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Full Length Research Paper

Total phenolic content and antioxidant activity of extracts of *Bridelia Retusa Spreng* Bark: Impact of dielectric constant and geographical location

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The aim of the present investigation was to determine the influence of solvents (possessing different dielectric constant values) and geographical location on the Total Phenolic Content (TPC) and antioxidant activity of extracts of *Bridelia retusa* bark. The bark was collected from two different geographical locations of India [Maharashtra (MAH) and Andhra Pradesh (AP)]. The highest extractive values were found to be as 20.70±0.56 %w/w and 18.43 ± 0.97 %w/w in case of methanolic extracts of *Bridelia retusa* bark collected from MAH and AP regions respectively while the total polyphenolic contents in the methanolic extract of *Bridelia retusa* bark were 32.52 ± 0.24 mg/100 gm and 28.04±0.67 mg/100gm in MAH and AP regions respectively. The methanolic extract of the species from MAH exhibited the highest radical scavenging activity while that from AP showed less scavenging activity. The extracts showed a positive correlation between the polyphenol contents and solvent's dielectric constant (R=0.728, P<0.05). This suggests that dielectric constant value significantly influences the choice of solvent for extraction as well as the total phenolic contents and subsequently the antioxidant activity of the plant extract.

Key words: Dielectric constant, total phenolic content (TPC), DPPH radical scavenging activity, *Bridelia retusa.*

INTRODUCTION

Bridelia retusa (B.retusa) [Euphorbiaceae] is a shrub or a tree up to 18 m in height armed with strong spines 7 cm long found through India up to an altitude of 1000 m, except in very dry regions. Bark is grey to brown exfoliating in irregular flakes. The genus *Bridelia* comprises shrubs, climbers or trees distributed in Asia, Africa and Australia (Anon., 1988). The plant is used in folk medicine to treat diabetes, rheumatism, dysentery and diarrhoea (Pawar et al., 2007; Kirtikar et al., 1996; Raju et al., 2005).

Polyphenolic compounds belong to a large heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom and have multiple applications in food, cosmetic and pharmaceutical industries (Strube et al., 1993; Kahkonen et al., 1999). The antioxidant capacity possessed by phenolic compounds is mainly due to their redox properties, which permit them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Besides their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (Balasundram et al., 2006).

A number of factors influence the concentration of the active constituent's particularly phenolic compounds present in the herbals. Some of the notable factors are time and period of collection, geographical origin and climatic conditions. Sometimes, the influence of these factors may such dominating leading to even absence of active constituents in the same plant collected from different regions, as evidenced by several research reports (Bilia, 2002; Houghton., 1998; Marcus et al., 2002).

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Selection of the appropriate solvent for extraction of phenolic compounds is a very important aspect that needs to be addressed in a judicious way so as to achieve the maximum concentration of desired phytoconstituents in plant extracts. Solubility of these active constituents in a particular solvent also plays vital role in context of selection of solvent. Some phenolic compounds like those present in creosote bush resin are insoluble in water but soluble in non-polar solvents. On the other hand, some phenolic glycosides are highly soluble in water (Waterman and Mole, 1994). Research studies have indicated that phenolic compounds can be extracted from plant material using a sequence of solvents with divergent polarity (Apak et al., 2007).

The basis of most of the antioxidant based assays is either electron transfer (ET) or hydrogen atom transfer (HAT). The dielectric constant of the solvent, intra-/inter-molecular hydrogen bonding associations and standard redox potential of phenolics and derived aryloxy radicals in a given solvent may be important for electron transfer kinetics in antioxidant assays (Huang et al., 2005; Prior et al., 2005).

Thus, the significant impact of the molecular chemical properties of the solvents especially the dielectric constant towards the selectivity of phenolic components in a particular part of the plant can be of vital interest from the research point of view. The present work is an attempt to investigate the impact of dielectric constant on the efficiency of a particular solvent to extract total phenolic components from the bark of *B. retusa* and thereby establishing the correlation with the subsequent antioxidant activity residing in the plant .The experimental approach relied on the use of 2,2-Diphenyl-1-picrylhydrazyl [DPPH] radical scavenging assay. Apart from this, the efforts have also been directed to observe the impact of geographical location in this regard.

MATERIALS AND METHODS

Sample collection

Young fresh bark of *B. retusa* having uniform size, firm texture and good appearance, were collected from two different locations (MAH and AP). The samples were properly authenticated in the Department of Botany by senior botanist and taxonomist. A voucher specimen has been maintained for future reference.

