

*Full Length Research Paper*

## Bioactive compounds and antioxidant activity of different extracts from *Vitex negundo* leaf

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The study was conducted to assess the antioxidant activity of methanol and hexane extract and essential oil from *Vitex negundo* leaf using different *in vitro* antioxidant assays. Antioxidant property was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity, ferric ion reducing antioxidant power (FRAP) and  $\beta$ -carotene-linoleic acid assays. Total phenolic contents (TPC) were measured using Folin-Ciocalteu method. Flavonoids, tocopherols,  $\beta$ -carotene and lycopene were analyzed using high performance liquid chromatography (HPLC). Results of the present study showed that methanol extract of *V. negundo* leaf exhibited significantly ( $p < 0.05$ ) higher antioxidant activity in terms of measurements of DPPH free radical ( $IC_{50}$ ), FRAP and  $\beta$ -carotene-linoleic assays than those of hexane extract and essential oil. Methanol extract of *V. negundo* leaf also contained high amounts of bioactive compounds including total phenolic compounds (363 mg GAE/g), epicatechin (16.98 mg/g), quercetin (13.45 mg/g), catechin (8.95 mg/g) and myricetin (3.32 mg/g) while the concentrations of tocopherol,  $\beta$ -carotene and lycopene were found to be lower.

**Key words:** *Vitex negundo* leaf, antioxidant activity, bioactive compounds, methanol extract, essential oil.

### INTRODUCTION

Free radicals such as singlet oxygen and hydrogen peroxide generated by oxidation process can eventually lead to damaging the body cells. Studies have shown that oxidative stress plays important role in the pathogenesis of certain cancers and atherosclerosis (Lee et al., 1999; Wang et al., 2009; Sun and Ho, 2005), Alzheimer diseases (Engelhart et al., 2002), diabetes (Sabu and Kuttan, 2002), and rheumatoid arthritis (Ostrakhovitch et al., 2001). Antioxidants are substances that are capable of slowing or preventing the oxidation of other molecules. Fruits, vegetables and herbs are rich sources of antioxidants such as phenolic compounds, flavonoids,

carotenoids, tocopherol and ascorbic acid. These compounds are reported to be well correlated with antioxidant capacity (Katalinic et al., 2004).

Nowadays, researchers have immense interest in investigating the effects of food-derived natural antioxidants on health. Many studies have confirmed that antioxidants, in particular, polyphenols and flavonoids are able to prevent or at least decrease injuries caused by oxidation reactions in the cells, such as coronary heart diseases, certain cancers and liver disorders (Lin et al., 2007; Imai and Nakachi, 1995; Madani et al., 2008).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), being effective in neutralization of free radicals, are used as inhibitors of lipid peroxidation to stabilize fat-containing foods. However, the uses of BHA and BHT have been associated with toxic effects and

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carcinogenicity. Carcinogenicity (Shahidi, 1997; Jeong et al., 2004), and toxicity effects like liver swelling have been reported (Siddhuraju and Becker, 2003) with synthetic antioxidants. Therefore, the use of synthetic antioxidants is doubted. In recent years, researchers are trying to replace synthetic antioxidants with natural antioxidants such as flavonoids, tocopherols and ascorbic acid (Hras, 2000; Bruni et al., 2004; Williams et al., 2004; Frutos and Hernandez-Herrero, 2005). Regular consumption of foods with appropriate amount of antioxidant may decrease risk of disease associated with free radicals such as heart and neurological diseases. Several medicinal plants have been shown to exhibit potential antioxidant activity due to presence various phytochemicals, e.g. flavonoids, phenolic diterpenes, phenolic acids, tannins, carotenoids and tocopherol (Dawidowicz et al., 2006). Due to the above reasons herbal plants have been regarded as a source of natural bioactive compounds. Since ancient times, the plants have been regarded as preventer and recuperatives of some of the diseases. In addition to the uses of natural antioxidants in medicine, these compounds have been used in many industrial applications, such as preservatives in foods and cosmetics.

