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# Antioxidant and HPLC analysis of an Indian Medicinal Herb: *Paederia foetida* L. (Prasarini)

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### **ABSTRACT**

**Background:** Paederia foetida (Prasarini) is a traditionally used herb majorly against diarrhoea and dysentery. It is well known for its antimicrobial and anti-inflammatory properties. They are also used for increasing male testosterone level.

**Aim**: This study was conducted to evaluate the antioxidant properties of the medicinal herb *P. foetida*. Further, High performance liquid chromatography (HPLC) of *P. foetida* extracts were done in order to determine the presence of phenolics and flavonoid compounds which essential for the antioxidant property of the plant.

**Methods**: The leaf extracts were prepared using 4 solvents, methanol, acetone, ethyl acetate and water. The antioxidant property was evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS method using Ascorbic acid as standard. In HPLC, Caffeic acid, Ferrulic acid, Galic acid, p-Coumaric acid, Quercetin, Myricetin, Kaemperol and Isorhamnetin were used for the analysis. The standards were dissolved in HPLC grade methanol. The dilutions of the standards were done from the range of  $20\mu g/ml$  to  $100\mu g/ml$  followed by making the standard curve.

**Results:** It was concluded from the DPPH assay that the ethyl acetate extract of *P. foetida* leaves had the highest free radical scavenging activity than other extracts, however, from the ABTS assay it was concluded that the acetone extract of *P. foetida* had the highest free radical scavenging activity. From the HPLC analysis it was confirmed that the ethyl acetate extract and the highest number of phenolic compounds, however they had moderate number of flavonoid compounds.

**Conclusion**: The antioxidant property of *P. foetida* leaves is very high as they are rich in phenolic compounds and can be extracted using ethyl acetate and acetone.

**Keywords**: *Paederia foetida*; Antioxidant assay; DPPH; ABTS; HPLC; Phenolic compounds

#### **INTRODUCTION**

Antioxidants prevent the slow damage to cells caused by the release of free radicals or free-radical scavengers or reactive oxygen species (ROS), which are unstable molecules produced in the body in reaction to defective metabolism, environmental and other related stress on the body. Antioxidants are both natural and artificial. Several plant-based products are well known antioxidant drugs (Wilson et al., 2017). Human body also produces few antioxidants, known as endogenous antioxidants. Antioxidants from external sources are called exogenous. If the body is unable to process and remove such free radicals efficiently, it results in oxidative stress and lead to defective metabolism and diseases (Ramana et al., 2018). The number of free radicals in the body escalates because of internal factors, such as inflamm-ation, or external factors like, environmental pollution, ultraviolet rays, indifferent food habits and tobacco use (Lobo et al., 2010). Oxidative stress may lead to cardiac defects, cancer, arthritis, strokes, respiratory problems and immunodeficiency or suppressed condition, emphysema, Parkinson's disease and other inflammatory or ischemic diseases (Pizzino et al., 2017). Therefore, antioxidants become much more important as they are required to neutralize free radicals and to keep the body healthy.

Medicinal plants since ancient times were known to be the potent source of antioxidants as they contain a mixture of different secondary metabolites and phytochemicals, that may act individually or in synergy as therapeutic agent against various health ailments (Xu et al., 2017). In last 4 decades, research on antioxidants and medicinal plants has significantly increased and the popularity of natural antioxidant products has escalated, particularly in the urban society. A single medicinal plant may have variety of phytochemicals, which may have several pharmacological properties (Kasote et al., 2015; Machado et al., 2018). Unfortunately, several herbal medicines which are in use today have not gone under proper scientific evaluation and some of them have life threatening side/toxic effects. Therefore, careful scientific and clinical studies of the herbal medicine for the safety and toxicity has become essential (Asif, 2012). With high prevalence of medicinal plants in India and their potential as a source of antioxidants need to be judiciously investigated (Sen and Chakraborty, 2016; Kumar et al., 2016).

