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Research Article

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Evaluation of Antioxidant Potential of *Passiflora foetida* Extract and Quantitative Evaluation of its Phytochemical Content- A Possible Natural Antioxidant

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Abstract The study aimed to investigate the quantitative evaluation of phytochemicals and the antioxidant potential of leaf extract of *Passiflora foetida.* The antioxidant activities were analyzed the methods of DPPH, ABTS and total antioxidant activity. The leaf extract of *P. foetida* recorded highest total phenolic (63.91 mg/g extract), flavonoids (30.98 RE mg/g extract; 25.03 QC mg/g extract) and saponin (32.27 mg/g extract) contents antioxidant activity. The IC_{50} value of DPPH was (614.405 µg/mL) and ABTS was (25.18 ug/ml). *P. foetida* leaves also possess total antioxidant potential comparable to ascorbic acid. The antioxidant potential of extract were also correlated with total phytochemical contents, which indicates that higher phenol, flavonoids and saponin suppose to support such potential property. Therefore, the results suggest that the leaf extract of *Passiflora foetida* have promising therapeutic value for as natural antioxidant.

Keywords Natural products, *Passiflora foetida,* Quantitative Phytochemicals, *Invitro* Antioxidant potential

Introduction

Medicinal plants contains some biologically active molecules, which are used in Ayurvedic preparations and usually these extracts were prepared in bulk for commercial purpose and for the societal benefits [1]. The genus *Passiflora* comprises of more diverse of the family Passifloraceae, comprising more than 560 species which are grouped into 21 subgenera, of vines, lianas, trees, and shrubs, commonly used for their edible fruits, ornamental decorative and therapeutic purposes. The species of this genus are distributed in the warm temperate and tropical regions of the world, but they are much rare in Asia, Australia, and tropical Africa. Dried leaves, fruits (ripe and unripe) and roots of these species have been traditionally used in several countries for the treatment of insomnia, anxiety and irritability [2]. Pharmacological studies carried out in the last 20 years reported distinct biological activities in the genus, including diuretic [3], anxiolytic, antiinflammatory, antioxidant, analgesic, antiviral, antihysteria [4] and antihyperglycemic [5-6], as well as antibacterial activity [7-10]. These pharmacological activities of this plant have been associated to the presence of alkaloids, flavonoids, saponins, cyanogenic compounds, essential oils and carotenoids [2].

Passiflora is also known by other names such as Passion flower, maypop, apricot vine, passion vine or krishnakamal in Hindi. It grows as much 30ft (10m) Tall, with a thick, woody stem. It is used for skin diseases with inflammation in countries like Brazil [11]. It shows antispasmodic, sedative, anxiolytic and hypotensive activities [2, 12-13]. The leaf paste of *Passiflora foetida* is applied for headache and to treat skin diseases [14].

Nowadays food industries are facing various problems due to deterioration of food and food products caused by the oxidation and microbial contaminations [15]. Also due to growing resistance against preservatives and antibiotics has created awareness for the identification of novel and natural compounds from plant sources as antioxidants. As it has been reported that, synthetic antioxidants are incorporated to prevent oxidation of food stuffs are found to cause severe adverse effects in human. Based on above reasons it is very essential to develop naturally occurring products with antioxidant and antimicrobial potential.

In the present paper we have investigated antioxidant potential of *P. foetida* extract, measured using various *in vitro* scavenging of DPPH, ABTS activity and Total antioxidant activity using ascorbic acid as standard. The total phenol, flavanoid, saponin, alkaloids and carbohydrate were also estimated. The aim of the current study was to report the phytochemical contents and antioxidant activities of leaf extract *in vitro*. The results from this study will possibly add to the merit of the therapeutic potential of *P. foetida* leaves, as there are very sparse studies (if any) to our knowledge that have investigated the phytochemical constituent and antioxidant activities for this plant ecotype in the area of current study

Material and Method

Plant material and Preparation of extract: The extracts were prepared according to method described by Patil et al. [16]. Leaves of *P. foetida* L. was collected from Departmental garden initially collected from Melghat forest area, Amravati, India. The plant was authenticated using standard flora and cross-checked with herbarium records at the NBRI, Lucknow, India as *Passiflora foetida* L. with an accession number- 98181. The leaves were cut into circular shape (about 1 cm) with sterile blade and washed with distill water and then by 70% alcohol. The extract were prepared from 100 gm cut leaves in 100 ml sterile distill water in 250 ml conical flask. The system was kept at 37°C and at 80 rpm. Harvesting was conducted by removing 100 ml of diffusate and replacing with an equal volume of sterile distilled water. For preparing the ethyl acetate extract, acidify the diffusate with 12N H_2SO_4 to pH of 1 to 1.5 and again extract with 0.35 volume of ethyl acetate using separating funnel for 10 min. It was further centrifuged and the pooled organic phase was evaporated to concentrate the extract.