*Vitex negundo* L. (Verbenaceae) is an aromatic shrub that can grow up to 4 to 5 m in height. The plant has been reported to exhibit medicinal properties including the curing of rheumatic pains and reducing swellings of the joints (Chopra et al., 1986). In Chinese traditional medicine, it has been used for the treatment of chronic bronchitis. An infusion of the twigs is considered to be an effective therapy for headaches, dizziness, convulsions, coughs, mental unrest and is said to promote wakefulness (Duke and Ayensu, 1985).

Preliminary studies showed that *V. negundo* leaf exhibited antioxidant properties and contain natural antioxidants. Thus, the objective of this study was to analyze the antioxidant activity of methanol and hexane extract and essential oil from *V. negundo* leaf using different *in vitro* antioxidant assays. In addition, total phenolic contents, flavonoids, tocopherol and carotenoid content of leaf of *V. negundo* were also quantified using high performance liquid chromatography (HPLC).

## MATERIALS AND METHODS

### Chemicals

Sodium acetate trihydrate,  $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  and  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  were supplied by Riedel-deHaën AG (Seelze, Germany). Glacial acetic acid, hydrochloric acid,  $\beta$ -carotene, linoleic acid, Tween 40, 2,6-ditert-butyl-4-hydroxytoluene (BHA), Lycopene standard (>95% purity), 2,6-di-tert-butyl-4-methylphenol (BHT), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, sodium bicarbonate, and Folin-Ciocalteu phenol reagent were purchased from Sigma chemical Co. (St. Louis, MO, USA). Methanol and hexane were obtained from Merck (Darmstadt, Germany). Chloroform was purchased from Fisher Scientific

(Loughborough, UK).

### Sample preparation

Mature leaves of *V. negundo* were collected from the University Agriculture Park, and Herbal unit at University Putra Malaysia (UPM), in November 2008. The voucher specimen of the plant was deposited in the Department of Food Science, University Putra Malaysia, Serdang, Malaysia. All of the petioles were cut and removed. The leaves were washed and dried utilizing oven dryer at  $40 \pm 1^\circ\text{C}$  for 48 h. The dried leaves were then ground into powder, stored in dark glass bottles and kept at  $-20 \pm 1^\circ\text{C}$  until further analysis.

### Methanol and hexane extraction

The finely ground *V. negundo* leaves (20 g) were extracted with methanol (200 ml) using a shaking water bath (Protech, Malaysia) for 2 h at  $40^\circ\text{C}$ . After filtration with Whatman filter paper No 1 using vacuum pump, the residue was re-extracted again in the similar way to get the maximum compounds. The filtrated samples were concentrated using a rotary vacuum evaporator (Buchi, Flavel, Switzerland) at  $40^\circ\text{C}$ . The concentrated extracts were kept in dark bottles at  $4^\circ\text{C}$  until used. Similar extraction procedure was used with that of hexane for hexane extract.

### Extraction of the essential oil

The freshly collected leaves of *V. negundo* were washed with running water and subjected to hydro-distillation using a Clevenger apparatus, British type (BT, Favorit, Germany) for 4 h as recommended by British pharmacopoeia (1988). The isolated essential oil was stored in sealed glass vials at  $4^\circ\text{C}$ , prior to analysis.

### Antioxidant activity assay

#### DPPH method

Antioxidant activity of methanol, hexane extract and essential oil of *V. negundo* leaves was determined using stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described by Brand-Williams et al. (1995). Different concentration of samples (0.5 ml) was added to 3.5 ml DPPH ( $6 \times 10^{-5}$  mol/L) in methanol and kept in room temperature ( $28 \pm 2^\circ\text{C}$ ) for 30 min. After incubation period, the absorbance was read at 515 nm. The percentage of radical scavenging activity was calculated according to the following equation:

$$\% = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

where  $A_{\text{blank}}$  is the absorbance of the control reaction (includes all reagents excluding the test compound), and  $A_{\text{sample}}$  is the absorbance of the test compound. The concentration of extract providing 50% inhibition ( $\text{IC}_{50}$ ) was calculated from the graph plotting inhibition percentage against extract concentration.  $\text{IC}_{50}$  is the concentration of the extract needed to inhibit half of the present free radicals. BHA and  $\alpha$ -tocopherol were used as positive controls and all tests were performed in triplicate.

