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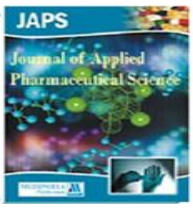

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Evaluation of antioxidant potential of different parts of wild edible plant *Passiflora foetida* L.

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ABSTRACT

The purpose of the present study was to investigate the in vitro anti oxidant activities of petroleum ether, ethanol and hot water extract of *Passiflora foetida* root, leaves, flower, fruit peel and seed. Plant material was extracted in soxhlet extractor successively with petroleum ether and ethanol. Extracts of *P.foetida* root, leaves, flower, fruit peel and seeds were analysed for the quantification of total phenolics, tannins and ascorbic acid (vitamin c). The antioxidant property was estimated using reducing power, metal chelating, hydroxyl radical scavenging, nitric oxide radical scavenging, $ABTS^{\cdot+}$, $DPPH^{\cdot}$, antihemolytic and β-carotene assays. P. foetida fruit peel was found to be much effective when compared to other parts taken for the present study. It is very clear that this is a plant with tremendous wide spread use and also with extraordinary potential for the pharmaceuticals.

Key words: *Passiflora foetida,* DPPH, $ABTS^{\cdot+}$, antihemolytic, β-carotene.

INTRODUCTION

Medicinal plants are nature gift to mankind. These plants have been used by man for centuries in various traditional system of medicine like Ayurveda, Siddha, Unani etc. (Ranganathan,1999). Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimum side effects and relatively low cost (Valiathan, 1998). Medicinal plants are the important source of life saving drugs for the majority of the world's population. *Passiflora* is the largest genus in the Passifloraceae family and comprises nearly 500 species (Vanderplank, 1996). *Passiflora foetida* L. (Stinking passion flower) is South American origin, which has been spread to many tropical areas. It is found in riverbeds, dry forest floors, and wayside thickets, covering the top thorny shrubs and also growing near hamlets. The mostly available wild species are *P. edulis, P. leschenaultia, P. mollissima* and *P. subpeltata*. The traditional medicines are used to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases. The ethanobotanical views of *P.foetida*, suggest that decoction of leaves and fruits is used to treat asthma and biliousness, leaves and root decoction is emmenagogue, used in hysteria and leaf paste is applied on the head for giddiness and headache (Chopra *et al.*, 1956). In Brazil, the herb is used in the form of lotions and skin diseases with inflammation (Chopra *et al.*, 1944). The major phytoconstituents of this plant are alkaloids, phenols, glycosides, flavonoids and cyanogenic compounds (Dhawan *et al.*, 2004) and passifloricins, polypeptides and alpha-pyrones in *P. foetida* (Echeverri *et al.*, 2001). Several years ago (Echeverri *et al.*, 1991) the resin of the leaves of *P. foetida* found. Some authors describe the use of *Passiflora* species in the popular medicine for the inflammatory diseases. About 294 volatile compounds have been identified in several passion fruit extracts (Shibamoto and Tang, 1990). The purpose of the present study was to investigate the *in vitro* antioxidant activities of

petroleum ether, ethanol and hot water extract of *Passiflora foetida* root, leaves, flower, fruit peel and seed.

MATERIALS AND METHODS

Chemicals

Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2'aninobis(3-ethylbenzothiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediamine tetracetic acid (EDTA) disodium salt, 2,2'-bipyridyl and hydroxylamine hydrochloride were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

Collection of plant materials

The fresh plant parts of *Passiflora foetida* L. were collected from Maruthamalai forest hills, Coimbatore. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical survey of India, Southern circle Coimbatore, Tamilnadu. The air-dried, powdered plant material was extracted in soxhlet extractor successively with petroleum ether and ethanol. Finally, the material was macerated using hot water with occasional stirring for 24 hr and the water extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator and then air-dried. The percentage yield was expressed in terms of air-dried weight of plant material. The extracts were freezing dried and stored in desiccators until further analysis

In vitro antioxidant studies

Determination of total phenolic and tannin contents

The total phenolic content of *P. foetida* plant extracts was determined by Folin ciocalteu method. Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). The amount of total phenolics and tannins were calculated as the tannic acid equivalents (TAE) as described by (Siddhuraju & Becker, 2003).

