

EVALUATION OF *BASELLA RUBRA* L., *RUMEX NEPALENSIS* SPRENG. AND *COMMELINA BENGHALENSIS* L. FOR ANTIOXIDANT ACTIVITY

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ABSTRACT

Basella rubra L., *Rumex nepalensis* Spreng. and *Commelina benghalensis* L., are commonly consumed as leafy vegetable in rural India. In the present study, acetone, methanol and water extracts of *B. rubra*, *R. nepalensis* and *C. benghalensis* were evaluated for their antioxidant and free radical scavenging potential by employing different *in vitro* assays such as reducing power, DPPH[•], ABTS^{••}, hydroxyl, superoxide and nitric oxide radical scavenging capacities, metal ion chelating ability and peroxidation inhibition activity through β -carotene/linoleic acid emulsion system. All the tested extracts contained considerable levels of total phenolics, tannin, flavonoids and vitamin C content. The extracts also exhibited increasing reducing activity with increasing concentration. Among the samples, the extracts of *R. nepalensis* registered higher activity in DPPH[•], hydroxyl and nitric oxide radical scavenging assays. Further, all the extracts registered noticeable superoxide and ABTS^{••} radical scavenging potential. At a concentration of 250 μ g in the final reaction mixture, all the tested extracts were found to have recognizable peroxidation inhibition and metal chelation. The results indicated that the selected plants may serve as potential source of natural antioxidants capable of offering protection against free radical mediated damages.

Keywords: Antioxidant; Free radicals; Total phenolics; *Basella rubra*; *Rumex nepalensis*; *Commelina benghalensis*

INTRODUCTION

The growing interest to survive a healthy life has led to the search for new natural antioxidants over the past few years. The products of reactive oxygen species (ROS) and oxidative stress has been implicated in the etiology of diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative disorders and ageing. Plant based foods containing many bioactive compounds contribute towards antioxidant activities which allow them to scavenge both ROS and electrophiles, inhibit nitrosation, chelate metal ions and modulate certain cellular enzyme activities. They include vitamins like α -tocopherol and ascorbic acid, carotenoids, polyphenols and some micronutrient elements like iron, zinc and selenium. Many commercially available products containing a large number of such compounds are very expensive. As such, providing modern healthcare to rural people is still a far reaching goal, due to the economic constraints. Hence people mainly depend on the locally available plant materials to cure various health disorders.

Green leafy vegetables (GLVs) are a good source of minerals and vitamins and constitute an important part in a well balanced diet. They are relatively inexpensive and are readily available without any formal cultivation. The ethnobotanical reports also offer information on the medicinal properties of GLV's^{1,2}. Hence attention has been devoted to commonly consumed GLV's, which though underexploited in most cases, possess tremendous potential to overcome the deadly diseases of modern society.

Basella rubra L. (Basellaceae), *Rumex nepalensis* Spreng. (Polygonaceae), and *Commelina benghalensis* L. (Commenlinaceae), consumed as fresh or pot vegetables, were selected for the present investigation. *B. rubra* (purple vine), commonly called as Malabar spinach, makes a good substitute for spinach. It is an excellent source of calcium and iron and has good roughage value characteristics of leafy vegetables. It has been used traditionally to treat ulcer, headache, gonorrhoea and balanitis¹. Earlier pharmacological studies on *B. rubra* indicated its hypoglycemic effect³. The tender leaves of *R. nepalensis* are cooked as vegetables, which impart an acidic-lemon flavor in dishes. The plant is reported to possess numerous medicinal properties like purgative, antifungal, antibacterial⁴⁻⁶, antihistaminic, anticholinergic, antibradykinin and antiprostaglandin⁷ activities. The presence of new seco-

