

Research Article

Standardized method to extract phenolic compounds from *Lagerstroemia speciosa* L. (Jarul) for enhanced antioxidant activity

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Abstract

Phenolic compounds contribute to the antioxidant property of plants and the efficient extraction of plant phenolics could enhance its antioxidant potential. *Lagerstroemia speciosa* (L.) Pers. has been investigated for its pharmacological activities, but comparing its antioxidant activities of phenolics derived from its various parts has a key role in developing natural antioxidants. This study was conducted to standardize the extraction of phenolics from leaves, pods and branches of *L. speciosa* plant, followed by determination of antioxidant activities of their solvent fractions. Phenolic compounds were extracted from the leaves, pods and branches under different parameters such as temperature, pH, type of solvent and volume of the solvent. The extracted phenolic compounds were subjected to solvent fractions and antioxidant assays were performed. Among the various extraction methods tested, the best method was 50% ethanol +1% HCl, refluxing temperature, 100 ml of solvent and 1 hour extraction time. The best solvent fractions were determined as NaHCO₃ + ethyl acetate for pods and ethyl acetate alone for leaves and branches with extracted phenolics content of 150.2 mg/g, 136.2 mg/g and 82.9 mg/g, respectively. The chloroform fraction was best among the fractions with maximum ascorbic acid equivalent (ASE) in all the parts of *L. speciosa* tested. Ferrous ion chelating capacity indicated that butanol fraction had the highest chelation and the same was recorded in ferric ion chelating assay with an EC₅₀ value of 28.2. Hydroxyl radical scavenging activity of the fractions indicated that NaHCO₃ + EtOAc fraction of pods had potential activity. Thus, the phenolic compounds from *L. speciosa* are excellent sources for future investigation on potent natural antioxidant compounds.

Keywords: Banaba, Jarul, *Lagerstroemia speciosa*, Phenolics, Polyphenols, Antioxidant

INTRODUCTION

Lagerstroemia speciosa Pers. Jarul or banaba is a medium-sized to large deciduous tree widely distributed in South East-Asian countries, the Philippines and India. Plants with considerable quantities of antioxidants help investigators to identify natural antioxidants in preventing diseases caused by free radicals and reactive oxygen species. Due to its antioxidant activities, great attention has been given to *L. speciosa* (Nurchayanti *et al.*, 2018). Glycosides, flavones, triterpenes, tannins, corosolic acid, ellagic acid and its derivatives have been isolated from various parts of *L. speciosa* (Stohs

et al., 2012; Tiwary *et al.*, 2017; Chan *et al.*, 2014). *L. speciosa* is reported to possess several polyphenolic compounds (Choi *et al.*, 2015). Leaves of the plant possess antioxidant and free radical scavenging activities (Unno *et al.*, 1997). Polyphenol from the leaves has many biological activities (Janggyoo *et al.*, 2015; Song *et al.*, 2013a). Flowers are a good source of phenolic compounds with antioxidant activities (Kaisoona *et al.*, 2011; Tiwary *et al.*, 2017) and had demonstrated DPPH scavenging activity when chloroform was used for extraction (Sharmin *et al.*, 2018). Corosolic acid, ellagitannins, ellagic acid from banaba leaves have hypoglycemic activity (Bai *et al.*, 2008; Stohs *et al.*,

2012; Guo *et al.*, 2020). Leaves of the plant have potential antitumor effects (Mousa *et al.*, 2019; Song *et al.*, 2013b) activity. The other biological activity of the plant includes antimicrobial (Chan *et al.*, 2014), anti-fibrotic (Prabhu *et al.*, 2010) and analgesic (Gupta *et al.*, 2017) effects.

Phenolic compounds are capable of scavenging free radicals of different origins (Qadir *et al.*, 2017; Moukette *et al.*, 2015; Lobo *et al.*, 2010; Bravo, 1998). Polyphenol content and composition depends on the plant part and processing conditions. Based on the potential biological activities of *L. speciosa*, this study was designed to determine the best methods to extract phenolic compounds from various parts of the plant and determine the effect of different parameters such as temperature, pH, type of solvent, and volume of the solvent. Solvent fractions of *L. speciosa* were prepared and tested for the antioxidant activities of polyphenols under *in vitro* conditions.

MATERIALS AND METHODS

Plant materials

The leaves, pods and branches of *L. speciosa* were collected from agronomical experimental trials at Aurawan Research Center of the Institute and Pantnagar. The samples were shade dried, ground to a fine powder and passed through a sieve (24 mesh).

