

Original Article

Comparative evaluation of antioxidant potential of extracts of *Vitex negundo*, *Vitex trifolia*, *Terminalia bellerica*, *Terminalia chebula*, *Embelica officinalis* and *Asparagus racemosus*

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Abstract

The antioxidant potential of six important Indian medicinal plants *V. negundo*, *V. trifolia*, *T. bellerica*, *T. chebula*, *E. officinalis* and *A. racemosus* were evaluated. Extracts prepared using solvents of varying polarity were screened for their free radical scavenging and reducing capacity by chemical assays. Total phenolic content was determined in order to assess its effect on the antioxidant activity of the extract. The properties of extracting solvents affected the measured total phenolic content and antioxidant activity. Extracts of all the six medicinal plants exhibited antioxidant potential but *T. bellerica*, *T. chebula*, *E. officinalis* proved more active. The presence of antioxidant activity in the extracts showed that these plants have the potential to be an alternate source of natural antioxidants. *In vivo* study is needed for successful commercialization and to benefit the food and pharmaceutical industries.

Keywords: Free radicals, reactive oxygen species, oxidative stress, antioxidant, total phenols, scavenger

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1. Introduction

Plants and herbs have been used an important contributor to the quality of human life for thousands of years. They provide abundant natural antioxidants which are virtually important for human health. Oxidants and antioxidants in humans are maintained in balance in a normal physiological state. However, the overproduction of oxidants in certain conditions may cause oxidative stress leading to oxidative damage to biomolecules and cells. [1, 2]. Reactive oxygen species (ROS) are formed during normal cellular metabolism but when present in high concentration they become toxic. Oxidative stress or excessive production of ROS is being implicated in

many diseases such as cancer, arthrosclerosis, aging, diabetes etc [3, 4]. The potential targets for ROS in cells are membrane lipids, DNA and proteins. Antioxidants are very important for human health. Natural antioxidants or other compounds such as vitamins, minerals or proteins can neutralize free radicals. Antioxidant supplementation is recommended to provide cellular protection from the deleterious effects of excessive reactive oxygen species concentration [5]. Antioxidants are our first line of defense against free radical damage and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even

more critical with increased exposure to free radicals. Pollution, cigarette smoking, drugs, illness, stress, and even exercise can increase free radical exposure [6]. Free radical scavenger may prevent the oxidative stress by peroxidation, inhibiting free radicals and also by other mechanism they prevent diseases. In addition to endogenous antioxidant defense systems, consumption of dietary and plant-derived antioxidants appears to be a suitable alternative as the synthetic antioxidants are reported to be harmful for human health. Further, synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have restricted use in food because of their carcinogenic properties [7]. Also, the use of the most potent synthetic food antioxidant tertiary butylated hydroquinone (TBHQ) is not permitted in Japan and Europe [8]. Therefore, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years [9]. Medicinal plants contain substantial amounts phytochemicals with capacity to scavenge the free radical and can serve as a potential source of natural antioxidants [10]. The majority of antioxidant belongs to flavonoids, isoflavones, anthocyanins, coumarins, lignans, catechins and isocatechins class of phytochemicals.

The genus *Vitex* belongs to the family Verbenaceae. It includes 80 genera and about 800 species. *Vitex* is a genus of trees and shrubs widely distributed in the tropic and warm region. *Vitex negundo* Linn is a shrub quite prolific in India, occurring up to an altitude of about 1500 m. It is one of the common plants used in traditional medicine and reported to have a number of biological activities [11]. Flavonoids, iridoids, terpenes and steroids are the major classes of compounds isolated from *V. negundo* [12]. A large number of compounds with varying structural classifications including glucononitol, camphene, α -pinene, citral, β -caryophyllene, casicin, orientin, isorientin, corymbosin, flavonoid glycosides, diterpenoid, triterpenoids, long chain unsaturated fatty acids, iridoid glycosides and lignin have been reported from the leaves, twigs, seeds and roots of *V. negundo* [13]. Although all parts of *V. negundo* are used as medicine in the indigenous system of medicine, the leaves are most potent for medicinal use. Decoction of the leaves of *V. negundo* is used as a bath in the puerperal state of women in India. The leaf extract of *V. negundo* has been reported to exhibit a wide range of pharmacological activities. The methanolic extract of *V. negundo* had been found to exhibit very potent antifeedent, anti-inflammatory, analgesic and anti

convulsant activities [13]. *V. negundo* is used in several commercial formulations and in the Ayurvedic System of Medicine, leaves of *V. negundo* is used as a drug of choice for pain, inflammation and related diseases [14, 15]. *Vitex trifolia* is a shrub or small tree, its leaves contains flavonoids, sterols and terpenoids. The leaves are considered useful as an external application to all rheumatic pain, sprains etc. The petroleum and ethanol extracts of leaves of *V. trifolia* exhibited moderate inhibiting activity against most of the tested Gram positive and Gram negative bacteria [13, 16]. Significant hepatoprotective activity of aqueous and ethanol extracts of *V. trifolia* in carbon tetrachloride induced liver damage of male wistar rats was also reported [17].