Determination of ascorbic acid (vitamin c)

The ascorbic acid was determined using the dichloro indophenols (DIP) method with a modification by (Yen and Chen, 1996). The concentrations of the ascorbic acid in the sample were determined through comparison with the absorbance of standard ascorbic acid at different concentration.

Reducing power

The reducing power of different solvent extracts of *P. foetida* was determined by the method reported by (Siddhuraju et al., 2002). 20-100 µg of extract was taken in 1 mL of phosphate buffer and 5 mL of 0.2 M phosphate buffer (pH 6.6) was added. To this, 5 mL of 1% Potassium ferricyanide solution was added and the mixture was incubated at 50˚ C for 20 min. After the incubation, 5mL of 10 % TCA was added. The content was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5mL) was mixed with 5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride. The absorbance of the reaction mixture was measured spectroscopically at 700 nm.

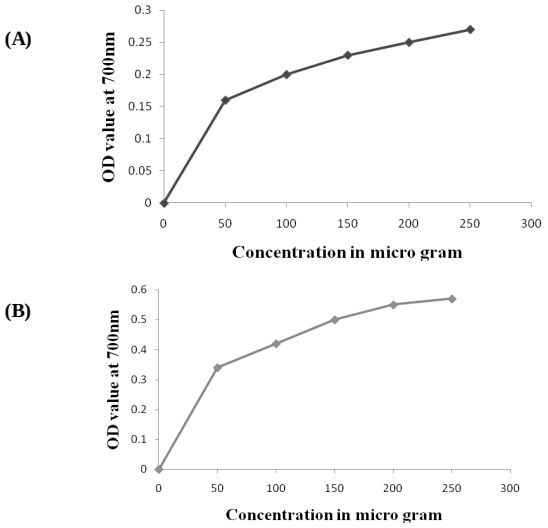


Fig: 1. Reducing power of *P.foetida* root & Reducing power of *P.foetida* peel. (A) Ethanol, (B) Aqueous extract.

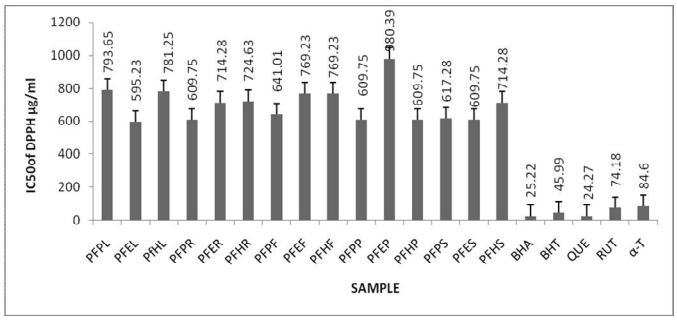
Metal chelating activity

The chelating of ferrous ions by various extracts of *P. foetida* parts were estimated by the method of (Dinis et al., 1994). Briefly the extract samples (250 µL) were added to a solution of 2 mmol/L FeCl2 (0.05 mL). The reaction was initiated by the addition of 5 mmol/ L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Hydroxyl radical scavenging activity

The scavenging activity of petroleum ether, ethanol and hot water extracts of *P. foetida* on hydroxyl radical was measured according to the method (Klein et al., 1991) various concentrations (50,100,150,and 200µg) of extracts were added with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of DMSO (0.85%, v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90^{o}C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1.0 mL of icecold TCA (17.5%, w/v). Three mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity (HRSA) is calculated by the following formula:

$$\%HRSA = [(Control\ OD - Sample\ OD)/Control\ OD] \times 100$$



PFPL - *P. foetida* petroleum ether extract of leaves; PFEL - P. *foetida* ethanol extract of leaves; PFHL - *P. foetida* hot water extract of leaves ; PFPR - *P. foetida* petroleum ether extract of roots; PFER - *P. foetida* ethanol extract of roots; PFHR - *P. foetida* hot water extract of roots; PFPF - *P. foetida* petroleum ether extract of flowers; PFEF - *P. foetida* ethanol extract of flowers; PFHF - *P. foetida* hot water extract of flowers; PFPP - *P. foetida* petroleum ether extract of peels; PFEP - *P. foetida* ethanol extract of peels; PFHP - *P. foetida* hot water extracts of peels; PFPS - *P. foetida* petroleum ether extract of seeds; PFES - *P. foetida* ethanol extract of seeds; PFHS - *P. foetida* hot water extract of seeds. BHA - Butylated hydroxyanisole; BHT - Butylated hydroxytoluene; QUE – Quercetin; RUT - Rutin; α-To – α-tocopherol.