Phytochemical screening of extract

The extracts of *P. foetida* were analyzed for the presence of Alkaloids, Saponins, phenol compound and Tannins, Glycoside, Steroid, carbohydrates, Protein and amino acid according to standard methods [17-20]. About 10 mg/ml mg extract prepared in distilled water and 15mg was dissolved in 10 ml dilute HCl (10%) for alkaloid determination. The extract was centrifuged at 4000g for 10 minutes, and supernatant was used for phytochemical screening.

Quantitative evaluation of Phyto-constituents

Total phenol-Quantitative evaluation of total phenol content of *P. foetida* extract was determined by using Folin-Ciocalteu method with slight modification. 200 µl of sample in the range of 10 µl/ml to 100 µl/ml was added to 100 µl Folin-Ciocalteu reagent dilute (1:10) and equilibrated for few minute. Then 800 µl of 2.5% aqueous Na_2CO_3 was added and mixture was allowed to stand for 60 minutes at room temperature with intermediate shaking. The absorbance of the tube color solution was measured at 765nm on UV-Visible spectrophotometer. Gallic acid (1mg/ml) was used as standard. The absorbance of solution was compared with Gallic acid calibration curve [21].

Flavonoids - 10mg of Rutin and Quercetin was dissolved in 10 ml of methanol. Dilution of standard was made as 10, 20, 40, 60, 80 and 100µg/ml (0.5ml) and make up each dilution up to 2ml ml with methanol, then 0.1 ml of aluminum chloride and 0.1 ml of potassium acetate and 2.8 ml of distilled water was added. Samples were incubated at room temperature for 30 min. The amount of aluminum chloride was substituted with the same amount of distilled water as a blank. Similarly 0.5 ml of extract was reacted with aluminum chloride for determination of flavonoid content. Absorbance of reaction was taken at 435nm [22].

Saponin- Diosgenine was used as a standard Saponin (10mg/10 ml of distilled water). Dilution of standard was made as 10, 20, 40, 60, 80 and 100µg/ml and volume was made up to (0.5ml) and 0.5 ml of 0.5% aniseldehyde was added Mixture was shaken and allowed to stand for 10 min then, 2ml of 50% H_2SO_4 was added and then mixed well. The reaction mixture was incubated at 60^o C for 10 min in water bath. Same procedure was followed for the extract. Absorbance was measured at 435nm [23].

Total alkaloid: *P. foetida* 5 gm powder was dissolved in 200ml of 10% ethanolic acetic acid for 4 hours at room temperature and allowed to stand. Further it was filtered and the filtrate was concentrated to 25 ml on water bath. Next it was precipitated by addition of concentrated ammonium hydroxide (ammonia solution). It was centrifuged and the supernatant was discarded the residue was further washed with 1% ammonium hydroxide and filtered through filter paper the dried residue was weighed and treated as total alkaloids.

Carbohydrate- For total carbohydrate estimation D-glucose was used as a standard and dilution was made as 10, 20, 40, 60, 80 and 100mg/ml and volume was made up to 0.5 ml. Then add 5ml of 96% H_2SO_4 and mixture was shaken and incubated for 40 min at room temperature after incubation added 1ml of 5% phenol in each tube. *P. foetida* extract was treated similarly as above method. The absorbance was measured at 490 nm [24].