anthraquinone glucosides, nepalensides and some known compounds such as torachryson, rumexoside, orientaloside, orcinol glucoside, aloesin, lyoniresinol 3 α -O- β -D-glucopyranoside, (-)-epicatechin-3-O-gallate, (3,5-dimethoxy-4-hydroxyphenyl)-1-O- β -D-(6-O-galloyl) glucose, and (-)-epicatechin have been previously reported in the roots of *R. nepalensis*⁸. *C. benghalensis*, commonly known as 'Benghal dayflower' or 'tropical spiderwort' is a perennial herb and its young leaves are eaten as vegetables. It is used as a folk medicine for the variety of ailments in the Indian subcontinent. It has antibacterial⁹, sedative, anxiolytic¹⁰, analgesic¹¹ and anticancer¹² properties and used against diuretic, febrifuge, inflammatory¹³ and leprosy² problems. Earlier phytochemical studies of *C. benghalensis* revealed the presence of lutein and its isomer zeaxanthin¹⁴. The plant is also reported to contain alkaloids, volatile oil, wax¹⁵, vitamin C and higher levels of vitamin A and β -carotene¹⁶. The DPPH radical scavenging activity of the hydro methanol extract of *C. benghalensis* has been reported by Hasan *et al.*¹⁷.

Although some ethnobotanical and phytochemical information are available on these plants, their nutraceutical values have not yet been exposed. Therefore the antioxidant potential of these plants was investigated by employing different *in vitro* free radical scavenging assays.

MATERIALS AND METHODS

Chemicals

Potassium ferricyanide, ferric chloride, 2,2'-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediamine tetracetic acid (EDTA) disodium salt, trichloroacetic acid (TCA), naphthyl ethylenediamine dihydrochloride (NEED), sodium nitroprusside and sulphanilamide were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

Plant material

The fresh aerial parts of *B. rubra* and *C. benghalensis* were collected during the month of July, 2011 from in and around Coimbatore,

Tamil Nadu, India and the fresh aerial part of *R. nepalensis* were collected from the Nilgiri hills, Tamil Nadu, India. The whole plants were immediately washed in running tap water to remove dirt and other foreign materials and wiped with tissue paper. They were frozen (Operon, Korea) to be further lyophilized (Virtis, USA). The lyophilized samples were ground into powder and kept at -20°C for subsequent use.

Preparation of extracts

The lyophilized plant samples were successively extracted in soxhlet extractor with acetone and methanol. Each time before extracting with the next solvent, the material was dried in hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 hr and the water extract filtered. The different solvent extracts were concentrated by rotary vacuum evaporator (Yamato BO410, Japan) and then dried. The dry extract obtained with each solvent was weighed to determine the yield of soluble components. The percentage yields were expressed in terms of the air dried weight of sample material. The extracts thus obtained were used directly to assess certain non-enzymatic antioxidants and antioxidant potential through various chemical assays.

Determination of non-enzymatic antioxidants

Vitamin C

The ascorbic acid content was determined using 2, 6, dichloroindophenol (DIP) method with a modification¹⁸. 1 mg of solvent extract was dissolved in 10 mL of 1% metaphosphoric acid and filtered using Whatmann No.1 filter paper. 1 mL of this filtrate was added with 9 mL of 50 µM DIP and incubated at room temperature for 15 sec. The developed colour was measured at 515 nm. The analysis was performed in triplicate and the results were expressed as ascorbic acid equivalents (AAE).

Total phenolics and tannins

The total phenolic content of different solvent extracts of the selected plants was determined by Folin ciocalteu method. Using the same extract, the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). The amount of total phenolics and tannins were calculated as the tannic acid equivalents (TAE) via, Siddhuraju and Becker¹⁹ and Siddhuraju and Manian²⁰.

Total flavonoid content

The flavonoid content was determined according to the method described by Zhishen *et al.*²¹. A 0.5 mL aliquot of appropriately diluted sample solution (2 mg/2 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% NaNO₂ solution. After 6 min, 0.15 mL of 10% AlCl₃ solution was added and allowed to stand for 6 min, and then 2 mL of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent (RE).

Total antioxidant activities

Reducing power

The reducing power of different solvent extracts of the plants was determined by the method reported by Siddhuraju *et al.*²². 20-100 µg of different solvent extracts or tannic acid or quercetin were taken in 1 mL of phosphate buffer and 5 mL of 0.2 M phosphate buffer (pH 6.6) was added further. To this, 5 mL of 1% potassium ferricyanide solution was added and the mixture was incubated at 50°C for 20 min. After the incubation, 5 mL of 10% TCA was added. The content was then centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 mL) was mixed with 5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the reaction mixture was read spectroscopically at 700 nm.