Fig. 2. $DPPH^{\cdot+}$ free radical scavenging activity of *P.foetida*. *Values are means of three replicate determinations (n=3) ± standard deviation.

Nitric oxide radical scavenging activity

The nitric oxide scavenging activity of different solvent extracts of *P. foetida* on nitric oxide radical was measured according to the method of (Sreejayan and Rao, 1997). Sodium nitroprusside (10 mM) in phosphate buffered saline, was mixed with different concentrations (50- 250 µg) of P. *foetida* extracts and incubated at room temperature for 150 min. Griess reagent (0.5mL), containing 1% sulphanilamide, 2% H3PO4 and 0.1% N-(1 – naphthyl) ethylene diamine dihydrochloride was added to the mixture after incubation time. The absorbance of the chromophore formed was read at 546 nm. Gallic acid and quercetin and the same mixture of the reaction without *P. foetida* extracts were employed as positive and negative control. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample.

Total antioxidant activity assay by radical cation ($ABTS^{\cdot+}$)

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hr before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30ºC to give an absorbance at 734 nm of 0.70±0.02 in a 1-cm cuvette (Re et al., 1999). The concentration of the extracts that produced between 20-80% inhibitions of the blank absorbance was determined and adapted. After the addition of 1 mL of diluted ABTS•+ solution to 10 µL of plant extract or Trolox standards (final concentration 0-15 µM) in ethanol, optical density (OD) was taken at 30ºC exactly 30 min after the initial mixing. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

Radical scavenging activity using $DPPH^{\cdot}$ method

The DPPH• radical scavenging activity of *P. foetida* plants parts extracts was measured according to the method of (Blios, 1958). IC50 values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Antihemolytic activity

The antihemolytic activity was determined according to the method described by (Naim et al., 1976). The erythrocytes from cow blood were separated by centrifugation and washed with phosphate buffer (pH 7.4) until the supernatant become colorless. The erythrocytes were diluted with saline phosphate buffer to give 4 % suspension. 500 µg of sample and 1 mL saline buffer were added to 2 mL of the suspension of erythrocytes and the volume was made up to 3.5 mL with saline buffer. This mixture was preincubated for 5 min and then 0.5 mL H_2O_2 solution of appropriate concentration in saline buffer was added .The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring about 90% haemolysis of blood cells after 240 min. After the incubation time the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of haemolysis was determined by measurement of the absorbance at (540 nm) corresponding to haemoglobin liberation.

β-carotene/linoleic acid peroxidation inhibition activity

The antioxidant activity of *P. foetida* was evaluated by the β-carotene linoleate model system. One milliliter of a β-carotene solution in chloroform (1 mg/10 ml) was pipetted into a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator (Yamato RE300, Japan) at 45 °C for 4 min and, 50 ml of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 200 µl of the antioxidant (extracts, BHA or α-tocopherol) solution at 1 mg/mL concentration and the absorbance was measured at 470 nm immediately, against a blank consisting of the emulsion without β-carotene (Taga, Miller, & Pratt , 1984). The tubes were placed in a water bath at 50 °C and the absorbance was monitored at 15 min intervals until 180 min. All determinations were carried out in triplicate.

Statistical analysis

The values are mean of triplicate analysis of the samples (n=3) and ± indicates standard deviation. Analysis of variance and significant differences ($P<0.05$) among means were tested by one-way ANOVA and Dunnet multiple range test.

RESULTS

Total yield percentage

The ethanol extracts of all the parts used for the analysis, showed higher percentage recovery over other solvent extracts. On the other hand, petroleum ether extracts of all the parts used, showed lower percentage recovery compared to other solvent extracts. Thus, the maximum percentage recovery was observed in

P. foetida ethanolic extract of root while the minimum one was shown by *P. foetida* petroleum ether extract of peel.

Total phenolics and **Tannins**

The total phenolic content determination showed that *P. foetida* ethanolic peel extract possess higher (10.09 %) phenolic content over the other extracts such as ethanol extract of root (9.3%), petroleum ether leaf extract (7.80 %), (6.95 %), hot water extract of seed (5.42%), and petroleum ether extract of flower (5.26 %). Total phenolics content of different parts of the solvent extract of *P. foetida* were shown in the table.1. Among the different samples analysed, the tannin content was found to be maximum (4.73%) in ethanol root extract, whereas the other sample extracts showed minimum tannin content. The results are showed in the (Table 1).

