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ORIGINAL ARTICLE

Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn.

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KEYWORDS

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Total phenolics;
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Abstract The objectives of this study were to determine phenolic content and antioxidant activities of chloroform, acetone, methanol and hot water extracts of *Bauhinia vahlii* leaves. The hot water extract afforded the highest yield (6.3%) while the lowest yield was obtained from the chloroform extract (2.1%). The methanol extract contains higher levels of total phenolics (48.7 ± 0.7 g GAE/100 g extract), tannins (21.7 ± 0.7 g GAE/100 g extract) and flavonoids (10.3 ± 0.2 RE/100 g extract). The extracts were subjected to assess their antioxidant potential using various *in vitro* systems such as DPPH$^{\cdot}$, ABTS$^{\cdot+}$, FRAP, OH$^{\cdot}$, β-carotene linoleic acid bleaching system, phosphomolybdenum reduction and Fe^{2+} chelation. It is concluded that the methanolic extract of *B. vahlii* leaves have strong antioxidant potential. Further study is necessary for isolation and characterization of the active antioxidants, which may serve as a potential source of natural antioxidants.

1. Introduction

Phenolic compounds are widely distributed in plants and in recent years they have gained much attention, due to their antioxidant activity and free radical-scavenging ability with potential beneficial implications in human health (Ross and Kasum, 2002). When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Jadhav et al., 1996). These antioxidants may help to relieve oxidative stress, i.e. preventing free radicals from damaging biomolecules such as proteins, DNA, and lipids (Shahidi and Naczk, 2004). The antioxidant activity of extracts of several plants, including their leaves, bark, roots, fruits, and seeds has been extensively studied (Mariod et al., 2008). However, many researchers reported the adverse effects of synthetic antioxidants such as toxicity and carcinogenicity. Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference.

Bauhinia vahlii Wight & Arn. is a very large, usually evergreen, climber and is distributed in deciduous forests of India from Gujarat Southwards to Maharashtra and Northern Andhra Pradesh, commonly on hillsides and in forest valleys (Parrotta, 2001). The ripe seeds, when eaten raw or fried, taste like cashew-nuts (NISC, 1986). The cooked and roasted mature seeds of *B. vahlii* are eaten by the tribes, Kondakapulu and Baagethalu of Araku valley, Visakhapatnam, Andhra Pradesh and Mundari group of tribes in India (Rajaram and Janardhanan, 1991; Vadivel and Janardhanan, 2000). Our previous

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study demonstrated that phenolic content and antioxidant activity in *B. vahlii* seeds were varied significantly during processing (Sowndhararajan et al., 2010). Tender pods and leaves are cooked as vegetables. The leaves are demulcent and mucilaginous. A decoction of the leaf is given to treat diarrhea and dysentery. Leaves are used for fodder to make mats and containers for food stuffs sometimes for thatching, also for tobacco wrappers for smoking (Manandhar, 2002; Nadkarni, 2005). Moreover, there is no information pertaining to the antioxidant potential of *B. vahlii* leaves. Based on the traditional knowledge of medicinal system, the present study was carried out to evaluate the antioxidant activity of different solvent and aqueous extracts of leaves of *B. vahlii*.

2. Materials and methods

2.1. Preparation of extracts

Fresh leaves of *B. vahlii* were collected from Coimbatore, Tamil Nadu state, India during the month of February 2012. The plant was authenticated by The Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. The plant leaves were washed thoroughly in tap water, shade dried at room temperature (25 °C), powdered and used for solvent extraction.

The powdered plant samples were packed into a soxhlet apparatus and were extracted sequentially with petroleum ether (for disposing lipid and pigments), chloroform (BLC), acetone (BLA) and methanol (BLM) and the air dried residue was further extracted with hot water (BLH) by the method of maceration. Each time before extracting with the next solvent, the material was dried in a hot air oven at 40 °C. The solvents were evaporated using a rotary vacuum-evaporator at 50 °C and the remaining water was removed by lyophilization. The extract recovery in different solvents was expressed as percent of the plant sample dry matter. The freeze-dried extracts thus obtained were dissolved in the respective solvents at the concentration of 1 mg/1 ml and used for assessment of antioxidant capacity through various chemical assays.

