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Original Article

Extraction of Total Phenolics, Flavonoids and Tannins from *Paederia foetida* L. Leaves and their Relation with Antioxidant Activity

Shaswat Ojha, Adarsha Raj, Alokesh Roy, Sudipta Roy*

ABSTRACT

Introduction: *Paederia foetida* L. is a climbing shrub that possesses several ethnomedicinal uses with immense pharmacologic relevance. **Objective:** The study aims to determine an efficient extraction condition for phenolic compounds with substantial antioxidant activity. **Materials and Methods:** Solvent (aqueous, methanol, ethanol and acetone) extracts were made from fresh leaves (FL) and shade dried leaves (SDL) of *P. foetida* with different durations (12, 24, 36 and 48 h). Quantitative estimations of all extracts were made for total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) along with their total antioxidant activity (TAA). The phenolic contents of different extracts were correlated with their TAA. Extracts with highest phenolic yield and TAA estimates for each solvent were assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2´-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), superoxide (SO) and ferric reducing antioxidant power (FRAP) assays. **Results:** Methanol exhibited the highest extraction ability for TPC, TFC and TAA while aqueous extractions are superior for TTC. The maximum estimates of all the studied components are noted at 48 h. Mostly, extracts of SDL are superior to FL. TPC and TFC are interrelated between themselves as well as showed positive and significant correlation with TAA. The antioxidant assays (DPPH, ABTS, SO and FRAP) reveal higher antioxidant activity with methanolic extracts compared to other studied solvents. **Conclusion:** The study highlights antioxidant properties of phenolics, particularly flavonoids in *P. foetida*, which can be further explored for its pharmacological importance.

Key words: Antioxidants, Extraction conditions, Fresh and shade dried leaves, *Paederia foetida*, Polyphenols.

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INTRODUCTION

Paederia foetida L., belonging to the family Rubiaceae is a perennial climbing shrub, extensively used in folk medicine for the remedy of stomach disorders, gastritis, indigestion, rheumatic pain, improvement of liver and kidney function[1,2,3] among others. The species possesses antiarthitic,[3] anti-inflammatory,[4] hepatoprotective,[5] antitussive,[6] antidiarrhoeal,[7] activities and also manifests aphrodisiac potentiality by increasing testosterone levels in experimental rats.[8] *P. foetida* is reported to consist of different phytoconstituents like iridoid glycosides (asperuloside, scandoside and paederoside), volatile oils (linalool, geraniol, α-terpineal), triterpenoids (urosolic acid, oleanolic acid) β-sitosterol, arachidic acid[1,9,10] which are implicated for diverse bioactivities.[11,12,13] It also contains alkaloids (paederine a and b), flavonoids and high proportion of mineral elements.[1,14] However, reports on phenolics in the species are rather meagre.[1] The foetid smell of *P. foetida* is due to release of methyl-mercaptan from the injured plant tissues following enzymatic cleavage of paederoside.[15]

Plant phenolics, an important class of secondary metabolites, consist of structurally heterogeneous group ranging from simple phenolic acids to much complex polymeric structure like tannins.[16] Flavonoids are the most abundant phenolics in plants that exhibit diverse bioactivities mostly due to their antioxidant potential. The plant derived antioxidants are significant due to their ability to inhibit or delay oxidative damages caused during many degenerative diseases.[17]

Polyphenols are extracted from plants by various ways differing in solvent composition and conditions. Among the different extraction methods, solvent extractions by cold maceration are widely used due to its convenience and efficacy.[18] Efficient extraction of bioactive phytochemicals involves a wide range of solvents of varying polarities of which solvents with higher polarities are preferable for phenolics.[16] Among several conditions that control extraction efficacy, extraction time appreciably influence quality and quantity of the extracts.[19]

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The present study highlights extraction of total phenolics, flavonoids and tannins under different solvents (water, methanol, ethanol, acetone) at different durations (12, 24, 36 and 48 h) using fresh and shade dried leaves of *P. foetida*. Furthermore, an interrelationship is ascertained between/among the phenolic compounds and antioxidant activity. The objective of the work is to determine an efficient extraction condition for phenolics with higher antioxidant activity in the studied plant species.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteau's (FC) reagent, aluminum chloride ($AlCl_3$), ammonium molybdate, ascorbic acid, ethylenediaminetetraacetic acid (EDTA), ferric chloride ($FeCl_3$), ferrous sulphate ($FeSO_4.7H_2O$), potassium acetate, potassium ferricyanide, potassium persulphate, riboflavin, sodium nitrite ($NaNO_2$) and sulfuric acid were obtained from Merck, India. DPPH, ABTS, gallic acid, quercetin, nitro blue tetrazolium (NBT), tannic acid and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma–Aldrich, (St. Louis, MO, USA). All other reagents were of analytical grade.

