RESEARCH ARTICLE

EFFICACY OF AQUEOUS AND ETHANOLIC EXTRACTION ON PHENOLICS AND ANTIOXIDANT ACTIVITY OF *PAEDERIA FOETIDA* L. AND *SPERMACOCE STRICTA* L.F.

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Abstract: Plant phenolics, particularly flavonoids are rich source of antioxidants. Efficient extraction of phenolics with solvents safe for human health is sought for dietary formulations. The study deals with the efficacy of aqueous (temperature 30, 50, 80, and 100°C; duration 10, 20 and 30 min) and ethanolic (concentration 50, 70, and 90%; duration 30, 60 and 90 min) extractions of total phenolic content (TPC) and flavonoid content (TFC), and total antioxidant activity (TAA) in *Paederia foetida* L. and *Spermacoce stricta* L.f. (Family; Rubiaceae). The observations are statistically analyzed and results reveal that the phenolic and flavonoid contents and antioxidant activity is higher in *P. foetida* than *S. stricta*. Furthermore, ethanolic extraction is better than aqueous extraction in terms of antioxidant activity. The result highlights the potential use of the two plant species in dietary formulations to defend oxidative stress.

Keywords: Phenolics, Flavonoid, Antioxidant activity, *Paederia foetida*, *Spermacoce stricta*.

INTRODUCTION

Plants produce a plethora of phytochemicals which is a boon for both the plants and humans (Ruskin *et al.*, 2002). These phytochemicals are useful to defend the plant from different biotic and abiotic stresses due to their biochemical activities (Dicko *et al.*, 2005; Ramakrisna and Ravishankar, 2011). Plants as a whole or their parts have been utilized by humans for their wellbeing including the remedy of different ailments and diseases (Calabrese *et al.*, 2012). The medicinal property of plants has thoroughly been investigated in relation to their phytochemical constituents and their antioxidant activity (Cao *et al.*, 1996; Prior and Cao, 2000). The growing knowledge in this field indicates that an increased intake of antioxidant rich food can lower the risk of many degenerative diseases like cardiovascular diseases and cancer (Liu, 2003; Su *et al.*, 2013). Plants produce three major classes of secondary metabolites or natural products i.e. terpenes, phenolics and alkaloids (Taiz and Zeiger, 2010). Among these natural products phenolics are the most important for dietary formulations and are most widely studied (Crozier *et al.*, 1997). Plant phenolics or polyphenols constitute a chemically diverse group among which flavonoids are of importance by their ability to defend oxidative damage to the plant (Svobodova *et al.*, 2003). The search for new and safe phytoconstituents with higher antioxidant potential from different plants have gained attention to develop natural antioxidant formulations for food, cosmetic, and other applications (Trautinger, 2001; Eshun and He, 2004). *Paedaria foetida* L. and *Spermacoce stricta* L.f. both belong to the family Rubiaceae are medicinally important and widely used in traditional folk medicine for curing various ailments and diseases. The reported photochemical constituents from these plants are chemically diverse and mainly found from the leaves of the plant (Conserva and Ferreira, 2012; Chanda *et al.*, 2013).

A number of extraction methods are usually employed for phenolic extraction out of which solvent extractions are commonly used due to its ease, efficiency and wider applicability. The efficacy of phenolic extraction depends upon the varying polarities of the solvent, extraction time, temperature, sample to solvent ratio, chemical composition and physical properties of the sample (Turkmen *et al.*, 2006). Solvents like methanol, ethanol, acetone, ethyl acetate and their combinations along with varying proportions of water have been used for extraction of phenolics (Robbins, 2003). Aqueous methanol is a preferred solvent for extraction of low molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone (Dai and Mumper, 2010). However, a dietary formulation requires use of non-toxic solvents safe for human consumption (Shi *et al.*, 2005). To address the problem, the extraction efficacy of phenolics from *P. foetida*, and *S. stricta* has been studied utilizing aqueous extraction at different temperatures and also with different concentrations of ethanol. Furthermore, the interrelationship between phenolics and antioxidant capacity has also been assessed to predict the efficacy of extraction in the plant species.

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MATERIAL AND METHOD

Chemicals

Gallic acid, quercetin and 2, 2- diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich, (St. Louis, MO, USA). Folin–Ciocalteau's (FC) reagent, aluminum chloride, ammonium molybdate, ascorbic acid, sodium phosphate, sodium carbonate, and potassium acetate were obtained from Merck, India. All other chemicals were of analytical grade.