2.2. Determination of total phenolic and flavonoid contents

The total phenolic content of leaves was determined by Folin Ciocalteu method. The amount of total phenolics and tannins was calculated as gallic acid equivalents (GAE) as described by Siddhuraju and Becker (2003). The total flavonoid content was determined by the method described previously by Zhishen et al. (1999) and expressed as gram of rutin equivalent (RE)/100 g of extract.

2.3. Ferric-reducing/antioxidant power (FRAP) assay

The antioxidant capacity of extracts was estimated according to the method described previously by Pulido et al. (2000). The absorbance of the reaction mixture was read at 593 nm. The values are expressed as mmol Fe (II)/g extract.

2.4. Antioxidant activity by the $ABTS^{\cdot+}$ assay

Radical scavenging activity of extracts was assessed spectrophotometrically by [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] $ABTS^{\cdot+}$ cation decolorization assay and the absorbance was taken at 734 nm (Re et al., 1999). The unit of total antioxidant activity is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μmol/g extracts.

2.5. Metal chelating activity

The chelating activity of ferrous ions by different extracts of *B. vahlii* leaves was estimated by the method described by Dinis et al. (1994). Absorbance of the solution was measured spectrophotometrically at 562 nm. The results were expressed as mg ethylenediaminetetraacetic acid (EDTA) equivalent/g extract.

2.6. Phosphomolybdenum assay

The antioxidant activity of extracts was evaluated by the green phosphomolybdenum complex formation according to the previously described method of Prieto et al. (1999). The absorbance of the mixture was measured at 695 nm. The results reported are mean values expressed as grams of ascorbic acid equivalents (AAE) per 100 g extract.

2.7. Free radical scavenging activity on $DPPH^{\cdot}$

The $DPPH^{\cdot}$ radical scavenging activity of different extracts of *B. vahlii* leaves was measured according to the method of Blios (1958). IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

2.8. Hydroxyl radical scavenging activity

The scavenging activity of different extracts of *B. vahlii* leaves (20, 40, 60 and 80 μg) on hydroxyl radical activity was measured according to the previously described method (Klein et al., 1991). The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The hydroxyl radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity.

2.9. The β-carotene/linoleic acid antioxidant activity

The β-carotene/linoleic acid antioxidant activity of the antioxidant (leaf extracts, or α-tocopherol (α T), 100 μL) solution was measured according to the previously described method (Taga et al., 1984).) at 470 nm. And the antioxidant activity of the bark extracts and standard was evaluated as % of antioxidant activity.

2.10. Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$) using statistica (Statsoft Inc., Tulsa, USA). Values expressed are means of three replicate determinations ± standard deviation.

3. Results and discussion

3.1. Recovery percent, total phenolics, tannins and flavonoid content

Plant phenolics present in the fruits and vegetables have received considerable attention because of their potential biological activity. Phenolic compounds such as flavonoids, phenolics acid, and tannins possess diverse biological activities including anti-inflammatory, anti-carcinogenic, and antiatherosclerotic activities. These activities might be related to their antioxidant activity (Chung et al., 1998). The yield percent, total phenolics, tannins and flavonoid content of the extracts obtained from *B. vahlii* leaves are shown in Table 1. Generally, the values of all the said parameters tended to increase with the increasing polarity of the solvents used as extraction medium. As such, the maximum extract yield was obtained in the hot water (6.3%) followed by methanol (5.9%). The extractable total phenolics (48.7 g/100 g extract), tannins (21.7 g/100 g extract) and flavonoids (10.3 g/100 g extract)(10.3 g 100 g^{-1} extract) were found to be higher in the methanol extract of *B. vahlii*. However, among the sample extracts the lowest concentrations of phenolics (18.9 g/100 g extract) tannins (7.1 g/100 g extract) and flavonoids (4.1 g/100 g extract) were observed in chloroform extract. Phenolics are powerful antioxidants and act in a structure-dependent manner; they can scavenge reactive oxygen species (ROS), and chelate transition metals which play vital roles in the initiation of deleterious free radical reactions (Fresco et al., 2006). Obviously, total phenolic content could be regarded as an important indication of antioxidant properties of plant extracts (Liu et al., 2008). Crude extracts of fruits, vegetables, and other plant materials are rich in phenolics. The importance of the antioxidant constituents of plant material in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers. There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds (Surh, 2002).