Plant materials

Healthy and mature leaves of *Paederia foetida* L. were collected in the month of September 2015 from the medicinal plant garden of Department of Botany, University of Kalyani, Kalyani (22.9750°N and 88.4344°E; 9.75 m above mean sea level), Nadia, West Bengal, India. The plant species was identified following suitable manual.[20] and an authentic voucher specimen was preserved at the herbarium repository of Department of Botany, University of Kalyani.

Extract Preparation

The extractions were made from fresh leaves (FL) and shade dried leaves (SDL). The SDL were obtained by drying the FL at room temperature (27± 2°C) for 10 d in shade (2g FL were dried to 1g SDL). The FL were washed with deionised water to remove the adherent debris and 2g leaves were cut into small pieces and extracted with double distilled water (aqueous), methanol, ethanol, and acetone for 12, 24, 36 and 48 h. The SDL samples (1g) were pulverised by a mechanical grinder and extracted similarly. During solvent extraction by cold maceration the sample to solvent ratio was kept constant at 1:20 (w/v). The extractions were made at room temperature with occasional shaking and subsequently the extracts were filtered through Whatman No 1 filter paper to remove the plant remains. The filtrates were concentrated under reduced pressure using rotary vacuum evaporator at 50°C (Büchi, Rotavapor R-II-HB; V-700; Switzerland), dried, estimated the final yield and stored at 4°C for further study.

Determination of total phenolic content (TPC)

TPC was determined by modified FC method.[21] The extracts (0.5 ml; 1mg/ml stock solution) were mixed with 0.5 ml of distilled water and 1ml of FC reagent (pre-diluted, 10 times, with distilled water) and incubated for 5 min at room temperature (27± 2°C). After incubation, 2 ml of 700 mM sodium carbonate was added in the reaction mixtures, mixed and kept in dark for 45 min at room temperature. The absorbances of the samples were measured at 765 nm using a UV–Vis spectrophotometer (CECIL, CE 7200; Cambridge, UK). A calibration curve was prepared using standard solutions of gallic acid ranging from10 to 80 μg/ml (r^2=0.983). The amount of phenolics in different extracts was calculated from the calibration curve and was expressed as mg gallic acid equivalent (GAE) per gm of FL and SDL.

Determination of total flavonoid content (TFC)

TFC were quantified according to the method of Chang *et al.*[22] with minor modifications. The samples (0.5 ml extract; 1mg/ml stock) were mixed with 1.5 ml distilled water and 0.2 ml 5% $NaNO_2$ and the resultant solution could stand for 2min at room temperature (27± 2°C). Subsequently, 0.2 ml of 10% $AlCl_3$ in ethanol and 0.6 ml 1N sodium hydroxide were added successively with vortexing in each step. The samples were incubated in dark at room temperature for 10 min and the absorbance was measured at 510 nm using a spectrophotometer. The amount of total flavonoid in the samples was quantified from the calibration curve of quercetin (ranging from 10 to 400 μg/ml; r^2=0.990) and was expressed as mg quercetin equivalent (QE) flavonoid per g of leaf samples (FL and SDL).

Determination of total tannin content (TTC)

TTC were estimated using the modified method of Price and Butler.[23] The extracts (500 μl; 1mg/ml stock solution) were mixed with distilled water (8 ml), 0.5 ml of 0.1M $FeCl_3$ and 0.5 ml of 8mM potassium ferricyanide sequentially and incubated at room temperature (27± 2°C) for 10 min. The absorbance was measured at 720 nm using a spectrophotometer. Reagent blanks for each solvent were prepared similarly without adding the sample. The amount of total tannin in the samples was quantified from a calibration curve of tannic acid (ranging from 1.5 to 20 μg/ml; r^2= 0.999) and expressed as mg tannic acid equivalent (TAE) per g of leaf samples (FL and SDL).