#### Ferric reducing antioxidant power (FRAP) test

The FRAP assay was conducted according to the method

described by Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g  $C_2H_3NaO_2 \cdot 3H_2O$  and 16 ml  $C_2H_4O_2$ ), pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM  $FeCl_3 \cdot 6H_2O$  solution. The fresh working solution (FRAP reagent) was prepared by mixing 50 ml acetate buffer, 5 ml TPTZ solution, and 5 ml  $FeCl_3 \cdot 6H_2O$  solution (ratio 10:1:1). 200  $\mu$ l sample was added to 3 ml of the FRAP reagent. After incubation in the dark at 37°C for 30 min, the absorbance was read at 593 nm. The standard curve was linear between 0 and 49.9  $\mu$ M Trolox. The final results were expressed as micromole Trolox equivalents (TE) per gram extract basis ( $\mu$ M TE/g). The difference in absorbance between the blank and each sample is comparative to the total ferric reducing antioxidant power (FRAP value) of the antioxidants in the sample. A high FRAP value indicated higher antioxidant activity of the sample.

### $\beta$ -carotene-linoleic acid assay

In  $\beta$ -carotene-linoleic acid assay system, an aqueous emulsion of linoleic acid and  $\beta$ -carotene under heat-induced oxidation were used (Miller, 1971). In this process, an atom of hydrogen separates from active methylene group of linoleic acid generating a free radical of pentadienyl that attacks the unsaturated  $\beta$ -carotene molecules (Frankel, 1998). Consequently,  $\beta$ -carotene molecules lose their trait orange colour, which can be measured using spectrophotometer.

Antioxidants molecules can delay  $\beta$ -carotene degradation. In this process, linoleate free radicals formed during the reaction are being neutralized by the antioxidants. Briefly, 200 mg linoleic acid (99% purity) and 20 mg Tween 40 were placed in a flask. Two mg of  $\beta$ -carotene (95% purity) was dissolved in 10 ml chloroform and added to the solution. Chloroform was evaporated using a vacuum evaporator apparatus. Then 50 ml of distilled water saturated with oxygen by shaking for 30 min was added. This mixture is used as a stock solution. Aliquots (200  $\mu$ l) of each sample dissolved in methanol (2.0 mg/ml) were added to 2.5 ml stock solution in the test tubes. Afterwards, the samples were placed in an oven at temperature 50°C for 3 h. The absorbance was read at 470 nm in the spectrophotometer. The percent of antioxidant activity was calculated from the following equation:

$$AA\% = 100 \times [1 - (A_0 - A_t/A_{00} - A_{0t})]$$

where AA% is percent of antioxidant activity,  $A_0$  is the absorbance at beginning of reaction,  $A_t$  is the absorbance after 3 h, with compound,  $A_{00}$  is the absorbance at beginning of reaction, without compound (200  $\mu$ l of methanol and 2.5 ml of stock solution), and  $A_{0t}$  is the absorbance after 3 h without compound. A blank containing the stock solution minus  $\beta$ -carotene was used to bring the spectrophotometer to zero. As a control positive BHT was used with same condition and concentration (2.0 mg/ml). Each assay was done in triplicate and the results averaged.

### Determination of total phenolic contents

Total phenolic contents (TPC) were measured calorimetrically using the Folin-Ciocalteu method (Sigleton et al., 1999). One milliliter of appropriately diluted extract (using 80% ethanol) of *V.negundo* was added to a 25 ml volumetric flask and then 9 ml distilled water was added. Five milliliter of 5%  $Na_2CO_3$  solution and 0.5 ml Folin-Ciocalteu phenol reagent were added to the sample and shaken vigorously. The solution was then immediately diluted to 25 ml with distilled water and mixed thoroughly. The mixtures were incubated for 1 h at room temperature and dark condition. The absorbance was then measured at 765 nm. Quantification was based on the calibration curve constructed by running series of gallic acid

standard solutions, and TPC was expressed as milligram gallic acid equivalent per gram (mgGAE/g).