Free radical scavenging activity on DPPH•

The DPPH• radical scavenging activity of different solvent extracts of the plants was measured according to the method of Blois²³. IC₅₀

values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Antioxidant activity by radical cation (ABTS•+)

The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Re *et al.*²⁴ described by Siddhuraju and Manian²⁰. ABTS•+ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 hr at room temperature. Prior to assay, this solution was diluted in ethanol (about 1: 89, v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.70 ± 0.02. The stock solution of the sample extracts were diluted such that after introduction of 10 µL aliquots into the assay, they produced between 20-80% inhibitions of the blank absorbance. After the addition of 1 mL of diluted ABTS•+ solution to 10 µL of sample or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extract as dry matter.

Hydroxyl radical scavenging activity

The scavenging activity of acetone, methanol and water extracts of the plant samples along with the reference standard tannic acid and quercetin on hydroxyl radical was measured according to the method of Klein *et al.*²⁵ The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC₅₀) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Nitric oxide radical scavenging activity

The nitric oxide scavenging activity of different solvent extracts along with the reference standard tannic acid and quercetin was measured according to the method of Sreejayan and Rao²⁶. The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC₅₀) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Superoxide radical scavenging activity

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich²⁷. The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC₅₀) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Metal chelating activity

The chelation of ferrous ions by different solvent extracts of the plant samples, standard tannic acid and quercetin was estimated by the method of Dinis *et al.*²⁸. Briefly, 50 µL of 2mM FeCl₂ was added to 250 µg sample extracts. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

β-carotene/linoleic acid peroxidation inhibition activity

One millilitre of β-carotene solution in chloroform (1 mg/10 mL) was pipetted into a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45°C for 4 min and 50 mL of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5 mL aliquot of the emulsion was

added to the tubes containing 250 μ L of different solvent extracts (10 mg/10 mL) and standard antioxidants tannic acid and quercetin (1 mg/1 mL). The absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without β -carotene²⁹. The tubes were placed in a water bath at 50°C and the absorbance was monitored at 15 min interval until 180 min. All determinations were carried out in triplicate. The antioxidant activity of the sample extracts and standard compounds were evaluated in terms of bleaching of β -carotene and the inhibition percentage was calculated as follows:

$$\% \text{ Peroxidation Inhibition} = [1 - (A_0 - A_t) / (A_0^1 - A_t^1)] \times 100$$

where, A_0 and A_0^1 are the absorbance measured at zero time of incubation for the sample extract and control respectively, and A_t and A_t^1 are the absorbance measured in the sample extract and control respectively, after the incubation for 180 min.

Statistical analysis

The data were subjected to 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 \pm standard deviation.

RESULTS AND DISCUSSION

Per cent yield and non-enzymatic antioxidant contents

The per cent yield of extractable compounds in acetone, methanol and water of the selected leafy vegetables are presented in Table 1.

The water extract of *R. nepalensis* (16.5%) recorded the maximum yield followed by the methanol extract of *B. rubra* (14.8%), while it was the minimum in the water extract of *C. benghalensis* (2.7%). The antioxidant vitamin C, total phenolics, tannin and flavonoid contents of the solvent extracts were estimated and presented in Table 1. Vitamin C (ascorbic acid), a monosaccharide antioxidant found in both plants and animals, is implicated in the prevention of some cancers, heart disease and common cold. Almost all the extracts displayed appreciable amounts of vitamin C that ranged between 31.1 and 42.8 mg/g extract in terms of ascorbic acid equivalent (AAE). The tested extracts also contained relatively higher amounts of extractable total phenolics that ranged between 31.1 and 51.2 mg/g extract in terms of tannic acid equivalent (TAE). The methanol extracts of *R. nepalensis* (51.2 mg/g extract) contained considerably higher total phenolic content. Unlike the more or less equal contents of vitamin C and total phenolics, the levels of tannins and flavonoids showed much variation between plant samples and extraction medium. Methanol extracts of *C. benghalensis* and *R. nepalensis* and water extracts of *R. nepalensis*, *B. rubra* registered higher and comparable levels of tannins while other extracts showed significantly lower contents. In the case of flavonoids, however, methanol extract of *R. nepalensis* gave the highest concentration followed by acetone extracts of *C. benghalensis* and *B. rubra*. Many of these phenolics have been shown to contain high levels of antioxidant activities³⁰. As antioxidants, they may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress, by acting directly on reactive oxygen species or by stimulating endogenous defense systems³¹.

