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Research Article

IN VITRO* FREE RADICAL SCAVENGING ACTIVITY AND SECONDARY METABOLITES IN *PASSIFLORA FOETIDA L.

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ABSTRACT:

Investigation of the total phenol content, total flavanoid content, *in vitro* lipid peroxidation and radical scavenging activities from *Passiflora foetida* L. Extraction from various solvents were used for the estimations. Among the five extracts, the flavanoid and phenol content was found to be high in aqueous and ethanol extract of leaf when compared to root extract. Different concentrations of plant extracts were used (100 – 500 µg/ml) and results showed strong reducing power at the concentration of 500µg/ml and the activity increases in dose dependent manner as to that of standard BHT. That the highest free radical scavenging activity was found in ethanolic extract of leaf of *Passiflora foetida* L. Consumption of *P. foetida* L. will reduce the free radical formation and afford protection against reactive oxygen species produced during diabetes which may be due to the presence of phenol and flavanoids.

Keywords: Total flavanoid, total phenols, antioxidants, lipid peroxidation, *Passiflora foetida* L.

INTRODUCTION:

Medicinal plants have played a significant role in various ancient traditional systems of medicine. They are rich sources of bioactive compounds and thus serve as an important raw material for drug production and have become a target for the search of new drugs[1]. Natural bioactive compounds like phenols and flavonoids are the important secondary metabolites in plants having intrinsic properties that affect appearance, taste, odor and oxidative stability of plant based foods. These compounds also posses biological properties like antioxidant, anti-aging, anti-carcinogen, protection from cardiovascular, immune/autoimmune diseases and brain dysfunctions viz. Parkinson's, Alzheimer's, Huntington's diseases, etc[2]. Like in case of laxatives, blood thinners, antibiotics and antimalaria medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively[3]. The plants are the invaluable, incredible and traditional sources for the curability of various diseases in the form of medicines. Plants are the main source of drugs that being used from the ancient times as a herbal remedies for the health care, prevention and cure of various diseases and ailments[4]. Plants secondary metabolites have been implicated for most of the plants therapeutic activites[5]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use the natural products with antidiabetic activity.

Passiflora is the largest genus in the Passifloraceae family and comprises nearly 500 species. The mostly available wild species are *P. edulis, P. incarnata, P. leschenaultia, P. mollissima* and *P. subpelta*. *Passiflora foetida* L. (Stinking passion flower) is South American origin, which has been spread to many tropical areas in India. It is found in riverbeds, dry forest floors, covering the top thorny shrubs and also growing near hamlets[6]. It is commonly called as Mupparisavalli, Siruppunaikkalli in Tamil, Tellajumiki in Telugu, Kukkiballi in Kannada and Chadayan, Poochapazham in Malayalam[7].

The ethanobotanical views of *P.foetida* L. suggest that decoction of leaves and fruits are used to treat asthma, leaves and root decoction is emmenagogue, used in hysteria and leaf paste is applied on the head for giddiness and headache and skin disease. Traditionally, the plant has been used for its properties like antiproliferative, sedative, anti-anxiety, antibacterial, leishmanicidal, antispasmodic, emetic, dressing for wounds and antiulcer[8]. The major phytoconstituents of this plant are alkaloids, phenols, glycosides, flavonoids and cyanogenic compounds, passifloricins, polypeptides and alpha-pyrones[6].

MATERIALS AND METHODS

Collection, identification and powdering of *Passiflora foetida* L.

The leaves, root, fruit peel (both ripened & un ripened) & seed of *Passiflora foetida* L. were collected from in and around Karpagam University, Coimbatore, Tamilnadu and authenticated by Dr.M. Palanisamy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore. The Voucher No is BSI/SRC/5/23/2012-13/Tech-108. All the parts of plant were

washed well with water. They were air dried at 25^0C for 10 days in the absence of sunlight and powdered coarsely using a mixer. They were then weighed and kept in an airtight container and stored in refrigerator for future use.

Preparation of plant extracts Twenty grams of powdered plant material were mixed with 100 mL of various solvents (petroleum ether, chloroform, ethyl acetate, ethanol and distilled water). The plant extracts were prepared by using soxhlet extraction and an orbitory shaker apparatus. After extraction the samples were collected and stored in a vial for further studies.

