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Antioxidant evaluation of ethanolic extract of *Fumaria parviflora* Lam. obtained from root, stem, leaf and fruit and measurement of their total Phenols and flavonoids

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Abstract

Present study deals with antioxidant capacity of different ethanolic extracts obtained from various parts of *Fumaria parviflora* Lam. The antioxidant activity of ethanolic extract of root, stem, leaf and fruit was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method from concentration 125 µg/mL to 1000 µg/mL. In this method, the ethanolic extract of leaf of *Fumaria parviflora* Lam. was found to be the most active and reference was used as quercetin. Total phenols and flavonoids substance of the ethanolic extracts were determined by UV- visible spectrophotometer using Folin-Ciocalteu's and AlCl₃ reagents, respectively. The data revealed that antioxidant activity of ethanolic extract of leaf was found to be more effective followed by stem, fruit, and root. From study it can be concluded that the ethanolic extract of leaf of *Fumaria parviflora* Lam. have more polyphenols and flavonoids compounds and these are responsible for the antioxidant activity.

Keywords: quercetin, *F. parviflora* Lam., antioxidant, ethanolic extract

Introduction

Antioxidant (free radical scavengers) ^[1] agents are present in different part of the plant which have been used as an important protective components for human health ^[2]. Antioxidants potential of the plants or their crude extracts due to the presence of flavonoids and phenolic acids which are present in different parts of medicinal plants ^[3]. In the pharmaceutical and food industries used synthetic antioxidants to preserve the formulations and food that is butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG), and tert-butyl hydroquinone (TBHQ) as a food preservative ^[4]. Synthetic BHA and BHT as antioxidants are produced some toxicity in the human body and manufacturing cost is more than natural antioxidants ^[5]. Main motive of this study is isolating the natural antioxidants from the vegetable sources and substitute of synthetic with natural antioxidants may be useful for our community.

Material and Method

Chemicals and Standards

All chemicals and reagents used were analytical grade. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, quercetin, gallic acid, ethanol, ferric chloride, potassium ferricyanide, trichloroacetic acid, Folin- Ciocalteus phenol reagent.

Plant material

The whole plant of *Fumaria parviflora* Lam. was collected from the region of Sirsa, Haryana (India). The collected plant was authenticated from Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR, New Delhi. A sample has been deposited in the Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR, reference no. NISCAIR/RHMD/Consult/2015/2811/04.

Preparation of extract

The different parts (root, stem, leaf and fruit) of the plant were dried under the shade and ground the coarse powder in grinding machine. Ethanolic extracts were prepared by extracting the ground powders of root, stem, leaf and fruit on soxhlet extraction apparatus. The each ethanolic extract was dried on rotary evaporator and stored in desiccators for further study ^[6-7].

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Measurement of total phenol and flavonoid substances of the extracts

The total phenolic substances of extracts were determined by Folin- Ciocalteus reagent by Singleton and Ross's method, 1965 [8]. Folin- Ciocalteu's reagent (750 µl) and sodium carbonate (600 µl) was mixed with sample in test tubes. The tubes were sonicated and incubated for 30 min at 40 °C. Absorbance was taken at 760 nm on UV- visible spectrophotometer. Total flavonoid substances were measured by aluminium chloride colorimetric method. Quercetin was used as a standard in this method. To prepare the calibration curve from different dilutions of the quercetin (10 µg/mL to 50 µg/mL) and same dilutions of the sample were also prepared. 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate as well as 2.8 mL of distilled water. Following incubation for 30 min at room temperature, absorbance of the reaction mixture was measured at wavelength of 415 nm with a UV-visible spectrophotometer. The total phenol and flavonoid substances of the various ethanolic extracts were measured as the percentage of gallic acid and quercetin equivalents per mg/g extract (mg/g) respectively [9-10].

Antioxidant activity studies

The following antioxidant methods were performed on the all ethanolic extracts of different parts of plant.