Antioxidant potential screening of *Passiflora foetida*

DPPH method: The antioxidant activity by free radical scavenging activity was estimated by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. The reaction mixture contained 100µl of test extract with varying concentration and 1.9 ml of methanolic solution of 0.1mm DPPH radical. The mixture was then shaken vigorously and incubated at 37^0C for 30 min. The absorbance was measured at 517 nm using ascorbic acid (1mg/ml) as positive control lower absorbance of the reaction mixture indicated higher free radical scavenging activity which was calculated using the following equation:

$$(\%)\text{DPPH scavenging effect} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

ABTS radical scavenging assay-Antioxidant activity was measured by ABTS decoloration method [25]. The method is based on the capacity of different component to scavenge the ABTS radical cation (ABTS+) compared to standard antioxidant compound (ascorbic acid or trolox). The stock solutions included 7.4mM ABTS solution and 2.6mM potassium persulphate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12hr at room temperature in the dark. The solution was then diluted with distilled water to obtain an absorbance of 0.710 at 734nm using spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (0.01ml) were allowed to react with 2.9ml of the ABTS solution for 2 hr in a dark condition. Then the absorbance was taken at 724nm using the spectrophotometer [26].

Total antioxidant activity: *P. foetida* extract was dissolved in methanol (1mg/ml) various concentration of extract and standard ascorbic acid was prepared as 10, 20, 40, 60, 80 and 100µg/ml in triplicate, to a mixture of ammonium molybdate (4 mM) and sodium phosphate (28 mM) in 0.6 M H_2SO_4in total volume of 2 ml in eppendorff tubes and kept at 95 $\pm 2^o$C for 90 min and the absorbance was measured at 695 nm after cooling at 25 ± 2^oC [23].

Result and Discussion:

Phytochemistry research area do possess are many reports, which support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with many diseases [27]. Therapeutic plants are the potential source of antioxidants for example Tannin, saponin, Terpenoid, Polar flavanoid, phenol, ascorbic acid, Non-polar flavanoid as secondary metabolites having the capability to scavenge the free radicals [28]. The phytochemical screening was carried out of *P. foetida* extract; it resulted in presence and absence of main classes of phytometabolites. The strong intensity of metabolites had been

represented by ++ sign, while + shows Normal intensity and – sign indicates absence of compounds. The results are summarized in Table 1.

Table 1

Sr. No.	Compound	Name of test	Observation	Test result
1	Alkaloid	Dragendroff's test	Yellow precipitate	+ ve
		Wagner's tests	Reddish brown precipitate	+ ve
		Mayer's test	White precipitate	+ ve
		Hager's test	Yellow precipitate	+ ve
		Barfoeds	No change	-ve
2	Phenolic compound and tannin	Ferric chloride test	Dark green color	++ve
		Gelatin test	White precipitate	+ve
		Lead acetate test	White precipitate	++ve
3	Glycosides	Born tragers test	No change	-ve
		Legals test	No change	-ve
4	Protein and amino acid	Biuret test	No change	-ve
		Ninhydrin test	No change	-ve
5	Carbohydrate	Molish test	Violet ring	+ve
		Fehlings test	red precipitate	+ve
		Benedicts test	No change	-ve
6	Steroid	Steroid	A reddish brown color	++ve

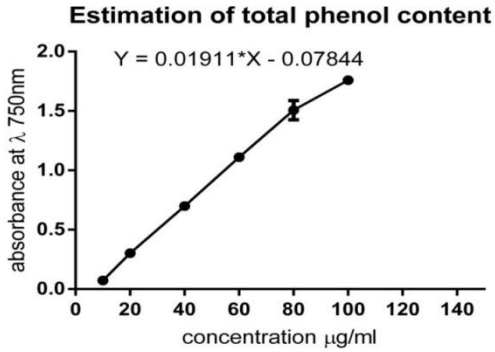
The bioactive compounds from plants in broad range has drawn the attention of many experimental works. It has been proven that these mechanisms may be important in the pathogenesis of certain diseases and aging [29]. There are many reports that support the use of antioxidants supplementation in the reducing level of the oxidative stress and in slowing or preventing the development of complication associated with disease [30]. Many synthetic antioxidants have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plants constituents have proved to show free radical scavenging / antioxidant activity [27]. Flavanoid and other phenolic compounds of plant origin have been reported in scavengers and inhibitors of lipid peroxidation can act as antioxidants.

P. foetida extract was subjected to total phenol content evaluation, showed that extract content 63.91 GAE mg/g dry weight of extract. It was deduced by Gallic acid calibration graph (Graph no.1.) using equitation [Y=0.01911xX0.07844] and R^2 value =0.9946. Previous report also correlate with present resultant data as (Sasikala et al. 2011) found 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 %) using different solvent extraction.

