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EFFECT OF VARIOUS SOLVENTS ON THE EXTRACTION OF ANTIOXIDANTS FROM DRIED *Basella alba* LEAVES

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ABSTRACT

***Basella alba*, an edible perennial vine, is an underutilized plant which possesses many potential health benefits including antioxidant property. Isolation of antioxidant compounds from the plant was attempted through extraction with different solvents. The solvents of varying polarities, using six extracting solvents (distilled water, ethyl acetate, hexane, ethanol, methanol and acetone) were evaluated to assess the extraction of antioxidant phenolics from dried leaves of *B. alba*. Each extract was analyzed for total phenols, flavonoids and total antioxidant activity using 2-diphenyl-1-picrylhydrazyl radical scavenging activity assay (DPPH-RSA), 2,2, azinobis, 3 ethyl benzothiazolin, 6-sulphonic acid assay (ABTS-RSA), reducing power assay (RPA) and ferric reducing antioxidant power (FRAP). Results revealed that methanol could extract maximum phenols (779.58 mg GAE 100 g^{-1}) while ethanol yielded maximum flavonoid (2726.43 mg RE 100 g^{-1}) content. Methanol extract was found the most potent scavenger of DPPH-RSA and ABTS-RSA (92.97 and 196.47 mg TE 100 g^{-1}, respectively) as well as depicted maximum RPA and FRAP values (2303.66 and 5.46 mg TE 100 g^{-1}, respectively). Pearson correlation indicated a positive significant relationship of total phenols and flavonoids with DPPH-RSA, ABTS-RSA and RPA. FRAP revealing a strong correlation with flavonoids. Overall, methanol was the most effective solvent for the extraction of antioxidant phenolics from *B. alba* leaves and can be a useful source of natural antioxidants.**

Keywords: Antioxidants, *Basella alba*, solvents

INTRODUCTION

Reactive oxygen species (ROS) and free radicals cause cellular oxidative stress that leads to various chronic diseases like diabetes, neurodegenerative diseases, cancer, cardiovascular disease, etc. (Iqbal *et al*., 2012; Olaniyi *et al*., 2013). These ROS and free radicals can be scavenged by various antioxidants that are present in plant-based foods. These antioxidants prevent oxidation of macro-molecules such as DNA, proteins and lipids by quenching ROS and reactive nitrogen species (RNS) as well as scavenge oxygen from various foods and biological system (Choi *et al*., 2007; Siddhuraju and Becker, 2007). Thus, plant antioxidants are beneficial in preventing the ill effects of oxidative stress. To prevent oxidation in food system various synthetic antioxidants like butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT), added as a food additive, were used in past but as they pose various health hazards so their use has been restricted and even banned in many developed countries (Yanishlieva and Marinova, 2001; Taghvaei and Jafari, 2015). These concerns have encouraged food scientist and health professionals to search some alternative sources of naturally occurring antioxidants that are easily available and show minimum or negligible side effects.

Basella alba (family: Basellaceae), known as creeping spinach, red vine spinach, malabar spinach, is a climbing perennial vine which is mostly found in tropical regions of world (Deshmukh and Gaikwad, 2014). *B. alba* leaves (BAL) possesses various health benefits and medicinal characteristic such as anti-inflammatory, antioxidant, anticancer, analgesic and red cell membrane stabilizing properties (Baskaran *et al.*, 2015). BAL has laxative property and is used for treating cataract. The flowers are used as an antidote for poisons (Olaniyi *et al.*, 2013).

Phytochemical studies of BAL have revealed the presence of steroids, alkaloids, terpenoids, glycoside, tannins, saponins, carotenoid, phenols and flavonoids that possess antioxidant capacity (Phadungkit *et al.*, 2012; Adegoke and Ojo, 2017). The BAL leaves contain protein, fat, vitamin A, thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), folic acid (vitamin B_9), vitamin C, vitamin E, vitamin K and minerals such as iron, calcium and magnesium (Jaiswal and Rao, 2016). The flavonoid present in BAL is kaempherol (Yang *et al.*, 2008). However, no much research has been carried out on the effect of various types of solvents on the extraction of antioxidants from BAL. Therefore, the present study was aimed to assess the efficacy of various solvents *viz*., methanol, ethanol, acetone, distilled water (D/W), ethyl acetate and hexane on the extraction of antioxidants from BAL.