Paederia foetida is an Indian edible herb that has traditionally been used for a various health ailment, particularly against diarrhoea and dysentery and in male infertility problems. It has both antioxidant and anti-inflammatory properties, like most medicinal plants (Wang et al., 2014). Some scientific reports also validate in erotogenic and aphrodisiac properties. The medicinal properties are majorly due to presence of phytochemicals present in this herb, which acts in combinations or in solitary mode against various human ailments. Hence, investigating the major bioactive chemicals of the plant has become essential (Wang et al., 2014; Kumar et al., 2014). No report of toxicity has been reported for this plant, however, very few studies has been conducted in this aspect (Chanda et al., 2015). This study was conducted to evaluate the antioxidant properties of the medicinal herb *P. foetida*. Further, High performance liquid chromatography (HPLC) of P. foetida extracts were done in order to determine the presence of phenolics and flavonoid compounds which are essential for the antioxidant property of the plant.

#### **MATERIALS AND METHODS**

# Processing of plant material

Fresh young leaves of *P. foetida* were collected from the Chandaka forest region of Bhubaneswar, Odisha in September 2018 and it was initially identified by its morphological features. The fresh leaves were washed properly with pyrogen free water and were shed dried and powdered in a mixer grinder. Further the powdered leaves were stored in airtight polybags for future use.

#### **Extraction**

A total of 15-gram of powdered leaf samples was taken and extraction was carried out with the help of a Soxhlet apparatus by using different solvent on their polarity basis. Different solvents used for extraction process were ethyl acetate, acetone, methanol and water respectively. After the extraction, solvent evaporation was done by using a rotary evaporator, where the solvent and extract got separated resulting in the yield of pure extract. Further the final crude extracts were stored in a glass airtight container and were kept at 4°C until further use.

#### In vitro Antioxidant Assay

The *in vitro* antioxidant properties of the 4 solvent leaf extracts from *P. foetida* were determined by using 2 methods: (a) The DPPH method; (b) The ABTS method

#### The DPPH method

One ml of DPPH solution in methanol (0.1 mMol/L<sup>-1</sup>) was mixed with 3.0 ml of leaf extract in several concentrations (10  $\mu$ g/ml, 20  $\mu$ g/ml, 30  $\mu$ g/ml, 40  $\mu$ g/ml and 50  $\mu$ g/ml) and the mixture was incubated for 30 min at room temperature in dark and the absorbance was recorded at 517 nm using a UV - Visible spectrophotometer. Ascorbic acid was used as standard to plot the standard curve. The antioxidant potentiality of these extracts was recorded as IC<sub>50</sub>. The IC<sub>50</sub> value is the concentration (in  $\mu$ g/ml) of extract that inhibits the formation of DPPH radicals by 50 (Kedare and Singh, 2011).

#### ABTS + Radical scavenging assay

ABTS  $^+$  was prepared by mixing 1:1 ratio of ABTS and potassium per sulphate and kept in dark for 12 hours to generate the free radicals. Then its absorbance was adjusted up to  $0.700 \pm 0.025$  at 734 nm before use. 0.5 ml of sample of different concentration was added with 2.5 ml of ABTS was prepared. Then control is prepared without sample 0.5 ml of methanol with 2.5 ml of ABTS. According to the sample type, blank is prepared. The absorbance was measured at 734 nm. The scavenging activity of the extract based on the % of ABTS radical scavenged (I%) using equation,

$$DPPHs cavenging/ABTS scavenging = \frac{Acontrol - Asample}{Acontrol}X100$$

A control - Absorbance of the control sample.

A  $_{\mbox{\scriptsize sample}\mbox{\scriptsize -}}$  Absorbance in the presence of the extracts.

Ascorbic acid was taken as the standard antioxidant compound. A standard curve was plotted using different concentrations of ascorbic acid and  $IC_{50}$  value were determined for both the test samples as well as standard sample and the  $IC_{50}$  value of test samples were compared (Rajurkar and Hande, 2011).

# HPLC ANALYSIS

**Standards used:** Caffeic acid (CA), Ferrulic acid (FA), Galic acid (GA), p-Coumaric acid (p-CA), Quercetin, Myricetin, Kaemperol and Isorhamnetin were used for the analysis. The standards were dissolved in HPLC grade methanol. The dilutions of the standards were

done from the range of  $20\mu g/ml$  to  $100\mu g/ml$  followed by making standard curve.

**Samples:** 5mg/ml stock solutions were prepared for ethyl acetate, acetone and methanol leaf extract of *P. foetida* with HPLC grade methanol. But the stock solution of water extract was prepared with mili Q water. All the stock solutions were filter sterilised properly with 0.2 mm filter.