Selection of best solvent, temperature, time and volume of extraction

Effect of solvent

To find out the suitable solvent for extraction of total phenolics, solvents of varying polarity like ethyl acetate, methanol and ethanol in combination with water and acid like hydrochloric acid were selected: 50% MeOH + 1% HCl, 3:1 EtOH/EtOAc+0.5% HCl, 75% MeOH, 100% MeOH, 50% EtOH, 50% EtOH +1% HCl, 50% MeOH, 10% MeOH, 3:1 EtOH /EtOAc. For this study, 25 mg of plant sample was extracted with 10 ml of above-mentioned solvents for 12 hours.

Effect of temperature

In order to find out the optimum temperature, sample was extracted with the best-selected solvent at room temperature, 50°C, 75°C, 100°C in five different conical flasks and the last one was refluxed.

Effect of time of extraction (single extraction)

The pods, branches and leaves were used for the selection of appropriate time of extraction. For this study, 25 g of sample was kept for 1, 2, 3 and 24 hours in 100 ml of the selected solvent at appropriate temperatures optimized in the above studies.

Effect of volume of solvent

The pods, branches and leaves were used for the selection of the appropriate volume of extraction. For this study, 1g of sample was kept for 1 hour in 10, 20, 40, 60, 80 and 100 ml of solvent at appropriate temperatures optimized in the above studies.

Total phenolic content (TPC) estimation

TPC in different extracts was measured with the slightly modified method of Ragazzi and Veronese (1973). In brief, 1ml plant extract was added with 1 ml of Folin's Reagent (1N) and 2 ml of Na₂CO₃ (20%). The contents were mixed well and kept at room temperature for 30 min and the final volume was made upto 25 ml with distilled water. The absorbance of mixture was measured at λ_{\max} 725 nm on UV-Vis spectrophotometer. TPC were expressed as gallic acid equivalent (GAE) mg/g on a dry weight basis.

Effect of solvent fractions and on antioxidant activity of *L. speciosa* extracts

After selecting the best solvent and conditions on the basis of their TPC, polyphenols were extracted in the dried and powdered sample (10 g) by using 50 ml of 50% (v/v) aqueous ethanol +1% HCl. The mixture was shaken in a rotary shaker at a constant stirring rate at room temperature (25°C) for 1 h. Solids were separated by filtration by washing them with 50% methanol/water. The solvent from the extract was removed under reduced pressure at 150 PSI and 40°C in a Buchi Rotavapor. The viscous residue thus obtained is called crude plant extract. The crude extract was redissolved in water and refluxed for 1 hour to convert the esters into the free phenolic acid. The resulting extract (100 ml) was partitioned with 50 ml of hexane. The obtained defatted hexane fraction was divided into 5 parts of 20 ml each and was extracted with ethyl acetate, ether, chloroform and butanol as such and pH of the remaining 20 ml is brought to 7.0-8.0 by the addition of 5% sodium bicarbonate. The ether, chloroform, butanol and both the ethyl acetate and their respective aqueous fractions were washed with 10 ml water and dried on 2 g of anhydrous sodium sulphate separately for 2 hours, followed by filtration. The solvent was removed under reduced pressure at 50 pounds per square inch (PSI) at 40°C. The viscous residues thus obtained were analyzed for their antioxidant activities.

Antioxidant activity (AOA)

AOA was estimated as described by Emmons and Peterson (1999) by monitoring the coupled autoxidation of β -carotene and linoleic acid. 2 mg of β -carotene was dissolved in 20 ml of CHCl₃ and its 3 ml was added to 40 mg of linoleic acid and 400 mg of Tween 40. After removing CHCl₃ under reduced pressure, 100 ml of

oxygenated water was added and mixed properly to obtain a stable emulsion. 3 ml aliquots of emulsion were mixed with 40 µl of plant extracts and incubated for 1 hour at 50°C. The absorption (λ_{\max} 470 nm) of test mixture was recorded at 0 and after 60 minutes of incubation at 50°C. The AOA was expressed as per cent of inhibition relative to control after 60 min. Activity of plant extracts was compared with standard curve of butylated hydroxy toluene (BHT) over a concentration range of 0 to 1.4 µg in 40 µl of sample. Antioxidant activity was expressed as percent inhibition relative to control after a 60 minutes incubation period and calculated by the following formula:

$$AOA = \frac{100 \times (DR_c - DR_s)}{DR_c} \dots \dots \text{Eq. 1}$$

Where: DR_c = rate of degradation of control = $\ln(a/b)/60$

DR_s = degradation rate of sample = $\ln(a/b)/60$
a and b = absorbance at 0 and 60 minutes.