Table 1: Yield per cent and contents of vitamin C, total phenolics, tannins and flavonoids of the selected leafy vegetable extracts

Sample	Yield (%)	Vitamin C (mgAAE/g extract)	Total phenolics (mgTAE/g extract)	Tannins (mgTAE/g extract)	Flavonoids (mgRE/g extract)
BRA	10.5	39.8 \pm 0.8 ^c	34.5 \pm 4.8 ^d	2.2 \pm 1.1 ^d	161.9 \pm 1.6 ^c
BRM	14.8	42.8 \pm 0.2 ^a	31.1 \pm 2.3 ^d	0.9 \pm 0.7 ^d	22.0 \pm 1.2 ^g
BRW	9.5	39.5 \pm 0.6 ^c	34.5 \pm 9.3 ^c	10.3 \pm 4.7 ^b	33.9 \pm 0.7 ^f
RNA	10.6	39.0 \pm 0.5 ^c	31.9 \pm 4.5 ^d	1.1 \pm 1.2 ^d	46.3 \pm 7.2 ^e
RNM	6.5	39.2 \pm 0.5 ^c	51.2 \pm 2.5 ^a	13.5 \pm 2.8 ^a	222.5 \pm 4.8 ^a
RNW	16.5	40.8 \pm 0.5 ^b	37.2 \pm 4.3 ^c	12.1 \pm 3.0 ^{ab}	31.2 \pm 1.2 ^f
CBA	4.7	33.5 \pm 0.2 ^e	40.9 \pm 4.2 ^b	6.3 \pm 3.3 ^c	168.3 \pm 3.4 ^b
CBM	11.2	35.4 \pm 0.9 ^d	42.9 \pm 0.9 ^b	14.4 \pm 0.9 ^a	51.8 \pm 1.7 ^d
CBW	2.7	31.1 \pm 0.4 ^f	36.9 \pm 1.4 ^c	6.5 \pm 1.6 ^c	23.1 \pm 1.3 ^g

Values are means of three independent analyses \pm standard deviation ($n = 3$). Mean values followed by different superscript letters indicate significant statistical difference ($P < 0.05$). BRA - *B. rubra* acetone extract; BRM - *B. rubra* methanol extract; BRW - *B. rubra* water extract; RNA - *R. nepalensis* acetone extract; RNM - *R. nepalensis* methanol extract; RNW - *R. nepalensis* water extract; CBA - *C. benghalensis* acetone extract; CBM - *C. benghalensis* methanol extract; CBW - *C. benghalensis* water extract.

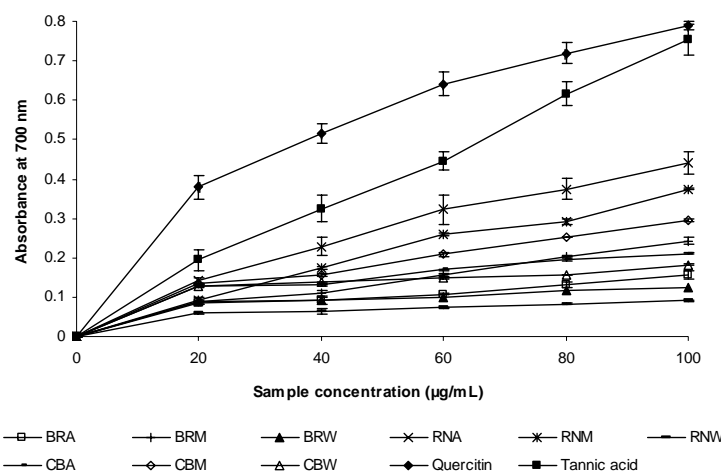


Fig. 1: Reducing power of acetone, methanol and water extracts of the selected leafy vegetables

Values are means of three independent analyses \pm standard deviation ($n = 3$). BRA - *B. rubra* acetone extract; BRM - *B. rubra* methanol extract; BRW - *B. rubra* water extract; RNA - *R. nepalensis* acetone extract; RNM - *R. nepalensis* methanol extract; RNW - *R. nepalensis* water extract; CBA - *C. benghalensis* acetone extract; CBM - *C. benghalensis* methanol extract; CBW - *C. benghalensis* water extract; T - Tannic acid; Q - Quercetin.

Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. It is necessary to determine the reducing power of the leafy vegetable to elucidate their antioxidant activity. The presence of antioxidants in the extract causes a reduction of the Fe^{3+} /ferricyanide complex to its ferrous form. The extent of the reduction is explained in terms of absorbance value at 700 nm for the tested samples ranging in concentration from 20-100 $\mu\text{g/mL}$. All the tested extracts exhibited increasing reducing activity with increasing concentration (Figure 1). The acetone and methanol extracts of *R. nepalensis* exhibited higher levels of reducing power. Though all the extracts did not exhibit a strong reducing power, as compared to the standards tannic acid and quercetin they did have an activity that reveals the selected leafy vegetables are electron donors and can react with free radicals and convert them to stable products thus terminating the free radical chain reactions.

DPPH radical scavenging activity

The free radical scavenging properties of the extracts were determined by the DPPH assay (Figure 2) wherein the DPPH radical is reduced by the antioxidant compound to its hydrazine derivative. All the extracts were capable of scavenging the DPPH radical, which decolorized with the increasing concentration of the extracts. The methanol and acetone extracts of *R. nepalensis* (IC_{50} 121.7 $\mu\text{g/mL}$ and IC_{50} 122.9 $\mu\text{g/mL}$ respectively), followed by the methanol extract of *C. benghalensis* (IC_{50} 138.7 $\mu\text{g/mL}$) exhibited strong DPPH radical scavenging activities. Yokozawa *et al.*³² suggested that the flavonoids and tannins reduce and decolorize DPPH \cdot by their hydrogen donating ability. The higher content of total phenolics, tannin, flavonoid and antioxidant vitamins C in the extracts of *R. nepalensis* (Table 1) might have contributed towards the scavenging of free radicals, thereby forming the stable end product. Similarly, Guerra *et al.*³³ also reported that the higher content of phenolic compounds from *R. induratus* revealed a strong radical scavenging activity.

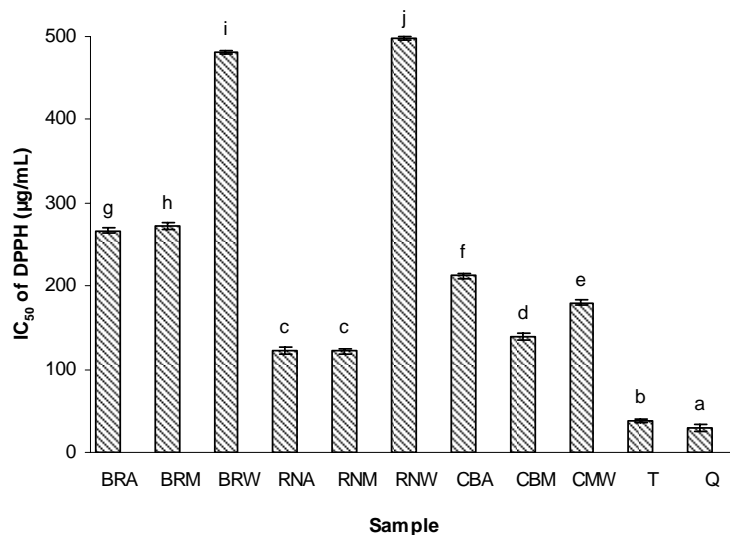


Fig. 2: DPPH \cdot scavenging activity of different solvent extracts of *B. rubra*, *R. nepalensis* and *C. benghalensis*

Values are means of three independent analyses \pm standard deviation ($n = 3$). Bars having different letters are significantly different ($P < 0.05$). BRA - *B. rubra* acetone extract; BRM - *B. rubra* methanol extract; BRW - *B. rubra* water extract; RNA - *R. nepalensis* acetone extract; RNM - *R. nepalensis* methanol extract; RNW - *R. nepalensis* water extract; CBA - *C. benghalensis* acetone extract; CBM - *C. benghalensis* methanol extract; CMW - *C. benghalensis* water extract; T - Tannic acid; Q - Quercetin.