Estimation of total antioxidant activity (TAA)

TAA of the extracts was determined using phospho-molybdenum method.[24] with modifications. The reaction mixture consisted of 0.5 ml sample (from 1mg/ml stock solution), 0.5 ml distilled water and 3ml reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The resultant mixtures were incubated in a water bath at 90±1°C for 90 min, cooled down to room temperature (27± 2°C) and the absorbance were recorded at 695 nm using a spectrophotometer. The estimates were done using a calibration curve of ascorbic acid (ranging from 10 to 140 μg/ml; r^2= 0.979) and expressed as mg ascorbic acid equivalent (AAE) per g of leaf samples (FL and SDL).

DPPH radical scavenging assay

The antioxidant activity of the superior extracts (FL and SDL; all solvents: 48 h) of different solvents was determined by measuring the reducing ability of antioxidants towards $DPPH^{\bullet}$ radical. The radical scavenging assay was carried out following Brand Williams *et al.*[25] with modifications. An aliquot (50 μl) of extract of different concentrations (50 μg/ml to 1000 μg/ml) was added to 1.2 ml of 6 x 10^{-5} M DPPH solution. The ingredients were mixed by vortexing, incubated at dark for 15 min at room temperature (27± 2°C) and the absorbance was measured at 517 nm against a blank (methanol) using a spectrophotometer. Results were expressed as percentage scavenging activity of the $DPPH^{\bullet}$ radical which was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ and A_{sample} are the absorbance of the control and the test sample, respectively. The 50% scavenging of the DPPH radical (IC_{50}) was estimated from a graphical plot where percent scavenging (inhibition) was plotted against varying concentrations of an extract.

ABTS radical scavenging assay

The radical scavenging activity of the extracts was determined by $ABTS^{\bullet+}$ radical decolourization assay described by Re *et al.*[26] with minor modifications. The $ABTS^{\bullet+}$ radical was generated by reacting 7 mM ABTS in deionized water with 2.45 mM potassium persulfate, incubated in the dark for 16 h at room temperature (27± 2°C). Prior to use, the ABTS solution was diluted with ethanol to get an absorbance of 0.700 ± 0.02

at 734 nm. The antioxidant assay was performed by adding 50 μl of the sample (in varying concentrations ranging from 100 to 1000 μg/ml) in 950 μl of the diluted ABTS solution and the absorbance was measured at 734 nm. The sample absorbance was compared with a blank (50 μl ethanol and 950 μl of diluted ABTS solution) to detect spontaneous degradation of ABTS, if any, without antioxidant. The percent scavenging activity and IC_{50} value of the samples were determined similarly that was described for DPPH scavenging assay.

Superoxide (SO) scavenging activity

The SO radicals were generated by modified method based on Beauchamp and Fridovich.[27] The assay was based on the potentiality of the samples to inhibit blue formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system.[28] The samples of different concentrations were prepared in 50 mM sodium phosphate buffer (pH 7.6). The total volume of reaction mixture was 3 ml which was prepared by sequential addition of 1 ml of sample solution, 1.8 ml of 50 mM sodium phosphate buffer pH 7.6, 20 µl 2.66 mM riboflavin, 80 μl 12 mM EDTA and 100 μl 1.22 mM NBT. The photo-induced reactions were initiated by illuminating the reaction mixtures with a 20 W luminous bulb within an aluminium lined box for 90 sec at room temperature. The non-illuminated reaction mixture was used as blank. After completion of reaction, the absorbances were measured at 590 nm. The IC_{50} values were determined from the percent SO radical scavenging and that was obtained from the formula represented in previous sections.

Determination of ferric reducing antioxidant potential (FRAP)

The ferric reducing power of extracts was determined by a modified method of Benzie and Strain.[29] The method relies on reduction of colourless ferric complex (Fe^{3+}) to a blue-coloured ferrous complex (Fe^{2+}), at low pH, by electron donating antioxidants. The FRAP reagent was prepared fresh by mixing 10 volumes of 300 mM sodium acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ in 40 mM hydrochloric acid and with 1 volume of 20 mM $FeCl_3$. Following preparation, the reagent was pre-warmed at 37°C before use. The reaction mixture, consisted of 300 μl of extract preparations with 2.7 ml of FRAP reagent, was incubated at 37°C for 5 min and absorbance were measured at 594 nm. FRAP values were expressed as mM Fe^{2+}/ mg of sample and calculated using a calibration curve of ferrous sulphate (r^2=0.981) of different concentrations.