MATERIALS AND METHODS

Procurement of raw materials

Fresh BAL was procured from the ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujrat (India). The identity of the plant was further authenticated by the Department of Biosciences, Sardar Patel University, V.V. Nagar, Anand, Gujarat.

Sample preparation

Six extracting solvents *viz*., distilled water (D/W), ethyl acetate (extra pure AR), hexane (extra pure AR 99%), ethanol (99.6% pure), methanol (99.8% pure) and acetone (extra pure AR) [purchased from Sisco research laboratories (SRL)] were used for sample extraction.

Dried powdered BAL (2 g) was taken and to it 15 mL of each solvent added separately, followed by crushing the contents to a fine paste using a mortar and pestle. The contents were kept in a shaker for 30 min and centrifuged for 15 min at 6000 rpm. The supernatant was collected in a sugar tube. Then to the residue, 15 mL of solvent was added and again crushed thoroughly, followed by shaking for 30 min and then centrifugation. The process was repeated three times and the supernatant collected was pooled. A known volume was made with the respective solvents (Singleton and Rossi, 1965).

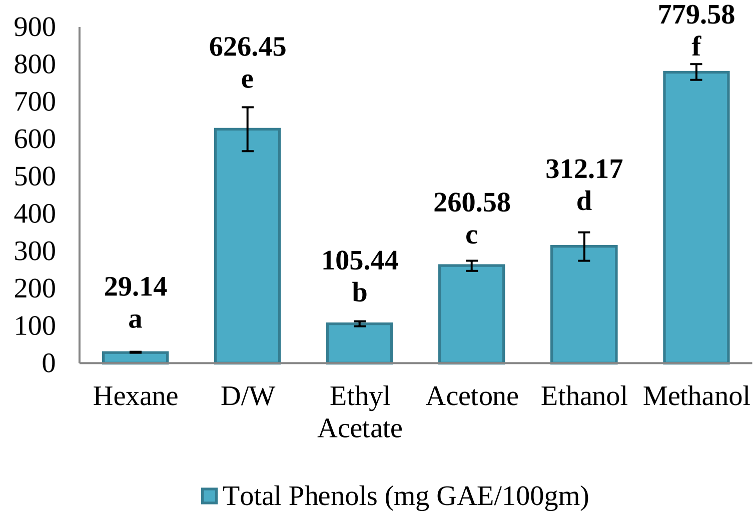
Each extract was analyzed for total phenols (Singleton and Rossi, 1965), flavonoids (Singleton *et al.*, 1999), total antioxidant capacity using FRAP [ferric reducing antioxidant power assay] (Benzie and Strain, 1996), DPPH-RSA [2,2-diphenyl-1-picrylhdrazyl radical scavenging assay] (Brand-Williams *et al.*, 1995), ABTS [2,2-aziinobis,3-ethyl benzo-thiazolin,6-sulphonic acid assay] (Miller and Rice, 1997) and RPA [reducing power assay] (Oyaizu, 1986). The protocols given by Jayswal *et al*. (2019) was followed.

Statistical analysis

One-way ANOVA and Pearson correlation was carried out to analyze the data by using SPSS (version 20). Graph was prepared using M.S. Excel (2007).