Free radical scavenging activity (FRSA) and reducing capacity

10 mg of plant extract residue was dissolved in 10 ml MeOH and its 0.1 ml was added to 2.9 ml freshly prepared 1,1-diphenyl-2-picryl-hydrazil (DPPH) solution (6×10^{-5} M in MeOH). The absorbance was recorded at λ_{\max} 517 nm after 1 hour of incubation. The FRSA was calculated according to the method of Yen and Duh (1994). The inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti-radical power (ARP) were estimated and calculated as described by Kroyer (2004). Reducing capacity of plant extracts (1 mg/ml in MeOH) was determined using a modified ferric reducing - antioxidant power assay and quercetin as reference standard. 100, 200, 300, 400 and 500 µl aliquots of samples were maintained to 1 ml with MeOH, followed by the addition of 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml of 1% w/v potassium ferric cyanide in each. The reaction mixtures thus obtained were incubated at 50°C in water bath for 20 min. After incubation, reaction was terminated by the addition of 2.5 ml of 10% w/v trichloroacetic acid (TCA) solution. 2.5 ml of above solution from each reaction was diluted with equal amount of deionized water. 0.5 ml of $FeCl_3$ (0.1% w/v) was added in each and absorbance (λ_{\max} 700 nm) was recorded after 10 min. Reducing power was expressed as ascorbic acid equivalent (1mM = 1 ASE). The ASE/ml value is inversely proportional to reducing power.

Ferrous ion chelating capacity test

10 mg of plant extract residue was dissolved in 10 ml MeOH and its ferrous ion chelating capacity test was determined by the method of Decker and Welch (1990). Aliquots (100, 200, 300, 400 and 500 µl) of samples

were maintained to 1 ml with MeOH, followed by the addition of 0.1 ml of 2 mM ferrous chloride, 0.2 ml of 5 mM ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine) and 3.7 ml methanol. The reaction mixture was kept at room temperature for 10 minutes and absorbance was measured at λ_{\max} 562 nm. A lower absorbance indicates a higher ferrous ion chelating capacity.

Ferric thiocyanate Assay

Ferric thiocyanate assay was performed by mixing 400 µl of plant extract (0.01 to 2 mg/ml), 200 µl of diluted linoleic acid (25 mg/ml, 99% ethanol) and 400 µl of 50 mM phosphate buffer (pH 7.4) followed by incubation at 40°C for 15 min. A 100 µl aliquot was then mixed with a reaction mixture containing 3 ml of 70% EtOH, 100 µl of ammonium thiocyanate (300mg/ml dry weight) and 100 µl of ferrous sulphate. The developed red color was measured at λ_{\max} 500 nm (Tsuda et al., 1993).

Scavenging effect on hydroxyl radicals

Hydroxyl radical scavenging activity was measured according to the method of Halliwell et al., (1987). Reaction mixture consisting 500 µl of sample at different concentrations ranging from 0.05 to 1.25 mg/ml and 100 µl each of 2 mM H_2O_2 , 2 mM ferric chloride, 0.05 mM ascorbate, 6 mM deoxyribose and 0.05 mM EDTA prepared in 0.02 M phosphate buffer (pH 7.4) was incubated at 37°C for 60 minutes. After addition of 1 ml of 2.8% (w/v) trichloroacetic acid and 1 ml of 1% (w/w) 2-thiobarbituric acid, the reaction mixture was further heated in a boiling water bath for 15 minutes. The colour developed was measured at λ_{\max} 532 nm against a blank containing phosphate buffer only. Hydroxyl radical scavenging activity was measured with the inhibition of deoxyribose oxidation and the hydroxyl radical scavenging activity was calculated as below;

$$\% \text{ scavenging} = [A_0 - (A_1 - A_2)] \times 100 / A_0 \dots \dots \text{Eq. 2}$$

Where, A_0 is the absorbance of the control. A_1 is the absorbance of sample with 2-deoxy-D-ribose. A_2 is the absorbance of the sample without 2-deoxy-D-ribose.