**Apparatus:** Reverse phase HPLC apparatus consisting of 2 pumping systems, injector, C18 column and UV visible detector of Shimadzu JAPAN was used. The data were collected and analysed by using "LC solution" software.

**Method:** Quantitative analysis of phenolic compounds was carried out with GA, FA, Caffeic acid and p-CA as the standards. The mobile phase was consisted of Solvent A (1% ortho-phosphoric acid in mili Q water) and Solvent B (1% ortho-phosphoric acid in acetonitrile). The ratio of solvent was 63:37. That means solvent A consisting of 63% where solvent B is 37%. The flow rate of the mobile phase was 0.5 ml/min and the injection volume 20µl. The peaks were monitored at 280nm for GA, 324nm for CA and FA, 310nm for p-CA. Quantitative analysis of flavonols was carried out by using Quercetin, Myricetin, Kaemferol and Isorhamnetin as standards. The mobile phase was methanol, acetonitrile and water (40:15:45). The flow rate of the mobile phase was kept at 0.5ml/min and the injection volume were 20µl. The peaks were monitored at 368nm for all (El-Nabi et al., 2018).

#### **RESULTS**

# **DPPH** assay

Leaf extracts of P. foetida exhibited a concentration response relationship in DPPH scavenging. The increase in concentration was synchronous with the increase in the scavenging capacity. Lower the IC<sub>50</sub> value greater of its scavenging activity. As the positive control, Ascorbic acid had the highest scavenging activity with IC<sub>50</sub> value 6.758 µg/ml. Comparing IC<sub>50</sub> values of all the extracts, it was recorded, that the acetone extract had more scavenging activity than rest three extracts. The IC50 value of acetone extract was 6.964 ± 16. Further, the ethyl acetate, methanol and aqueous extracts had the scavenging activity of IC<sub>50</sub> value 27.363 ± 3.093363, 13.336 ± 0.456353 and  $16.308 \pm 0.636684$  respectively. From the above readings and the graph, it was concluded that the ethyl acetate extract of P. foetida leaves had the highest scavenging activity than others (Table 1, Figure 1).

Table 1. Antioxidant activity of P. foetida leaf extracts in DPPH method

Extracts/ Standard	IC <sub>50</sub> value in μg/ml
Ethyl acetate	27.363 ± 3.093363
Acetone	6.964 ± 1.634197
Methanol	13.336 ± 0.456353
Aqueous	16.308 ± 0.636684
Ascorbic acid	2.52 ± 0.81

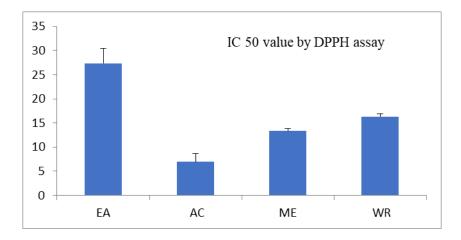


Figure 1: IC<sub>50</sub> value of different extracts of *P. foetida* leaf extracts by DPPH method

Table 2. Antioxidant activity of P. foetida leaf extracts in ABTS method

Extracts/ Standard	IC <sub>50</sub> value in μg/ml
Ethyl acetate	118.4562 ± 3.410
Acetone	28.55942 ± 0.367
Methanol	38.66273 ± 0.106
Aqueous	78.04537 ± 3.096
Ascorbic acid	24.52042 ± 0.02

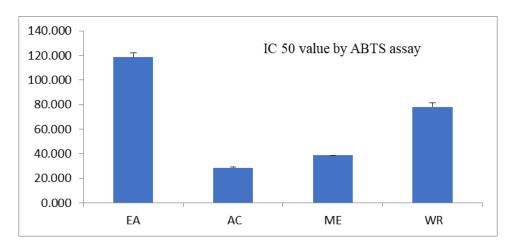


Figure 2. IC<sub>50</sub> values of different extracts of *P. foetida* leaf extracts by ABTS method

#### **Determination of ABTS assay**

P. foetida leaf extracts exhibited a concentration response relationship in ABTS radical scavenging. An increasing in concentration is synchronous with an increasing in scavenging capacity. Lower the IC<sub>50</sub> value greater of its scavenging activity. As the positive control, Ascorbic acid exhibited high scavenging activity with IC50 value 24.52042µg/ml. Comparing IC<sub>50</sub> value of all the leaf extracts of *P. foetida*, acetone extract recorded the highest scavenging activity than other extracts with an IC<sub>50</sub> value of  $28.55942 \pm 0.367$ . It was followed by methanol and aqueous extracts have the scavenging activity of IC50 value 38.66273 ± 0.106 and  $78.04537 \pm 3.096$  respectively. Ethyl acetate extract had and IC<sub>50</sub> value of 118.4562 ± 3.410. Hence, it was concluded that the acetone extract of P. foetida had the highest scavenging activity than others (Table 2, Figure 2).

