Research Article

Effect of solvents and extraction methods on the phenolic content, flavonoid content, and antioxidant activity of *Bauhinia variegata* and *Leptadenia reticulata*

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Abstract

Objective: In the present study, we investigated the total phenolic content, total flavonoid content and antioxidant activity of the crude extracts of *Bauhinia variegata* and *Leptadenia reticulata*. **Materials and Methods:** The extracts were prepared using three experimental conditions [elevated and 25°C temperature; ultrasound vibrations] and four solvent systems [water, methanol, water: methanol (1:1), chloroform: methanol (1:1)]. The total phenolic content was estimated by the Folin-Ciocalteu method; total flavonoids was estimated by $AlCl_3$ method; antioxidant activity was determined using DPPH and phosphomolybdenum assay. **Results:** For *Bauhinia variegata*, the methanol extract prepared at 25°C showed highest phenolic content (69.39 ± 1.22 mg GAE/g of dry extract), flavonoid content (44.50 ± 1.11 mg RE/g of dry extract) and free radical scavenging capacity (57.01 ± 1.57%) and methanol extract prepared at elevated temperature showed highest total antioxidant capacity (135.24 ± 4.37 mg AAE/g of dry extract). For *Leptadenia reticulata*, the water: methanol extract showed highest phenolic content (24.60 ± 0.65 mg GAE/g of dry extract), flavonoid content (6.44 ± 0.21 mg RE/g of dry extract) and free radical scavenging capacity (66.83 ± 0.91%) and methanol extract prepared by sonication showed highest total antioxidant capacity (52.88 ± 1.34 mg AAE/g of dry extract). **Conclusions:** These results indicate that these medicinal plants may have potential application in reducing oxidative stress due to presence of strong antioxidant activity. Further isolation and identification of bioactive component from these plants could have application in health and pharmaceutical industry.

Keywords: Bauhinia variegata, Leptadenia reticulata, antioxidant activity, phenolic content and flavonoid content

Introduction

Free radicals in the form of hydroxyl radical (OH), superoxide anion ($O2^-$), nitric oxide (NO), nitrogen dioxide (NO2), peroxyl (ROO) and lipid peroxyl (LOO) are by products of our body's metabolism (Alam et al., 2013; Pisoschi and Negulescu, 2011; Seifu et al., 2012). An imbalance between free radical generation and antioxidant defences produces oxidative stress which damages biomolecules like lipids, proteins and DNA and RNA (Amarowicz et al., 2004). It is now well established that oxidative stress has implications in pathogenesis of various diseases like cardiovascular, neurodegenerative, cancer and aging among others (Szymanska et al., 2016). Antioxidants are stable molecules that neutralize free radicals by donating an electron and thus reducing the damage caused by these free radicals (Brewer, 2011; Lobo et al., 2010). Medicinal plants are a great source of natural antioxidants due to the presence of secondary metabolites such as phenolic and flavonoid compounds which possess strong free radical scavenging property (Abdel-lateif et al., 2016).

For the analysis of medicinal plants, extraction plays an extremely crucial role as it is desirable to extract phytochemicals from the plant material (Sasidharan et al., 2011). During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity (Pandey and Tripathi, 2014). Additionally, as the phytochemicals to be extracted may be non-polar to polar and thermally labile, the suitability of the methods of extraction (Sasidharan et al., 2011) and various conditions such as time

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and temperature must also be considered (Doughari, 2012). Thus, it is recommended that solvents and conditions that enable isolation of wide range of compounds must be employed for efficient extraction (Romanik et al., 2007). Various methods, such as soxhlet extraction, ultrasound assisted extraction etc. are commonly used for the extraction from plant material (Azmir et al., 2013; Azwanida, 2015; Stalikas, 2007). Polar solvents are frequently employed for the recovery of phytochemicals such as polyphenols from a plant material. The most suitable of these solvents are water, methanol, ethanol, chloroform and aqueous mixtures containing ethanol and methanol (Peschel et al., 2006).