Test, standard and extraction procedure

The bark was cleaned and dried at 40°C employing procedure described by (Khamsah et al., 2006). The dried barks were grounded separately into fine powder using a dry grinder. Two hundred grammes of dried powder of bark of selected species was extracted with 300 mL of solvent with increased polarity using soxhlet extractor. Each extract was filtered using Whatman no. 1 filter paper. The solvents were removed from the extract under reduced pressure at 40°C using rotary evaporator and the extractive values were calculated. The extracts were filled in the bottles and stored at 4°C until further use. During the storage period, the extracts were periodically inspected. No changes in color were observed in the samples.

Total phenolic content (TPC)

Total phenolic content of the extracts obtained from solvents having different dielectric constant values was measured at 725 nm using Folin-Ciocalteau spectrophotometric method as described by Amin et al. (2004). All readings were taken in triplicate. Gallic acid was used as standard. The results were expressed as Gallic Acid Equivalents [(GAE) mg/100 gm]

DPPH radical scavenging assay

The DPPH radical scavenging activity assay used by (Chan et al., 2007) was adopted with slight modification. DPPH solution was prepared by dissolving 6 mg of DPPH in 100ml of methanol. To 1mL of various concentrations of the extracts (0.020, 0.040, 0.060, 0.080, 0.100 mg/ml), 2 mL of DPPH solution (0.1 mM) was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The experiments were performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{1- Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

DPPH radical scavenging activities of the extracts were expressed as IC_{50} values. $IC_{50,}$ the effective concentration of the extract required for 50% scavenging of DPPH radical was calculated from the graph of scavenging activity plotted against sample concentration using Microsoft Excel software.

Statistical analysis

Extracts were prepared for each kind of solvent with varying dielectric constant values. All chemical analytical procedures of antioxidant attributes and components were performed in triplicate. Solvent properties, such as dipole moment, dielectric constant, relative polarity and solubility parameters were taken as per reported by (Reichardt, 1988). Data were expressed as mean ± standard deviation. Significant differences among the means were determined by Fisher's least significant difference test after ANOVA ($P<0.05$). Correlations between content of components and antioxidant attributes were determined by linear regression analysis employing Microsoft excel software.

RESULTS AND DISCUSSION

Effect of geographical location

The bark of *B. retusa* exhibited variations in their contents of phytoconstituents depending upon the geographical location from where they were collected. It is quite evident and supported from their extractive values (Table 1) and total polyphenol content (Table 2). The methanolic extract of the plant collected from MAH and AP regions exhibited the highest extractive values of 20.70 ± 0.56 %w/w and 18.43 ± 0.97 %w/w, respectively. The effect of

Table 1. Extraction yield of bark of *B. retusa* with different solvents.

Samples	Solvents	Dielectric Constant [ε]	Extractive values (% w/w±SD)	
			MAH	AP
Bark of *B. retusa*	Methanol	32.7	20.70±0.56	18.43±0.97
	Ethanol	24.3	18.21±0.42	17.23±0.58
	Water	80.1	18.45±0.65	16.27±1.31
	Ethyl acetate	6.02	3.51±0.69	2.27±0.28
	Acetone	20.7	17.1±0.48	16.21±0.79
	Chloroform	4.81	2.92±0.73	2.12±0.91

The values are expressed as Mean ± Standard deviation (n=3), MAH- Maharashtra AP- Andhra Pradesh.

Table 2. Polyphenol content of *B. retusa* extracts obtained with solvent of different dielectric constant.

Solvent	Total polyphenolic content (mg/100 gm) [MAH]	Total polyphenolic content (mg/100 gm) [AP]
Methanol	32.52±0.24	28.04±0.67
Ethanol	26.34±0.72	24.46±0.46
Water	23.21±0.91	22.84±0.37
Ethyl acetate	3.82±0.59	2.94±0.48
Acetone	22.21±0.84	21.35±0.38
Chloroform	1.57±0.24	1.43±0.12

The values are expressed as Mean ± Standard deviation (n=3), MAH- Maharashtra AP- Andhra Pradesh.

geographical location on the content of polyphenol was also evaluated. The level of polyphenol in the methanolic extract from MAH region was found out to be 32.52 ± 0.24 mg/100 gm as compared to AP which was found out to be 28.04 ± 0.67 mg/100 gm. In totality, the plant collected from the region of Maharashtra was found to be superior with respect to extraction yield and total polyphenol content.