#### **HPLC ANALYSIS**

HPLC method was developed for the analysis of GA, ferrulic acid, caffeic acid and p-coumaric acid in the leaf extracts of *P. foetida*. The polyphenolic content list of P. foetida leaves are recorded in table 3. The quantification of polyphenolic compounds by HPLC exhibited that ethyl acetate extract had GA (316.83 mg/gm), FA (37.459mg/gm), CA (111.225mg/gm) and p-CA (31.52mg/gm) by comparing the chromatogram of the reference standards. Retention time (RT) of the ethyl acetate extract 7.441, 10.185, 8.288, 9.818 min corresponded well with the standard chromatogram peaks of GA, FA, CA and p-CA at 7.652, 10.169, 8.062 and 9.827 min respectively. From the results it was concluded that ethyl acetate extract of *P. foetida* leaves has a high concentration of phenolic compounds (Table 3).

Similarly, the quantification of polyphenolic compounds by HPLC exhibited that acetone extract of

P. foetida leaves had GA (766.73mg/gm), FA CA (85.842mg/gm) (27.00 mg/gm),and (55.27mg/gm) by comparing the chromatogram of the reference standards. RT of the acetone extract of P. foetida leaves at 7.442, 10.152, 8.275 and 9.808min corresponded well with the standard chromatogram peaks of GA, FA, CA and p-CA at 7.652, 10.169, 8.062 and 9.827 min respectively. From the results it was concluded that acetone extract of P. foetida leaves has a high concentration of phenolic compounds (Table 3). Likewise, the quantification of polyphenolic compounds by HPLC exhibited that methanol extract of P. foetida leaves had GA (785.964mg/gm), FA (0), CA (113.94mg/gm) and p-CA (65.12mg/gm) by comparing the chromatogram of the reference standards. RT time of the acetone extract of P. foetida leaves at 7.435, 10.968, 8.279, 9.840 min corresponded well with the standard chromatogram peaks of GA, FA, CA and p-CA at 7.652, 10.169, 8.062 and 9.827 min, respectively. From the results it has been shown that methanol extract of P. foetida leaves had a rich content of GA, CA, p-CA and comparatively less amount of FA (Table 3).

Further, the quantification of polyphenolic compounds by HPLC exhibited that aqueous extract of *P. foetida* leaves had GA (121.22mg/gm), FA (0), CA (8.56mg/gm) and p-CA (0) by comparing the chromatogram of the reference standards. RT time of the aqueous extract of *P. foetida* leaves at 7.024, 8.261, 9.831min corresponded well with the standard chromatogram peaks of GA, FA, CA and p-CA at 7.652, 8.062 and 9.827 min respectively. From the results it has been shown that aqueous extract of *P. foetida* leaves had a rich amount of GA, caffeic acid, however, p-CA and FA is absent in case of aqueous extract. Phenolic compounds are a category of phytonutrients with strong antioxidant properties (Table 3).

Table 3. HPLC analysis for phenolic content

Standards	Std. Equation	Ethyl Acetate extract	Acetone extract	Methanol extract	Aqueous extract
GA	Y= 41355X+10355	316.837	766.732	785.964	121.225
Ferrulic acid	Y=1402X-21127	37.459	27.009	0	0
Caffeic acid	Y= 12936X-36524	111.225	85.842	113.942	8.560
Pcoumaric acid	Y= 17581X+26496	31.523	55.271	65.123	0

Table 4. HPLC analysis of flavonoid content

Standards	Standard Equation	Ethyl acetate extract	Acetone extract	Methanol extract	Aqueous extract
Quercetin	Y=68662X-35060	0	0	0	0
Myricetin	Y= 89948X+39064	0	2.806855072	0	0.247843198
Kaemferol	Y= 10861X-55166	0	7.080287266	5.975876991	5.69349047
Isorhamnetin	Y= 15595X+48534	0	0	0	0