Statistical analysis

Critical difference (CD) at 0.05 probability level was performed to assess the significant level, if any, between and among the estimates of phenolics (TPC, TFC, and TTC) and TAA for different extraction conditions. CD at 0.05 probability level was also ascertained between/among the estimates of different antioxidant assays (DPPH, ABTS, SO and FRAP) in FL and SDL (48 h, all solvents) to assess significant variation, if any. Pearson correlation coefficient (r) was determined between the studied attributes like TPC, TFC, TTC and TAA considering extraction conditions at 47 degrees of freedom to ascertain whether there exists any interrelationship between and among them or not.

RESULTS

Extraction efficacy of phenolic components

The extraction efficacy of TPC (GAE/g; FL and SDL), TFC (QE/g; FL and SDL) and TTC (TAE/g; FL and SDL) from leaf samples (FL and SDL) under different extraction conditions (solvents used and duration of extraction) is presented in Table 1 and Figure 1. Results demonstrate that maximum quantity of TPC (FL: 4.090± 0.11; SDL: 4.957± 0.17) and TFC (FL: 28.002± 1.86; SDL: 71.221± 3.08) is recorded following methanolic extraction for 48 h duration. The yield of TPC and TFC is found to enhance in a time dependent manner. The quantified amount of

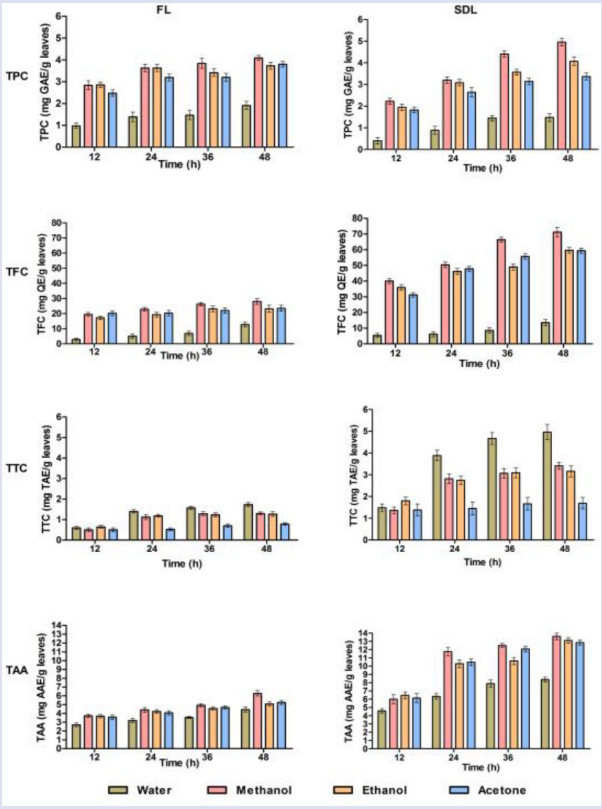

Figure 1: Phenolic yield (TPC, TFC and TTC) and total antioxidant activity (TAA) in FL and SDL of *P. foetida*.

TFC is mostly two-fold higher in all cases in SDL than FL. However, the estimates noted in TPC are rather higher mostly in FL than SDL expecting for 36 h and 48 h durations with methanolic and ethanolic extractions. Irrespective of the leaf types used, quantity of phenolics (TPC and TFC) mostly varied significantly ($p<0.05$) between/among the solvents used, and durations of extraction. For both TPC and TFC the efficacy of extraction is in the order of methanol > acetone > ethanol for FL and methanol > ethanol > acetone for SDL. Results highlight that maximum yield of TTC is obtained following aqueous extraction for 48 h in both FL (1.733± 0.10) and SDL (4.961± 0.35) with significant enhancement in SDL than FL. Thus, irrespective of the solvents used, extraction efficiency of TPC, TFC, and TTC is best in 48 h, SDL.

Correlation analyses (Table 2) reveal positive and significant interrelationship only between TPC and TFC (FL: $p< 0.001$, $r = 0.961$, DF 47; SDL: $p< 0.001$, $r = 0.950$, DF 47).