Table 2: Total phenol content in *P. foetida* using Gallic acid as standard

S. No	Gallic acid Standard (µg/ml)	Absorbance at 750nm. (±S.E.M.)	Y=0.01911xX-0.07844 R^2=0.9946
1	10	0.073±0.0011	63.91
2	20	0.303±0.0167	
3	40	0.699±0.0058	
4	60	1.110±0.0035	
5	80	1.507±0.0813	
6	100	1.760±0.0111	

*±S.E.M represents standard error of mean values (n=3)



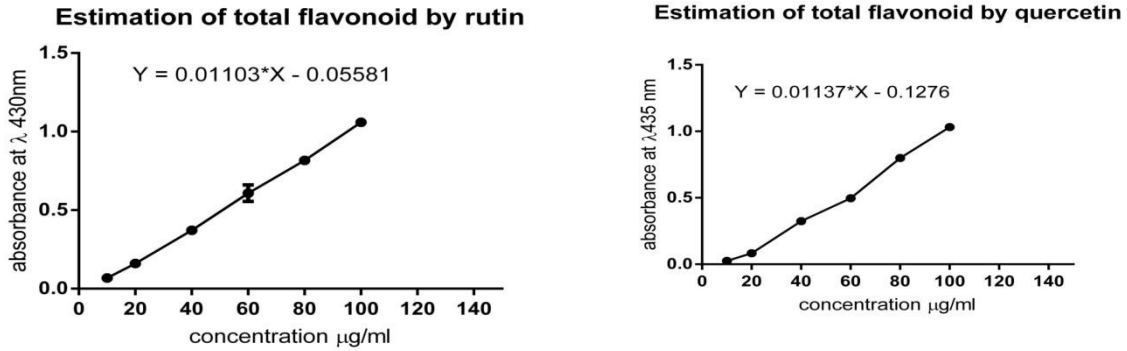
Graph- 1 Estimation of total phenol content

Total flavonoid content of *P. foetida* extract was determined using Aluminum chloride method with Rutin and Quercetin standards. It was found that *P. foetida* extract shows 30.98 RE mg/g dry weight of extract, while in quercetin 25.03 QC mg/g dry weight of extract (Table no. 3). It was calculated by Rutin and Quercetin calibration graphs (Graph no. 2 and 3) with equation Y=0.01103×X-0.05581 and R^2=0.9992 and Y= 0.01137×X-0.1276 respectively. Very few reports are available in case with *P. foetida* total flavonoid content estimation, while similar method was used in other plants shows flavanoids (17.10 ± 0.02 mg (RE)/g sample) are present in leaves of *Hypericum perforatum* L. [31].

Table 3: Total Flavonoid content estimation

S. No.	Concentration µg/ml	Absorbance of Rutin at 435nm (±S.E.M.)	Y=0.01103×X-0.05581 R^2=0.9992	Absorbance of quercetin at 435 nm(±S.E.M)	Y= 0.01137×X-0.1276
1	10	0.0683±0.00033	30.98	0.0253±0.0072	25.030
2	20	0.161±0.00317		0.083±0.0133	
3	40	0.372±0.00523		0.3245±0.0017	
4	60	0.608±0.05226		0.497±0.00655	
5	80	0.817±0.00850		0.799±0.00560	
6	100	1.059±0.0129		1.032±0.01178	

*±S.E.M represents standard error of mean values (n=3)



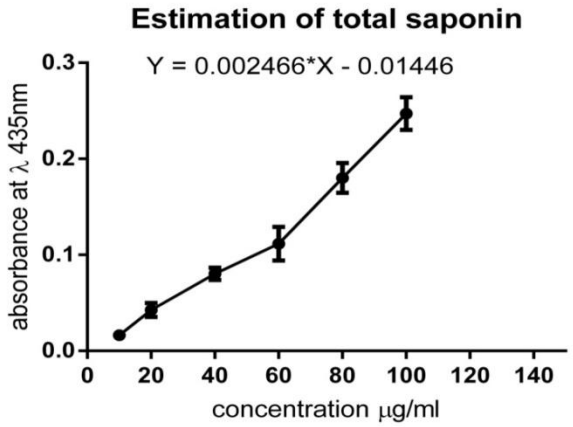
Graph 2-3: Calibration graph of Rutin and Quercetin standard

In order evaluate other phytochemicals in quantitative estimation of *P. foetida* extract total saponin content was assessed. It results in 32.27 DGE mg/g dry weight of extract represented in Table no. 4 and deduced from Diosgenine calibration graph (Graph no. 4) using equation Y=0.002466×X-0.01446 and R^2= 0.9783. Earlier reports claim similar results showing presence of saponins in *Passiflora* species [32]. *Passiflora alata* consist of primarily saponins in the leaves showing qudranguloside as major saponin [33].

