63* 32.86 ± 1.58 24.31 ± 0.88
4	*n*-Butanol soluble fraction	250 125 60	68.03 ± 0.96* 45.58 ± 0.67 17.99 ± 0.44
5	Aqueous fraction	1000 500 250 125	76.48 ± 1.49* 58.57 ± 0.76* 53.43 ± 0.61 45.71 ± 0.54
6	Ascorbic acid[b)]	125 60 30	79.45 ± 0.25* 59.09 ±0.39* 30.11 ± 0.55

[a)] All results are presented as mean ± standard mean error of three assays.
[b)] Standard antioxidant.
*p < 0.05 when compared with negative control i.e. blank/solvent (p<0.05 is taken as significant).

Table 3. IC_{50}, total phenolics, total antioxidant activity, FRAP values and lipid peroxidation inhibition values of different fractions of *Fumaria indica* Pugsley.

Sr. No.	Sample	DPPH-radical scavenging activity(IC_{50};µg/mL)	Total antioxidant activity	FRAP value (TE M/mL)	Total phenolics (GAE µg /g)	Inhibition of lipid peroxidation (%)
1	*n*-Hexane soluble fraction	ND***	0.316 ± 0.06	35.71 ± 0.98	29.14 ± 0.68	8.57 ± 0.08
2	Chloroform Soluble fraction	141.04 ± 1.78**	0.839 ± 0.07**	148.52 ± 1.69*	104.18 ± 1.94*	43.27 ± 0.75**
3	Ethyl acetate soluble fraction	79.15 ± 0.87**	1.029 ± 0.08**	249.66 ± 1.83*	123.23 ± 0.41*	51.16 ± 1.48**
4	*n*-Butanol soluble fraction	169.47 ± 1.39**	0.741 ± 0.11**	178.43 ± 1.41*	96.37 ± 0.83*	36.52 ± 0.69**
5	Aqueous fraction	217.27 ± 1.42	0.523 ± 0.28	32.64 ± 0.95	46.58 ± 0.61	18.22 ± 0.63
6	Ascorbic acid[a)]	58.9 ± 0.97	–	–	–	–
7	BHT[b)]	–	1.22 ± 0.53	–	–	62.96 ± 1.18
8	Blank[c)]	–	–	22.73	14.96	–

All results are presented as mean ± standard mean error of three assays.
a) expressed relative to ascorbic acid.
b) expressed relative to BHT.
c) expressed relative to blank.

*p< 0.05 when compared with negative control i.e. blank/solvent (p<0.05 is taken as significant).
**p < 0.05 when compared with reference standards (BHT/Ascorbic acid).
***not detected.

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