### Analysis of flavonoids

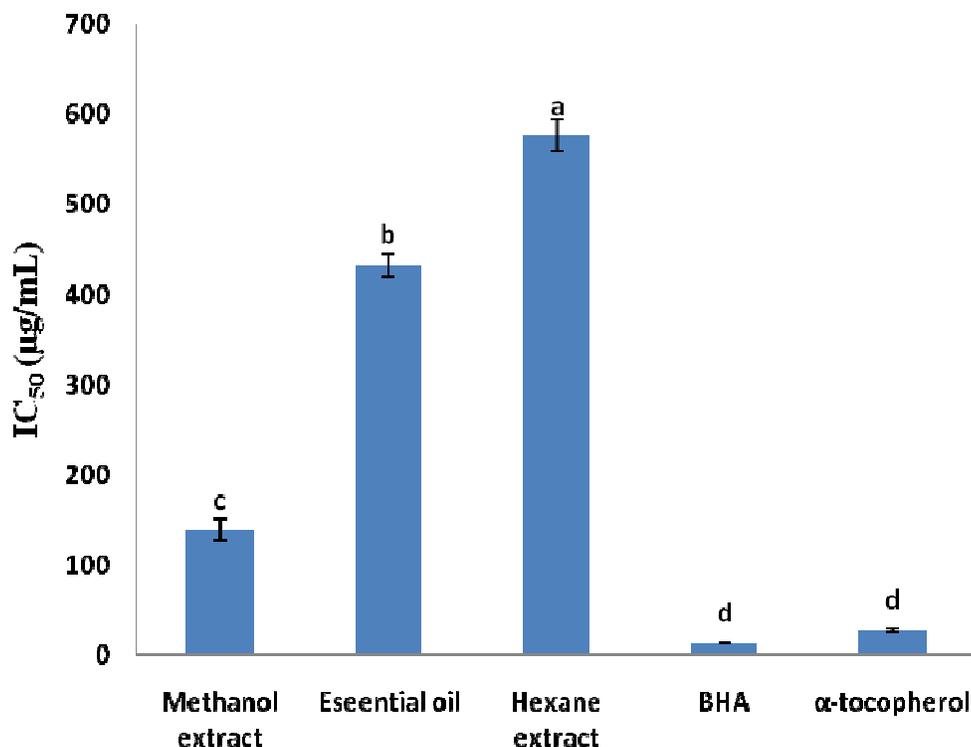
Flavonoids were extracted and hydrolyzed to their aglycones based on Mikkonen et al. (2001). The *V.negundo* extract was dissolved in methanol and centrifuged at 4000 rpm for 10 min, and then 10 ml of supernatant was transferred into a glass tube. Ten millilitre methanol and 5 ml hydrochloric acid was then added. The mixture was refluxed in a water bath at 85°C for 2 h with tert-butylhydroquinone (TBHQ) as an antioxidant. The hydrolyzed solutions were extracted five times using ethyl acetate. The layers of ethyl acetate were collected and placed at 35°C to dryness. The residue was dissolved in 10 ml methanol and filtered through (0.22  $\mu$ m) Millipore membrane filter. The HPLC system was used for detection of flavonoids in *V. negundo*. The HPLC system consisted of Waters 2487 Dual Wavelength Absorbance Detector, Waters 600 pump and controlled by waters Empower 2 software (Water, Milford, MA). Separations were carried out using Waters RP  $\mu$ Bondapak C18 column (300 x 3.9 mm, 10  $\mu$ m). The mobile phase include deionized water with TFA (PH=2.5) as solvent A and absolute methanol as solvent B. The gradient used was as follows: 100 to 50% solvent A (0 to 20 min), 50 to 40% solvent A (20 to 30 min) and 40 to 100% solvent A (30 to 40 min). The mobile phase flow rate was 1.0 ml/min and detection was performed at 280 nm. Identification of the unknown flavonoids was based on matching their retention times with those of pure standards and amounts quantified using standard calibration curves.