Hydroxyl radical scavenging activity

Hydroxyl radicals are the most reactive and one of the quick initiators of lipid peroxidation process and induce severe damages to adjacent biomolecules, especially lipids, polypeptides, proteins and DNA in the living cells. Therefore it is important to assess the protective ability of the leafy vegetables against these free radicals. All the solvent extracts exhibited good hydroxyl radical scavenging ability (Table 2). The acetone, methanol and water extracts of *R. nepalensis*, (IC_{50} 38.3 ± 1.4 $\mu\text{g/mL}$, 44.9 ± 3.9 $\mu\text{g/mL}$ and 32.9 ± 2.8 $\mu\text{g/mL}$ respectively), exhibited an effective inhibition of the hydroxyl radical compared to the extracts of *B. rubra* and *C. benghalensis*. As such, *R. nepalensis* has proved to be more potent inhibitor of chain reactions, which might be attributed to the presence of anthroquinone glucosides (nepalensides), epicatechingallate and epicatechin reported in this plant⁸.

Nitric oxide radical scavenging activity

Nitric oxide formed from L- arginine by NO synthase, is associated with initiation of lipid peroxidation, production of other free radicals and involved in the neurodegenerative and chronic inflammatory diseases. In the present study, the nitric oxide generated at physiological pH, interacts with oxygen to produce nitrite ions, which can be estimated using the Griess reagent. Among the

samples, the water extract of *B. rubra* (IC_{50} 29.2 ± 1.6 $\mu\text{g/mL}$), methanol and acetone extracts of *R. nepalensis* (IC_{50} 29.4 ± 1.0 $\mu\text{g/mL}$ and IC_{50} 30.3 ± 3.0 $\mu\text{g/mL}$, respectively) possessed the maximum radical scavenging activities (Table 2). On the other hand, the lowest NO scavenging activities were recorded for the extracts of *C. benghalensis*. These leafy vegetables which have established their capability to counteract the effects of NO formation, in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body³⁴.

Superoxide radical scavenging activity

Superoxide anion, a highly reactive toxic species, induces oxidative damages in lipids, proteins and DNA in the living system. It has been implicated in several pathophysiological processes due to its transformation into more reactive species such as hydrogen peroxide, hydroxyl and singlet oxygen. The superoxide radical scavenging activity of the selected green leafy vegetables, determined using riboflavin- NBT- light system *in vitro* are presented in Table 2. The photochemical reduction of flavin generates superoxide radical which reduced NBT, resulting in the formation of blue formazine. The inhibition of formation of blue formazine was higher in the methanol extract of *C. benghalensis* (IC_{50} 163.7 ± 3.2 $\mu\text{g/mL}$) followed by the acetone extract of *C. benghalensis*, (IC_{50} 191.6 ± 4.4 $\mu\text{g/mL}$). The water extract of *R.*

nepalensis and the acetone extract of *B. rubra* exhibited a slightly lower but equal activity of IC₅₀ 213.9 ± 2.4 µg/mL and IC₅₀ 213.1 ± 3.1 µg/mL, respectively. The results were in accordance with Dasgupta and De³⁵ and Ferreres *et al.*³⁶ who also reported superoxide radical scavenging activity for certain green leafy

vegetables. The activity of the tested extracts against superoxide radical is of significance because superoxide can decrease the activity of other antioxidant defense enzymes such as catalase and glutathione peroxidase as well as being cytotoxic by generating more reactive species like peroxy nitrite³⁷.