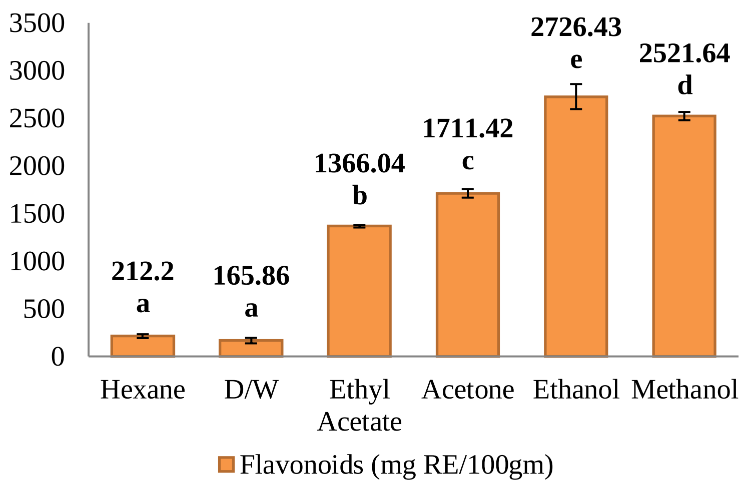
RESULTS AND DISCUSSION

The total phenols and flavonoid contents of dried BAL extracts using different solvents are presented in Fig. 1 and 2, respectively. Total phenols of BAL extracts varied from 779.58 to 29.14 mg GAE 100 g^{-1}. The results revealed significantly ($p \leqslant 0.05$) higher phenolic content in methanol followed by D/W, ethanol, acetone, ethyl acetate and hexane. These results are in conformity with Razali *et al*. (2012) who reported methanol as the most effective solvent for the extraction of phenolic content from various parts of *Tamarindus indica* and the antioxidants were mainly polar compounds. Routray *et al*. (2013) while evaluating the antioxidant potential of BAL with six leafy vegetables from different solvent extracts found methanolic extracts as maximum yielders of crude extract and phenolic contents. Methanolic extraction of vegetable residues had higher phenolic compound as compared to the other solvents (hexane, chloroform, ethyl acetate and methanol) tested (Babbar *et al*., 2014). Methanol extracted maximum bioactive compounds from *Severinia buxifolia* especially phenolics and flavonoids (Truong *et al*., 2019). Flavonoid in different extracts varied widely from 165.86 to 22726.43 mg RE 100 g^{-1}. The flavonoid in present study was significantly higher in ethanolic extract, followed by methanol, acetone, ethyl acetate, hexane and D/W. Kostic *et al*. (2013) also found highest flavonoid content in ethanolic extract of *Morus nigra* fruit. Tongco *et al*. (2015) observed high flavonoid content in ethanol extract of BAL as compared to the water extracts. The hydrogen bonds between or among water molecules do not break easily in phenolic molecule and are thus less water soluble. In addition, the presence of bulky phenyl ring in phenol structure makes it less soluble in water.



[a]Values are mean of four observations; [b]Bars not sharing the same alphabets are significantly different ($p \leqslant 0.05$)

Fig. 1: Total phenols content of extracts of dried *Basella alba* leaves using different solvents



[a]Values are mean of four observations; [b]Bars not sharing the same alphabets are significantly different ($p \leqslant 0.05$)

Fig. 2: Flavonoid content of extracts of dried *Basella alba* leaves using different solvents

Table 1: Total antioxidant capacity of extracts of dried *Basella alba* leaves using different solvents

Parameters	DPPH-RSA (mg TE 100 g^{-1})	ABTS-RSA (mg TE 100 g^{-1})	RPA (mg TE 100 g^{-1})	FRAP (mg TE 100 g^{-1})
Methanol	92.97 ± 2.27^{d}	196.47 ± 12.04^{d}	2303.66 ± 150.95^{c}	5.46 ± 0.64^{c}
Ethanol	75.65 ± 8.94^{c}	80.70 ± 2.84^{c}	2196.75 ± 229.15^{c}	4.63 ± 0.79^{b}
Acetone	52.59 ± 7.11^{b}	81.21 ± 3.23^{c}	1096.38 ± 12.09^{b}	4.25 ± 0.33^{b}
Distilled water (D/W)	54.20 ± 0.46^{b}	66.29 ± 4.93^{b}	992.03 ± 65.13^{b}	1.22 ± 0.09^{a}
Ethyl acetate	68.87 ± 0.17^{c}	60.70 ± 1.44^{b}	189.87 ± 3.18^{a}	1.88 ± 0.10^{a}
Hexane	40.64 ± 0.63^{a}	17.70 ± 2.19^{a}	63.36 ± 4.84^{a}	1.20 ± 0.05^{a}
F-value ($p \leqslant 0.05$)	62.26^{*}	441.48^{*}	274.34^{*}	72.24^{*}

[a]Values are mean ± SD of four observations; [b]Mean values with different superscripts within a column differ significantly ($p \leqslant 0.05$); [c] *Indicates significant difference ($p \leqslant 0.05$)