Bauhinia variegata Linn (Caesalpiniaceae) has been used in treating bronchitis, leprosy, tumors and ulcer (Mishra et al., 2013; Rajkapoor et al., 2006). Also, chemopreventive, anticarcinogenic and antimutagenic potentials of the plant has been reported (Agrawal and Pandey, 2009; Rajkapoor et al., 2003). *Leptadenia reticulata* Wight & Arn (Asclepiadaceae) possesses various pharmacological activities like antibacterial (Kalidass et al., 2009), antifungal (Mishra et al., 2010), analgesic, anti-inflammatory, anti-lipoxygenase (Mohanty et al., 2015). Also, anticarcinogenic potential of the ethanolic extracts have been reported against Dalton's Ascitic Lymphoma (Sathiyanarayanan et al., 2007). However, reports on the phenolic content, flavonoid content and antioxidant activity are sparse. Thus, in the present study, we have evaluated the phenolic content, flavonoid content, free radical scavenging and total antioxidant activity of these two plants.

Materials and Methods

Chemicals

Methanol, Chloroform, Sodium Carbonate, Sodium Hydroxide, Sulfuric Acid and Aluminium Chloride were procured from Merck chemicals. Dimethyl Sulphoxide (DMSO), Gallic acid, Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and Rutin were procured from HiMedia Laboratories Pvt. Ltd. Ascorbic acid and Sodium Nitrite was ordered from S. D. Fine Chemicals Ltd. Sodium Phosphate and Ammonium Molybdate were procured from Sisco Research Laboratory. All chemicals used in the experiments were of analytical grade.

Instrumentations

The bath sonicator used was from Chicago electric power tools, temperature controlled water bath was from Shivtronics and rotary shaker was from Bright Instruments. The Multiskan Go plate reader was purchased from Thermo Fischer Scientific. Ultrapure water was collected from Milli Q system. Deep freezer (-20°C) was purchased from Blue Star.

Collection of plant material and extraction

The leaf part of *Bauhinia variegata* and *Leptadenia reticulata* was collected from Xavier Residence c/o St. Xavier's College Campus, Ahmedabad. The plant was verified and confirmed by Botanist Dr. Hitesh Solanki from Botany Department of Gujarat University. Leaves were thoroughly washed with tap water to remove the dirt particles followed by distilled water and were then shade dried. The dried leaf was then crushed into fine powder and subjected to extraction using three experimental conditions (elevated temperature, 25°C temperature and ultrasound waves) and four solvent systems [water, methanol, water: methanol (1:1), chloroform: methanol (1:1)]. During extraction the powder to solvent (w/v) ratio was kept 1:10 (Tiwari et al., 2011). The elevated temperature extraction was carried out in hot water bath below the boiling point of respective solvent used for extraction. The temperature for extraction was for water: 90°C; for methanol: 55°C; for water: methanol- 65°C; for chloroform: methanol- 50°C. The extraction using ultrasound waves was performed (60 Hz frequency) with the aid of a bath sonicator (Romanik et al., 2007). For elevated temperature and sonication extraction, one gram powder was taken in a stoppered tube and 10 ml of solvent was added to it which was then incubated in hot water bath or bath sonicator for one hour, as relevant. At the end of the incubation period, the filtrate was collected in a pre-weighed beaker. This procedure was repeated until the solvent became colourless. For cold extraction, the solvents were changed every 24 hours and constant agitation was provided at 100 rpm (Daniel, 1991). The extracts were filtered using Whatman no. 1 filter paper. The collected filtrate was pooled and evaporated to dryness until a constant weight was achieved. Then stocks of the extract at a concentration of 100 mg/ml were prepared in DMSO and stored at -20°C until further use.

Total phenolic estimation

The total phenolic content was estimated by the Folin-Ciocalteu method as described by Herald et al., 2012, and Vyas and Braganza, 2018. Briefly, 20 µl plant extract (*Bauhinia variegata*- 1 mg/ml; *Leptadenia reticulata*- 5 mg/ml) was added into the wells of 96 well plate followed by 80 µl Folin Ciocalteu reagent (1:10 dilution [v/v] with MilliQ water). At the end of the 6th minute, 100 µl of 7.5% Na_2CO_3 was added to the mixture in each well. The plate was covered and incubated in the dark for 90 minutes. The absorbance was measured at 765 nm against methanol blank in Multiskan Go plate reader. Gallic acid was used as a standard to generate a calibration curve (12.5- 200 µg/ml). The results were expressed in terms of Gallic acid equivalent (mg GAE/g dry extract). Each standard and sample solution was analysed in triplicate. The samples were assayed against a sample control (i.e. sample solution without F–C reagent and Na_2CO_3).