#### For flavonoids

HPLC method was developed for the analysis of Quercetin, Myricetin, Kaemferol and Isorhamnetin in the extracts of *P. foetida* leaves. The flavanols profile of P. foetida leaves are shown in the table 4. The quantification of flavonoid compounds by HPLC exhibited that ethyl acetate extract of P. foetida leaves did not had Quercetin, Myricetin, Kaemferol and Isorhamnetin by comparing the chromatogram of the reference standards. In the acetone extract, Myricetin (2.806mg/gm) and Kaemferol (7.0802mg/gm) were present. RT of the acetone extract at 11.283 and 26.345 min corresponded well with the standard chromatogram peaks of myrecetin and kaemferol at 11.626 and 26.787 min respectively. In the methanol extract, Kaemferol (5.97mg/gm) was present. RT time of the methanol extract at 26.635min corresponded well with the standard chromatogram peaks of kaemferol 26.787 min. Others are absent. In the aqueous extract, Myricetin (0.2478mg/gm) and Kaemferol (5.693mg/gm) were present. RT time of the aqueous extract of P. foetida leaves at 11.603 and 26.836 min corresponded well with the standard chromatogram peaks of myrecetin and kaemferol at 11.626 and 26.787 min respectively. Quercetin and Isorhamnetin were absent in the aqueous extracts (Table 4).

# **DISCUSSION**

The present investigation study discusses about the antioxidant potential effect of the 4-leaf extract of P. foetida. In general, phenolic compounds and flavonoids exhibits a wide range of medicinal activities in vivo. Previous studies suggest that P. foetida extracts can prevent the oxidation of  $\beta$ -carotene and linoleic acid and exhibit antioxidant property when tested in ABTS free radical scavenging assay system (Osman *et al.*, 2009; Upadhyaya, 2013). In this study, we concluded that P. foetida leaf extract also have strong antioxidant activity in DPPH radical scavenging assay. DPPH

antioxidant assay is primarily depending upon the presence of antioxidants, a stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) will decolorize. The DPPH radical has free electron, which is responsible for the absorbance at 515-517 nm and develops a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Sahoo and Bhatnagar, 2016). *P. foetida* exhibited scavenging activity of DPPH radicals. This activity was increased with the increasing concentration of sample extract.

Phyto-polyphenols are secondary metabolites/ their derivatives/ isomers of flavones, isoflavones, flavonols, catechins and phenolic acids. More than 9000 structural versions of polyphenols and 3,000 flavonoids are distributed as unique elements of plant life. Considering, their structure, their solubility varies in polar to non-polar solvents. Most of the phenolic extracts are soluble in organic polar solvents, e.g. methanol, ethanol and acetone. A correct mixture of polar and non-polar solvents results in higher extraction of these phytophenols (Ojha et al., 2018). Absence of phenolic constituents can be attributed the physical properties of a specific solvent. The polarity of solvents and physical/ chemical houses of active components play an essential role on number of phenolic compounds at some point of extraction. Phenolic and flavonoid components like quercetin, sebiferine and litseferine are extracted with water in 80% polar solvents. So, increased numbers of peaks in natural solvent systems are due to presence of these additives (Wang et al., 2014). Phenolic components as glycosides/ aglycones has lower Rf values (zero. 00- 0. 25) and oligo-hydroxylated and methylated additives has more Rf values (0, 50, 75). The versions in quantity of peaks of phenolics between solvents can be because of their prevalence with appreciate to Rf values. Quercetin-3-glucosides, quercetin-three-arabinosides, quercetin-3-rhamnoglucosides and kaempferol

glucoside has 0. 33, 0.35, 0. 29 and zero. 54 Rf values respectively (Wang *et al.*, 2014; Kumar *et al.*, 2014).

#### **CONCLUSION**

From the study, it was concluded that the antioxidant property of *P. foetida* leaves is very high as they are rich in phenolic compounds and can be extracted using ethyl acetate and acetone. The results of this study justify the use of this plant as food product and in the pharmaceutical industry. It also validates the ethnomedicinal values of this plant.

**Conflict of Interest:** The authors declare no conflict of interest.

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