Terminalia chebula belongs to the family Combretaceae and is found throughout India chiefly in deciduous forests. It occurs abundantly in North India and range extends southwards at 300 to 900 m altitude [18]. Its yellowish brown fruits are included in the Indian pharmacopoeia under the category astringent. It possesses laxative, diuretic, cardio tonic and hypoglycemic properties. The dried fruit of *T. chebula* is used extensively in the indigenous system of medicine (Ayurvedic) for its homeostatic, antitussive, laxative, diuretic and cardio tonic activities [19]. Gallic acid, ellagic acid, tannic acid, ethyl gallate, chebulic acid, chebulagic acid, corilagin, mannitol, ascorbic acid and other compounds had been reported from *T. chebula* [20]. *Terminalia bellirica* plant is also common throughout India in the plains and lower hills, chiefly in the deciduous forests at 900 m elevation where the climate is not very dry [21]. *Embllica officinalis* is a medium sized tree found cultivated or growing wild in mixed deciduous forests of India ascending up to 1300 m on hills [22]. *Triphala*, a combination drug is a composite mixture of *T. chebula*, *T. bellirica* and *E. officinalis* and used in popular traditional medicine for the treatment of many chronic diseases such as ageing, heart ailments, hepatic diseases etc. [23, 24]. Fruit of *E. officinalis* is a rich source of phenolic compounds [25]. *A. racemosus* is distributed in tropical and subtropical parts of India including the Andamans and ascending in the Himalayas up to an altitude of 1500 m. The root of *A. racemosus* is the source of the drug 'shatavar' and is highly mucilaginous, antidiarrhoeal, refrigerant, diuretic, antidysenteric, nutritive, tonic, and demulcent, galactagogue, aphrodisiac and antispasmodic. The root contained proteins, saponins, carbohydrates, crude fibre, inorganic matter and mucilage. The saponin present in the root was identified as sarsapogenin [26, 27].

Medicinal plants have been extremely studied for their antioxidant assay. Selection of the plants in the present study was based on their use in traditional Indian Systems of Medicine (ISM). Extraction solvent significantly alter the antioxidant estimation, therefore, selection of extracting solvent of matching polarity is one of the most important factors in the extraction of antioxidants. Water, methanol and ethanol have been widely used for extraction of antioxidants.

Polar solvents and alcoholic solutions frequently provide satisfactory extraction [28, 29]. However, more than one extragent or sequential multi solvent extraction are preferred in very few cases. Herbs are traditionally ingested as hot water infusion. Recovery of antioxidant phytochemicals from plant materials is controlled by their chemical properties and distribution in plant matrix. Because of the presence of different groups of antioxidants with differing chemical behaviour in plant matrix, extract yield and resulting antioxidant activities are strongly dependent on the nature of extracting solvent as polarity of the solvent used for extraction purposes may or may not be matching. Plants extracts made with water are nutritionally more relevant and also required for clinical usages.

In the present report, antioxidant potential of six important medicinal plants *V. negundo*, *V. trifolia*, *T. bellerica*, *T. chebula*, *E. officinalis* and *A. racemosus* was determined with non-polar (hexane, chloroform), semipolar (ethyl acetate) and polar (methanol, n-butanol and water) solvents. Although, there have been numerous researches on crude extraction and bioactive compounds of potential plants for natural and possibly economic and effective antioxidants to replace the synthetic ones. These sources particularly leaves, bark and roots have not been received much attention as antioxidant sources as compared to the other plants/fruits. The aim of the present research work was to determine the best solvent for extraction of antioxidants from six important Indian medicinal plants and to make a comparative evaluation of the antioxidant potential of the extracts. Plant materials of six medicinal plants were extracted with five solvents hexane, chloroform, ethyl acetate, n-butanol and water using conventional extraction method. Total phenols in the extracts were determined and antioxidant potential of the extracts was assayed using chemical assays. The result would be useful for the optimization of the antioxidant yielding fractions for development of up scaling process from these medicinal plants.

2. Material and Methods

Plant material and chemicals:

Leaves of *V. negundo*, *V. trifolia*, barks of *T. bellerica*, *T. chebula*, fruits of *E. officinalis* and roots of *A. racemosus* were collected from the herbal garden of Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India during the year 2011. Analytical grade solvents hexane, chloroform, ethyl acetate, methanol and n-butanol were purchased from Sisco Research Laboratories (SRL), Mumbai, India. Reagent grade trichloroacetic acid (TCA), ammonium persulphate, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, potassium ferricyanide, ferric chloride, ascorbic acid were also obtained from Sisco Research Laboratories (SRL), Mumbai, India. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS), butylated hydroxy toluene (BHT), tertiary butylhydroquinone (TBHQ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were purchased from Sigma-Aldrich, Bangalore, India.

Preparation of extract:

Plant materials were divided into small pieces and dried at room temperature under shade. Further, the powder of suitable mesh size of dried plant materials was prepared using an electric grinder and powder samples were used for preparation of the extracts. The powder samples of plant materials were extracted with methanol at room temperature for 24 hrs. This procedure was repeated for five times and the solvent was removed from the combined extract under vacuum. A part of the methanol extract obtained was dissolved in water and sequentially extracted with hexane, chloroform, ethyl acetate and n-butanol to obtain hexane, chloroform, ethyl acetate, n-butanol and aqueous extracts respectively.

Measurement of total phenolics:

Total phenolics in the extracts were quantified using Folin-Ciocalteu reagent and expressed as gallic acid equivalent [30, 31]. Extract (0.5ml) was mixed with Folin-Ciocalteu reagent (0.5ml) and distilled water (7.5 ml). The resulting mixture was incubated at room temperature for 10 minutes. Thereafter, sodium carbonate solution (1.5ml, 20%) was added to the mixture and further incubated in a water bath for 20 min at 40°C. The absorbance of the solution was measured using a UV-visible spectrophotometer at 755 nm. A calibration curve was prepared by plotting concentration vs absorbance of gallic acid as standard. Total phenolics in the extract samples were quantified from the calibration curve and were

expressed as gallic acid equivalent (GAE) per gm of dry extract.