The impact of geographical location on the extraction of total phenol content is also supported by the fact that variety of diverse factors such as worldwide changes in seasonal patterns, weather episodes, temperature changes, biotic and abiotic stresses may affect the production of secondary metabolites in plants. In the context of the biotic and abiotic stresses, a special mention should be made to production of stress induced Phenyl propanoid compounds. Nutritional stress such as low iron levels can cause increased release of phenolic acids, presumably to help solubilize metals and thereby facilitate their uptake (Marschner, 1991).

The amount of Camptothecin was evaluated in methanolic extract of various parts of *Nothapodytes nimmoniana* collected in the month of February from different regions (Mahabaleshwar and Patan regions of Maharashtra state and Sirsi region of Karnataka state) of Western Ghats, India, using high performance liquid chromatography. The study indicated that geographical and climatic conditions have phenomenal influence in the content of Camptothecin in *N. nimmoniana* (Namdeo et al., 2010)

The results of the present study and the references cited above showed that the geographical topography and climatic conditions have a profound effect on the extraction yield and level of Polyphenols in the bark of *B. retusa.*

Impact of solvent on the TPC and antioxidant activity

Several studies have indicated that phenolics are responsible for the variation in the antioxidant activity of the plant (Cai et al., 2004). They possess antioxidant activity by there ability to inactivate lipid free radicals or by preventing decomposition of hydroperoxides into free radicals (Pokorney et al., 2001 and Pitchaon et al., 2007). During the process of oxidation of phenol, Folin-Ciocalteu reagent which is a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids is reduced to blue oxides of tungstene (W_8O_{23}) and molybdene (Mo_8O_{23}). This reaction occurs under alkaline condition provided by sodium carbonate. The intensity of blue colour is indicative of the quantity of phenolic compounds, which can be measured using spectrophotometer (Conforti et al., 2006). In the present

investigation, the content of total phenolics in extracts of *B.retusa* obtained from solvents with varying dielectric constant values was experimentally determined. The polyphenol content in *B. retusa* extracts was found to be in decreasing order as per the extractive solvents used that is, Methanol>Ethanol>Water>Acetone>Ethyl acetate>Chloroform (Table 2). The highest concentration of total phenolics was 32.52 ± 0.24 mg/100 gm obtained from methanol as solvent of *B.retusa* species collected from MAH and the lowest concentration of 1.57 ± 0.24 mg/100gm was observed in the chloroform. Also, the extracts showed a positive correlation between the polyphenol contents and solvents relative polarity ($R=0.842$, $P<0.05$), and between the polyphenol contents and solvents dielectric constant ($R=0.728$, $P<0.05$).

The results suggest that extraction by methanol could give higher phenolic content. The findings were likely to be in accordance with (Pérez et al., 2007) who found that methanol was the most efficient solvent as compared to ethanol and water for extracting phenolic compounds from control rosemary leaves and from those decontaminated by gamma irradiation. In addition, (Yang et al., 2007) reported that methanol extract of lotus rhizome had the highest yield and total phenolic recovery. The ability to inhibit polyphenol oxidase that causes the oxidation of phenolics and its ease of evaporation compared to water makes methanol a suitable choice of solvents for extraction of variety of phenolic compounds (Yao et al., 2004). However, (Moure et al., 2000) suggested that both methanol and ethanol offered the best results to extract phenolic compounds from *Gevuina avellana* hulls as compared to acetone. Similar findings proving the effectiveness of both methanol and ethanol for extracting phenolic compounds was shown by (Siddhuraju and Becher, 2003; Azlim Almey, 2010). Among the various phenolics exhibiting antioxidant properties, certain classes of compounds such as phenolic acids, hydroxycinnamic acids, flavonoids, and carotenoids require a decreasing order of solvent polarity for extraction, respectively, although, suitable solvent combinations may be adopted for specific purposes. Due to the diversity of phenolic antioxidant phytochemicals in botanicals, certain compromises have to be made in solvent selection (Prior et al., 2005; Apak et al., 2007).