Table 4: Total saponin content using Diosgenine as standard

S. No.	Sample	Concentration µg/ml	Absorbance at 435nm. (±S.E.M.)
1		10	0.0163±0.00166
2		20	0.0416±0.00717
3		40	0.0803±0.00635
4	Diosgenine	60	0.1116±0.0176
5		80	0.1800±0.0155
6		100	0.247±0.0170

*±S.E.M represents standard error of mean values (n=3)



Graph 4: Calibration graph of Diosgenine standard

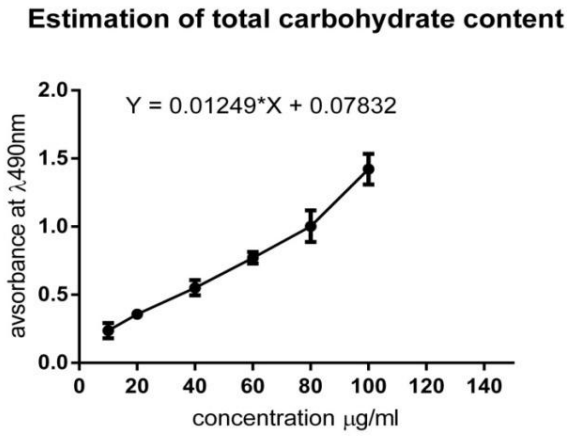
The total alkaloids were also estimated from *P. foetida* extract, it was found that from 5 gm of extract subjected for alkaloid estimation 32.4 mg alkaloid residue was obtained. Hence *P. foetida* shows 6.48 mg/g of powder analyzed. Presence of Harmala alkaloids were reported earlier [34], it shows *P. foetida* has 0.78 % w/w Harmaline analysed by HPTLC technique.

P. foetida extract was further analyzed for total carbohydrate estimation by Phenol sulphuric acid method. Table no. 5 represents the total carbohydrate content using Glucose as standard. The *P. foetida* extract shows low quantity of carbohydrates i.e. 3.897 G mg/dry weight of extract. The estimation was calculated using equation $Y=0.01249\times X+0.07832$ and $R^2=0.9789$ (Graph no. 5). Reports on carbohydrate content of *P. foetida* are not much discussed or attempted. Although other species of *Passiflora* shows high content of carbohydrate in fruit pulps. *P. edulis* fruits show 23.38 gm/100 gm, carbohydrate as reported in previous studies [35]. The complete phytochemical profile including Phenol, Flvonoids, Saponin, carbohydrates and alkaloids is shown in Graph no. 6.

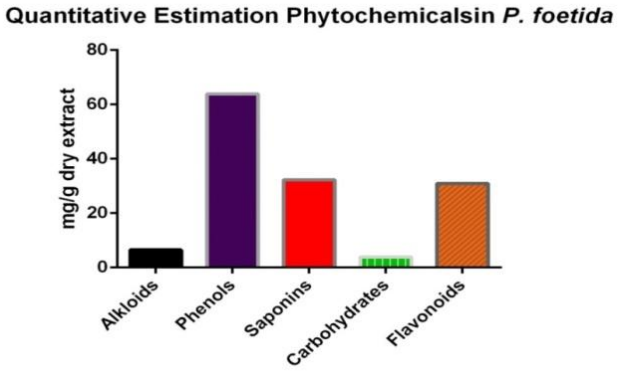
Table 5: Total Carbohydrate content using D-Glucose as standard

S. No.	D-Glucose Standard Concentration µg/ml	Absorbance at 490nm (±S.E.M.)	Y=0.01249×X+0.07832 R^2= 0.9789
1	10	0.237±0.056	
2	20	0.357±0.0216	
3	40	0.551±0.0558	
4	60	0.771±0.0424	3.897 G mg/dry extract
5	80	1.003±0.119	
6	100	1.422±0.1128	

*±S.E.M represents standard error of mean values (n=3)