Antioxidant Activity Assay

DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging assay:

The antioxidant activity of the plant extracts was determined using the DPPH free radical scavenging method with some modifications [32]. DPPH solution (0.1mM litre⁻¹) was prepared in methanol. Stock solution (1mg ml⁻¹) of extracts and the standard antioxidants (ascorbic acid, BHT, BHQ and Trolox) were also prepared in methanol. Working concentration of the standard antioxidants and extracts were prepared using appropriate dilution. Freshly prepared DPPH solution (1 ml) was added to standard antioxidants and extracts solution (3ml). The mixture was incubated in the dark for 30 min and thereafter, absorbance was recorded at 517 nm against the blank. For control, DPPH solution (1 ml) was mixed with methanol (3 ml) and used for measuring the absorbance value. The decrease in absorbance of DPPH solution on addition of test samples in relation to the control was used to calculate the antioxidant activity in terms of percentage inhibition of DPPH radical. The capability of scavenging DPPH radical (%RSA) was calculated using the following equation as described by Lee et al [33]

$$\text{Radical scavenging percentage} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

The IC₅₀ values of the standard antioxidants as well as the plant extracts corresponded to concentration inhibiting fifty percent of initial concentration of DPPH and values were obtained from the graph of the percentage inhibition versus the concentration. Obtained IC₅₀ values of the standards and extracts were verified experimentally.

ABTS radical assay:

ABTS radical cation decolorization assay was performed as described by Re et al [33]. ABTS cation was generated by the reaction between ABTS (7 mM) and potassium persulfate (2.45 mM) at room temperature in the dark for 12–16 h before use. ABTS solution was diluted with methanol to get a final absorbance of 0.7 ± 0.02 at 734 nm. For measuring the decrease of absorbance of blue- green colour of cation generated, ABTS solution (990 µl) was mixed with test solution (10 µl). The reduction in absorbance was recorded after 6 min and compared with the control.

Reducing power assay:

The total reducing power of standard antioxidants and extracts were determined as described by Oyaizu [34]. Different concentrations of extracts were mixed with distilled water (2.5 ml), phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The resulting mixture was incubated at 50°C for 20 min in a water bath. After cooling, trichloroacetic acid (2.5ml, 10 %,) was added to the mixture. The upper layer of solution (2.5ml) was taken and mixed with distilled water (2.5 ml) and ferric chloride (0.5ml, 0.1%). The absorbance was measured using a UV-visible spectrophotometer at 700 nm. The increasing absorbance value was interpreted as increased reducing activity [35]. The extract concentration providing absorbance value of 0.5 (EC₅₀) was calculated from the graph.

3. Results and Discussion

Solvent polarity plays a key role in increasing phenolic solubility [36]. Phenolic compounds such as BHT, propyl gallate etc. are known to be effective antioxidants. Phenolic compounds have been known to act as antioxidants not only because of their ability to donate electrons also because of their stable radical intermediates which can effectively prevent the oxidation at cellular and physiological level [37]. In the present study five solvents with different polarity were used and they can be arranged as following (starting from more non- polar solvent): hexane< chloroform< ethyl acetate< n- butanol< methanol< water. Even though it is unclear whether active compounds are active against free radicals after being absorbed and metabolized by cells in the body, radical scavenging assays have gained acceptance for their capacity to rapidly screen materials of interest [38]. Antioxidant activity of plants is well correlated with the content of their phenolic compounds [39]. The most commonly used antioxidant methods DPPH. And ABTS.+ were used to assess the free radical scavenging activity of the different extracts.

Extract yield of plant material:

The extraction yield varied depending on the type of extraction solvent and extract yield of plant materials of *V. negundo*, *V. trifolia*, *T. bellerica*, *T. chebula*, *A. racemosus* and *E. officinalis* has been described in Figure 1. For *V. negundo* and *V. tripholia* leaves, extract yield was maximum for aqueous extract and minimum for chloroform extract and it varied in the following order: aqueous>methanol>hexane>ethyl acetate>chloroform. In case of *T. chebula* and *T.*

bellerica bark extract, aqueous extract had highest yield. The extract yield for *T. chebula* and *T. bellerica* varied in the following order: aqueous> methanol> n-butanol> ethyl acetate> hexane> chloroform. For fruits of *E. officinalis*, yield of methanol extract was highest followed by aqueous and n-butanol extract. The extract yield varied in the following order: methanol> aqueous> n-butanol> ethyl acetate> hexane> chloroform. For *A. racemosus*, methanol was used for preparation of the extract.