Table 2: Hydroxyl, nitric oxide and superoxide radical scavenging activity of *B. rubra*, *R. nepalensis* and *C. benghalensis* extracts

Sample	IC ₅₀ •OH (µg/mL)	NO• (µg/mL)	•O ₂ ⁻ (µg/mL)
BRA	47.3 ± 5.0 ^d	37.0 ± 1.4 ^c	213.1 ± 3.1 ^e
BRM	67.7 ± 4.8 ^f	32.6 ± 2.8 ^b	315.7 ± 2.4 ^g
BRW	53.1 ± 2.2 ^e	29.2 ± 1.6 ^b	400.6 ± 1.7 ^h
RNA	38.3 ± 1.4 ^c	30.3 ± 3.0 ^b	426.1 ± 2.0 ⁱ
RNM	44.9 ± 3.9 ^d	29.4 ± 1.0 ^b	254.8 ± 2.5 ^f
RNW	32.9 ± 2.8 ^b	44.4 ± 4.4 ^d	213.9 ± 2.4 ^e
CBA	78.3 ± 1.4 ^h	69.4 ± 1.3 ^f	191.6 ± 4.4 ^d
CBM	93.7 ± 3.2 ⁱ	58.7 ± 2.3 ^e	163.7 ± 3.2 ^c
CBW	71.9 ± 2.8 ^g	71.9 ± 9.3 ^g	468.2 ± 1.6 ^j
TA	2.2 ± 1.0 ^a	4.4 ± 1.3 ^a	27.5 ± 2.1 ^b
Q	5.5 ± 3.2 ^a	3.0 ± 0.7 ^a	7.9 ± 1.6 ^a

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference (P<0.05). BRA - *B. rubra* acetone extract; BRM - *B. rubra* methanol extract; BRW - *B. rubra* water extract; RNA - *R. nepalensis* acetone extract; RNM - *R. nepalensis* methanol extract; RNW - *R. nepalensis* water extract; CBA - *C. benghalensis* acetone extract; CBM - *C. benghalensis* methanol extract; CBW - *C. benghalensis* water extract; TA - Tannic acid; Q- Quercetin.

ABTS radical cation scavenging activity

Proton radical scavenging is an important attribute of antioxidants. The ability of the selected extracts to quench the ABTS^{•+} green chromophore with characteristic absorption at 734 nm are presented in Table 3. The results are expressed as Trolox equivalent (the micromolar trolox solution having an antioxidant capacity equivalent to 1 g dry matter of the substance under investigation). The methanol extract of *C. benghalensis* (13538.5 ± 200.2 µmol/g extract) followed by its acetone extract (9719.7 ± 551.1 µmol/g extract) displayed strong antioxidant activity. On the other hand, the methanol extract of *R. nepalensis*, exhibiting uniformly higher levels of ascorbic acid, total phenolics, tannins and flavonoids registered significantly lower ABTS radical scavenging activity. Hagerman *et al.*³⁸ have reported that high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS^{•+}) and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group substitution than the specific functional groups. Further, the acetone and methanol extracts of a medicinal leafy vegetable, *Melothria maderaspatana* exhibiting higher scavenging of ABTS^{•+} has also been reported earlier³⁹.

Metal chelating property

The extremely reactive metals such as iron play an important role in the initiation and propagation steps of lipid oxidation. Metal chelators form complex ions or coordination compounds with metals, thereby preventing the interaction between metals and lipid intermediates. The chelation of ferrous ion by the leafy vegetable extracts was estimated by the ferrozine assay and the results are presented in Table 3. The chelating ability was higher in water extract of *C. benghalensis* (10.1 ± 1.9 mg EDTA/g extract), followed by the acetone extract of *C. benghalensis* and water extract of *B. rubra*, (9.52 ± 9.2 mg EDTA/g extract and 6.78 ± 6.7 mg EDTA/g extract respectively). The chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Gowda *et al.*⁴⁰ observed that the lutein, isolated from methi leaves, exhibited higher ferrous ion chelation activity.

β-Carotene/ linoleic acid peroxidation inhibition activity

The antioxidant assay using the discoloration of β-carotene is a widely used system because β-carotene is extremely susceptible to free radical mediated oxidation. β-carotene undergoes rapid discoloration with the oxidation of linoleic acid in the absence of an antioxidant. In the present study, the antioxidant activity of the

tested extracts was measured by inhibiting the bleaching of β-carotene. The different solvent extracts exhibited various degrees of antioxidant activity (Fig. 3). At a concentration of 250 µg, the water extract of *B. rubra* exhibited a strong peroxidation inhibition activity (57.4%). However, the inhibition of β-carotene bleaching by all the extracts was lower than those of standards, tannic acid (74.5%) and quercetin (61.5%). These plant extracts could reduce the extent of β-carotene destruction by neutralizing the linoleate-free radical and other free radicals formed in the system.