Graph 5: Calibration graph of D-Glucose standard



Graph 6: Quantitative estimation of major phytochemicals in P. foetida extract

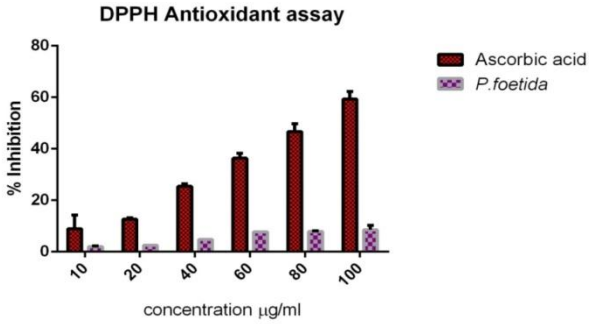
Antioxidant consumption is important for health because of the ability of redox molecules in scavenging free radicals. There are different methods to evaluate the antioxidant capacity of plants extracts, and at least three standards assays are necessary to prove the efficiency, such as ABTS, DPPH, FRAP and ORAC [26, 36]. Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione [37].

In this work, we analysed the extracts of *Passiflora* spp. leaves using DPPH, ABTS and total antioxidant activity demonstrating that antioxidant capacity depends on the efficiency of the method and the solvent used for extraction. The antioxidant methods have shown different results among previous reports, but with a high correlation between ABTS and DPPH methods [38].

In DPPH radical scavenging method, the activity of tested extracts was expressed as Percent Inhibition (I %) of standard ascorbic acid and *P. foetida* extract. Table no. 12 and Graph no. 10 represents the comparative DPPH percent inhibition potential of Ascorbic acid and *P. foetida* extract. The IC_{50} (50 percent Inhibition) of Ascorbic acid 84.40 µg/ml it was calculated using equation, Y= 0.5630×X+2.480 and R^2 value is 0.9983, whereas *P. foetida* exhibits low DPPH antioxidant activity, i.e. IC_{50} = 614.405 µg/ml, calculated using equation Y=0.07893×X+1.505 and R^2 = 0.9173. Sasikala et al. [21] showed that 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 for the DPPH radical scavenging activity. 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.

Table 6: Antioxidant activity by DPPH assay

S. No.	Conc. (µg/ml)	Absorbance at (±S.E.M.)			
		Ascorbic acid	**Y= 0.0.5630×X+2.480 R^2=0.9983**	***P. foetida***	**Y=0.07893×X+1.505 R^2=0.9173**
1	10	8.9547±5.289		2.025±0.2155	
2	20	12.631±0.907		2.551±0.000	
3	40	25.4344±0.9708		4.7413±0.000	
4	60	36.3634±1.898		7.7586±0.000	
5	80	46.6462±3.1257	IC_{50}=84.40	7.8879±0.000	IC_{50}=614.405
6	100	59.3808±2.933		8.5344±1.7241	



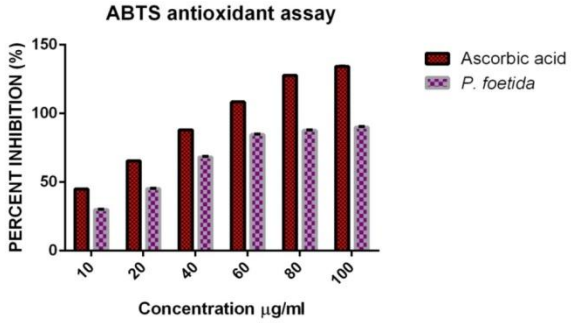
Graph 7: Comparative DPPH antioxidant activity of Standard ascorbic acid and P. foetida

In ABTS˙+ cation radical scavenging method, the activity of tested extracts was expressed as Percent Inhibition (I %) of Standard ascorbic acid and *P. foetida* extract. The results are summarised in Table 7 and Graph 8, it shows that *P. foetida* has IC_{50} (50% Inhibition) value is 25.18 µg/ml using equationY=0.6664×X+33.22 and the R^2 value is 0.8714 while Ascorbic exhibited high potential with IC_{50} 6.49 µg/ml using equation Y= 0.9921×X+43.56 and R^2 value is 0.9645. Sasikala et al. [21] reported that even though the *P. foetida* leaf samples exhibited good ABTS radical scavenging activity, the petroleum ether extract of flower showed the highest activity (3991.9µmolg^{-1}).