### Determination of $\beta$ -carotene and lycopene

Determination of  $\beta$ -carotene was carried out following the method of Tee and Lim (1991) with some modifications. Five grams dried leaves of *V. negundo* was hydrolyzed with 20 ml of 95% ethanol and 5 ml of 100% KOH and refluxed for 30 min. The hydrolysate was then extracted 3 times with hexane until the extract is colorless and then dried over anhydrous sodium sulphate. Extracted sample was then filtered through nylon membrane filter (0.45  $\mu$ m) and was analyzed using reversed phase HPLC. The separation was performed under isocratic separation on a  $\mu$ Bondapak C<sub>18</sub> column (3.9 x 300 mm) using a ternary mixture of acetonitrile-methanol-ethyl acetate (80:18:2 v/v) mobile phase at a flow rate of 1.0 mL/minute.  $\beta$ -carotene and lycopene eluted were detected at 436 nm using a UV-VIS detector attached to the HPLC and quantified from calibration curve by running standard solutions.

### Analysis of tocopherol

Alpha tocopherol was extracted and analyzed according to the method of Konings et al. (1996). Five grams of dried extract was suspended in 90 ml of 95% ethanol containing 0.25 g of ascorbic acid and 30 ml of 6% aqueous potassium hydroxide solution. The mixtures were flushed with nitrogen and connected to air cooler. The resulting mixture was saponified at 80°C for 40 min. Water was added to bring ethanol / water ratio to 0.3. The analogues of  $\alpha$ -tocopherol were extracted two times with 100 ml of extraction solvent (9:1 hexane-ethyl acetate) in a separating funnel. The upper layer fraction was collected and washed with 100 ml portion of water until reaction of the washed fraction with phenolphthalein was neutral and dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum at 40°C and the residues were re-dissolved in appropriate volume of hexane-BHT solution. The solution was then filtered using 0.45  $\mu$ m membrane filter paper (Whatman, England) and 20  $\mu$ l was injected onto HPLC (Waters



**Figure 1.** Antioxidant activity of different extracts of *V. negundo* leaves using DPPH method. Different alphabets show significant ( $p < 0.05$ ) variations among extracts.

600, USA). The mobile phase consisted of methanol: water in the ratio of 94: 6 (v/v) at flow rate of 1 ml/min and detection was 292 nm using UV detector (Waters 486, USA).

#### Statistical analysis

All data was analyzed using SPSS version 16. Analysis of variance (ANOVA) was performed according to ANOVA procedure and significant ( $p < 0.05$ ) difference between means were determined according to Duncan's multiple range tests.