Total phenol content (TPC):

Total phenol content expressed as gallic acid equivalent (mg /g GAE) in different extracts of *V. negundo*, *V. trifolia*, *T. bellerica*, *T. chebula*, *A. racemosus* and *E. officinalis* has been summarized in Table 1. For *V. negundo* and *V. trifolia* leaves extract, total phenol content was maximum in ethyl acetate extract (172.1, 150.3 mg g⁻¹ GAE) followed by methanol (90.1, 74.5 mg g⁻¹ GAE), chloroform (56.3, 74.1 mg g⁻¹ GAE), aqueous (53.6, 62.2 mg g⁻¹ GAE) and hexane extract (35.5, 33.8 mg g⁻¹ GAE). Kumar et al [12] reported total phenol content (27.7 + 0.3 mg g⁻¹ GAE) in aqueous - methanol extract of *V. negundo* leaves. However, in case of *T. chebula*, aqueous extract had highest total phenol content (595.4 mg g⁻¹ GAE) followed by methanol (531.5 mg g⁻¹ GAE), ethyl acetate (410.8 mg g⁻¹ GAE) and n-butanol (380.5 mg g⁻¹ GAE). Similar trend was reported for fruit extracts of *T. chebula* by Chang and Lin [40]. For *T. bellerica*, n-butanol extract had highest total phenol content (500.7 mg g⁻¹ GAE) followed by aqueous (376.4 mg g⁻¹ GAE), ethyl acetate (367.4 mg g⁻¹ GAE) and methanol (362.5 mg g⁻¹ GAE). Here, *T. chebula* and *T. bellerica* bark extracts had higher total phenol content than the total polyphenolic compounds in the methanol extracts of fruits from *T. horrida*, *T. chebula* and *T. bellerica* [41]. Ethyl acetate extract of *E. officinalis* had highest total phenol content (404.0 mg g⁻¹ GAE) followed by n-butanol (362.3 mg g⁻¹ GAE), methanol (221.6 mg g⁻¹ GAE). Aqueous extract (153.8 mg g⁻¹ GAE) and chloroform extract (153.1 mg g⁻¹ GAE) had similar total phenolic content. *A. racemosus* extracts had very low total phenolic

content in the following order: n-butanol (25.1 mg g⁻¹ GAE)> methanol (10.0 mg g⁻¹ GAE)> aqueous (4.8 mg g⁻¹ GAE).

Free radical scavenging activity by stable DPPH radical:

For DPPH assay, four synthetic antioxidants such as ascorbic acid, BHT, BHQ and trolox were used as reference and their IC₅₀ values were found to be 4.2, 4.3, 1.7 and 2.1 µg mL⁻¹ respectively, these values are comparable to their reported IC₅₀ values [42, 43]. Results of DPPH assay of different extracts of *V. negundo*, *V. trifolia*, *T. bellerica*, *T. chebula*, *A. racemosus* and *E. officinalis* has been summarized in Table 1. For *V. negundo* and *V. trifolia*, ethyl acetate extract had the lowest IC₅₀ value followed by methanol, aqueous and chloroform extract. IC₅₀ value of ethyl acetate extract of *V. negundo* (4.2 µg mL⁻¹) was found to be comparable to the IC₅₀ value of ascorbic acid and BHT and comparatively lower than reported value for ethanol extract of *V. negundo* leaves extract [44]. All the four extracts of *T. chebula* and *T. bellerica* exhibited very low IC₅₀ value. For *T. chebula*, the IC₅₀ varied in the following order: aqueous (1.6 µg mL⁻¹) < methanol (2.0 µg mL⁻¹) < n-butanol (2.4 µg mL⁻¹) < ethyl acetate (2.7 µg mL⁻¹). These values were comparable to the IC₅₀ of standard antioxidants such as trolox and BHQ. However, in case of *T. bellerica* extracts, IC₅₀ values varied in the following order: n-butanol (2.7 µg mL⁻¹) < ethyl acetate (2.8 µg mL⁻¹) < methanol (3.1 µg mL⁻¹) < aqueous (3.5 mL⁻¹). IC₅₀ value of ethyl acetate extract of *T. chebula* bark was observed to be lower than the IC₅₀ values reported for the methanol extract of the fruits of *T. chebula* and *T. bellerica*. Also for *E. officinalis*, IC₅₀ values comparable to standard antioxidants were recorded for n-butanol (1.5 µg mL⁻¹), methanol (1.6 µg mL⁻¹) and aqueous extracts (4.1 µg mL⁻¹). For *A. racemosus* extracts very high IC₅₀ values (methanol extract: 614 µg mL⁻¹, n-butanol extract: 348.6 µg mL⁻¹) were in agreement to the values reported by Dohare et al [45].

Plant	Extract	TPC (GAE gm ⁻¹)	DPPH (IC ₅₀ , µgmL ⁻¹)	ABTS (IC ₅₀ , µgmL ⁻¹)	RPA (IC ₅₀ , µgmL ⁻¹)
<i>V. negundo</i>	methanol	90.1	23.4	547.2	161.5
	hexane	35.5	107.8	>1000	851.7
	chloroform	56.3	51.3	632.9	561.7
	ethyl acetate	172.1	4.2	211.5	40.1
	aqueous	53.6	36.5	465.2	193
<i>V. trifolia</i>	methanol	74.5	16.8	493.1	381.7
	hexane	33.8	78.9	>1000	605
	chloroform	74.1	60.4	418.8	477
	ethyl acetate	150.3	9.8	286.9	215
	aqueous	62.2	27.9	506.9	549.5
<i>T. chebula</i>	methanol	531.5	2.0	34.5	37.9
	ethyl acetate	410.8	2.7	56.5	49.4
	n- butanol	380.5	2.4	48.5	44.9
	aqueous	595.4	1.6	29.2	27.1
<i>T. bellerica</i>	methanol	362.5	3.1	42.9	64.2
	ethyl acetate	367.4	2.8	40.8	53.0
	n- butanol	500.7	2.7	38.8	43.1
	aqueous	376.4	3.5	52.6	66.7
<i>E. officinalis</i>	methanol	221.6	1.6	68.8	52.7
	hexane	129.2	5.4	135.6	113.6
	chloroform	153.1	6.7	119.3	101.3
	ethyl acetate	403.9	1.8	29.8	35.5
	n- butanol	362.3	1.5	39.6	33.9
	aqueous	153.8	4.1	80.5	71.7
<i>A. racemosus</i>	methanol	10.0	613.9	>1000	1655.9
	n- butanol	25.1	348.6	871.9	384.9
	aqueous	4.8	>1000	>1000	>1000