Table 7: Antioxidant activity by ABTS assay

S. No.	Conc. (µg/ml)	Percent Inhibition I% (±S.E.M.)			
		Ascorbic acid	**Y= 0.9921×X+43.56 R^2=0.9645**	***P. foetida***	**Y=0.6664×X+33.22 R^2=0.8714**
1	10	45.068±0.01210		30.048±0.120	
2	20	65.43331±0.00123	IC_{50}= 6.49	45.31433±0.168	IC_{50} = 25.18
3	40	88.0156±0.0412		68.30933±0.412	
4	60	108.456±0.0164		84.638±0.29	
5	80	127.63267±0.0235		87.63267±0.25	
6	100	134.3125±0..125		89.995±0.51	

*±S.E.M represents standard error of mean values (n=3)'



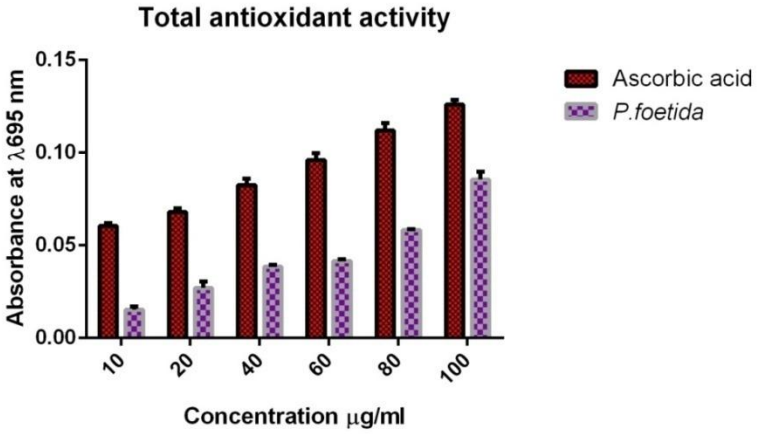
Graph 8: Comparative ABTS antioxidant activity of Standard ascorbic acid and P. foetida.

The *P. foetida* extract was further subjected for evaluation of antioxidant activity; it results in to good antioxidant potential of *P. foetida* extract as compared with standard ascorbic acid. The Graph no. 9 shows comparative evaluation, both the sample and standard exhibit increasing antioxidant activity as the concentration increases Table no. 8. Hence highest activity was shown by 100µg/ml *P. foetida* extract with OD 0.085 at 695nm by *P. foetida* extract and ascorbic acid shows OD 0.126 at 695 nm. Proposed method was not adopted for analysis of total antioxidant activity in *Passiflora* earlier. Although other reports are available in case with different plant samples using similar method [1] have determined the total antioxidant activity in 35 ug and fruits and vegetables. They found that *Cleome gynandra* had this activity as 1.56 ± 0.73, that for *Amaranthus* spp. 1.00 ± 0.32, for *Solanum macrocarpon* L. 0.87 ± 0.17 and that for *spinacia oleracea* L. as 0.98 % TAC [23].

Table 8: Total Antioxidant activity of *Passiflora foetida* extract

S. No.	Concentration	Absorbance at (±S.E.M.)	
		Ascorbic acid	***P. foetida***
1	10	0.0606±0.00133	0.0153±0.00176
2	20	0.0680±0.00208	0.027±0.00342
3	40	0.0826±0.00333	0.0386±0.00088
4	60	0.096±0.00377	0.0416±0.000881
5	80	0.112±0.00409	0.0583±0.00176
6	100	0.126±0.00240	0.0856±0.00321

*±S.E.M represents standard error of mean values (n=3)



Graph 9: Comparative total antioxidant activity of P. foetida and Ascorbic acid

Conclusions

Natural products with potential bioactive compounds have received major attention nowadays due to their wide applications. In recent years, the studies have been increased with phenolic compounds for the development of new natural drugs. The choice of solvent/method for extraction of phenolic compounds is crucial.

In present study the extraction of leaves were done by leaf cutting and extracting in water confirms that method is suitable for extracting the phytochemicals present in *P. foetida* leaves. The results confirms that *P. foetida* leaves extract do content higher phenolics, then flavonoids and Saponins which can contribute for their antioxidant potential. The evaluation of the antioxidant capacity (DPPH, ABTS and total antioxidant) was analysed in, which confirms the presence of suitable potential to be used as natural antioxidant.

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