Table 1. Total phenolics, tannins and vitamin C of *P.foetida.*

Extraction medium	Sample	Total Phenolics	Tannins	Vitamin C
Petroleum ether	Leaf	7.8 ± 0.1	3.1 ± 0.2	2.3 ± 0.0
	Root	4.7 ± 0.0	0.1 ± 0.3	1.7 ± 0.0
	Flower	5.2 ± 0.2	0.5 ± 0.0	1.3 ± 0.0
	Peel	6.9 ± 0.3	0.6 ± 0.3	2.4 ± 0.0
	Seed	5.0 ± 0.0	0.6 ± 0.1	1.3 ± 0.0
Ethanol	Leaf	4.8 ± 0.2	0.004 ± 0.3	1.6 ± 0.0
	Root	9.3 ± 0.1	4.7 ± 0.2	1.5 ± 0.0
	Flower	4.3 ± 0.0	0.8 ± 0.2	1.3 ± 0.0
	Peel	10.0 ± 0.0	2.7 ± 0.3	1.5 ± 0.0
	Seed	4.5 ± 0.1	0.7 ± 0.1	1.4 ± 0.0
Hot water	Leaf	4.3 ± 0.0	0.1 ± 0.2	1.3 ± 0.0
	Root	4.9 ± 0.1	0.3 ± 0.2	1.2 ± 0.0
	Flower	4.8 ± 0.0	1.1 ± 0.2	1.3 ± 0.0
	Peel	4.8 ± 0.1	0 ± 0.0	1.2 ± 0.0
	Seed	5.4 ± 0.2	0.5 ± 0.0	1.4 ± 0.0

Values are means of three replicate determinations (n=3) ± standard deviation.

Vitamin C

The ascorbic acid content of *P foetida* was found to be higher in petroleum ether extract of peel (2.4%) and petroleum ether extract of leaf (2.3%), while the other extracts showed Vitamin C content ranging from 1.2% - 1.7%. The results are showed in the (Table 1).

Reducing power

In the reducing power assay, the presence of reductants (antioxidants) in tested samples would result in the reduction of Fe^{3+}/ ferricyanide complex to the ferrous form. The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussion blue at 700 nm (Chung et al., 2002). The reducing power of different solvent extracts of the samples was shown in (Figure 2). A strong reducing power was noted for the samples ethanol extract of root and hot water extract of peel (0.57 OD_{700}). Much lower reducing power was found for the sample petroleum ether extract of root (0.27 OD_{700}).

Metal chelating activity

The transition metal iron is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular diseases (Chung et al.,2002). Since Fe^{2+} also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. In the metal chelating assay, ferrozine can quantitatively form complexes with Fe^{2+}. The chelating effects on the ferrous ions by the various solvent extracts of different parts of the *P. foetida* were shown in (Table 2). All the sample extracts exhibited the ability to chelate metal irons. Among the different samples extracts, the ethanol extract of flower showed higher metal chelating activity (6748.1mg/g) than other sample extracts.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radical formed by the oxidation will react with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals, by treatment, with Nash reagent (Singh, Murthy & Jayaprakasha, 2002). The scavenging activities of different plant parts were shown in (Table 2). The $OH^{\cdot}$ scavenging activities of all the samples were investigated at the concentration of 250µg in the reaction mixture. The ethanol extracts of peel showed the highest level of scavenging activity (92.5%). The flower samples exhibited moderate scavenging activity (62.5%, 64.2%, and 65.5%) compared to other sample extracts.