DPPH free radical scavenging activity [11-12]

The DPPH solution (0.1mM) was prepared by dissolving in ethanol. 3 mL of DPPH solution was added in different dilutions of samples range 125 µg/mL to 1000 µg/mL. Quercetin used as reference was made same way as sample. The control was prepared by without adding the referece/ sample. Absorbance of the different dilutions of extracts/reference was taken at 520 nm on UV- visible spectrophotometer. The free radical scavenging activity was calculated by using the following formula

$$\text{Inhibitory percentage scavenging activity} = [(A_c - A_d)/A_c] \times 100$$

Ac = Absorbance of control, Ad = Absorbance of sample/reference

Results

Quantitative determination of total phenols and flavonoids content

The TPC and TFC of ethanolic extract of root, stem, leaf and fruit of *Fumaria parviflora* Lam. were calculated on the basis of a standard curve of gallic acid and quercetin shown in Figure 1 and 2. As is evident from the Table 1 and 2, the highest percentage of phenols as well as flavonoids content was found in ethanolic extract of leaves followed by stem, fruit and root. Negligible flavonoids content were observed in ethanolic extract of root and fruit.

DPPH free radical scavenging assay

The DPPH radical scavenging activity of ethanolic extract of root, stem, leaf and fruit of *Fumaria parviflora* Lam. were shown in Table 3 as comparable with known antioxidant quercetin. The scavenging effects of ethanolic extracts of various parts of plant on DPPH radical were in the following order: EEL > EES > EEF > EER. At a concentration of 125 µg/ml - 1000 µg/ml, the values for % scavenging of DPPH for

ethanolic extract of leaves ranged between 58 -82.64%. Comparatively lower activity was observed with stem, fruit, and root crude ethanol extract where the % scavenging of DPPH ranged between 42.24 -74.82%, 32.06% and 29.65 - 58.32% respectively.

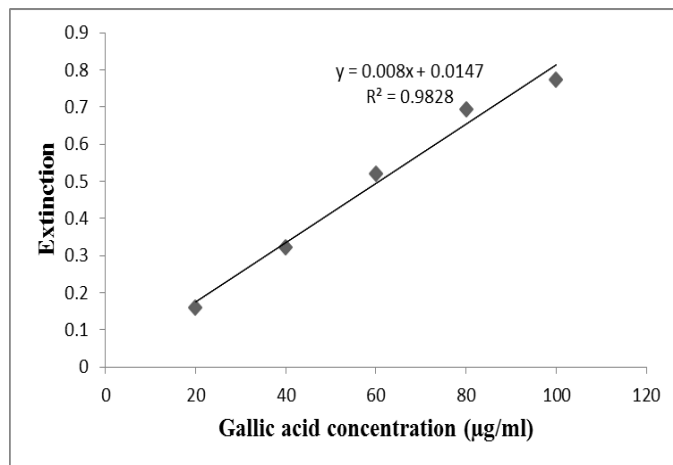


Fig 1: Calibration curve of absorbance against Gallic acid concentration

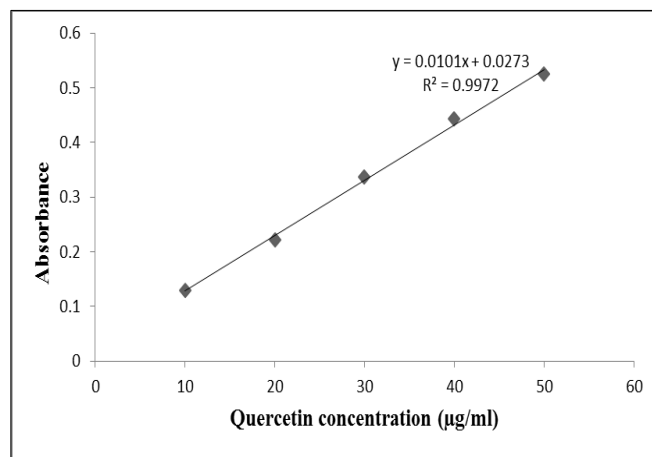


Fig 2: Calibration curve of absorbance against Quercetin concentration

Table 1: Total Phenolic content in ethanolic extract of root, stem, leaf and fruit of *Fumaria parviflora* Lam.