## RESULTS

### Antioxidant activity

#### 1,1-diphenyl-2-picrylhydrazyl (DPPH) method

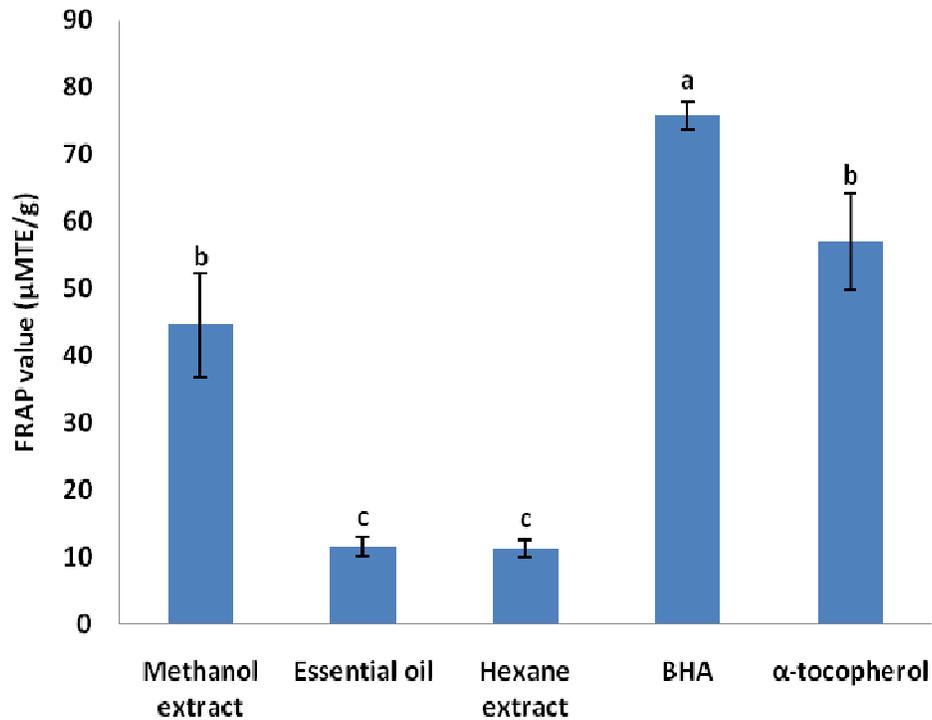
DPPH method measured the ability of antioxidant in scavenging free radicals present. Antioxidant activity of *V. negundo* leaf was expressed as the concentration that inhibits 50% DPPH free radical (IC<sub>50</sub>). Results obtained in the study showed that the IC<sub>50</sub> of methanol extract of *V. negundo* (138 ± 11.68 µg/ml) was significantly ( $p < 0.05$ ) lower than that of both essential oil (432 ± 12.65 µg/ml) and hexane extract (567 ± 17.37 µg/ml), revealing its higher antioxidant activity than those of hexane extract and essential oil (Figure 1).

#### FRAP method

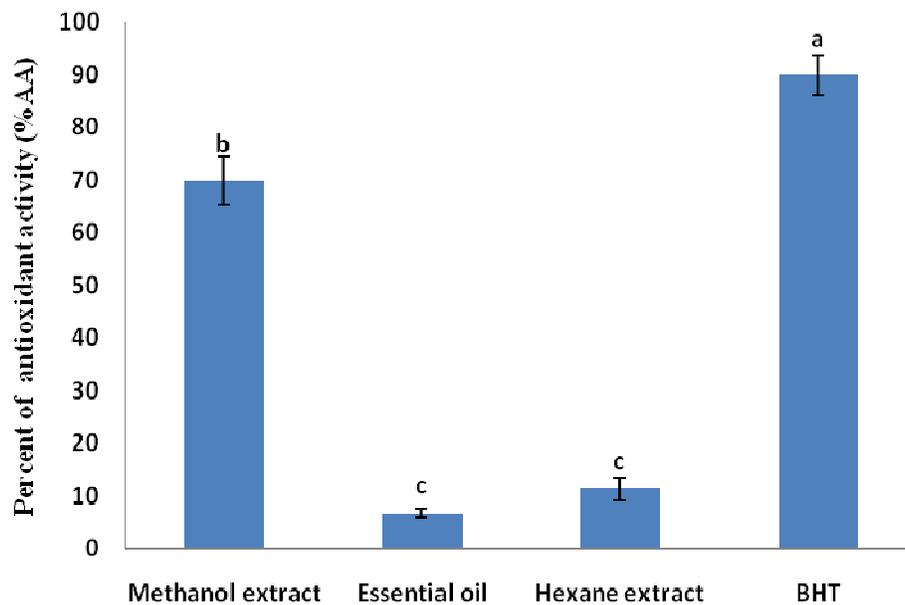
The FRAP test measures the ability of samples to reduce ferric ion to the ferrous form of TPTZ (2,4,6-tripyridyls-triazine). Arbitrarily, one FRAP unit is defined as the reduction of 1 mol of Fe<sup>3+</sup> to Fe<sup>2+</sup>. Similarly, result of the study showed that the antioxidant capacity of methanol extract (44.6 ± 7.8 µM TE/g) was significantly ( $p < 0.05$ ) higher than that of hexane (11.30 ± 1.3 µM TE/g) and essential oil (11.53 ± 1.35 µM TE/g) of leaves of *V. negundo* (Figure 2). However, there was no significant ( $p < 0.05$ ) difference on the antioxidant capacity between hexane extract and essential oil. The antioxidant capacity of methanol extract was noted to be four times higher than that of hexane extract and essential oil. It is interesting to note that the trend of antioxidant activity obtained from FRAP assay was similar to that obtained in DPPH assay.