Table 1: Total phenol content (TPC) and IC₅₀ values of extracts of *V. negundo*, *V. trifolia*, *T. chebula*, *T. bellerica*, *E. officinalis* and *A. racemosus* in DPPH, ABTS and reducing power assay (RPA)

Free radical scavenging activity by stable ABTS radical cation:

In the ABTS assay, standard antioxidants ascorbic acid, BHT, BHQ and trolox had IC₅₀ corresponding to 42.7, 116.5, 64.4 and 47.4 µg mL⁻¹ respectively. Here also, IC₅₀ value of *V. negundo*, *V. trifolia*, *T. bellerica*, *T. chebula*, *A. racemosus* and *E. officinalis* exhibited similar trend as in DPPH assay (Table 1). Ethyl acetate of *V. negundo*, *V. trifolia* exhibited lowest IC₅₀ value and hexane extract exhibited the maximum IC₅₀ value. In ABTS assay for IC₅₀ value

five *V. negundo* leaves extract, the following order of scavenging activity in terms of IC₅₀ value was reported by Tiwari et al [46] : total ethanolic>methanolic>water>ethyl acetate>hexane extract. For *T. chebula*, aqueous extract had lowest IC₅₀ value and it varied in the following order: aqueous (29.2 µg mL⁻¹) < methanol (34.5 µg mL⁻¹) < n-butanol (48.5 µg mL⁻¹) < ethyl acetate (56.5 µg mL⁻¹). Also for *T. bellerica*, the IC₅₀ values were found in the following order: n-butanol (38.8 µg mL⁻¹) < ethyl acetate (40.8 µg mL⁻¹) < methanol (42.9 µg

mL-1) < aqueous (52.6 µg mL-1). Although, IC50 concentration of *T. bellerica* extract had higher values than the corresponding values of *T. chebula* extracts but the trend was found to be similar as observed in DPPH assay. Also the IC50 values of *T. chebula* and *T. bellerica* extracts were comparable to the four standard antioxidants. For *E. officinalis*, ethyl acetate (29.8 µg mL-1) and n-butanol (39.6 µg mL-1) extracts had IC50 lower than the corresponding values for all the four standard antioxidants. As observed in DPPH assay, here also, IC50 values of *A. racemosus* extracts were found to be very high.

Reducing power assay:

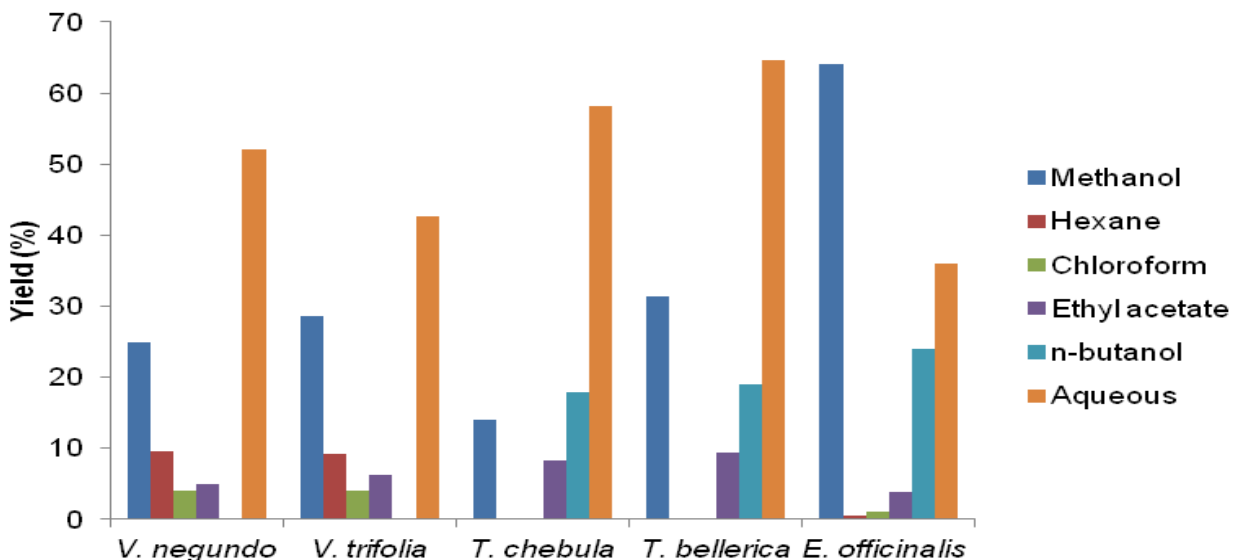
The reducing power of an extract may serve as a significant indicator of its potential as an antioxidant and reducing power activity was calculated in terms of EC50 concentration. Ascorbic acid, BHT, BHQ and trolox had EC50 concentration 35.5, 59.8, 31.3 and 65.9 µg mL-1 respectively. For *V. negundo*, EC50 varied in the following order: ethyl acetate (40.1 µg mL-1) < methanol (161.5 µg mL-1) < aqueous (193 µg mL-1) < chloroform (561.7 µg mL-1) < hexane (851.7 µg mL-1). Ethyl acetate extract of *V. trifolia* (215 µg mL-1) had the lowest EC50 concentration followed by methanol (381.7 µg mL-1), chloroform (477 µg mL-1), aqueous (549.5 µg mL-1) and hexane (605 µg mL-