Estimation of total flavonoids

The flavonoid content was examined by adopting the method developed by Ordon *et al*.,[9] Briefly, 0.5 mL of 2% AlCl 3 in ethanol solution was added to 0.5 mL of sample solution. After one h incubation at room temperature, yellow colour was developed. This was measured at 420 nm with a UV-visible spectrophotometer. A standard graph was prepared using the quercetin and the total flavonoid content was expressed as quercetin equivalent (mg/g).

Estimation of total phenols

Total phenolic content of the ethanolic extract of E. alsinoides was measured based on the Folin-Ciocalteu assay[10]. Briefly, 0.5 mL of the ethanolic extract was first mixed with 2.5 mL of distilled water, and then 0.5 mL of Folin-ciocalteu reagent was added. After 3 min, 2 mL of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for exactly 1 min. It was then cooled and the absorbance was measured at 650 nm using a spectrophotometer against the reagent blank. Total phenolic content was expressed as mg gallic acid equivalents (GAE)/g fresh weight.

Reducing power

Various concentrations of the extracts (100, 200, 300, 400 and 500 mg/ml) were prepared. To all the extracts in test tubes 2.5ml of sodium phosphate buffer was added followed by 2.5 ml of 1% potassium ferricyanide [K_3 Fe (CN) $_6$] solution. The contents were vortexed well and then incubated at 50ºC for 20 minutes. After incubation, 2.5ml of 10% trichloroacetic acid (TCA) was added to all the tubes and centrifugation was carried out at 3000 g for 10 minutes. To 5ml of the supernatant, 5 ml of deionised water was added. To this about 1 ml of 1% ferric chloride was added to each test tube and incubated at 35ºC for 10 minutes. The reducing power of the extract was linearly proportional to the concentration of the sample. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated Hydroxyl Toluene (BHT) was taken as reference standard[11].

DPPH radical scavenging assay

Various concentrations (100-500 µg/ml) of samples and ascorbic acid were taken in different test tubes. The volume was adjusted to 500 µl by adding methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 minutes. The control was prepared as above without any extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. The radical scavenging activity was expressed as the inhibition percentage. The inhibition percentage was calculated using the following formula:

% Radical scavenging activity = [Control OD – Sample OD)/ Control OD] × 100.

Ascorbic acid was taken as reference standard. The percentage inhibition versus concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC_{50} value[12].

Inhibition of *in vitro* lipid peroxidation

Rat liver was processed to get 10% homogenate in cold phosphate buffered saline, pH 7.4 using homogeniser and centrifuged. The degree of lipid peroxidation was assayed by estimating the Thio Barbituric Acid Reactive Substance (TBARS) by using the standard methods with minor modifications. Different concentrations of extracts (100 -500µg /ml) were added to 0.1 ml of liver homogenate. Lipid peroxidation was initiated by the addition of 100µl of 15mM ferrous sulphate solution to the 3 ml of reaction mixture. After 30 minutes, 100 µl of this mixture was taken in a tube containing 1.5 ml of 10 % TCA, mixed well and kept for 10 minutes. The tubes were then centrifuged and supernatants were separated and mixed with 1.5 ml of 0.67 % TBA in 50 % acetic acid. Then the mixture was heated for 30 minutes in a boiling water bath. The intensity of the pink coloured complex formed was measured at 535 nm. The results were expressed as n moles of MDA formed/ mg protein[13].