Antioxidant activity-DPPH and its correlation with TPC

The methanolic and the ethanolic extracts at various concentrations ranging from 0.020-0.100 mg/ml were tested for their antioxidant activity using DPPH radical scavenging assay method keeping in view the suggestion from the studies discussed in the earlier sections of this paper. DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and on reacting with an antioxidant compound which can donate hydrogen, it is reduced to diphenylpicrylhydrazine (DPPHH). The switch in colour (that is, from deep-violet to light-yellow) can be measured spectrophotometrically.

Figures 1 and 2 depict the DPPH radical scavenging activity of the methanolic and ethanolic extract of both plant species. The result revealed that the methanolic extract of the species from MAH exhibited the highest radical scavenging activity (%) with 80.55 ± 0.32 followed by its ethanolic extract with 66.5 ± 0.50. In comparison to the species from MAH the species from AP showed less scavenging activity. The value of scavenging activity for methanolic and the ethanolic extracts for AP species were observed to be 61.46 ± 0.37 and 54.9 ± 0.25, respectively. The DPPH radical scavenging activities of methanolic and the ethanolic extract increased gradually in a dose dependent manner. Decrease in absorbance of DPPH solution (that is, from purple to yellow) depends on intrinsic antioxidant activity of antioxidant as well as on the acceleration of reaction between DPPH and antioxidant.

Smaller IC_{50} value corresponds to a higher antioxidant activity of the plant extract (Maisuthisakul et al., 2007). The methanolic extract decolorized the purple colour of DPPH to the yellow of DPPHH with an IC_{50} value of 0.048 mg/ml in *B. retusa* of MAH region and a IC_{50} value of 0.069 mg/ml in *B. retusa* of AP region. The IC_{50} value of the standard ascorbic acid was observed to be 0.024 mg/ml. The IC_{50} value observed for the ethanolic extract of MAH and AP region were 0.057 mg/ml and 0.086 mg/ml respectively.

It is evident from the observations that the methanolic extract showed higher proton donating ability on DPPH to form stable DPPHH molecules. Both methanolic and ethanolic extracts of *B. retusa* from MAH and AP region had the lowest IC_{50} value, which indicated its powerful free radical scavenger ability.The DPPH free radical scavenging activity of the methanolic and ethanolic extract was significantly related to their total phenolic content (R_{DPPH}(MeOH) = 0.9405, R_{DPPH}(EtOH) = 0.8520).

Conclusion

The present study highlights the significant differences in terms of composition and contents of phenolics extract of the bark of *B. retusa* when dielectric constant of the solvents and geographical locations are taken into consideration. These differences may possibly be related to the natural climatic differences which occur over a particular geographical area to be influenced by several climatic factors. An in-depth study comprising the recording of climatic data *in-situ* and agricultural practices at different geographical locations incorporating seasonal and climatic variation for a longer period of time is therefore recommended to pull out deeper conclusions in this regard. Our results also suggest that the more efficient extraction of antioxidant compounds from bark of

