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

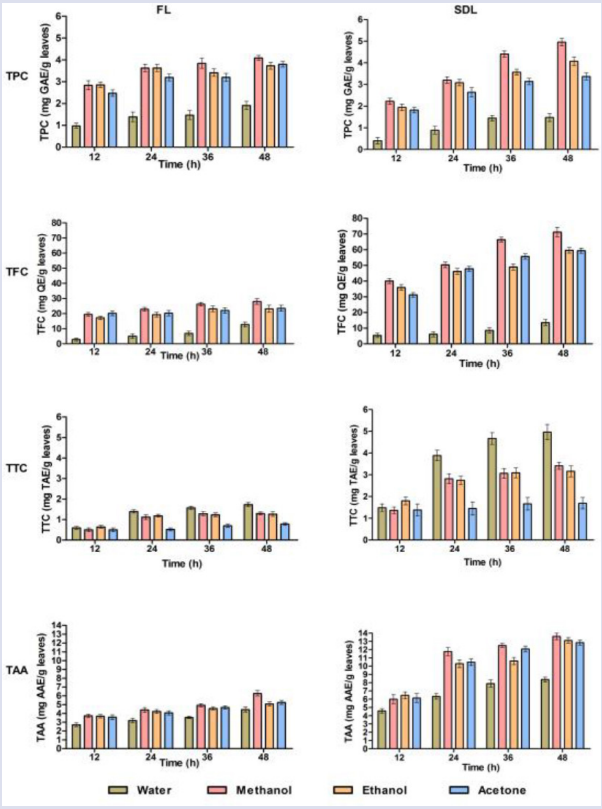

Figure 1: Phenolic yield (TPC, TFC and TTC) and total antioxidant activity (TAA) in FL and SDL of *P. foetida*.

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	

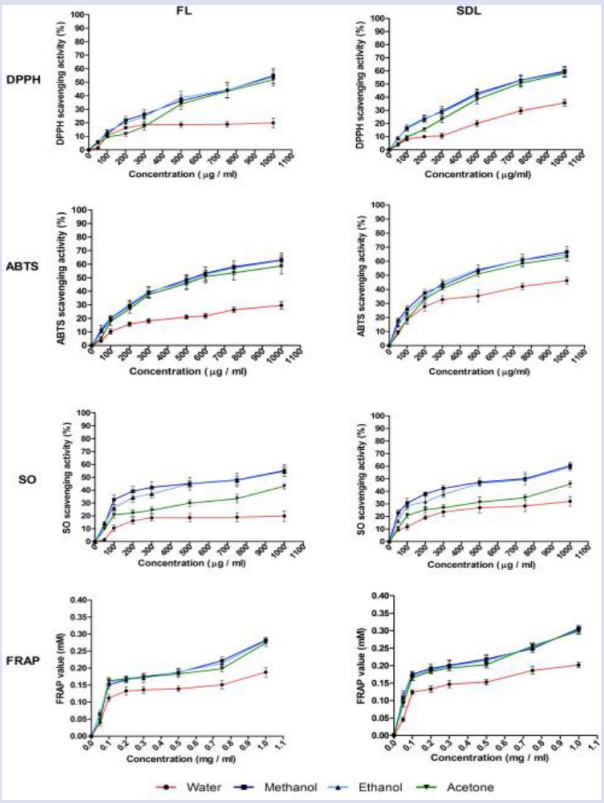

Figure 2: Radical scavenging (DPPH, ABTS and SO) and reducing power (FRAP) activity of extracts from FL and SDL using different solvents at 48 h duration.

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**

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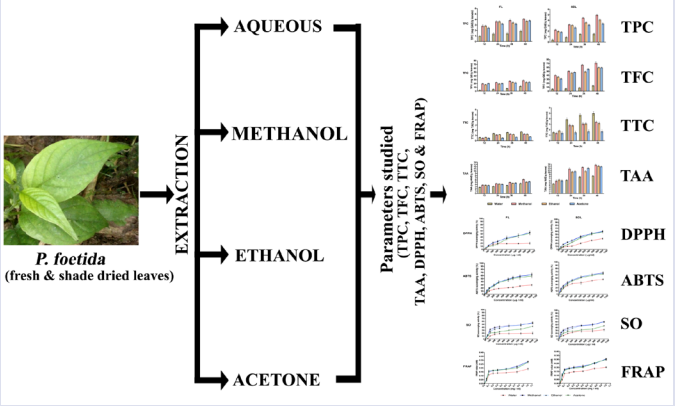
GRAPHICAL ABSTRACT



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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