Materials

Paederia foetida L., and *Spermacoce stricta* L.f., belonging to the family Rubiaceae were collected from Kalyani University campus, Kalyani (22.9750° N and 88.4344° E, 9.75 m above mean sea level), Nadia, West Bengal. The identification of plants was done following the use of appropriate manual (Prain, 1903). The plant specimens were preserved at the herbarium repository of Department of Botany, University of Kalyani as voucher specimen.

Sample Preparation

The leaves were collected from the plant and washed under running water to remove the adherent dirt from the leaves. These leaves were then shade dried for 15 days at room temperature (30 ±1°C). The shade dried leaves were pulverized by a mechanical grinder and extracts were made with water at different temperatures (30, 50, 80, and 100°C) and ethanol at different concentrations (50, 70, and 90%). The sample to solvent ratio was kept constant at a ratio 1:20 (w/v) and extractions were done at different time (aqueous- 10, 20, and 30 min and ethanolic-30, 60, and 90 min) intervals. During the process, samples were occasionally shaken and subsequently the extracts were filtered through Whatman filter paper to remove the debris. The extracts were kept at 4ºC until further use.

Determination of total phenolic content

The total phenolic content (TPC) was determined using modified Folin–Ciocalteu (FC) method (Ainsworth and Gillespie, 2007). The assay mixture was prepared using 0.5 ml of sample solution, 0.5 ml of ethanol, 1ml of FC reagent (1:10), followed by addition of 2 ml 700 mM sodium carbonate (Na_2CO_3). The reaction mixtures were incubated in dark for 45 min at room temperature (30 ±1ºC) and the absorbance was measured in a UV–Vis spectrophotometer (CECIL, CE 7200; Cambridge, England) at 765 nm. The amount of total phenolics was quantified by comparing the standard curve of gallic acid and was expressed as milligram (mg) of gallic acid equivalent (GAE) in per gram (g) of dry sample.

Determination of total flavonoid content

The total flavonoid (TFC) contents were quantified using the method of Chang *et al*., (2002) with modifications. The reaction mixtures were prepared by sequential addition of 0.5 ml of sample, 1.5 ml of 90% ethanol, 0.2 ml 10% aluminum chloride ($AlCl_3$) in ethanol and 0.2 ml 1M potassium acetate. Finally the volume of each reaction mixture was made up to 3 ml by adding distilled water. The reaction mixtures were incubated in dark at room temperature (30 ±1ºC) for 20 min and the absorbance was measured at 415 nm in a spectrophotometer. The amount of total flavonoid was quantified by comparing the standard curve of quercetin and was expressed as mg quercetin equivalent flavonoid (QE) per gram (g) of dry sample.

Estimation of total antioxidant activity

The total antioxidant activity (TAA) of the extracts was determined by the phospho-molybdenum method adopting the procedure of Prieto *et al*., (1999). The assay mixtures were prepared by addition of 3ml reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and 1 ml sample extract. The assay mixtures were incubated in a water bath at 90 ±1ºC for 90 min, cooled down to room temperature and the absorbance was recorded at 695 nm in a spectrophotometer. The TAA was estimated by comparing ascorbic acid as standard and expressed as mg ascorbic acid equivalent (AAE) per gram (g) of dry sample.

Statistical analysis

All analyses are done in triplicate, and the average value is presented in table 1 and 2. In order to determine the association between TPC-TAA and TFC-TAA (recorded at maximum level mostly), correlation analysis has been performed. Test of significance (t-test analysis) is also made to assess significant variation, if any, between mean values in a species as well as between species for maximum level of TAA.

RESULT AND DISCUSSION

Total phenolic (TPC) and flavonoid (TFC) contents, and total antioxidant activity (TAA) are represented in table 1 (aqueous extracts) and table 2 (ethanolic extracts) of *P. foetida*, and *S. stricta*.

Aqueous extraction of *P. foetida*

TPC ranges from 3.60 ± 0.36 mg GAE/g (temperature 30ºC, time 10 min) to 4.71 ± 0.25 mg GAE/g (temperature 50ºC, time 30 min); while TFC varies from 0.36 ± 0.74 mg QE/g (30ºC, 10 min) to 4.95 ± 0.29 mg QE/g (50ºC, 30 min). TAA also represents the similar trend with an activity ranging from 5.01 ± 0.85 mg AAE/g (30ºC, 10 min) to 10.8 ± 0.53 mg AAE/g (50ºC, 30 min). The result indicates

that all three parameters in the study increase with an increase of extraction time. However, the trend is not similar with increasing temperature. Maximum amount (TPC and TFC) and activity (TAA) have been recorded at 50ºC; 30 min. The decreasing antioxidant activity at 80ºC and 100ºC in *P. foetida* may be due to degradation or inactivation of phenolics at higher temperature. Temperature treatment above 70ºC during extraction has shown substantial degradation of phenolics like anthocyanin (Havlikova and Mikova, 1985).