Total flavonoid estimation

The total flavonoid content was estimated by the Aluminium chloride method as described by Herald et al., (2012) and Vyas

and Braganza, 2018. Briefly, 25µl plant extract (*Bauhinia variegata*- 1 mg/ml; *Leptadenia reticulata*- 5 mg/ml) followed by 10µl 5% $NaNO_2$ was added to the wells of 96 well plate. After 5 min, 15µl 10% $AlCl_3$ was added to the reaction mixture. At the 6th min, 50µl 1M NaOH was added to the wells followed by 150 µl MilliQ water. The absorbance was read against methanol blank at 510 nm using Multiskan Go plate reader. Rutin was used to generate calibration curve (100 to 700µg/ml) and the results were expressed as Rutin equivalent (mg RE/g dry extract). The samples were measured against sample control.

Free radical scavenging activity

The free radical scavenging activity of the extracts was estimated using free radical DPPH as described by Blois, (1958) and Vyas and Braganza, (2018). DPPH is a stable free radical and loses its deep purple colour and turns to yellow upon reaction with any oxidising compound. It is a rapid, simple, and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors (Kedare and Singh, 2011). A freshly prepared 0.1 mM DPPH in methanol was used for the estimation. 25µl of plant extract (*Bauhinia variegata*- 0.5 mg/ml; *Leptadenia reticulata*- 2.5 mg/ml) was added to wells of a 96 well plate followed by 200 µl DPPH was added and the mixture was incubated for 30 minutes in dark. The absorbance (A) was measured at 517 nm at the end of the incubation period using Multiskan go plate reader. A standard curve was prepared using Ascorbic acid (10µg/ml to 50µg/ml). Appropriate blank and controls were also used in the experiment. The results were expressed as percentage DPPH quenched and was calculated using the following formula:

% DPPH quenched

$$= [1 - (A_{sample} - A_{blank}) / (A_{control} - A_{blank})] \times 100 \quad .....(1)$$

Total antioxidant activity

The total antioxidant capacity was performed as per the method described by Prieto et al., 1999. Phosphomolybdenum assay is a quantitative method used for evaluation of the antioxidant capacity indicated by electron donating capacity. This method is based on the reduction of Mo (VI) to Mo (V) and the formation of a green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity of the extracts is expressed as the number of equivalents of ascorbic acid. Briefly, 100µl plant extract (*Bauhinia variegata*- 1 mg/ml; *Leptadenia reticulata*- 5 mg/ml) was taken in a tube and 1 ml phosphomolybdenum reagent (0.6 M Sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to this. The samples were then incubated at 95°C for 90 minutes in a water-bath. After the incubation period was completed, samples were allowed to cool at room temperature. The absorbance of the samples was measured at 695 nm. A calibration curve was generated using Ascorbic acid (50µg/ml to 250µg/ml) and results were expressed as Ascorbic acid equivalents mg AAE/g of dry extract.

Statistical analysis

All the experiments were performed in triplicates. Results were expressed as the Mean ± SD of values obtained in triplicate from three independent experiments.

Results

The results of the total phenolic and flavonoid content, free radical scavenging activity and total antioxidant activity for *Bauhinia variegata* Linn and *Leptadenia reticulata* (Retz.) Wight & Arn were as shown in table 1 and 2 respectively.

Table 1. Total phenolic content, total flavonoid content, free radical scavenging activity and total antioxidant activity of *Bauhinia variegata* plant extracts.

Treatment	Solvent systems	TPC* (mg GAE/g)	TFC*(mg RE/g)	FRS* (% DPPH Quenched)	TAA* (mg AAE/g)
Elevated temperature	W	26.01 ± 0.89	8.46 ± 0.66	17.04 ± 0.88	56.39 ± 1.57
	M	59.70 ± 1.52	38.62 ± 1.40	50.37 ± 1.63	135.24 ± 4.37
	W: M	51.61 ± 1.78	37.64 ± 1.11	45.43 ± 1.57	70.96 ± 1.54
	C: M	49.10 ± 1.63	34.15 ± 1.93	40.85 ± 1.12	110.12 ±1.71
25°C	W	24.04 ± 1.15	3.86 ± 0.63	26.24 ± 0.92	30.14 ± 1.34
	M	69.39 ± 1.22	44.50 ± 1.11	57.01 ± 1.57	117.35 ± 2.47
	W: M	53.63 ± 1.59	24.09 ± 1.33	42.01 ± 1.55	65.77 ± 1.17
	C: M	32.41 ± 1.17	27.04 ± 0.55	28.40 ± 1.23	74.69 ± 1.76
Sonication	W	45.84 ± 1.82	18.40 ± 0.74	29.13 ± 1.24	52.79 ± 1.20
	M	54.11 ± 1.97	32.58 ± 1.20	41.59 ± 1.08	85.40 ± 2.98
	W: M	62.46 ± 0.55	36.56 ± 1.43	50.02 ± 1.27	73.65 ± 2.94
	C: M	41.56 ± 0.77	29.17 ± 0.94	42.68 ± 1.44	80.85 ± 1.60