3.2. Ferric reducing antioxidant power (FRAP) assay

Antioxidants can be explained as reductants, and inactivators of oxidants (Siddhuraju and Becker, 2007). Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be related to reducing power. Therefore, the antioxidant potential of different extracts of *B. vahlii* leaves was estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) (Table 2). The ferric reducing ability of the extracts revealed that all of them gave good FRAP activity (734.4–3257.8 mmol Fe (II)/g extract). Among the extracts, the highest activity was noted for methanol extract (3257.4 mmol Fe (II)/g extract) followed by acetone extract (3028.9 mmol Fe (II)/g extract) of *B.vahlii* leaves. The chloroform extract had significantly ($P < 0.05$) lower FRAP activity (734.4 mmol Fe (II)/g extract). FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples (Halvorsen et al., 2006; Pellegrini et al., 2003). Halvorsen et al. (2006) suggested most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay.

3.3. Antioxidant activity by the $ABTS^{\bullet+}$ assay

The chloroform, acetone, and methanol extracts from the leaves of *B. vahlii* were fast and effective scavengers of the

Table 1 Extract yield percentage, total phenolics, flavonoid and tannin contents of *B. vahlii* leaves.

Sample	Extract yield (%)	Total phenolics (g GAE/100 g extract)	Tannins (g GAE/100 g extract)	Flavonoids (g RE/100 g extract)
BLC	2.1	$18.9 \pm 1.2^{d,*}$	7.1 ± 0.3^{d}	4.1 ± 0.1^{d}
BLA	3.6	38.2 ± 1.4^{b}	16.5 ± 1.1^{b}	8.9 ± 0.4^{b}
BLM	5.9	48.7 ± 0.7^{a}	21.7 ± 0.8^{a}	10.3 ± 0.2^{a}
BLH	6.3	23.7 ± 1.3^{c}	10.9 ± 0.4^{c}	5.9 ± 0.2^{c}

BLC – chloroform extract of *B. vahlii* leaves; BLA – acetone extract of *B. vahlii* leaves; BLM – methanol extract of *B. vahlii* leaves; BLH – hot water extract of *B. vahlii* leaves.

* Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$).

Table 2 FRAP, $ABTS^{\bullet+}$ scavenging, metal chelating and phosphomolybdenum reduction activity of *B. vahlii* leaves.

Sample	FRAP (mmol Fe(II)/g extract)	ABTS (μmol Trolox/g extract)	Metal chelating property (mg EDTA/g extract)	Phosphomolybdenum (g AAE/100 g extract)
BLC	$734.4 \pm 19.4^{c,*}$	8569.7 ± 994.5^{d}	0.9 ± 0.1^{d}	25.4 ± 0.8^{c}
BLA	3028.9 ± 43.4^{a}	21760.1 ± 1311.2^{b}	6.2 ± 0.2^{a}	38.0 ± 1.4^{b}
BLM	3257.8 ± 53.8^{a}	25435.2 ± 1798.1^{a}	5.7 ± 0.3^{b}	40.5 ± 1.1^{a}
BLH	2109.4 ± 44.7^{b}	12356.5 ± 1234.5^{c}	3.6 ± 0.3^{c}	32.5 ± 1.1^{b}

BLC – chloroform extract of *B. vahlii* leaves; BLA – acetone extract of *B. vahlii* leaves; BLM – methanol extract of *B. vahlii* leaves; BLH – hot water extract of *B. vahlii* leaves.

* Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$).