In *P. foetida*, significant correlation is found between TPC and TAA ($r = 0.979$, 2df, $P<0.05$) as well as between TFC and TAA ($r = 0.987$, 2df, $P<0.05$) at 50ºC for 30 min. Correlation analyses signify that both phenolics and flavonoids are interrelated with high antioxidant activity.

Aqueous extraction of *S. stricta*

TPC ranges from 1.41 ± 0.19 mg GAE/g (30ºC, 10 min) to 4.70 ± 0.24 mg GAE/g (100ºC, 30 min); while, TFC varies from 0.23±0.81 mg QE/g (30ºC, 10 min) to 1.58 ± 0.31 mg QE/g (100ºC, 30 min). TAA is estimated to be minimum at 30ºC, 10 min (2.17 ± 0.36 mg AAE/g) and maximum at 50ºC, 30 min (6.45 ± 0.29 mg AAE/g). Result also shows that the amount and activity of the observed parameters increase with an increase in temperature. Unlike *P. foetida*, *S. stricta* shows a higher TPC and TFC at 100ºC with 30 min extraction time. The maximum TAA observed in *S. stricta* is at 50ºC, 30 min, corresponding to *P. foetida*.

Correlation analyses (100ºC, 30 min) show a positive and significant relationship between TPC and TAA ($r = 0.999$, 2df, $P<0.05$) but such association is not found between TFC and TAA ($r = 0.863$, 2df, $P>0.05$). It reflects that flavonoid content is not showing maximum antioxidant capacity. It may be due to the inactivation or modification of flavonoids at very high temperature.

Ethanolic extraction of *P. foetida*

The components under study show a gradual increase with an increase in extraction time. TPC ranges from 2.24 ± 0.27 mg GAE/g (concentration 90%, time 30 min) to 4.71 ± 0.31 mg GAE/g (70%, 90min); while, TFC varies between 1.43 ±0.61 mg QE/g (90%, 30 min) and 4.50 ± 0.40 mg QE/g (70%, 90 min). Minimum TAA value is recorded at 90% concentration, 30 min (6.51 ± 0.38 mg AAE/g) and maximum at 50%, 30 min (11.2 ± 0.38 mg AAE/g). The interrelationship between TPC and TAA ($r = 0.899$, 2df, $P>0.05$) and TFC and TAA ($r = 0.918$, 2df, $P>0.05$) is non-significant (recorded at -70%, 90 min).

Ethanolic extraction of *S. stricta*

TPC ranges from 2.20 ± 0.17 mg GAE/g (90%, 30 min) to 4.63 ± 0.18 mg GAE/g (50%, 90 min); while TFC varies from 0.76 ± 0.28 mg QE/g (70%, 30 min) to 1.94 ± 0.19 mg QE/g (50%, 90 min). TAA value has been recorded minimum in 90%, 30 min (5.74 ± 0.47 mg AAE/g) and maximum at 50%, 90 min (9.92 ± 0.41 mg AAE/g) corroborating with that of TPC and TFC. Correlation analyses (50%, 90 min) reveal a positive and significant interrelationship between TPC and TAA ($r = 0.997$, 2df, $P<0.05$) and TFC and TAA ($r = 0.981$, 2df, $P<0.05$).

Test of significance (t-test) analysis:

The t-test analyses reveal significant variation in TAA ($t=12.424$, 4df, $P<0.001$- *P. foetida*; $t= 3.99$ 4df, $P<0.001$- *S. stricta*) between species irrespective of aqueous (50ºC, 30 min) and ethanolic (50%, 90 min) extraction method. In *P. foetida*, TAA activity is random ($t= 1.34$, 4df, $P>0.05$) between extractions; while, non-randomness has been studied ($t= 11.980$, 4df, $P<0.001$) for *S. stricta*. The test also suggests that TAA varied at maximum level between solvents (aqueous – $t = 6.790$, df 4, $P<0.001$; ethanolic – $t= 7.789$, df 4, $P<0.001$). The results also reflect that TAA is higher in *P. foetida* than *S. stricta*, and the efficiency of extraction is better in ethanol than water.

Table 1. Effect of temperature and time on total phenolic content, total flavonoid content and total antioxidant activity of aqueous extracts from *P. foetida*, and *S. stricta*.