#### β-carotene-linoleic acid assay

Beta-carotene-linoleic acid assay is used to assess the ability of the extracts in protecting β-carotene and the data is expressed as percent of antioxidant activity (AA%). Results of the present study showed that AA% of methanol extract (69.9 ± 4.6%) was significantly ( $p < 0.05$ )



**Figure 2.** Antioxidant activity of different extracts of *V. negundo* leaves using FRAP method. Different alphabets show significant ( $p < 0.05$ ) variations among extracts.



**Figure 3.** Antioxidant activity of different extracts of *V. negundo* leaves using linoleic acid- $\beta$ -carotene method. Different alphabets show significant ( $p < 0.05$ ) variations among extracts.

higher than that of hexane extract ( $11.3 \pm 2.2\%$ ) and essential oil ( $6.7 \pm 0.9\%$ ) (Figure 3). The AA% of methanol extract was found to be almost six times higher

than that of hexane extract and essential oil. This trend of antioxidant activity was similar to that observed in DPPH and FRAPS tests, where methanol extract exhibited the

**Table 1.** Bioactive compound in dried leaves of *V. negundo*.

Bioactive compounds	(mg/g)
Catechin	8.95 ± 0.26 <sup>d</sup>
Epicatechin	16.98 ± 0.81 <sup>b</sup>
Quercetin	13.45 ± 0.17 <sup>c</sup>
Myricetin	3.32 ± 0.03 <sup>e</sup>
Kaempferol	0.84 ± 0.04 <sup>f</sup>
Naringenin	0.39 ± 0.03 <sup>h</sup>
β-carotene	0.56 ± 0.002 <sup>g</sup>
Tocopherol	0.24 ± 0.002 <sup>i</sup>
Total phenolic contents	*363 ± 0.81 <sup>a</sup>

Data represents mean ± standard deviation. Means followed by the different alphabets are significantly (p < 0.05) different (n=3), \* = Value based on mg GAE/g.

highest antioxidant activity.

### Bioactive compounds in *V. negundo* leaves

Selected bioactive compounds from *V. negundo* leaf were determined and showed in Table 1. Results of the study showed that TPC of *V. negundo* leaf were 363 ± 0.81 mg GAE/g of extract. The major flavonoids identified were epicatechin (16.98 ± 0.81 mg/g) and quercetin (13.45 ± 0.17 mg/g). This was followed by catechin (8.95 ± 0.26 mg/g), myricetin (3.32 ± 0.03 mg/g), kaempferol (0.84 ± 0.04 mg/g) and naringenin (0.39 ± 0.03 mg/g). However, concentrations of β-carotene (0.56 ± 0.002 mg/g) and tocopherol (0.24 ± 0.002 mg/g) were quite low.

### DISCUSSION

In this study, methanol and hexane extracts, and essential oil of *V. negundo* leaf were evaluated for their antioxidant activities using DPPH, FRAP and β-carotene linoleic acid assays. The different methods used here are crucial due to the different array of bioactive components present in the plants that maybe responsible for the antioxidant activity of medicinal plants. Consequently, the mode of action of the different antioxidants in delaying oxidation process may be different.