Table 3: ABTS radical cation scavenging activity and metal chelating activity of the

Sample	TAA ^A (µmol g ⁻¹ extract)	Metal chelating activity (mg EDTA/g extract)
BRA	2526.4 ± 83.5 ^f	3.75 ± 3.5 ^e
BRM	925.7 ± 200.4 ^g	3.80 ± 1.0 ^e
BRW	655.7 ± 167.0 ^h	6.78 ± 6.7 ^c
RNA	2593.9 ± 417.5 ^f	5.13 ± 3.6 ^d
RNM	3837.8 ± 318.2 ^e	1.01 ± 3.0 ^g
RNW	4252.4 ± 238.8 ^c	3.68 ± 3.6 ^e
CBA	9719.7 ± 551.1 ^b	9.52 ± 9.2 ^b
CBM	13538.5 ± 200.2 ^a	2.98 ± 5.4 ^f
CBW	4030.7 ± 318.4 ^d	10.1 ± 1.9 ^a
TA	10254.3 ± 56.2 ^a	11.2 ± 0.5 ^a
Q	14984.2 ± 111.3 ^a	13.5 ± 1.3 ^a

^A Total antioxidant activity (µmol equivalent Trolox performed by using ABTS radical cation)

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference (P<0.05). BRA - *B. rubra* acetone extract; BRM - *B. rubra* methanol extract; BRW - *B. rubra* water extract; RNA - *R. nepalensis* acetone extract; RNM - *R. nepalensis* methanol extract; RNW - *R. nepalensis* water extract; CBA - *C. benghalensis* acetone extract; CBM - *C. benghalensis* methanol extract; CBW - *C. benghalensis* water extract; TA- Tannic acid; Q- Quercetin.

The present study demonstrated the antioxidant potential of the leafy vegetables, *B. rubra*, *R. nepalensis* and *C. benghalensis*. The inhibitory ability of these extracts against the free radicals, suggest that the consumption of these vegetables will prevent aging related diseases. The quantification of non-enzymatic components serves as a guiding factor in establishing their nutraceutical support.

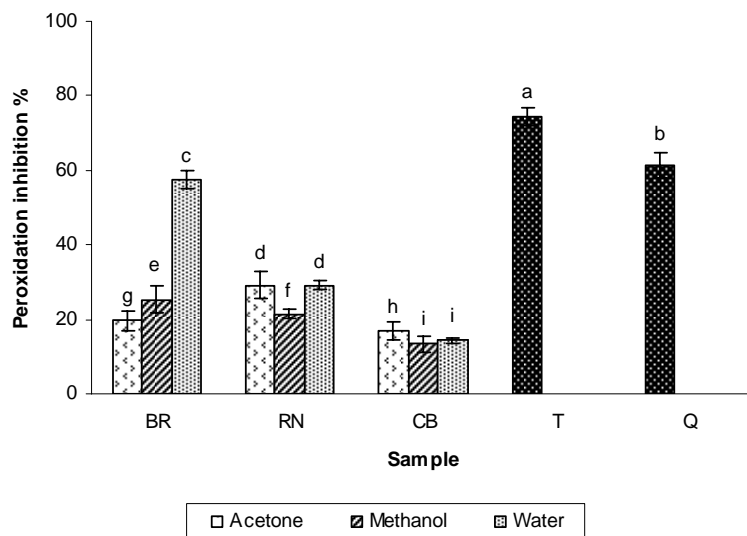


Fig. 3: β -carotene/ linoleic acid peroxidation inhibiting property of the selected leafy vegetable extracts

Values are means of three independent analyses \pm standard deviation (n = 3). Bars having different letters are significantly different ($P < 0.05$). BR - *B. rubra*; RN - *R. nepalensis*; CB - *C. benghalensis*; T - Tannic acid; Q - Quercetin.

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