1). For *T. chebula*, aqueous extract had lowest EC50 value and it varied in the following order aqueous (27.1 µg mL-1) < methanol (37.9 µg mL-1) < n-butanol (44.9 µg mL-1) < ethyl acetate (49.4 µg mL-1). Also for *T. bellerica*, the EC50 values were found in the following order: n-butanol (43.1 µg mL-1) < ethyl acetate (53.0 µg mL-1) < methanol (64.2 µg mL-1) < aqueous (66.7 µg mL-1). For *E. officinalis*, n-butanol (33.9 µg mL-1) had the lowest EC50 concentration followed by ethyl acetate (35.5 µg mL-1), methanol (52.7 µg mL-1), aqueous (71.7 µg mL-1), chloroform (101.3 µg mL-1) and hexane (113.6 µg mL-1). For *A. racemosus*, here also, EC50 concentration was found to be very high.

Correlation between antioxidant activity and phenolic content:

Different phenolic compounds may show different antioxidant activities depending on their structure as well as synergistic or antagonistic effect of other compounds present in the extract [47]. Correlation analysis were performed to determine the relationship between the TPC of the extracts and corresponding 1/IC50 values obtained in DPPH, ABTS and reducing power assay (Figure 2). Significant positive correlation between TPC and 1/IC50 values of DPPH, ABTS and reducing power assay was established with estimated coefficient of

Figure 1: Extract yield of different fractions of *V. negundo*, *V. trifolia*, *T. chebula*, *T. bellerica* and *E. officinalis*