**TPC- Total Phenolic Content, TFC- Total Flavonoid Content, FRS- Free Radical Scavenging and TAA- Total Antioxidant Activity. The TPC and TFC were expressed as mg GAE/ g and mg RE/g of dry extract respectively. The free radical scavenging activity was expressed as % DPPH scavenged. The total antioxidant activity was expressed as mg AAE/ g extract. Results were expressed in terms of Mean ± SD. The abbreviations used in table represent Water (W), Methanol (M), and Water: Methanol (WM) and Chloroform: Methanol (CM).*

Total phenolic estimation and flavonoid estimation

In the present study, the results obtained for the total phenolic and flavonoid content, free radical scavenging and total antioxidant activity were as shown in table 1 for *Bauhinia variegata* Linn crude extracts. In the methanol extracts prepared at elevated temperature, the total phenolic and flavonoid content ranged from 26.01 ± 0.89 to 59.70 ± 1.52 mg GAE/g of dry extract and 8.46 ± 0.66 to 38.62 ± 1.40 mg RE/g of dry extract, respectively. In the extracts prepared at 25°C, the phenolic and flavonoid content ranged from 24.04 ± 1.15 to 69.39 ± 1.22 mg GAE/g of dry extract and 3.86 ± 0.63 and 44.50 ± 1.11 mg RE/g of dry extract, respectively. In the extracts prepared using sonication, the phenolic and flavonoid content ranged from 41.56 ± 0.77 to 62.46 ± 0.55 mg GAE/g of dry extract and 18.40 ± 0.74 to 36.56 ± 1.43 mg RE/g of dry extract, respectively.

As shown in table 2 for the *Leptadenia reticulata* crude extracts prepared at elevated temperature, the total phenolic and flavonoid content ranged from 7.34 ± 0.10 to 24.60 ± 0.65 mg GAE/g of dry extract and 1.60 ± 0.04 to 6.44 ± 0.21 mg RE/g of dry extract, respectively. In the extracts prepared at 25°C, the phenolic and flavonoid content ranged from 9.47 ± 0.22 to 21.46 ± 0.34 mg GAE/g of dry extract and 1.41 ± 0.05 to 7.47 ± 0.30 mg RE/g of dry extract, respectively. In the extracts prepared using sonication, the phenolic and flavonoid content ranged from 3.68 ± 0.20 to 6.83 ± 0.19 mg GAE/g of dry extract and 3.68 ± 0.20 to 6.83 ± 0.19 mg RE/g of dry extract, respectively.

Free radical scavenging activity

For *Bauhinia variegata* extracts prepared at elevated temperature, the % free radical scavenging activity ranged from 17 % to 51 %. For the extracts prepared at 25°C, it ranged from 26 % to 57 %. For extracts prepared by sonication, it ranged from 29% to 50%.

For *Leptadenia reticulata* extracts prepared at elevated temperature, the % free radical scavenging activity ranged from 13 % to 67 %. For the extracts prepared at 25°C, it ranged from 18 % to 51%. For extracts prepared by sonication, it ranged from 30% to 65 %.

Total antioxidant activity

For *Bauhinia variegata*, the total antioxidant capacity for the extracts prepared at elevated temperature ranged from 56.39 ± 1.57 to 135.24 ± 4.37 mg AAE/g of dry weight of extracts. For the extracts prepared at 25°C, it ranged from 30.14 ± 1.34 to 117.35 ± 2.47 mg AAE/g of dry weight of extracts. The extracts prepared using sonication were in the range, 52.79 ± 1.20 to 85.40 ± 2.98 mg AAE/g of dry weight of extracts.

For *Leptadenia reticulata*, the total antioxidant capacity for the extracts prepared at elevated temperature ranged from 14.56 ± 0.52 to 40.89 ± 1.56 mg AAE/g of dry weight of extracts. For the extracts prepared at 25°C, it ranged from 18.42 ± 1.05 to 51.37 ± 2.06 mg AAE/g of dry weight of extracts. The extracts prepared using sonication were in the range, 20.44 ± 0.77 to 52. 88 ± 1.34 mg AAE/g of dry weight of extracts.