The antioxidant activity ability of the plant extracts basically depend on the composition of the extracts, hydrophobic or hydrophilic nature of the antioxidants, type of solvent used for extraction process, method of extraction, temperature and conditions of the test systems. Therefore, it is necessary to use more than one method for evaluation of antioxidant activity of plant extracts defining various mechanisms of antioxidant actions (Wong et al., 2006). The literature reveals different approaches for determination of antioxidant activity of plant extracts. These different approaches may lead to garning data offering no strong correlations

among results of different assays (Koleva et al., 2002; Mantle et al., 1998; Ruberto and Baratta, 2000).

Antioxidant activity (IC<sub>50</sub> values) of different fractions of *V. negundo* analyzed using 2, 2'-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid (ABTS<sup>•+</sup>) radical and lipid peroxidation assay showed that both ethanol fraction (235 µg/ml) and methanol fraction (260 µg/ml), were able to trap free radicals, and thereby inhibited lipid peroxidation (Tiwari and Tripathi, 2007). Similarly, the authors also showed that the hexane fraction has minimum trapping potential for free radicals and did not possess any scavenging potential for superoxidase, which were seen in the remaining polar fractions. They deduced that polar fractions of *V. negundo* leaf possess potent antioxidant properties. Antioxidant activities of methanol and hexane extracts of *Cordia wallichii* were compared and results showed that methanol extract (28.2%) was more effective than that of hexane extract (16.7%) as measured by DPPH radical scavenging assay (Makari et al., 2008). Sheikh et al. (2009) reported that antioxidant activity of some marine macro-algae depend on type of solvent used for extraction apart from other condition. Similar a weak antioxidant activity in some essential oil such as *Artemisia* essential oil has been reported previously (Lopes-Lutz et al., 2008).

In the present study, the antioxidant activity of *V. negundo* leaf is in agreement with that reported by Tiwari and Tripathi (2007) who claimed that the polar fraction of *V. negundo* demonstrated higher antioxidant activity than that of non-polar fraction. They have suggested that the leaves of *V. negundo* may contain various antioxidant components with different polarity. Therefore, the results obtained will depend on type of antioxidative components isolated by each of the solvent used. The results from this study revealed that the antioxidant activity demonstrated by leaf of *V. negundo* may probably be attributed to polar antioxidants in particular to phenolic compounds, including epicatechin, quercetin, catechin and other flavonoids.

Various studies showed strong correlations between

TPC and multiple biological functions such as antioxidant, anticancer, anti-aging effects of plants. Antioxidant capacity and health benefits of medicinal plants are often ascribed to their total phenolic contents (Hua-Bin, 2008; Nagai et al., 2003). Similarly, a positive significant linear relationship (all  $R^2$  values  $\geq 0.95$ ) was investigated between antioxidant activity and total phenolic content in 112 traditional Chinese medicinal plants (Yizhong et al., 2004). The same high correlation was also reported between antioxidant activity as measured by DPPH method and total phenolic compound and flavonoid content of 21 selected tropical plants (Mustapha et al., 2010).

Thus, the high antioxidant activity of methanol extract of *V. negundo* leaf found in this study may probably due to its high phenolic and flavonoid contents. The activity exhibited by the extract may be due to synergistic effect of these flavonoids. Various studies have shown that antioxidant activity of flavonoids was reported to be better than that of individual flavonoid (Pekkarinen et al., 1999). Similarly, green tea exhibited higher total antioxidant activity than that of pure catechins proportionately combined based on the green tea constituent, which is mainly due to its synergistic effect (Rice-Evans et al., 1995).

## Conclusion

The antioxidant activity of *V. negundo* leaf was found to be correlated with its polar compounds, such as TPC, catechin, epicatechin, quercetin and myricetin identified in the plant. In addition to phenolics, other polar compounds including ascorbic acid might have been responsible for the high antioxidant activity of *V. negundo* which were not measured in this study. In conclusion, *V. negundo* leaf can be an excellent natural antioxidant source that can be useful in functional food development.

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