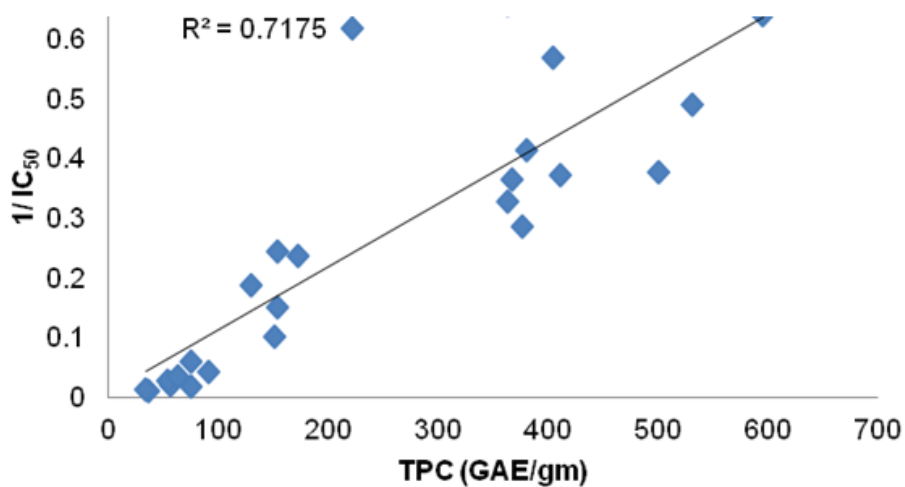


Figure 1 (A): Correlation between TPC and IC₅₀ value of extracts in DPPH assay

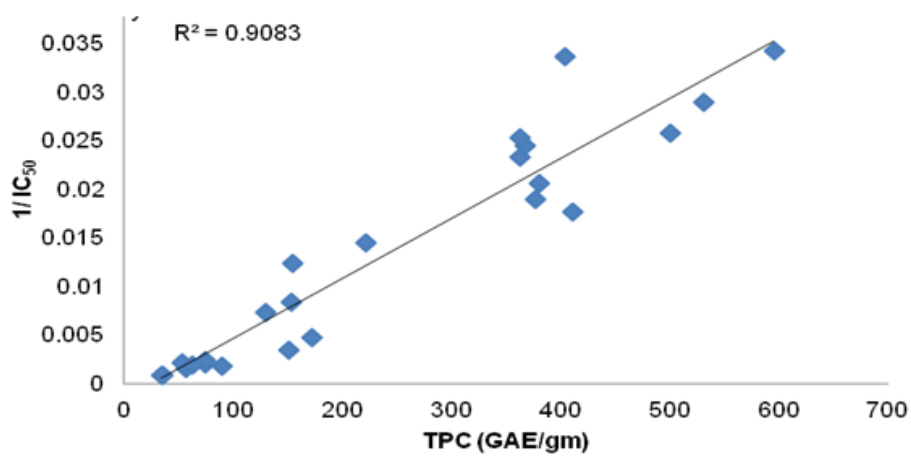


Figure 2 (B): Correlation between TPC and IC₅₀ value of extracts in ABTS assay

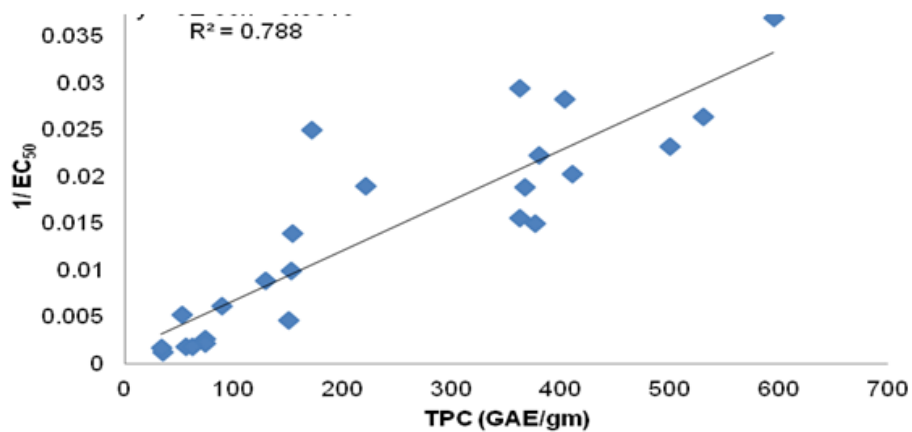


Figure 2 (C): Correlation between TPC and IC₅₀ value of extracts in reducing power assay

determination R² values 0.72, 0.91 and 0.79 respectively. The high correlation between TPC and antioxidant activity suggested that the phenolic compounds contributed significantly to the antioxidant capacity of studied medicinal plants.

Conclusions

Extraction yield varied significantly, depending on the extraction solvent and plant material used. Also TPC and free radical scavenging activity varied depending on the polarity of the solvent used. The results of the present study indicated the presence of compounds in the extracts possessing low (*A. racemosus*), moderate (*V. negundo* and *V. trifolia*) and high (*T. chebula*, *T. bellerica* and *E. officinalis*) antioxidant activity. Also the lowest value IC₅₀ values of extracts of *T. chebula*, *T. bellerica* and *E. officinalis* were below the maximum permissible level (200 ppm) for the synthetic antioxidants [8].

Conflict of Interest: None

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4. References

1. Temple, N J, 2000, Antioxidants and disease: More questions than answers, *Nutr Res*, 20; 449-459.
2. Willett, W C, 2002, Balancing life style and genomics research for disease prevention, *Science*, 296; 695-698.
3. Finkel, T, Holbrook, N J , 2000, Oxidants, oxidative stress and the biology of ageing, *Nature*, 408; 239-247.
4. Halliwell , B, Gutteridge, J M C, 1985, Free radicals, ageing and disease. In *Free Radicals in Biology and Medicine*, Clarendon, Oxford, 279-315.
5. Yanai, N , Shiotani, S, Hagiwara, S, Nabetani, H, Nakajima, M, 2008, Antioxidant combination inhibits reactive oxygen species mediated damage. *Biosci Biotechnol Biochem*, 72 ; 3100-3106 .
6. Ablise, M , Mao, X M, Kasim, R, 2011, Antioxidant activities of Uyghur medicinal tea in human HL-60 cell line and rat hepatic microsomes. *J Med Plants Res*, 5; 2677-268.
7. Ito, N, Fukushima, S, Tsunda, H, 1986, Carcinogenicity and modification of the carcinogenic response by BHA, BHT and other antioxidants , *CRC Critical reviews in Toxicology*, 15; 109-150.

8. Suja, K P, Jayalekshmy, A, Arumugan, C, 2005, Antioxidant activity of sesame cake extract, *Food Chem*, 91; 213-219.
9. Lobo, V, Patil, A , Phatak, A , Chandra, N, 2010, Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacog Rev*, 4; 118-126.
10. Kratchanova, M , Denev, P, Ciz, M , Lojek, A, Mihailov, A, 2010, Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds : comparison of two extraction systems, *Acta Biochim Polonica* , 57; 229-234.
11. Cushnie, T P T, Lamb, A J, 2005, Antimicrobial activity of flavonoids, *Int J Antimicrob Agents*, 26; 343-356.
12. Kumar, M, Rawat, P, Dixit, P, Mishra, D, Gautam, A K, Pandey, R, Singh, D, Chattopadhyay, N, Maurya, R, 2010, Anti-osteoporotic constituents from Indian medicinal plants, *Phytomed*, 17; 993-999.
13. Sharma, R L, Prabhakar, A, Dhar, K L., Sachar, A, 2009, A new iridoid glycoside from *Vitex negundo* Linn (*Verbenaceae*), *Nat Prod Res*, 13; 1201-1209.
14. Tiwari, N, Luqman, S , Masood, N, Gupta, M M, 2012, Validated high performance thin layer chromatographic method for simultaneous quantification of major iridoids in *Vitex trifolia* and their antioxidant studies , *J Pharm Biomed Anal*, 61; 207-214.
15. Lokhande, P J, Verma, J K, 2009, Quantification of negundoside in *Vitex negundo* Linn. Leaf by high performance thin layer chromatography, *J Planar Chromatogr*, 22; 225-228.
16. Hossain, M M, Paul, N, Sohrab, N H , Rahman, E, Rashid, M A, 2001, Antimicrobial activity of *Vitex trifolia*, *Fitoter*, 72; 695-697.
17. Manjunatha, B K, Vidya, S M, 2008, Hepatoprotective activity of *Vitex trifolia* against carbon tetrachloride induced hepatic damage, *Ind J Pharm Sci*, 70 ; 241-245.
18. Aiyer, K N, Kolammal, M, 1963. *Pharmacognosy of Ayurvedic drugs*, University of Kerala, p. 112-116.
19. Barthakur, N N, Arnold, N P, 1991, Nutritive value of the chebulic myrobalan (*Termanalia chebula* retz.) and its potential as food source, *Food Chem*, 40; 213-219.
20. Grover, I S, Bala, S, 1992, Antimutagenic activity of *T. chebula* (myrobalan) in *Salmonella typhimurium*, *Ind J Exp Biology*, 30; 339-341.
21. *Wealth of India, Raw Materials*, 1956, CSIR, New Delhi, Vol. X, pp. 164-167.
22. *Quality Standards of Indian Medicinal Plants*, 2003. Indian Council of Medical Research, New Delhi.
23. Kaur, S, Arora, S, Kaur, K, Kumar, S, 1998, The in vitro antimutagenic activity of *Triphala* an Indian herbal drug, *Food Chem Toxicol*, 40 ; 527-534.