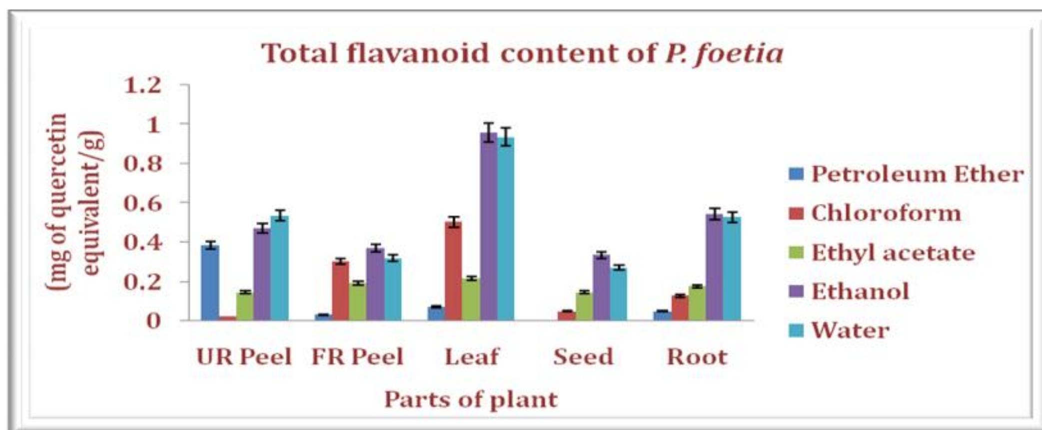
RESULTS AND DISCUSION

Total flavanoid content (TFC) of *P. foetida* L:

Flavanoids are group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. The mechanisms of action of flavanoids are through scavenging or chelating process[14]. The flavanoids have the ability to scavenge free radicals; superoxide and hydroxyl radicals by single-electron transfer[15]. Consumption of flavanoids may be beneficial because they interact with various biological systems and show anti-inflammatory, hypolipidemic, hypoglycemic and antioxidant activities[16]. Flavanoids can affect the functions of cells linked to inflammatory processes, acting on enzymes and pathways involved in anti-inflammatory processes[17].

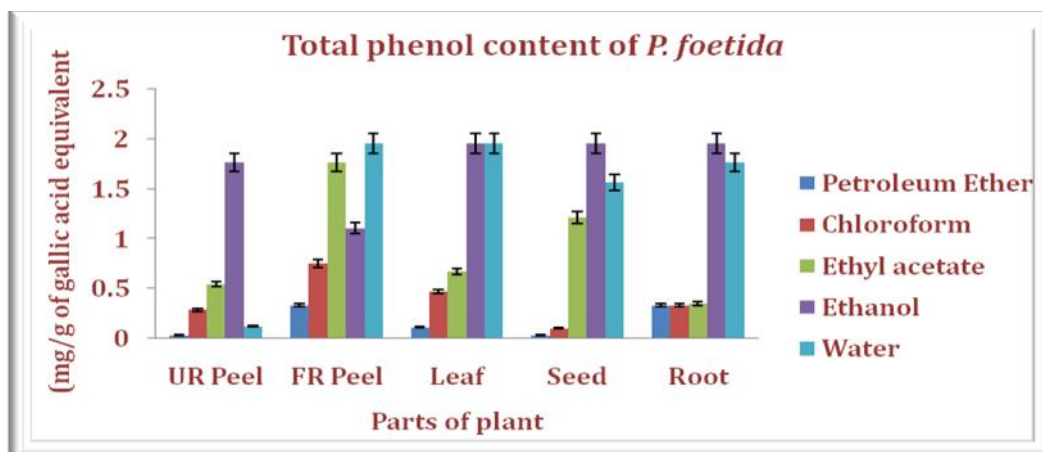
Flavanoid content of different extracts of *Passifolora foetida* L. was studied and the results were represented in figure 1a. Among the five extracts, the flavanoid content was found to be high in aqueous and ethanolic extract of leaf (0.936mg/g, 0.960 mg/g of quercetin equivalent respectively) & root (0.528mg/g, 0.544 mg of quercetin equivalent/g respectively) whereas, less amount of flavanoid content was present in petroleum ether extract of plant parts when compare to other extracts.

Figure 1(a): Estimation of total flavanoid content of *P. foetida* L:



Values are expressed as Mean±SD of three individual experiments

Figure 1(b): Estimation of total phenol content of *P. foetida* L:



Values are expressed as Mean±SD of three individual experiments

Total phenol content (TPC) of *P. foetida* L:

Phenolics compounds are commonly found in both edible and non-edible plants and they have multiple biological effects. 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.The total phenolic content of different extract of *Passiflora foetida* L. was studied. In that aqueous and ethanolic extracts of *P. foetida* L. showed high amount of total phenol content when compare to other extracts. Among the five extracts, the phenol content was found to be high in aqueous and ethanolic extract of leaf (1.960mg/g) & root (1.768mg/g, 1.960 mg/g of gallic acid equivalent respectively) whereas, less amount of phenol content was recorded in petroleum ether extract of plant parts when compare to other extracts (Figure 1b). Our results are supported by[6] who reported that the most of the phenolic content about 9.3% was found in the ethanolic extract of root of *P. foetida* L.