Extract of various plant part	Total phenols content (%w/w) values expressed in mean ⁿ ± S.D ^a .
Root ethanolic extract	2.14 ± 0.01
Stem ethanolic extract	3.20 ± 0.03
Leaf ethanolic extract	4.83 ± 0.02
Fruit ethanolic extract	2.30 ± 0.004

n = 3, a = Standard deviation

Table 2: Total Flavonoids content in ethanolic extract of root, stem, leaf and fruit of *Fumaria parviflora* Lam.

Extract of various plant part	Total flavonoids content (%w/w) values expressed in mean ⁿ ± S.D ^a .
Root ethanolic extract	0.01 ± 0.001
Stem ethanolic extract	0.09 ± 0.01
Leaf ethanolic extract	1.62 ± 0.02
Fruit ethanolic extract	0.02 ± 0.001

n = 3, a= Standard deviation

Table 3: Antioxidant activity of ethanolic extract of root, stem, leaf and fruit of *F. parviflora* Lam.

Extract of various plant part	Inhibition % mean values ⁿ ± S.D ^a . against DPPH			
	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
Root ethanolic extract	29.65 ± 0.15	35.67 ± 0.11	43.80 ± 0.04	58.65 ± 0.07
Stem ethanolic extract	42.24 ± 0.22	50.93 ± 0.15	59.99 ± 0.22	71.82 ± 0.11
Leaf ethanolic extract	58 ± 0.04	63.03 ± 0.07	74.13 ± 0.18	82.64 ± 0.04
Fruit ethanolic extract	32.06 ± 0.11	35.92 ± 0.07	48.17 ± 0.15	60.91 ± 0.11
Quercetin	63.15 ± 0.07	70.57 ± 0.01	77.84 ± 0.04	90.16 ± 0.02

n = 3, a= Standard deviation

Discussion

Polyphenols (TPC and TFC) contents

Polyphenols were found in all plant parts of ethanolic extracts. The obtained results for DPPH are in agreement with the phenol contents determined for each sample. These phenolics are important dietary antioxidants because they have ideal structural chemistry for free radical scavenging activities, and have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis. Our present study finding that leaves of *Fumaria parviflora* Lam. in phenolic and flavonoids contents which are the major component for antioxidant activity. It is also reported that secondary metabolites in the extracts such as polyphenols, flavonoids, flavonols, tannins, phytosterols, alkaloids and glycosides are important phytoconstituents of plant which are responsible for antioxidant activity [13]. Preliminary phytochemical evaluation of ethanolic extract of leaves also revealed the presence of above class of phytoconstituents. Therefore, the antioxidant activity may also come from other antioxidant present in the extract as well.

DPPH radical scavenging activity

DPPH radical scavenging model is widely used method to evaluate antioxidant activity of natural compound and plant extracts. The level of decolorizing indicates the scavenging potency of the antioxidant extract, which is due to the hydrogen donating ability. IC₅₀ value of 250µg/mL to 1000µg/mL for DPPH radical scavenging has been reported for the ethanol extract of this whole plant [14, 15]. The present study revealed that crude ethanol extract of leaves have the more effects of DPPH radical scavenging activity. Presence of flavonoids and Phenolic components -quercetin, tannins and steroids in leaves might be contribute towards the DPPH radical scavenging activity since these classes of compounds are known as free radical scavenger. Thus, the consumption of *Fumaria parviflora* Lam. leaves can be beneficial in preventing oxidative stress related numerous chronic diseases.

Conclusion

The natural antioxidants are many advantageous over the synthetic antioxidants because of that are more beneficial for human health. In the present study, analysis of free radical scavenging activity and total phenolic and flavonoid contents showed that the ethanolic extract of *Fumaria parviflora* Lam. leaves can be the more effective source of natural antioxidants. Though, further detailed study, mainly *in vivo* antioxidant studies and fractionation, isolation are needed to justify its use as a natural source of antioxidants to play a role in helping to prevent diseases.

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