24. Jagetia, G C, Baliga, M S , Malagi, K J, Sethukumar, K M, 2002, The evaluation of the radioprotective effect Triphala (an ayurvedic rejuvenating drug) in the mice exposed to gamma radiation, *Phytomed*, 9 ; 99-108.
25. Khan, K H, 2009, Roles of *Emblca officinalis* in medicine- a review. *Bot Res Int*, 2; 218-228.
26. Rao, P S, Budhiraja, R P, 1952. Studies on tuber mucilages. Part VI. Partial hydrolysis of the mucilage from the tubers of *Asparagus racemosus* wild. *J Sci Ind Res*, 11B; 209-210
27. Raisinghani, K H, 1962, Studies on *Luffa echinata* and other indigenous drugs. *Bombay Technol*,12, 133-135.
28. Hinneburg, I, Neubert, R H H, 2005. Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (*Fagopurum esculentum*) herb, *J Agric Food Chem*, 53; 3-7.
29. Sultana, B, Anwar, F, Ashraf, M, 2009, Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plants extract, *Molecules*, 14; 2167-2180.
30. Singleton, V L, Orthofer, R, Lamuela –Raventos, R M, 1999, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymol*, vol. 29, pp. 152-178.
31. Li, X, Wu, X, Huang, L, 2009, Correlation between antioxidant activities and phenolic contents of *radix angelicae sinensis* (Danggui), *Molecules*,14, 5349-5361.
32. Brand-Williams, W, Cuvelier, M E, Berset, C, 1995. Use of a free radical method to evaluate antioxidant activity, *LWT Food Sci Technol*, 28; 25-30.
33. Lee, S E, Hwang, H J, Ha, J S, 2003, Screening of medicinal plant extracts for antioxidant activity, *Life Sci*, 73; 167-179.
34. Re, R , Pellegrini, N , Proteggente, A , Pannala, A , Yang, M, Rice-Evans, C, 1999, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biol*, 26 ; 1231-1237.
35. Oyaizu, M, 1986, Studies on products of browning reaction: antioxidative activities of browning reaction prepared from glucosamine. *Jap J Nutr*, 44 ; 307-315.
36. Wong, P Y Y , Kitts, D D , 2003, Chemistry of buttermilk solid antioxidant activity. *J Dairy Sci*, 86; 1541-1547.
37. Chang, C L, Lin C S, 2012, Phytochemical composition, antioxidant activity and neuroprotective effect of *Terminalia chebula* Retzius extracts. *Evi Compl Alt Med*, article ID 125247, 7 pages.
38. Velioglu, Y S , Mazza, G , Gao, L, Oomah, B D, 1988, Antioxidant activity and total phenolics in selected fruits, vegetable and grain products, *J Agric Food Chem*, 46 ; 4113-4117.
39. Pfundstein, B , El Desouky, S K , Hull, W E , Haubner, R , Erben, G, Owen, R W. 2010, Polyphenolic compounds in the fruits of Egyptian medicinal plants (*Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrida*): characterization , quantification and determination of antioxidant capacities, *Phytochem*, 71; 1132-1148.
40. Ferreira, I C F R, Baptista, P, Vilas-Boas, M, Barros, L, 2007, Free radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity, *Food Chem*, 100 ; 1511-1516.
41. Naczki, M, Shahidi, F, 2006, Phenolics in cereals, fruit and vegetables: occurrence, extraction and analysis, *J Pharm Biomed Anal*, 41; 1523- 1542.
42. Paramapojn, S, Gritsanapan, W, 2009, Free radical scavenging activity determination and quantitative analysis of curcuminoids in *Curcuma zedoaria* rhizome extracts by HPLC method, *Current Sci*, 97; 1069-1072.
43. Sharma, O P, Bhatt, TK, 2009, DPPH antioxidant assay revisited, *Food Chem*, 113; 1202-1205.
44. Lakshmanashetty, R H , Nagaraj, V B , Hiremath, M G, Kumar, V, 2010, In vitro antioxidant activity of *Vitex negundo* L. leaf extracts. *Chiang Mai J Sci*, 37; 489-497.
45. Dohare, S, Shuaib, M, Naqvi, K J, 2011, In vitro antioxidant activity of *Asparagus racemosus* roots, *Int J Biomed Res*, 4; 228-235.
46. Tiwari, O P, Tripathi, Y.B, 2007, Antioxidant properties of different fractions of *Vitex negundo* Linn, *Food Chem*, 100, 1170-1176.
47. Niciforovic, N, Mihailovic, P, Maskovic, P, Solujic, S, Stojkovic, A, Pavlovic Muratspaphic, D, 2010, Antioxidant activity of selected plant species: potential new sources of natural antioxidants, *Food Che Toxicol*, 48; 3125-3130.

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