***In vitro* radical scavenging assays of *Passiflora foetida* L.**

The following free radical scavenging assays were performed by using different concentration of aqueous and ethanolic extract of *Passiflora foetida* L. which ranges from 100- 500 µg/ml and IC_{50} value was calculated.

Reducing power

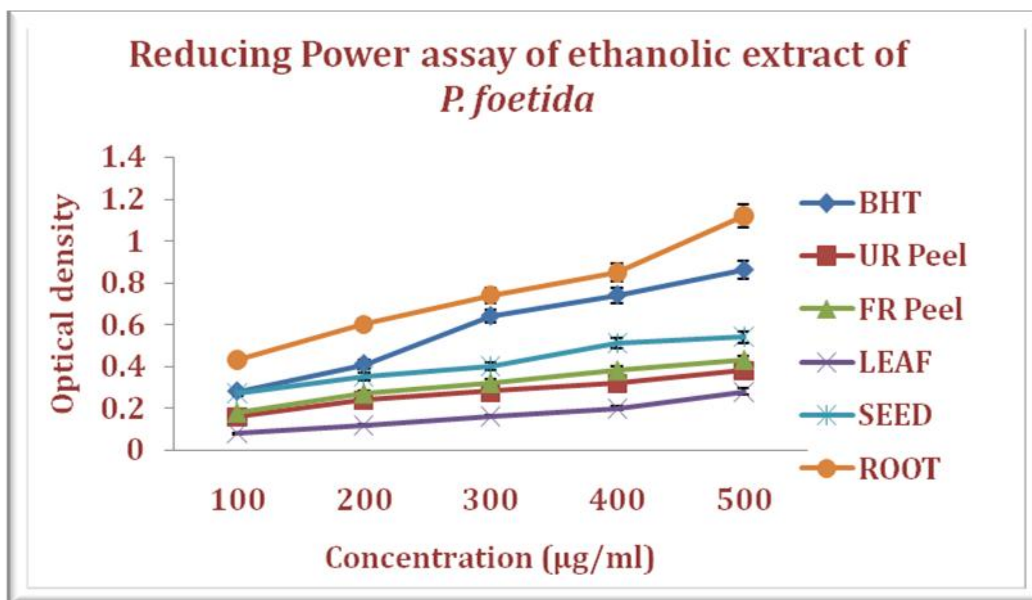
Reducing power Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action, can be strongly correlated with other antioxidant properties[18]. In the reducing power assay, the presence of reductants (antioxidants) in tested 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[19]. The reducing power ability of ethanolic and aqueous extract of *P. foetida* L. was shown in figure 2a & 2b. Different concentrations of plant extracts were used (100 – 500 µg/ml) and the ethanolic extract of root (1.12 µg/ml) showed strong reducing power at the concentration of 500µg/ml and the activity increases in dose dependent manner when compared with that of standard BHT. Similar results were reported by[6].

DPPH radical scavenging assayDPPH scavenging is widely used to test the free radical scavenging activity of several natural products[20]. The DPPH radical scavenging activity of various parts of *P. foetida* L. was measured and the results are represented in the table 3(a) and 3(b). Different concentrations were used (100 – 500 µg/ml) and the percent inhibition of the ethanolic extract of root and aqueous extract of unripe peel was found to be maximum when compared with the standard ascorbic acid. In[6] reported that the highest free radical scavenging activity was found in ethanolic extract of leaf of *Passiflora foetida* L. The highest radical scavenging activity in leaves may be due to its more phenolic content of *P. foetida* L. leaf. Phenols actively remove the freeradicals due to the presence of their free OH group and afford protection against oxidative damage[21].

Percentage inhibition of *invitro* lipid peroxidation with ethanolic & aqueous extract of *Passiflora foetida* L.