RESULTS AND DISCUSSION

Various solvents at different concentrations and combinations used to extract total phenolics from different parts of *L. speciosa* showed that leaves of the plant were rich with phenolics than the pods and branches. Among the solvents tested, 50% EtOH + 1% HCl had recovered maximum phenolics from the *L. speciosa* parts (Fig.1).

The results on the effect of temperature on the extraction of phenolics revealed that higher temperatures lead to the maximum recovery of phenolics (Fig. 2). However, refluxing the plant extract resulted in better

extraction of phenolics from the leaves of the plant. Similarly, the higher the volume of solvent used, the better was the extraction of the phenolics (Fig. 3). Extraction of phenolic with best solvent combination for 1 hour period resulted in maximum phenolic as expressed in gallic acid equivalents (mg/g). Total phenolics were also higher in overnight extraction, but in this study, 1 hour extraction was considered the optimal extraction time for *L. speciosa* (Fig. 4).

Organic solvents are employed to extract the free and soluble conjugate phenolic acids, whereas acid, alkali treatment releases bound phenolics (Krygier et al., 1982). Among the solvent fractions prepared using ethanol, ether, chloroform, butanol and ethyl acetate + NaHCO₃ as alkali treatment to release bound phenolics from *L. speciosa*, ethyl acetate + NaHCO₃ treatment

released maximum phenolics (150.2 mg/g) from pods, whereas ethyl acetate fraction resulted in the maximum release of phenolics from leaves (136.2 mg/g) and branches (82.9 mg/g) (Fig.5). Antioxidant activities of solvent fractions indicated that *L. speciosa* exhibited the suitable solvent fraction with highest antioxidant activity. Total antioxidant activity was highest in ethyl acetate fraction of leaves and branches (85.6%) followed by ethyl acetate + NaHCO₃ fraction of pods (82.3%) as depicted in Fig.6.

FRSA assay of different solvent fractions revealed that chloroform fraction was best among the fractions with maximum ascorbic acid equivalent (ASE) in all the parts of *L. speciosa* tested (Fig.7a-7d). Ferrous ion chelating capacity of *L. speciosa* indicated that butanol fraction had highest chelation capacity followed by chloroform

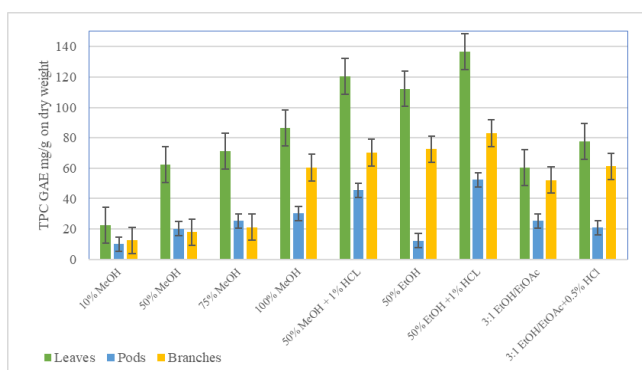


Fig. 1. Effect of time on total phenolics extraction.

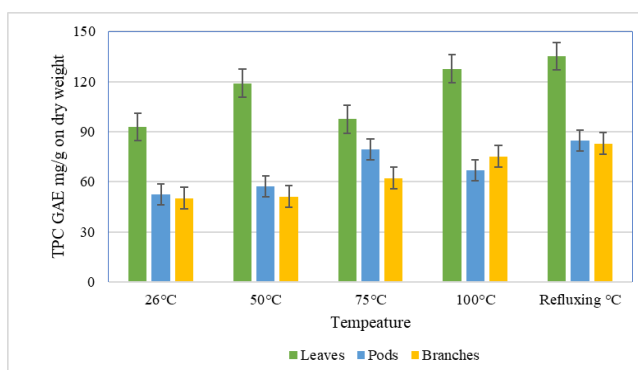


Fig. 2. Effect of temperature on total phenolics extraction.