ABTS radical (Table 2). In $ABTS^{\cdot+}$ scavenging activity the values are varied significantly ($P < 0.05$) and ranged from 8569.7 to 25435.2 μmol Trolox/g extract. Actually, the ABTS radical cation scavenging activity also reflects hydrogen-donating ability. Hagerman et al. (1998) reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{\cdot+}$). Since, the extracts from various samples have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction, they could serve as potential nutraceuticals when ingested along with nutrient.

3.4. Metal chelating activity

Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress (Hippeli and Elstner, 1999). An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton type reactions. Therefore, it is considered important to screen the iron (II) chelating ability of the extracts. All the extracts demonstrated a moderate level of ability to chelate metal ions (Table 2). The acetone extracts of *B. vahlii* leaves (6.2 mg EDTA/g extract) were found to have the maximum chelating ability. From the iron chelating data, it is evident that the extracts may be able to play a protective role against oxidative damage by sequestering Fe (II) ions that may otherwise catalyze Fenton type reactions or participate in metal catalyzed hydroperoxide decomposition reactions. The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups (Pazos et al., 2005).

3.5. Phosphomolybdenum assay

This assay has been routinely used to evaluate the antioxidant capacity of extracts (Prieto et al., 1999). Various extracts of *B. vahlii* were also used to determine their antioxidant capacities by the formation of green phosphomolybdenum complex. The formation of the complex was measured by the intensity of absorbance in extracts at a concentration of 100 μg/ml at 95 °C as shown in Table 2. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green phosphate/Mo(V) complex with the maximal absorption at 695 nm. Being simple and independent of other antioxidant measurements commonly employed, the assay was extended to plant polyphenols. In the ranking of the antioxidant capacity obtained by this method, the methanol extract of *B. vahlii* leaves showed higher phosphomolybdenum reduction (40.5 g AA/100 g extract) followed by acetone extract (38.0 g AA/100 g extract). This may be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants (Loo et al., 2008).

3.6. Free radical scavenging activity on DPPH·

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant (Lu and Yeap Foo, 2001). It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto et al., 2000; Soares et al., 1997). As presented in Fig. 1, the scavenging abilities of different solvent extracts of *B. vahili* were concentration-dependent and expressed as IC_{50} values. Concentration of the sample necessary to decrease the initial concentration of DPPH· by 50% (IC_{50}) under the experimental condition was calculated. Therefore a lower IC_{50} value indicates a higher antioxidant activity.

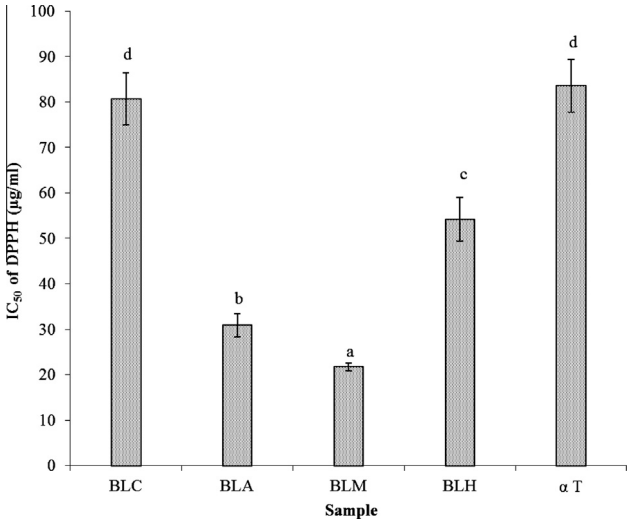

Figure 1 DPPH· radical scavenging activity of *B. vahlii* leaves. Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Bars having different letters are significantly different ($P < 0.05$). BLC – chloroform extract of *B. vahlii* leaves; BLA – acetone extract of *B. vahlii* leaves; BLM – methanol extract of *B. vahlii* leaves; BLH – hot water extract of *B. vahlii* leaves; α T – α-tocopehrol.