Antioxidant activity of extracts

The TAA (AAE /g; FL and SDL) activity of *P. foetida* leaf extracts is also depicted in Table 1 and Figure 1. The maximum activity of TAA in FL (6.273± 0.31) and SDL (13.587± 0.39) is recorded following extraction with methanol for 48 h. For both the leaf types, methanolic extraction show significantly ($p<0.05$) higher activities than the other solvent extractions for 24, 36 and 48 h durations. At 12 h duration, TAA activity is highest with ethanolic extraction. In all cases, SDL show pronounced (> 2-fold increase mostly) TAA activity than FL.

Correlation studies (Table 2) reveal that TAA is positively and significantly associated with TPC (FL: $p< 0.001$, $r = 0.797$, DF 47; SDL:

Table 1: Extraction efficacy of phenolics and total antioxidant activity in fresh leaves (FL) and shade dried leaves (SDL) of *P. foetida*.

Parameters		TPC (GAE/ g leaf sample)				CD at 5%	TFC (QE/ g leaf sample)				CD at 5%	TTC (TAE/ g leaf sample)				CD at 5%	TAA (AAE/ g of leaf sample)				CD at 5%
Durations		12 h	24 h	36 h	48 h		12 h	24 h	36 h	48 h		12 h	24 h	36 h	48 h		12 h	24 h	36 h	48 h	
Solvents																					
Water	FL	0.974 ± 012	1.388 ± 0.22	1.468 ± 0.20	1.907 ± 0.19	0.096	2.869 ± 0.96	5.022 ± 1.54	6.944 ± 1.42	12.819 ± 1.61	1.440	0.599 ± 0.07	1.399 ± 0.08	1.574 ± 0.07	1.733 ± 0.10	0.058	2.698 ± 0.21	3.186 ± 0.25	3.545 ± 0.14	4.428 ± 0.29	0.115
	SDL	0.402 ± 0.16	0.886 ± 0.18	1.445 ± 0.13	1.477 ± 0.19	0.165	5.349 ± 1.38	6.079 ± 1.62	8.580 ± 1.72	13.497 ± 1.86	4.013	1.491 ± 0.17	3.883 ± 0.24	4.664 ± 0.27	4.961 ± 0.35	0.302	4.582 ± 0.27	6.337 ± 0.32	7.891 ± 0.43	8.367 ± 0.30	0.292
Methanol	FL	2.840 ± 0.21	3.629 ± 0.17	3.844 ± 0.23	4.090 ± 0.11	0.029	19.483 ± 1.20	22.922 ± 1.20	26.216 ± 1.21	28.002 ± 1.86	0.740	0.503 ± 0.09	1.115 ± 0.11	1.285 ± 0.10	1.300 ± 0.07	0.153	3.725 ± 0.18	4.396 ± 0.27	4.923 ± 0.18	6.273 ± 0.31	0.304
	SDL	2.224 ± 0.14	3.203 ± 0.15	4.408 ± 0.14	4.957 ± 0.17	0.190	40.053 ± 1.62	50.325 ± 1.78	66.352 ± 1.55	71.221 ± 3.08	3.199	1.365 ± 0.15	2.818 ± 0.23	3.062 ± 0.23	3.409 ± 0.16	0.177	5.995 ± 0.53	11.761 ± 0.48	12.505 ± 0.24	13.587 ± 0.39	0.203
Ethanol	FL	2.858 ± 0.13	3.571 ± 0.16	3.410 ± 0.17	3.731 ± 0.16	0.045	17.202 ± 1.16	19.329 ± 1.52	23.079 ± 1.87	23.147 ± 2.32	0.929	0.644 ± 0.07	1.188 ± 0.07	1.230 ± 0.09	1.268 ± 0.11	0.045	3.666 ± 0.19	4.213 ± 0.22	4.553 ± 0.16	5.072 ± 0.23	0.053
	SDL	1.945 ± 0.15	3.085 ± 0.14	3.568 ± 0.14	4.072 ± 0.19	0.249	35.880 ± 1.87	46.161 ± 1.90	48.963 ± 1.96	59.617 ± 1.76	1.949	1.799 ± 0.18	2.741 ± 0.19	3.092 ± 0.23	3.156 ± 0.25	0.076	6.469 ± 0.41	10.300 ± 0.48	10.625 ± 0.43	13.113 ± 0.24	0.120
Acetone	FL	2.475 ± 0.14	3.195 ± 0.15	3.203 ± 0.18	3.803 ± 0.13	0.063	20.198 ± 1.54	20.267 ± 1.77	22.086 ± 1.90	23.515 ± 2.01	0.507	0.504 ± 0.09	0.522 ± 0.07	0.701 ± 0.09	0.784 ± 0.07	0.063	3.560 ± 0.27	4.053 ± 0.24	4.671 ± 0.18	5.246 ± 0.23	0.203
	SDL	1.821 ± 0.13	2.641 ± 0.21	3.138 ± 0.14	3.369 ± 0.15	0.242	31.161 ± 1.42	47.806 ± 1.58	55.601 ± 1.77	59.337 ± 1.62	1.433	1.382 ± 0.28	1.450 ± 0.30	1.667 ± 0.29	1.693 ± 0.26	0.124	6.140 ± 0.54	10.472 ± 0.41	12.078 ± 0.36	12.836 ± 0.29	0.148
CD at 5%	FL	0.036	0.036	0.063	0.061		0.719	0.744	0.775	0.978		0.058	0.115	0.045	0.089		0.052	0.100	0.198	0.330	
	SDL	0.138	0.304	0.202	0.251		1.789	2.012	2.279	2.631		0.115	0.045	0.096	0.421		0.196	0.109	0.260	0.165	