Discussion

Plants possess secondary metabolites belonging to different classes such as phenolics, terpenoids, alkaloids etc. Amongst these classes, phenolics comprise the largest

Table 2. Total phenolic content, total flavonoid content, free radical scavenging activity and total antioxidant activity of *Leptadenia reticulata* plant extracts

Treatment	Solvent systems	TPC* (mg GAE/g)	TFC* (mg RE/g)	FRS* (% DPPH Quenched)	TAA* (mg AAE/g)
Elevated temp.	W	7.34 ± 0.10	1.60 ± 0.04	15.82 ± 1.50	14.56 ± 0.52
	M	14.39 ± 0.63	2.13 ± 0.07	30.30 ± 1.86	31.73 ± 1.46
	W: M	24.60 ± 0.65	6.44 ± 0.21	66.83 ± 0.91	27.50 ± 0.59
	C: M	16.20 ± 0.16	4.40 ± 0.26	13.51 ± 0.89	40.89 ± 1.56
25°C	W	9.47 ± 0.22	1.41 ± 0.05	18.56 ± 0.82	18.42 ± 1.05
	M	17.40 ± 0.25	4.75 ± 0.11	27.59 ± 1.34	33.92 ± 0.97
	W: M	16.77 ± 0.66	2.22 ± 0.08	32.04 ± 0.45	23.33 ± 0.94
	C: M	21.46 ± 0.34	7.47 ± 0.30	51.29 ± 2.10	51.37 ± 2.06
Sonication	W	16.11 ± 0.34	4.26 ± 0.17	30.16 ± 0.40	20.44 ± 0.77
	M	23.44 ± 0.42	6.83 ± 0.19	65.32 ± 1.81	52.88 ± 1.34
	W: M	22.48 ± 0.84	5.73 ± 0.05	40.97 ± 1.95	27.96 ± 0.72
	C: M	16.40 ± 0.22	3.68 ± 0.20	47.82 ± 2.65	44.07 ± 1.23

**TPC- Total Phenolic Content, TFC- Total Flavonoid Content, FRS- Free Radical Scavenging and TAA- Total Antioxidant Activity. The TPC and TFC were expressed as mg GAE/ g and mg RE/g of dry extract respectively. The free radical scavenging activity was expressed as % DPPH scavenged. The total antioxidant activity was expressed as mg AAE/ g extract. Results were expressed in terms of Mean ± SD. The abbreviations used in table represent Water (W), Methanol (M), and Water: Methanol (WM) and Chloroform: Methanol (CM).*

group of compounds in plants and flavonoids are the largest subclass of phenolics (Liu, 2004). These compounds are known to possess a strong free radical scavenging property (Abdel-lateif et al., 2016). Reports are available where it has been clearly demonstrated that phenolic compounds present in the plant extracts have a major contribution to antioxidant activity (Cai et al., 2004; Li et al., 2008; Sun et al., 2002; Zou et al., 2011). It has also been hypothesized that the phenolics (Yang et al., 2001) and flavonoids (Kumar and Pandey, 2013) in medicinal plants might prevent cancer through antioxidant action and/or by inhibiting the initiation, promotion, and progression stages of carcinogenesis.

In the present study, the crude extracts of two medicinal plants *Bauhinia variegata* Linn and *Leptadenia reticulata* (Retz.) Wight & Arn prepared by different methods and solvent systems have yielded different phenolic contents, flavonoid contents and antioxidant potentials. It is well known that various parameters influence the extraction yield of phenolics such as polarity of solvent used as well as method of extraction (Do et al., 2014). Water, methanol, chloroform and aqueous methanol combinations have been found to be very effective in extracting a wide range of phytochemicals (Peschel et al., 2006; Sultana et al., 2009). From the present study, it was observed that methanol extracts and water: methanol were found to possess high amount of phenolics and flavonoids which was in agreement with previously reported studies (Siddhuraju and Becker, 2003; Sultana et al., 2009). Similar findings were reported for medicinal plant *Nyctanthes arbortristis* where methanol extract showed high phenolic content and strong antioxidant potential (Vyas and Braganza, 2018).