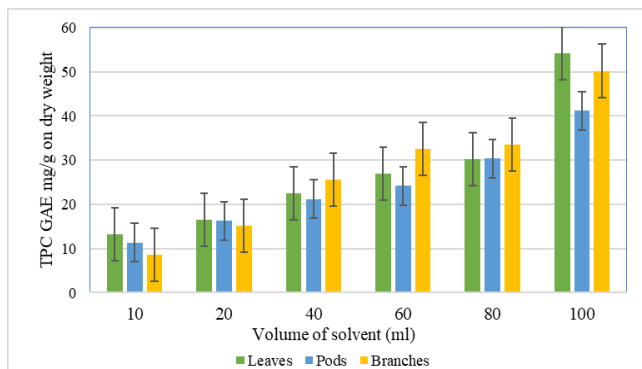


Fig. 3. Effect of volume of solvent on total phenolics extraction.

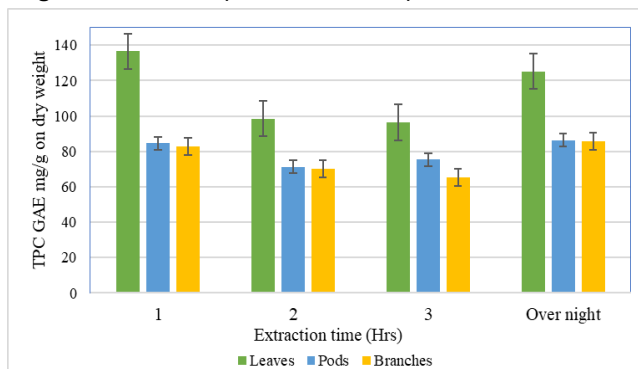


Fig. 4. Effect of extraction time on total phenolics extraction.

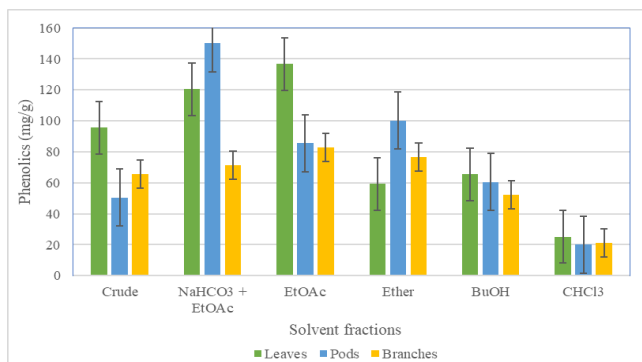


Fig. 5. Phenolic contents in solvent fractions.

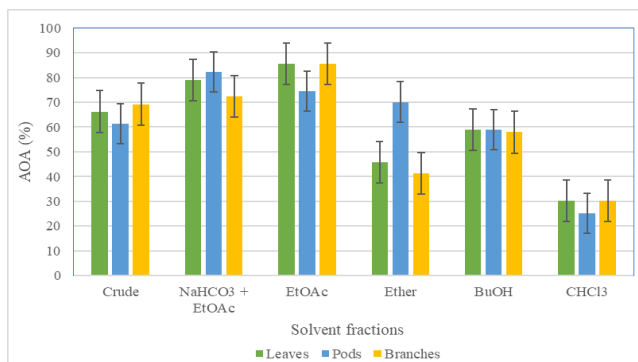


Fig. 6. Total antioxidant activity of solvent fractions.

fraction (Fig. 8). Butanol fraction from branches of the plant recorded the highest ferrous ion chelating capacity with IC_{50} value of 0.65. Similar results were also obtained for ferric ion chelating assay where butanol fraction of branches exhibited EC_{50} value of 28.2 followed by chloroform fraction of pods with EC_{50} value of 26.5 (Fig. 9).

Hydroxyl radical scavenging activity of the fractions indicated that $NaHCO_3 + EtOAc$ fraction of pods had potential activity followed by ethyl acetate fraction of branches.

Supplementation of antioxidants from plant origin helps in controlling the reactive oxygen species-mediated damage with minimal side effects (Dina et al., 2009). Plants are proven to be a good source of antioxidants. The nature of antioxidant activity depends on the plant part. Phenolic compounds are primarily responsible for the antioxidant activities of plants and the extraction of plant phenolics is influenced by various factors such as type of solvent and extraction conditions. Flower extract of *L. speciosa* exhibited higher antioxidant activities due to their phenolic compounds (Tiwary et al., 2017) and in this present study, antioxidant activities of leaves, pods and branches of *L. speciosa* were observed indicating the antioxidant properties of the apical parts of the plant.