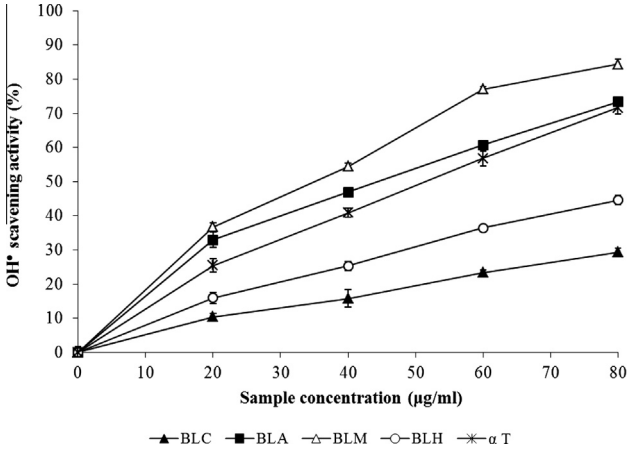

Figure 2 Hydroxyl radical (OH·) scavenging activity of *B. vahlii* leaves. Values are mean of three replicate determinations ($n = 3$) ± standard deviation. BLC – chloroform extract of *B. vahlii* leaves; BLA – acetone extract of *B. vahlii* leaves; BLM – methanol extract of *B. vahlii* leaves; BLH – hot water extract of *B. vahlii* leaves; α T – α-tocopehrol.

DPPH free radical scavenging effect of *B.vahili* extracts and standards was in this order: BLM > BLA > BHA > BLC > α T. Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation (Bamforth et al., 1993).

3.7. Hydroxyl radical ($OH^{\cdot}$) scavenging activity

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *in vivo* (Duan et al., 2007). Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides (Wang et al., 2008). Thus, removing $OH^{\cdot}$ is very important for the protection of living systems. The hydroxyl radical scavenging potential of various solvent extracts of *B. vahlii* leaves is shown in Fig. 2. Each extract showing hydroxyl radical scavenging activity was increased with increasing concentration of sample extracts. In the present investigation, the hydroxyl radical scavenging activity observed was in the range of 29.3–84.4% at the concentration of 80 μg/ml. While scavenging hydroxyl radical, the ability of methanol extract (84.4%) was found to be higher than other sample extracts.

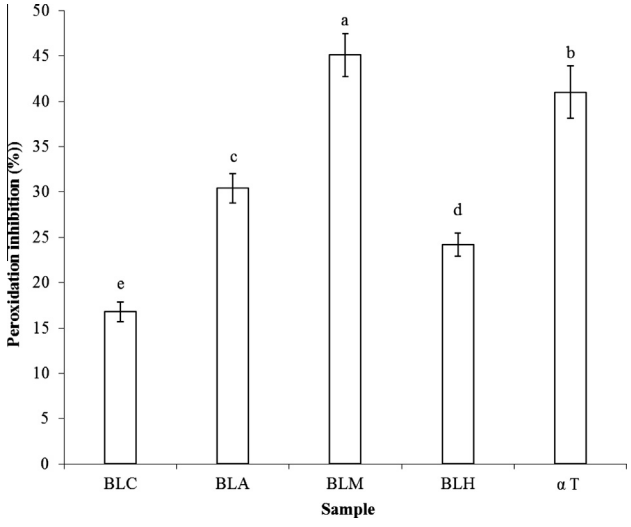

Figure 3 β-carotene/linoleic acid peroxidation inhibition activity of *B. vahlii* leaves. Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Bars having different letters are significantly different ($P < 0.05$). BLC – chloroform extract of *B. vahlii* leaves; BLA – acetone extract of *B. vahlii* leaves; BLM – methanol extract of *B. vahlii* leaves; BLH – hot water extract of *B. vahlii* leaves; α T – α-tocopehrol.