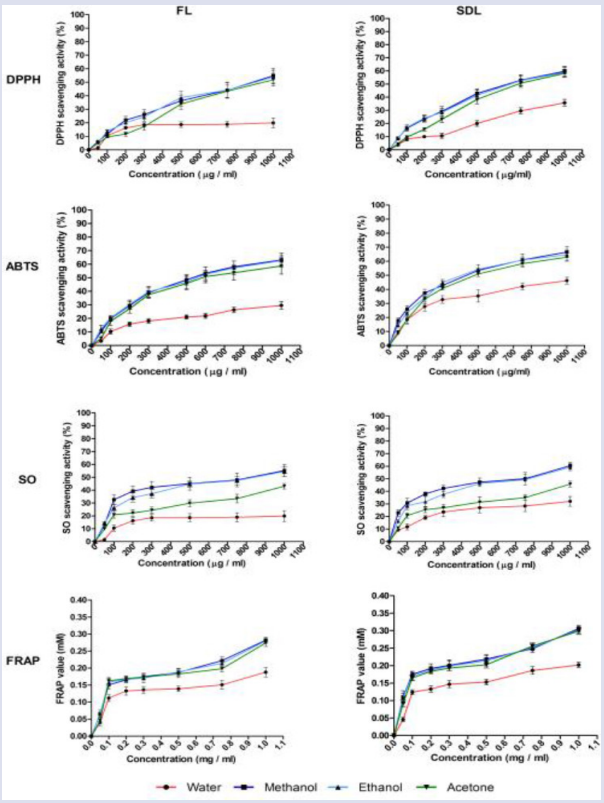

Figure 2: Radical scavenging (DPPH, ABTS and SO) and reducing power (FRAP) activity of extracts from FL and SDL using different solvents at 48 h duration.

p< 0.001, r = 0.900, DF 47) and TFC (FL: p< 0.001, r = 0.799, DF 47; SDL: p< 0.001, r = 0.845, DF 47).

From extraction efficacy it appears that 48 h duration is most productive for TPC, TFC, TTC yield and TAA activity in both FL and SDL for all the solvents studied. The data presented in Table 3 and Figure 2 documents antioxidant (DPPH, ABTS, SO and FRAP) activities ascertained from FL and SDL extracts at 48 h. The Figure 2 depicts higher antioxidant activity in SDL compared to FL in all cases with maximum efficacy in methanolic extracts followed by ethanol, acetone and water. The IC_{50} value is determined for DPPH, ABTS and SO by the radical scavenging activity of the antioxidants present in the extracts. The lower IC_{50} values indicate

Table 2: Correlation analysis showing relationship between the attributes.