Leaves extract of the plant alleviates oxidative stress through decreased non-enzymatic and enzymatic antioxidants (Mousa et al., 2019). The leaves were protective against oxidative stress (Song et al., 2013). Aerial

parts of the plant have inhibitory activities against nitric oxide production (Choi et al., 2015). In another study, leaves of this plant demonstrated significant antioxidant activities in vitro (Pareek et al., 2010). The major chemical constituents of the leaves are ellagic acid, ellagitannins, caffeic acid and corosolic acid (Bai et al., 2008). Although seeds, leaves and flowers of *L. speciosa* were studied individually for their antioxidant activity (Junaid et al., 2013; Saumya and Basha, 2011; Pavithra et al., 2013), no report exists on comparing antioxidant activities of phenolic compounds from leaves, pods and branches of this plant. Therefore, this study determined the best extraction methods to obtain phenolic compounds from various parts of this plant and examined their antioxidant activities.

To understand the antioxidant activities of its phenolic compounds from solvent fractions, various experiments on *in vitro* assays indicated varying results. Among the various extraction methods tested, the best methods were 50% ethanol +1% HCl, refluxing temperature, 100 ml of solvent and 1 hour extraction time. The results revealed the presence of the high amount of phenolic compounds in leaves, pods and branches of *L. speciosa*. Similarly, the best solvent fractions were determined as $NaHCO_3 +$ ethyl acetate for pods and ethyl acetate alone for leaves and branches. The present study revealed in general that solvent fractions were proven to exhibit higher antioxidant activities than the crude extract. Sharmin et al., (2008) used the solvent fractions of the flowers of *L. speciosa* and the chloro-

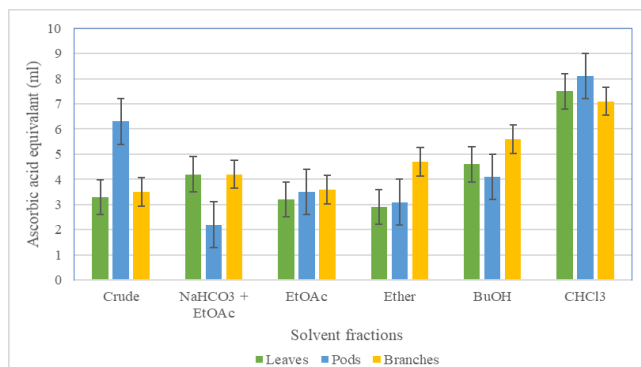


Fig. 7a. FRSA activity of solvent fractions.

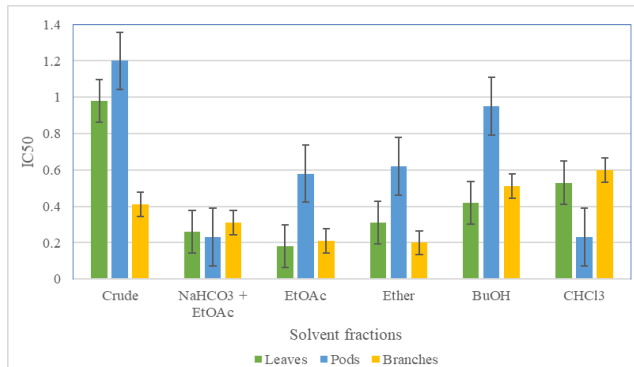


Fig. 7b. Inhibitory concentration (IC_{50}) of solvent fractions.

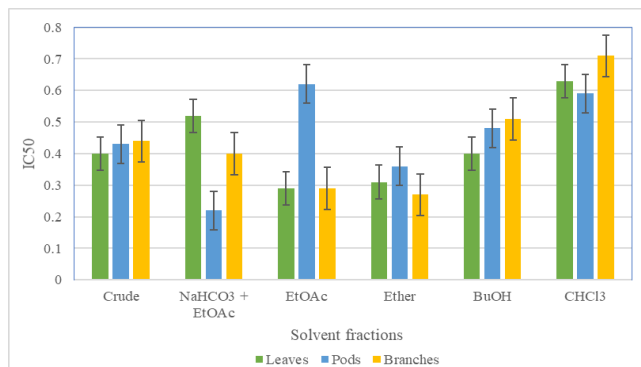


Fig. 7c. Efficiency concentration (EC_{50}) of solvent fractions.