Nitric oxide radical scavenging activity

Nitric oxide is a very unstable species, so under aerobic condition it can react with O_2 to produce its stable products such as nitrate and nitrite through intermediates NO_2, N_2O_4. The nitric oxide radical scavenging activity was estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease and can be measured at 546nm. The nitric oxide radical scavenging activity of different solvent extracts of all the samples were shown in (Table 2). The higher free radical scavenging activity was exerted by ethanol extract of peel (120.48µg) followed by ethanol extract of flower > hot water extract of leaf > hot water extract of peel > ethanol extract of root > ethanol extract of leaf > hot water extract of leaf > hot water extract of flower > petroleum ether extract of seed > ethanol extract of seed > hot water extract of seed > petroleum ether extract of leaf > petroleum ether extract of flower > ethanol

extract of leaf > petroleum ether extract of peel.

ABTS radical scavenging activity

In $ABTS^{\cdot+}$ cation radical scavenging method, the activity of tested extracts were expressed as micromolar equivalent of Trolox solution having an antioxidant equivalent to one gram dry matter of the sample under the experimental investigation. The effect of various solvent extracts of different plant parts of *P. foetida* is shown in (Table 2). Eventhough the samples exhibited good ABTS radical scavenging activity, the petroleum ether extract of flower showed the highest activity (3991.9µmolg^{-1}). Whereas the ethanol extract of peel showed lower level of activity (1649. 9µmolg^{-1}), when compare to other samples extracts.

Table 2. ABTS*, metal chelating and hydroxyl radical scavenging activities of *P.foetida*

Extraction medium	Sample	TAA (µmol/g DM)	Metal chelating Property (mg EDTA/g extract)	Hydroxyl radical scavenging activity	Nitric oxide radical scavenging activity
Petroleum ether	Leaf	3570.7±217.2	5903.7±325.1	49.9±0.4	204.0 ± 0.5.
	Root	3914.9±57.6	6270.3±89.8	55.8±0.5	259.0 ± 0.3
	Flower	3991.4±54.9	5777.7±86.7	64.2±0.7	206.0 ± 1.5
	Peel	3739.4±35.7	5985.1± 587.9	62.3±2.1	228.3 ± 1.9
	Seed	3140.9±237.0	6348.1± 193.1	58.6±0.6	141.2 ± 0.4
Ethanol	Leaf	3845.2±57.4	5540.7±511.8	25.0±1.2	227.0 ± 0.7
	Root	3725.9±128.6	5129.6±588.9	50.9±1.6	143.2 ± 1.0
	Flower	3710.2±89.1	6748.1±281.3	62.5±0.1	127.2 ± 0.3
	Peel	1649.9±157.0	6325.9± 83.3	92.5±0.6	120.4 ± 0.6
	Seed	3667.4±67.6	6451.8± 145.0	55.9±0.7	180.5 ± 1.3
Hot water	Leaf	3631.4±210.5	5574.0±409.2	44.0±10.0	131.5 ± 1.4
	Root	2094.7±295.7	5562.9±272.2	55.4±0.2	144.5 ± 0.4
	Flower	3680.2±218.2	6562.9±212.0	65.5±2.1	162.8 ± 0.9
	Peel	3543.7±201.2	6077.7± 539.8	78.2±0.5	132.6 ± 0.2
	Seed	2857.4±116.1	6340.7± 73.9	63.0±0.3	200.0 ± 0.6

Values are means of three replicate determinations (n=3) ± standard deviation.

DPPH radical scavenging activity

The free radical-scavenging activities of different parts of the *P. foetida* samples along with standards such as BHA, BHT, Quercitin, Tannic acid and α-Tocopherol were determined by the DPPH radical scavenging activity and the results were shown in (Figure 2). The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a colour change from purple to yellow. A lower value of IC_{50} indicates a higher antioxidant activity. The highest free radical scavenging activity was exerted by ethanol extract of leaf (595.23µg). The free radical scavenging activity was found to be least in petroleum ether extract of peels (980.39 µg). The scavenging effects of various solvent extracts with the DPPH radical in the ascending order is given by ethanol extract of leaf > petroleum ether extract of root > ethanol extract of peel > hot water extract of peel > ethanol extract of seed > petroleum ether extract of seed > petroleum ether extract of flower > hot water extract of seed > ethanol extract of root > hot water extract of root > ethanol extract of flower > hot water extract of flower > hot water extract of leaf > petroleum ether extract of leaf > petroleum ether extract of peel.