Parameters	TPC	TFC	TTC	TAA
TPC	1.000			
	1.000			
TFC	0.961***	1.000		
	0.950*	**1.000**		
TTC	-0.062	-0.123	1.000	
	0.019	**-0.259**	**1.000**	
TAA	0.797***	0.799***	0.225	1.000
	0.900*	**0.845***	**0.152**	**1.000**

*** Significant at 0.001 probability level.

Bold values represent SDL.

higher scavenging efficiency and with enhanced antioxidant potentiality. The IC_{50} value could not be determined precisely in aqueous extracts of DPPH, ABTS and SO and that of acetone extracts of SO as it is above the maximum concentration (1000 μg/ml) used in the present study (Table 3). The DPPH assay data represent lower IC_{50} values (740.60± 36.58 to 786.97± 39.23) for SDL than FL (859.20± 38.65 to 902.30± 37.73) following methanol, ethanol and acetone extractions. Similar trend is also followed in ABTS (SDL: 538.97± 43.64 to 609.63± 49.37; FL: 629.80± 44.62 to 690.03± 53.62) and SO (SDL: 673.93± 58.91 to 726.83± 48.64; FL: 769.03± 33.40 to 789.13± 48.88). Although significant (p<0.05) variation is noted in detectable IC_{50} values between SDL and FL, variations are not significant among the different solvents in either of the leaf types. In FRAP assay, higher values (mM/mg) are indicative of better antioxidant activity. Excepting aqueous extracts (FL: 0.626± 0.04; SDL: 0.673± 0.02), FRAP values are higher in other solvents with a maximum in methanol extracts (FL: 0.940± 0.04; SDL: 1.020± 0.03). The FRAP values are relatively higher and mostly significant (p<0.05) in SDL than FL.

DISCUSSION

The present study reaffirms that the leaves of *P. foetida* are rich source of phenolics as evinced from quantitative estimation of TPC, TFC and TTC.[30,31] Solvent extraction following maceration and enhanced duration softens and breaks the cell wall to release soluble phytochemicals. The present investigation demonstrates that the amount of phenolics is increased with time; with a maximum at 48 h. Estimates of TPC and TFC are higher with methanol compared to other studied solvents. Methanol is commonly used solvent for its higher polarity with higher dielectric

Table 3: Results showing antioxidant activity in FL and SDL in different solvents at 48 h duration.

Samples	IC_{50} value (µg / ml)			FRAP value (mM)/ mg sample
	DPPH	ABTS	SO	
FL 48 W	>1000	>1000	>1000	0.626 ± 0.04
FL 48 M	859.20 ± 38.65	629.80 ± 44.62	769.03 ± 33.40	0.940 ± 0.04
FL 48 E	890.77 ± 30.99	633.33 ± 55.16	789.13 ± 48.88	0.933 ± 0.04
FL 48 A	902.30 ± 37.73	690.03 ± 53.62	>1000	0.912 ± 0.05
SDL 48 W	>1000	>1000	>1000	0.673 ± 0.02
SDL 48 M	740.60 ± 36.58	538.97 ± 43.64	673.93 ± 58.91	1.020 ± 0.03
SDL 48 E	751.17 ± 29.86	556.47 ± 39.69	726.83 ± 48.64	1.007 ± 0.03
SDL 48 A	786.97 ± 39.23	609.63 ± 49.37	>1000	0.998 ± 0.03
CD at 5 %	**53.72**	**82.87**	**75.28**	**0.06**

constant (32.6 at 20ºC) for extraction of phenolics[32] from different plant species namely, *Amomum*,[33] *Thymus*,[34] *Moringa*[35] among others. Methanol extraction is reported to work more efficiently for low molecular weight polyphenols.[36] However, better extractability with ethanol[37] and acetone[38] are also reported in different plant species. The present study also reveals that TTC is extracted best in aqueous condition at 48 h. Efficacy of aqueous extraction for TTC is also reported earlier[23] suggesting more water-soluble tannins in the leaves of the plant species. Variation in the quantified amounts of different phenolic components in relation to solvent extraction may be attributed to varying solubility and the existence of a phenolic compound in different forms.[39] The present study also indicates higher estimates of the phenolics mostly in SDL than FL, which is in accordance to the earlier reports.[40] The higher amount of TPC in FL in most extractions is possibly due to interaction of FC reagent with interfering molecules present in higher amount in FL, causing an overestimation of total TPC.[39,41] However, a reversal of such situation is noted at higher durations of methanolic and ethanolic extracts of SDL which can be the consequences of masking of interfering compounds due to higher yield of TPC.