A significantly higher DPPH-RSA was found in methanolic extract (92.97 mg TE 100 g^{-1}) than other solvent extracts (Table 1). Ethanol and ethyl acetate as well as acetone and D/W extracts did not differ significantly in DPPH-RSA among themselves. Hexane extract had least DPPH-RSA (40.64 mg TE 100 g^{-1}). Highest value of ABTS-RSA was observed in methanolic extract, followed by acetone, ethanol, D/W, ethyl acetate and least in hexane. Ethanol and acetone as well as D/W and ethyl acetate extracts did not differ significantly in ABTS-RSA. The RPA values ranged from 2303.66 to 63.36 mg TE 100 g^{-1}. Methanolic extract had significantly higher RPA value than ethanol-, acetone-, D/W-, ethyl acetate- and hexane-based extracts. The order of potency of extracting the antioxidants by FRAP method is: methanol > ethanol > acetone > ethyl acetate > D/W > hexane. Routray *et al.* (2013) while evaluated antioxidant potential of BAL from different solvent extracts found methanolic extract yielding maximum antioxidant activity using DPPH-RSA. Razali *et al*. (2012) and Felhi (2017) also reported methanol extract of *Tamarindus indica* leaf and *Ecballium elaterium* seeds and peels fruits having highest antioxidant activities (FRAP, DPPH-RSA and ABTS-RSA).

The type of solvent used for extraction, the time required for extraction and the temperature employed are the three main factors which influence the recovery of antioxidants (Robards, 2003). Since the nature of extracting solvents, their chemical characteristics and polarities widely vary, this affects the solubility of phenolics in a particular solvent. Solvents with higher polarity are mostly used for the recovery of polyphenols from plant matrix (Peschel *et al*., 2006), mainly due to the interaction (hydrogen bonds) between the polar site of antioxidant molecules of the sample and the solvent used for extraction (Liu, 2007). Polyphenols that have low molecular weight are extracted in methanol (Guyot *et al*., 2001; Prior *et al*., 2001). Ethanol was found less effective than methanol in extracting antioxidant probably due to the presence of ethyl radical that is longer than methyl radical present in methanol, leading to a lower solvation of antioxidant molecules. Hexane extracted least antioxidants from BAL due to its non-polar nature (Kulkarni *et al*., 2004).

Table 2: Pearson correlation among different antioxidant parameters of extracts of dried *Basella alba* leaves using different solvents

Parameters	Total phenols	Flavonoid	DPPH-RSA	ABTS-RSA	RPA	FRAP
Total phenols	1.000	0.281	0.592**	0.798**	0.701**	0.434*
Flavonoid	0.281	1.000	0.797**	0.673**	0.790**	0.904**
DPPH-RSA	0.592**	0.797**	1.000	0.840**	0.734**	0.690**
ABTS-RSA	0.798**	0.673**	0.840**	1.000	0.774**	0.764**
RPA	0.701**	0.790**	0.734**	0.774**	1.000	0.847**
FRAP	0.434*	0.904**	0.690**	0.764**	0.847**	1.000

[a] **Correlation is significant at the 0.01 level (2-tailed); [b] *correlation is significant at the 0.05 level (2-tailed)

of antioxidants and due to high phenolic content, BAL can serve as a nutraceutical ingredient in various functional foods.

Pearson correlation indicated a significantly positive relationship of total phenols and flavonoid with DPPH-RSA, ABTS-RSA and RPA (Table 2). Total phenols showed a significantly positive correlation with FRAP. FRAP exhibited strong correlations with flavonoid content. Boeing *et al*. (2014) observed a significant positive relationship between TPC and antioxidant capacities measured by FRAP, DPPH and ORAC for different berries. Iqbal *et al*. (2012) reported a strong positive correlation of TPC with total flavonoid, FRAP, ABTS and DPPH content in *Artemisia annua* leaves. Similarly, Adegoke *et al.* (2017) found significant correlation between TPC and FRAP values in *Basella* plants and *Amaranthus caudatus*. The high values of Pearson correlation in present study depicted that phenolic and flavonoid compounds are the main contributors to the total antioxidant capacities of BAL. The study concludes that methanol could be used as the best solvent for extraction.

Conflict of interest: The authors declare that there is no conflict of interest with regard to publishing any aspect of this study.

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