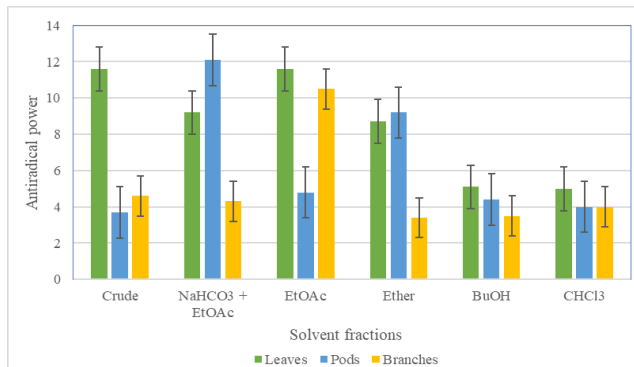


Fig. 7d. Antiradical power of solvent fractions.

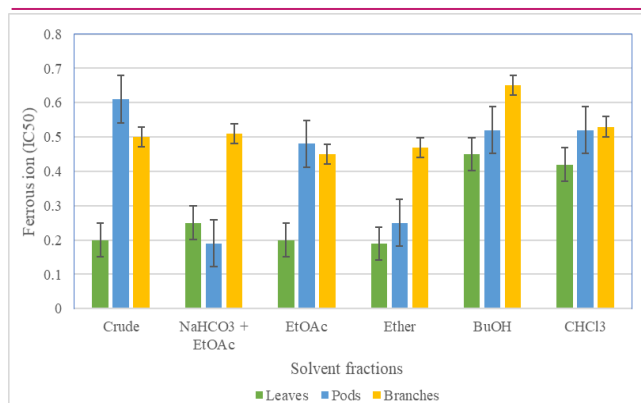


Fig. 8. Ferrous ion chelation activity of solvent fractions.

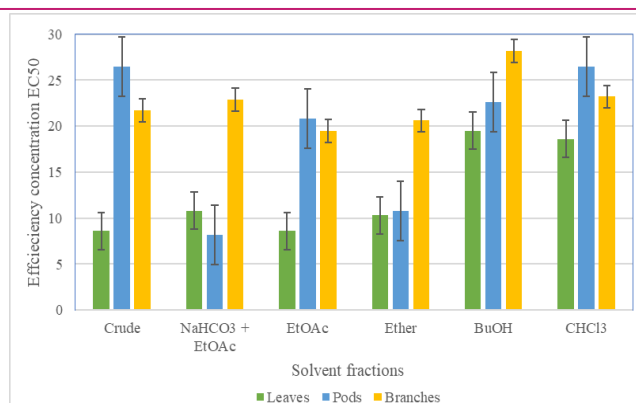


Fig. 9. Ferric ion chelation activity of solvent fractions.

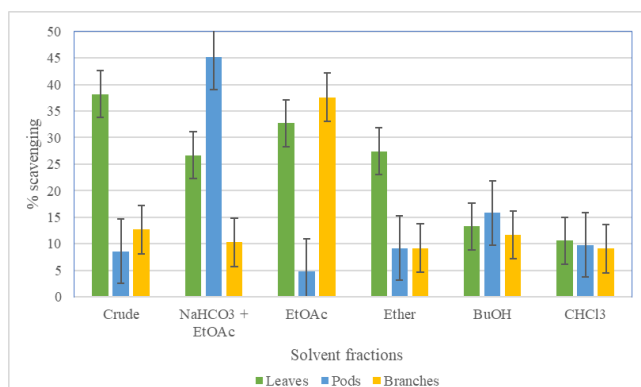


Fig. 10. Hydroxyl radical scavenging activity of solvent fractions.

form fraction demonstrated the highest antioxidant activity in terms of DPPH free radical scavenging. Similarly, subsequent solvent extracts were investigated for its antioxidant activities in an earlier study (Priya *et al.*, 2008). Based on the findings, it is clear that solvent fractions of *L. speciosa* leaves, pods and branches are better choice in terms of antioxidant activities rather than crude extract.

Conclusion

This study revealed the presence of higher amounts of phenolic compounds in leaves than the pods and branches of *L. speciosa* which can function as antioxidants. Standardized of extraction method using best solvent, volume of solvent, optimal temperature and extraction time to extract phenolics from various parts of the plant is reported in this study and extraction using 50% ethanol + 1% HCl has led to enhanced antioxidant activity of the plant. In conclusion, it can be said that *L. speciosa* is an excellent source of antioxidants and identification of phenolic compounds is necessary for its further use as potent antioxidant source.

Conflict of interest

The authors declare that they have no conflict of interest.

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