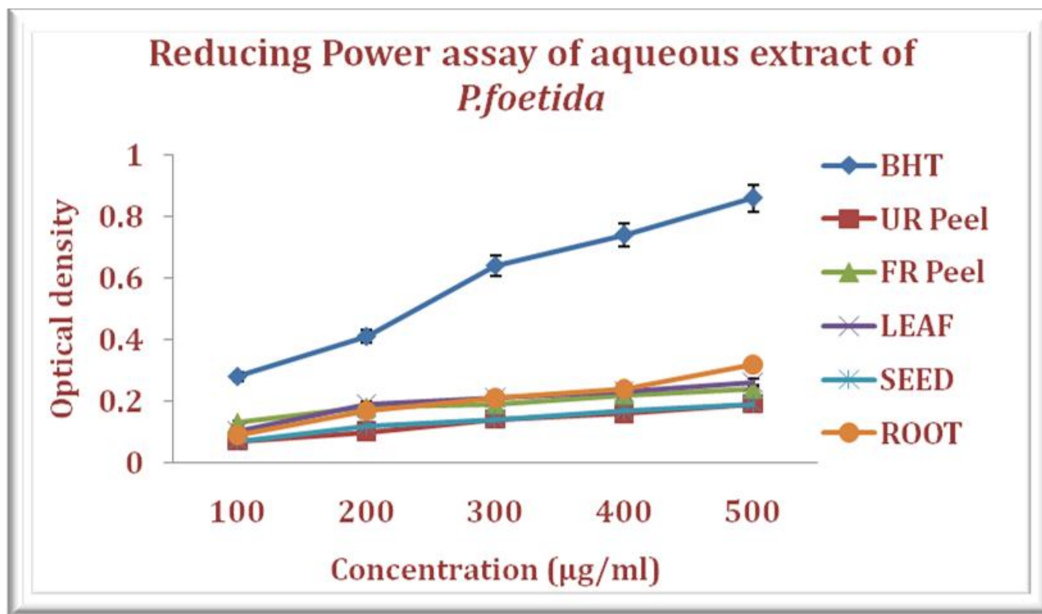
Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of free radicals, the uptake of oxygen and rearrangement of double bond in unsaturated lipids, which eventually result in destruction of membrane lipids[8]. Increased MDA content is an important indicator of lipid peroxidation[22]. The excessive generation of free radicals leads to peroxidative changes that ultimately result in enhanced lipid peroxidation. In figure 4a & 4b, *P. foetida* L. showed significant ($P < 0.01$) reduction *in vitro* lipid peroxidation. Some diseases have been reported to be associated with increased disruption of membrane lipids leading to subsequent formation of peroxide radicals. The process of lipid peroxidation generates hydroperoxides, aldehydes and other free radical intermediates which can react with essential proteins, enzymes and nucleic acids and render them inactive. Elevated lipid peroxidation and poor antioxidant systems have suggested that a lack of antioxidant defense is responsible for the elevated lipid peroxidation in erythrocytes[23]. Since the extract is able to inhibit the lipid peroxidation, it is assumed that the consumption of *P. foetida* L. will reduce the free radical formation and afford protection against reactive oxygen species produced during diabetes. This is also due to presence of phenol & flavanoid content in leaf and root of ethanolic and aqueous extract of *P. foetida* L.

Figure 2 (a): Reducing power assay of ethanolic extract of *Passiflora foetida* L.