In recent years, plant phenolics are gaining much importance for its health benefits due to their antioxidant properties.[39] The present work highlights significant enhancement of TAA in SDL than FL in a time dependent manner following different solvent extractions. Such observations are also reported earlier.[42] Methanol extracted samples of *P. foetida* are found superior in antioxidant activity over ethanol, acetone and water. TAA activity is found positively and significantly correlated with TPC and TFC. Similar findings are also noted in other plant taxa by earlier workers.[34,43,44] On the contrary, acetone and ethanol extracts are found effective in extracting antioxidant rich fractions from banana peel[45] and in *Gynura* leaf[46] respectively.

Plant derived antioxidants are also assessed by two distinct methods-ability to quench free radicals and reducing power assay.[47] In the present investigation, three radical scavenging (DPPH, ABTS, SO) and one reducing power (FRAP) assay are tested for antioxidant activity of leaf extracts obtained with different solvents at 48 h. Among all the assays, methanolic extract of SDL at 48 h shows higher activity over the extracts with ethanol, acetone and water. The superiority of methanol over other solvents is also evidenced for DPPH,[43] ABTS,[34] SO,[48] and FRAP[49] assays in different plant species. Thus, the methanolic extractions of SDL for 48 h duration are superior over other conditions for extracting phenolic components with substantial antioxidant activity in *P. foetida*.

CONCLUSION

The study documents the following: (i) the leaves of *P. foetida* possesses significant amount of phenolics; (ii) methanol is the better extraction solvent than ethanol, acetone and water for TPC and TFC; TTC estimates are highest in aqueous extraction for 48 h; (iii) the TAA estimates positively and significantly correlated with TPC and TFC indicating phenolic components, particularly flavonoids are the major contributors for antioxidant activity and (iv) the superiority of methanolic extracts is confirmed by the estimates of lower IC_{50} values in DPPH, ABTS and SO scavenging assays and higher estimates in FRAP assay.

The outcome of the study may be explored for identifying antioxidant rich flavonoids in *P. foetida*. Studies related to antioxidant components may provide pharmacologic importance signifying ethnomedicinal uses of the plant species.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS USED

TPC: Total phenolic content; **TFC:** Total flavonoid content; **TTC:** Total tannin content; **TAA:** Total antioxidant activity; **FL:** Fresh leaves; **SDL:** Shade dried leaves; **DPPH:** Diphenyl-1-picrylhydrazyl; **ABTS:** 2, 2´-azinobis-3-ethylbenzthiazoline-6-sulfonic acid; **SO:** Superoxide; **FRAP:** Ferric reducing antioxidant power; **NBT:** Nitro blue tetrazolium; **TPTZ:** 2, 4, 6-tripyridyl-s-triazine; **CD:** Critical difference.

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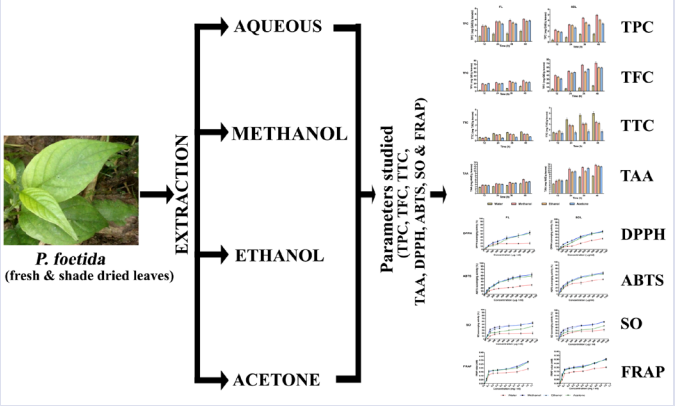
GRAPHICAL ABSTRACT



SUMMARY

- Leaf extracts of *P. foetida* contain substantial total phenolic, total flavonoid and total tannin contents.
- The yield of phenolics is higher in methanolic extractions, 48 h in shade dried leaves compared to fresh leaves.
- The polyphenol rich extracts manifest strong antioxidant activity.
- Total phenolics and flavonoids of *P. foetida* are important contributors for antioxidant property as evidenced from correlation analysis.

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