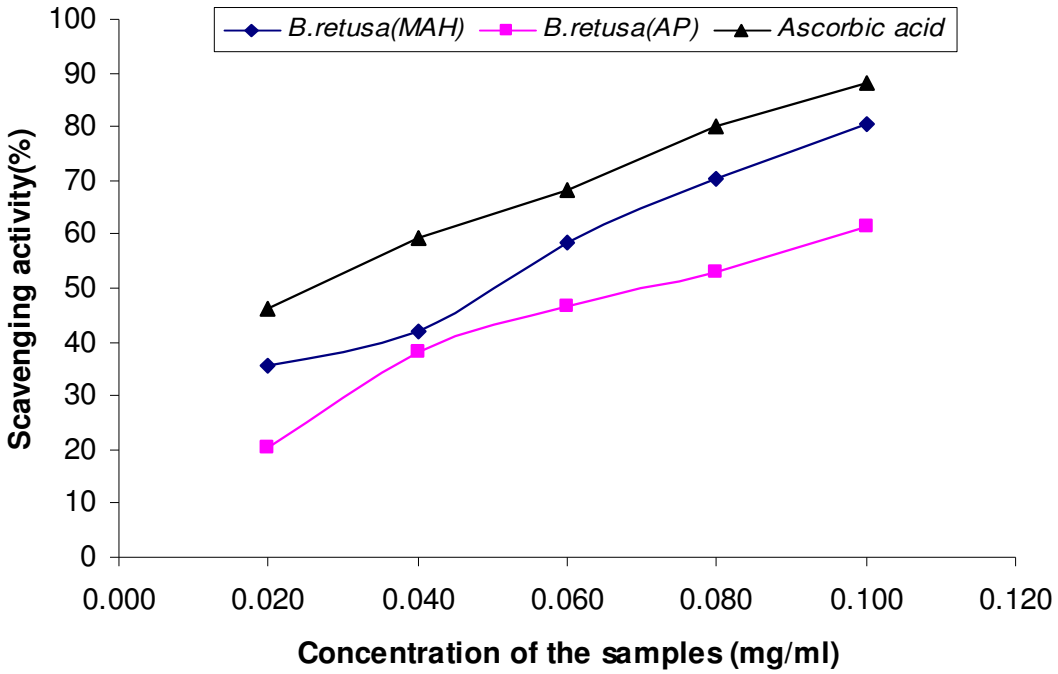

Figure 1. Scavenging activities of the methanolic extract of *B. retusa* on DPPH radical. The values are expressed as mean ± standard deviation (n=3), ascorbic acid was used as a standard, MAH- Maharashtra AP- Andhra Pradesh.

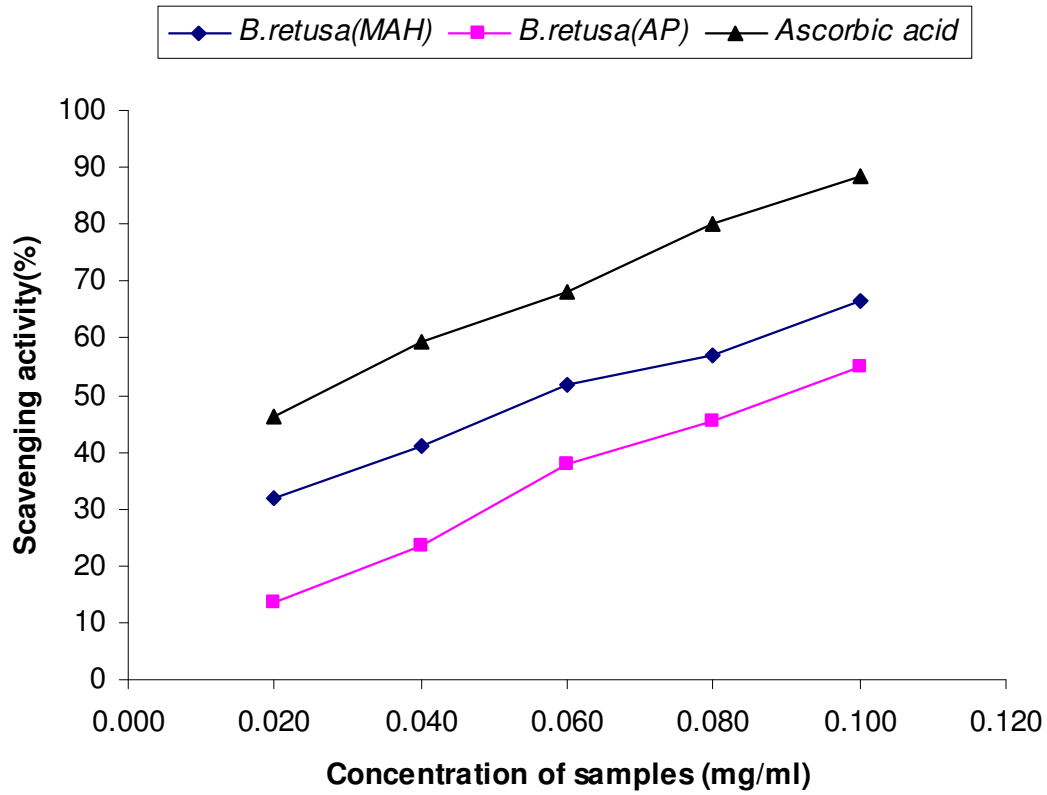

Figure 2. Scavenging activities of the ethanolic extract of *B.retusa* both on DPPH radical. The values are expressed as mean ± standard deviation (n=3), ascorbic acid was used as a standard, MAH- Maharashtra AP- Andhra Pradesh.

B. retusa may be carried out using methanol as solvent, resulting in an extract with the best overall antioxidant attributes. The selection of the appropriate solvent resulting in the maximum extractive yield in case of B. *retusa* is greatly influenced by dielectric constant value of the solvent.

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