Temperature (°C)	Attributes	***Paederia foetida***			***Spermacoce stricta***		
		Extraction Time (min)			Extraction Time(min)		
		10	20	30	10	20	30
30	TPC	3.60± 0.36	3.74± 0.43	3.94± 0.21	1.41± 0.19	2.48± 0.36	2.53± 0.09
	TFC	0.36± 0.74	0.45± 0.89	0.76± 0.73	0.23± 0.81	0.25± 0.54	0.50± 0.49
	TAA	5.01± 0.80	5.29± 0.73	5.41± 0.66	2.17± 0.36	3.65± 0.49	3.82±0.64
50	TPC	4.31± 0.31	4.43± 0.04	4.71± 0.25	4.09± 0.28	4.17± 0.51	4.35± 0.43
	TFC	3.10± 0.81	4.04± 0.35	4.95± 0.29	0.94± 0.47	1.26± 0.93	1.53± 0.73
	TAA	9.93± 0.54	10.3± 0.68	10.8± 0.53	5.63± 0.52	5.91± 0.34	6.45± 0.29
80	TPC	4.37± 0.42	4.48± 0.11	4.61± 0.37	3.94± 0.11	4.16± 0.26	4.24± 0.34
	TFC	1.73± 0.34	1.99± 0.67	2.41± 0.84	0.54± 0.18	0.58± 0.73	0.77± 0.84
	TAA	9.54± 0.61	9.81± 0.34	9.84± 0.22	4.13± 0.69	4.33± 0.43	4.35± 0.52
100	TPC	4.46± 0.21	4.52± 0.18	4.59± 0.37	4.60± 0.05	4.65± 0.15	4.70± 0.24

TFC	1.51± 0.67	2.17± 0.06	3.29± 0.93	0.89± 1.05	1.16± 0.83	1.58± 0.31
TAA	9.82± 0.49	9.15± 0.47	8.94± 0.64	5.27± 0.27	5.25± 0.54	5.06± 0.21

TPC: Total phenolic content TFC: Total flavonoid content, TAA: Total antioxidant activity
Data are presented as mean± SD (n=3), in mg/g dry sample.

Table 2. Effect of concentration and time on total phenolic content, total flavonoid content and total antioxidant activity of ethanol extracts from *P. foetida*, and *S. stricta*.

Ethanol Concentration (%)	Attributes	***Paederia foetida*** Extraction Time (min)			***Spermacoce stricta*** Extraction Time (min)		
		30	60	90	30	60	90
50	TPC	4.59± 0.07	4.63± 0.14	4.70± 0.13	4.56± 0.54	4.61± 0.26	4.63± 0.18
	TFC	3.30± 0.54	4.28± 0.56	4.45± 0.34	0.92± 0.77	1.74± 0.52	1.94± 0.19
	TAA	9.73± 0.4	10.5± 0.23	11.2± 0.38	7.58± 0.76	8.84± 0.35	9.92± 0.41
70	TPC	4.53± 0.21	4.68±0.08	4.71± 0.31	4.27± 0.48	4.55± 0.33	4.56± 0.1
	TFC	1.67± 0.63	4.11± 0.49	4.50± 0.40	0.76± 0.28	0.90± 0.33	1.86± 0.26
	TAA	6.78± 0.45	7.96± 0.74	8.83± 0.37	6.01± 0.56	8.11± 0.24	9.16± 0.39
90	TPC	2.24± 0.27	3.04± 0.21	3.40± 0.16	2.20± 0.17	2.77± 0.28	3.11± 0.49
	TFC	1.43± 0.61	1.85± 0.42	3.12± 0.08	1.24± 0.82	1.26± 0.43	1.28± 0.21
	TAA	6.51± 0.38	7.19± 0.57	7.93± 0.63	5.74± 0.47	6.20± 0.58	6.69± 0.46

TPC: Total phenolic content TFC: Total flavonoid content, TAA: Total antioxidant activity
Data are presented as mean± SD (n=3), in mg/g dry sample.

CONCLUSION

The total phenolic (TPC) and flavonoid (TFC) content, and antioxidant activity (TAA) of *P. foetida* and *S. stricta* are estimated following aqueous and ethanolic extracts. Aqueous and ethanolic extractions for the study are simple, efficient, cost-effective and safe. Results indicate that the presence of substantial antioxidant activity in both species which show high correlation with phenolic and flavonoid contents. These observations highlight the potential of *P. foetida* and *S. stricta* as source for antioxidant and also signify their role in dietary formulations to defend oxidative stress.

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