Antihemolytic activity

Lipid oxidation of cow blood erythrocyte membrane, mediated by H_2O_2 induces membrane damage and subsequently haemolysis. Moreover, the RBC haemolysis is a more sensitive system for evaluating the antioxidant properties of the phytoceuticals. The antihaemolytic activity of different solvent extracts was given in (Figure 3). At the concentration of 500µg in the final reaction mixture, all the sample extracts exhibited the antihaemolytic activity. Inhibition of haemolysis was found to be the highest in hot water extract of roots (89.09%).

β- Carotene/linoleic acid peroxidation inhibition activity

The β-carotene bleaching method estimates the relative ability of antioxidants compounds to scavenge the radical of linoleic acid peroxide that oxidizes β-carotene in the emulsion phase. In the *β*-carotene bleaching system, the highest antioxidant activity was shown by ethanol extract of leaf with percentage

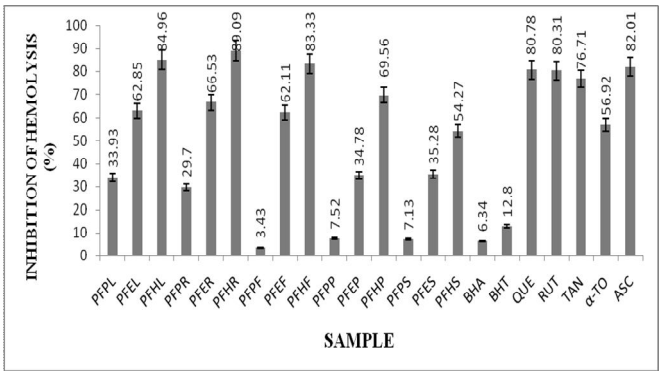


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Fig. 3. Antihemolytic activity of different solvent of *P. foetida*. Values are means of three replicate determinations (n=3) ± standard deviation.

inhibition of 18.52 when compared to other extracts. The results were shown in the (Figure 4).

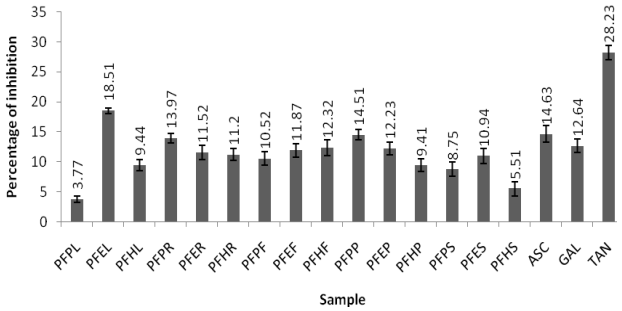

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Fig. 4. Antioxidant activity of β- carotene bleaching method of *P. foetida.*
Values are means of three replicate determinations (n=3) ± standard deviation.

DISCUSSION

Phenolics compounds are commonly found in both edible and non-edible plants, and they have multiple biological effects. Among the different sample extract, total phenol content present in the peel ethanol extract was found to be maximum. Phenolic compounds are widely distributed in plants, which have gained much attention, due to their antimutegenic, antitumor, antioxidant