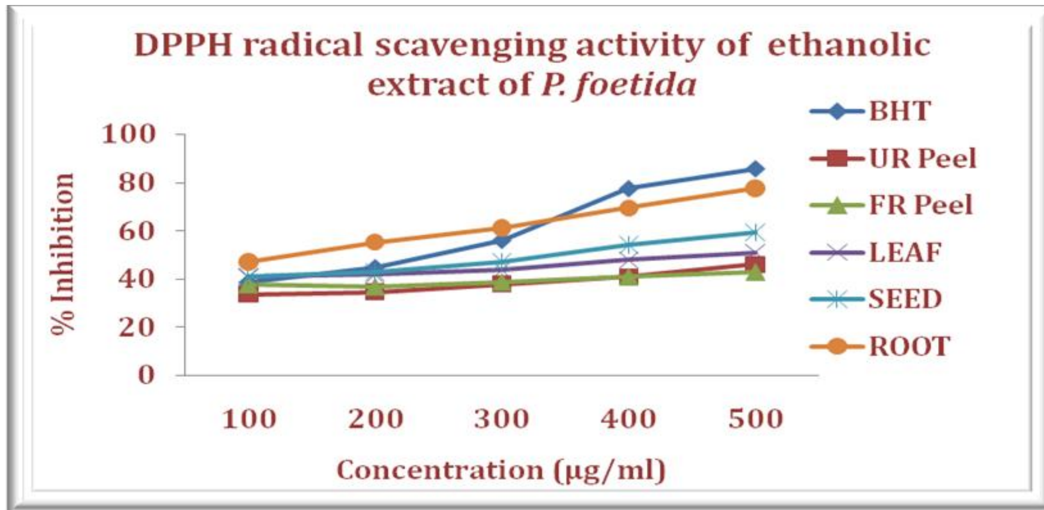
Values are expressed as Mean±SD of three individual experiments

Figure 2 (b): Reducing power assay of aqueous extract of *Passiflora foetida* L.



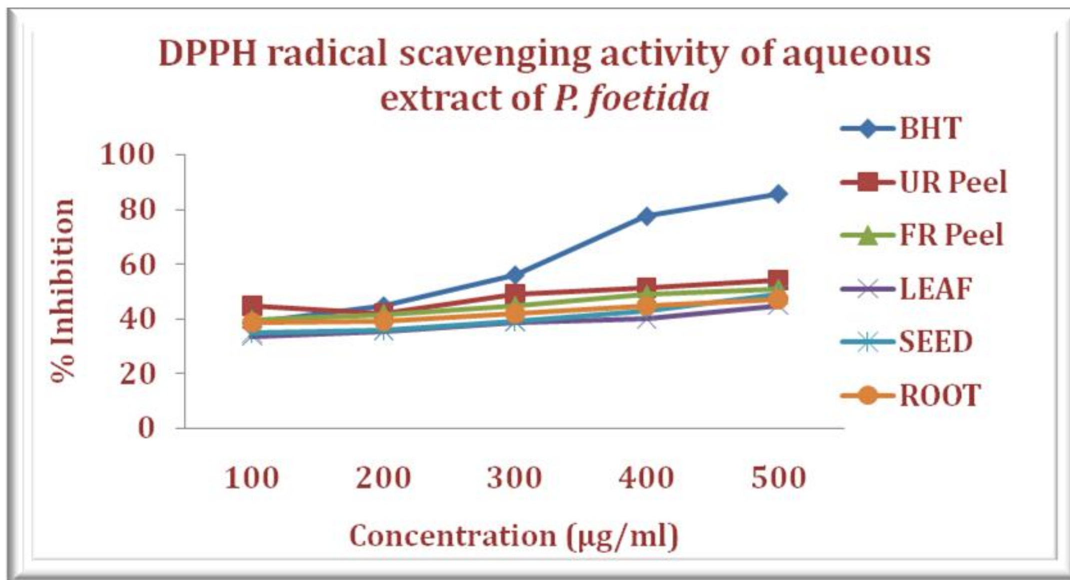
Values are expressed as Mean±SD of three individual experiments

Figure 3(a): DPPH radical scavanging activity of ethanolic extract of *Passiflora foetida* L:



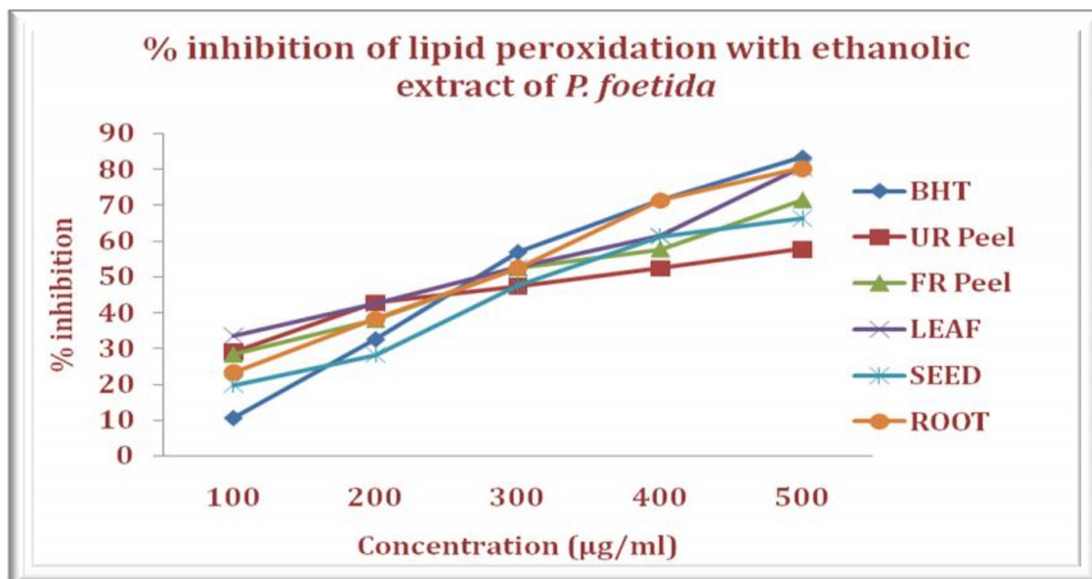
Values are expressed as Mean±SD of three individual experiments

Figure 3(b): DPPH radical scavanging activity of aqueous extract of *P. foetida* L:



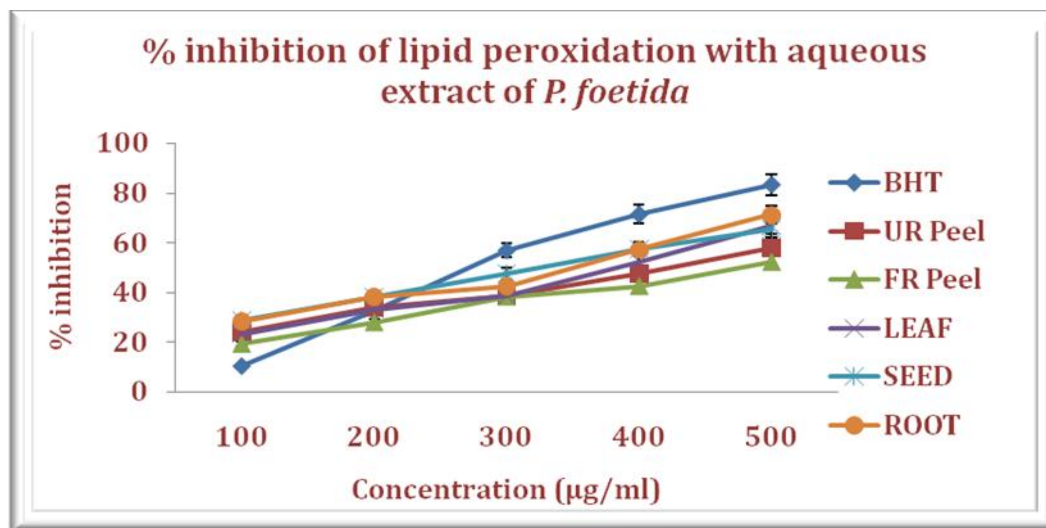
Values are expressed as Mean±SD of three individual experiments

Figure 4(a): % inhibition of *invitro* lipid peroxidation with ethanolic extract of *P. foetida* L:



Values are expressed as Mean±SD of three individual experiments

Figure 4(b): % inhibition of *invitro* lipid peroxidation with aqueous extract of *P. foetida*:



Values are expressed as Mean±SD of three individual experiments

CONCLUSION

In conclusion aqueous and etanolic extract of *Passiflora foetida* L. leaf & root recorded high amount of total phenol and flavanoid whereas less amount of phytochemicals were found in petroleum ether extract. A strong reducing power was noted in ethanolic extract of *P. foetida* L. root whereas less activity in aqueous extract of *P. foetida* L. unripe peels. The highest DPPH free radical scavenging activity was found in ethanolic extract of root and aqueous extract of unripe peel of *Passiflora foetida* L. when compared with other parts of plant. The ethanolic extract of leaf and aqueous extract of *P. foetida* L. root showed maximum percent inhibition of lipid peroxidation when compare to other parts of *P. foetida* L.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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