3.8. β-carotene/linoleic acid antioxidant activity

The antioxidant assay using the discoloration of β-carotene is widely used to measure the antioxidant activity of bioactive compounds. In this assay, oxidation of linoleic acid produces hydroperoxyl radicals evolving toward lipid hydroperoxides, conjugated dienes, and volatile by-products, which simultaneously attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion (Frankel, 1998). In this test, β-carotene undergoes rapid discoloration in the absence of antioxidant, which results in a reduction in absorbance of the test solution with increasing reaction time. The presence of antioxidant hinders the extent of bleaching by neutralizing the linoleic hydroperoxyl radicals formed (Kulisic et al., 2004). Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. In the present study antioxidant capacity of extracts was in the range of 16.8–45.1% (Fig. 3) at the concentration of 100 μg/ml. The antioxidant capacity of the studied extracts was in the order BLM > α T > BLA > BLH > BLC. Interestingly, methanol extract of *B. vahlii* leaves registered significantly higher antioxidant capacity than positive control α T (41%). Hydroperoxides formed in this system will be decomposed by the antioxidants from the *B. vahlii* extracts.

3.9. Comparison between different antioxidant assays by correlation analysis

The results of the different antioxidant assays used in the present investigation of different extracts were compared and correlated with each other. Correlation between the results of different antioxidant assays is represented in Table 3. The content of total phenolics showed a good correlation with most of the antioxi-

Table 3 Comparison between different antioxidant assays as represented by correlation coefficient.

	TPC	TFC	FRAP	$ABTS^{\cdot+}$	Metal chelating	AEAC	$DPPH^{\cdot}$	$OH^{\cdot}$	β-Carotene
TPC	–	0.974	0.828	0.981	0.756	0.890	0.900	0.970	0.959
TFC	0.974	–	0.928	0.995	0.879	0.965	0.972	0.998	0.969
FRAP	0.828	0.928	–	0.895	0.971	0.991	0.988	0.924	0.907
$ABTS^{\cdot+}$	0.981	0.995	0.895	–	0.856	0.938	0.948	0.997	0.949
Metal chelating	0.756	0.879	0.971	0.856	–	0.944	0.947	0.885	0.808
AEAC	0.890	0.965	0.991	0.938	0.944	–	0.999	0.960	0.952
$DPPH^{\cdot}$	0.900	0.972	0.988	0.948	0.947	0.999	–	0.968	0.951
$OH^{\cdot}$	0.970	0.998	0.924	0.997	0.885	0.960	0.968	–	0.957
β-Carotene	0.959	0.969	0.907	0.949	0.808	0.952	0.951	0.957	–

TPC – Total phenolic content; TFC – Total flavonoid content; FRAP – Ferric-reducing/antioxidant power assay; AEAC – Ascorbic acid equivalent antioxidant capacity (Phosphomolybdenum reduction); $OH^{\cdot}$ – Hydroxyl radical.

dant assays, such as FRAP (R^2 = 0.828), $ABTS^{\cdot}+$ (R^2 = 0.981), metal chelating (R^2 = 0.756), AEAC (R^2 = 0.890), $DPPH^{\cdot}$ radical scavenging assay (R^2 = 0.900), $OH^{\cdot}$ (R^2 = 0.970) and β-carotene bleaching assay. (R^2 = 0.959). Some authors have reported excellent linear correlations between antioxidant activity tests and total phenolic content (Sultana et al., 2007; Oliveira et al., 2009). There was also a close correlation between TPC and TFC (R^2 = 0.974). In addition, the content of total flavonoids showed a good correlation with most of the antioxidant assays. While, DPPH radical scavenging assay showed a close relation with total phenolics, flavonoids and all antioxidant assays which were ranged from R^2 = 0.900 to R^2 = 0.999. This may be due the fact that many other compounds such as carotenoids, tocopherol and vitamin C other than total phenols and total flavonoids also contribute to antioxidant activity (Mccune and Johns, 2002). The differences in correlation coefficient among different antioxidant methods indicate the fact that single assay may not be used to assess the total antioxidant activity (Silva et al., 2006).

4. Conclusion

Higher level of antioxidant activity is observed in the methanol extract of *B. vahlii* leaves when compared with other tested extracts. Thus, these extracts can be considered as new sources of natural antioxidants. Our findings now provide a basis for developing a valuable food additive to enhance human nutrition via their phenolic composition and antioxidant activity.

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