activities and free radical-scavenging abilities, which potentially have beneficial implication for human health (Li et al., 2006). Among the different samples, the tannin content was found to be maximum in the ethanol extract. Tannins were identified as another large class of phenolics in the tested medicinal herbs. Vitamins are the small molecule dietary antioxidant, quickly moving into an important position in medicinal and nutritional circles (Milton, 1999). Vitamin C is highly bio-available and is consequently one of the most important water-soluble antioxidants in cells. In the present study, vitamin C content was strongly correlated with antioxidant capacity. This supports the observation that vitamin C may be one of the major antioxidants in *P.foetida* fruits. Reducing power Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action, can be strongly correlated with other antioxidant properties (Dorman et al., 2003). *P.foetida* root and peel extracts showed the highest reducing power and the values were comparable to that of ascorbic acid standard. Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron was an extremely reactive metal and will catalyze oxidative change in lipid, protein and other cellular components (Decker & Hultin, 1992). An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton-type reaction. Therefore, it was considered of importance to screen the iron (II) chelating ability to the extracts. In the present study, all the extracts demonstrated the ability to chelate iron. From the iron (II) chelating data, the extracts may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyze Fenton-type reaction or participate in metal-catalyzed hydroperoxide decomposition reactions (Dorman, et al., 2003) Among the different sample extract of *P. foetida*, the ethanol extract of flower showed higher activity. Hydroxyl radical scavenging activity was esrimated by generating the hydroxyl radical using ascorbic acid iron EDTA. The hydroxyl radical formed by the oxidation will react with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals, by treatment, with Nash reagent (Singh, Murthy & Jayaprakasha, 2002). The hydroxyl radical is an extremely reactive free radical formed in biological system and has been implicate as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). In *P.foetida* peel samples a significant correlation between hydroxyl radical scavenging activity and total phenolic content was observed. Nitric oxide is a free radical with a single unpaired electron. Nitric oxide is formed from L-argenine by NO synthase (Fang, Yang, & Wu, 2002). Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and initiate lipid peroxidation (Halliwell, 1996). The nitric oxide scavenging assay procedure is based on the principle that the sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. In the present study, all the extract of *P.foetida* exhibited dose dependent nitric oxide radical scavenging activity. The $ABTS^{\cdot+}$ radical assay can be used to measure the substances, i.e., both aqueous phase radicals and lipid peroxyl radicals (Robert et al., 1999). The scavenging activity of the extract on the radical ABTS, generated by potassium persulfate was compared with a standard amount of trolox. In general, medicinal plants may play an important role in chemical protection from oxidative damage by possessing endogenous antioxidants such as phenolic compounds. Hagerman et al., (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{\cdot+}$). In the present investigation, all the plant extract possessed the free radical property. The ethanol extract of peel exhibited the highest radical scavenging activity. Li et al., (2006) had recently reported that the peels of pomegranate have higher antioxidant activity than its pulp and seed. Regarding these result, it could be considered that the peel extracts of *P.foetida* contain strong antioxidative agents, and had the highest potential. DPPH scavenging is widely used to test the free radical scavenging activity of several natural products (Ahn et al. 2007). DPPH is a stable free radical and any molecule that can donate an electron or hydrogen to DPPH* can react with it and bleach the DPPH* absorption at 517 nm (Huang, Ou, & Prior, 2005). There is a reverse correlation between IC_{50} values and DPPH scavenging activity. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. In the present study, the highest free radical scavenging activity was extracted by ethanol extract of leaf. The result indicates that medicinal plants have significant effects on scavenging free radicals. Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidant but only few studies have been performed with erythrocytes in whole blood. Zhu et al., (2002). Demonstrated a dose dependent activity for three fraction of Oolong tea effective against lipid oxidation in the erythrocytes membrane. In this study, we used a biological test based on the free radical-induced erythrocytes lysis in cow blood. This assay is useful either for screening studies on various molecules and their metabolites, especially on the one hand molecules have an oxidizing or antioxidizing activity and on the other hand molecule having a long-term action (Djeridane et al., 2006). More over the RBC hemolysis is a more sensitive system for evaluating the antioxidant properties of the phytoceuticals. The higher antihemolytic potential was shown by *P.foetida* hot water extract of root, which might attribute to the presence of Vitamin C. β-carotene bleaching method estimates the relative ability of antioxidants compounds to scavenging the radical of linoleic acid peroxide that oxidizes β-carotene in the emulsion phase. The antioxidant assay using the discoloration of β-carotene is widely used because β-carotene is extremely susceptible to free radical mediated oxidation. Due to its 11 pairs of double bonds, which are extremely sensitive to oxidation, β-carotene decolorized easily with the oxidation of linoleic acid (Unten, Koketsum & Kim, 1997). The protective effect of β-carotene can be explained by scavenging of free radicals before they cause damage to cellular macromolecules because

these are known antioxidant with the capacity for free radical trapping. This indicates that the *P.foetida* possess antioxidant activity. Hence, the result justifies the employment of *P. foetida* in treating various ailments and their related disorders..

CONCLUSION

This study supports the idea that the medicinal plants of *P.foetida* have a good source of natural antioxidants. A good correlation between total phenols content and the FRAP values also support the idea that phenols may be the principal contributor of the antioxidant power of botanical materials. It is very clear that this is a plant with tremendous wide spread use and also with extraordinary potential for the pharmaceuticals. Hence further studies should be considered in isolating bioactive compounds from *P.foetida* and it will pave a way for promoting natural drugs for various health benefits.

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