For *Bauhinia variegata* Linn, the highest total phenolic and flavonoid content was observed in methanol extracts whereas water extract had lowest amount of phenolics. As per the study by Badgujar et al., (2017), the phenolic and flavonoid content in the methanol extracts of leaves were 112.0 ± 2.43 mg GAE/g and 20.73 ± 1.43 mg QE/g dry extract. In the same study, the IC_{50} value of methanol extract for DPPH radical scavenging activity was reported to be 117±3.33µg/ml (Badgujar et al., 2017). This is in agreement with our study where methanol extracts were found to possess high amount of phenolics and flavonoids as well as free radical scavenging property in comparison to extracts prepared using other solvents. In this study, for methanol extract the phenolic content ranged from 67.85 ± 6.14 to 54.11 ± 1.97 mg GAE/g and the flavonoids content ranged from 52.41 ± 6.62 to 38.50 ± 4.51 mg RE/g dry extracts. In the present study, the DPPH radical scavenging activity ranged from 50 to 56% for methanol extracts prepared by three methods. To the best of our knowledge, the antioxidant capacity of water, water: methanol and chloroform: methanol extracts of *Bauhinia variegata* leaf has not been reported.

For *Leptadenia reticulata* Wight & Arn, the highest total phenolic and flavonoid content was observed in water: methanol and chloroform: methanol, respectively, whereas water extract had lowest amount of phenolics. In a study by Hewageegana et al., (2014), the total phenolic content and total flavonoid contents of methanolic extracts were reported to be 55.6 ± 0.50 mg GAE/g extract and 22.9 ± 0.80 mg QE/g extract respectively. In our study, for methanolic extract the phenolic content ranged from 14.39 ± 0.63 to 23.44 ± 0.42 mg GAE/g and the flavonoids content ranged from 11.59 ± 2.00 to 28.24 ± 3.05 mg RE/g dry extracts. The radical scavenging activity of methanolic extract was reported to be having IC50 value 18.56 ± 0. 29µg/mL (Hewageegana et al., 2014). In our study, the DPPH scavenging activity of methanol extracts prepared by different methods ranged from 30% to 50%. In another study, the IC50 value of methanol extracts of *L. reticulata* was reported to be 56.66µg/ml (Sonara et al., 2013). In a study by (Mohanty et al., 2014), the IC50 value of methanol and aqueous extracts of *L. reticulata* were reported to be 510.15µg/mL and 130.92 µg/mL, respectively. To the best of our knowledge, the antioxidant capacity of water, water: methanol and chloroform: methanol extracts of *L. reticulata* leaf has not been reported. In the study it was observed that there are significant differences in the phenolic and flavonoid content of the extracts. Similarly, it was also noted that the free radical scavenging activity and total antioxidant activity varied among all the extracts. These differences may be attributed to the fact that different solvents and experimental conditions were employed while preparing the extracts.

Conclusion

In the present study, the total phenolic content, total flavonoid content and antioxidant potential of two medicinal plants *Bauhinia variegata* Linn and *Leptadenia reticulata* (Retz.) Wight & Arn was estimated. A combination of three treatment methods (elevated temperature; 25°C temperature and ultrasound waves) and four solvent systems [water, methanol, water: methanol (1:1) and chloroform: methanol (1:1)] for extraction from plant material yielded a total of 24 extracts. This approach has helped in selecting effective methods and solvent systems for optimal extraction of phytochemicals from the plant material. For *Bauhinia variegata*, methanol extract prepared at 25°C showed highest phenolic 69.39 ± 1.22 mg GAE/g of dry extract and flavonoid content 44.50 ± 1.11 mg RE/g of dry extract. Furthermore, same extract exhibited strong free radical scavenging activity (57%) and total antioxidant activity (117.35 ± 2.47 mg AAE/g of dry extract). For *Leptadenia reticulata*, the water: methanol extract prepared at elevated temperature exhibited highest phenolic 24.60 ± 0.65 mg GAE/g of dry extract and

flavonoid content 6.44 ± 0.21 mg RE/g of dry extract as well as free radical scavenging activity (67%). The methanol extract prepared by sonication demonstrated highest total antioxidant activity (52.88 ± 1.34 mg AAE/g of dry extract). Based on this study, it can be concluded that solvent systems and extraction conditions play important roles in preparation of crude extracts containing phenolic compounds and antioxidant activity. Also, both plants possess high amounts of phenolics and strong free radical scavenging activity. This suggests that these plants might have potential application as antioxidant agents.

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Conflict of interest

There are no conflicts to declare.

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