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Research Article

ANTIOXIDANT AND FREE RADICAL SCAVENGING POTENTIAL OF LEAF AND STEM BARK EXTRACTS OF *BAUHINIA MALABARICA* ROXB.

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ABSTRACT

Aim: Crude acetone and methanolic extracts of *Bauhinia malabarica* leaf and stem bark were analyzed for their *in vitro* antioxidant and free radical scavenging properties.

Methods: The extracts were screened for their antioxidant activities using ferric reducing antioxidant power, DPPH$^{\bullet}$,NO$^{\bullet}$, hydroxyl radical scavenging, metal iron chelating, ABTS$^{\bullet+}$, β-carotene-linoleate model system and antihemolytic activity.

Results: Acetone extracts of stem bark exhibited remarkable antioxidant activity in terms of all the assays tested. In antihemolytic activity, the extracts exhibited efficient antioxidant activity and the values were considerably higher than the natural and synthetic antioxidants tested. Overall, the acetone was found to be the best solvent for the extraction of antioxidant compounds.

Conclusion: Considering all the activities performed, it is known that both the leaf and stem bark extracts of *B. malabarica* were found to be the most effective free radical quencher and a potent source of natural antioxidants, thus justifying their traditional use in green therapeutics.

Keywords: *Bauhinia malabarica*, Stem bark, ABTS$^{\bullet+}$, Radical scavenging activity, Lipid peroxidation.

INTRODUCTION

Bauhinia malabarica (Caesalpiniaceae) is a small or moderate sized deciduous tree, distributed throughout India, mainly on the sub-Himalayan tracts, Bengal, Assam and in south India. It is also found in peninsular India and in the western sub-Himalayan forests, deciduous and semi-evergreen forests, areas receiving 1000 to 3000 mm annual rainfall. The leaves of the plant are consumed in India, Indonesia and Thailand. It is used in traditional medicine for wound healing, as diuretic, to cure dysentery, headache and fever and as an emmenagogue[1]**.** Leaves are acrid and are considered to be a febrifuge; it is also used as a flavoring agent for meat and fish[2]**.**The mineral content of the leaves shows that they are an excellent source of calcium and a very good source of iron. Tender leaves and young pods of the species are consumed by the local population of India. Furthermore, the stem bark and leaves of *B. malabarica* are used respectively to treat jaundice and diabetes by the local population of Siruvani hills, Tamil Nadu, India. Young shoots are also edible and are used to treat worm infestations, leprosy, wounds, menorrhagia, gout, scrofula, wasting diseases, cough, haemorrhage, urinary disorders, glandular swellings and goitre[3]**.** Certain flavonols were isolated from the methanol extract of leaves[1]**.** An optically active isomer of tartaric acid has been identified and quantitatively analyzed from the leaves of *B. malabarica* [4]**.** Despite several ethnobotanical and ethnopharmacological surveys on the therapeutic uses of *B. malabarica,* laboratory data on their bioactivity is still in paucity. Therefore to address these lacunae, the present study was carried out to elucidate the *in vitro* antioxidant potential of leaf and stem bark extracts of *B. malabarica*. The antioxidant activities were measured using ferric reducing antioxidant power, ABTS$^{\bullet+}$, DPPH$^{\bullet}$,NO$^{\bullet}$, hydroxylradical scavenging, iron chelating, β – carotene- linoleate model system and antihemolytic activity.

MATERIALS AND METHODS

Procurement and preparation of plant materials

Fresh leaves and stem bark of *B. malabarica* were collected from the protected area of Siruvani hills, Coimbatore, Tamil Nadu, India. The authenticity of the selected plant materials was duly identified and confirmed by comparison with reference specimens preserved in the herbarium at Botanical Survey of India, Southern Circle, Coimbatore. The voucher specimens (vide no: BSI/SC/5/23/08-09/Tech.-1718) were lodged in the departmental herbarium for further reference. The plant materials were cleaned, washed with copious amounts of distilled water, shade dried, chopped into bits, and coarsely powdered in a Willy mill (Nippon Electricals, Chennai, India) to 60-mesh size for extraction.

Preparation of crude plant extracts

50 g coarsely powdered plant samples were exhaustively extracted with acetone/water (70/30, v/v), followed by methanol/water (50/50, v/v) using a round bottom flask with an attached reflux condenser for 3 h at a controlled temperature. The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE300; Yamato, Japan), lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) to remove traces of water molecules and the lyophilized powders were stored at -20°C until used directly for the assessment of various *in vitro* antioxidant activities.

Reducing power

The Fe^{+++} reducing power of the extract was determined according to the method suggested by Oyaizu [5]. Various concentrations of the extracts (dissolved in the appropriate solvents) were dissolved in 1.0 mL of phosphate buffer and 5.0 mL of 0.2 M phosphate buffer to adjust the pH 6.6. Subsequently, 5.0 mL of 1% potassium ferricyanide was added. The mixture was incubated at 50°C for 20 min and then cooled. The reaction was terminated by adding 5.0 mL of 10% TCA solution (w/v), and the mixture was centrifuged (REMI, India) at 1000 rpm for 10 min. The upper layer of the supernatant (5.0 mL) was taken and mixed with 5.0 mL of distilled water and 1.0 mL of 0.1% (w/v) ferric chloride. The absorbance was read spectrophotometrically at 700 nm against water blank. Rutin, quercetin, BHA and BHT were served as positive controls for comparison. All the tests were carried out in triplicate. A higher absorbance indicates a higher reductive capability.

DPPH radical scavenging activity

The antiradical efficiency was assessed using the DPPH$^{\bullet}$ method as described by Blois[6]. In this method commercially available methanol soluble stable free radical DPPH was used. In its radical form, DPPH has an absorption band at 515 nm, which disappears upon reduction by an antioxidant compound or a radical species. For the photometric assay, different volumes of the extracts were taken in different test tubes. The volume was adjusted to 100 µL with methanol. 5.0 mL of 0.1 mM methanolic solution of DPPHwas added

to these tubes and shaken vigorously. The tubes were allowed to stand for 20 min at 27ºC. The control was prepared as above but without the test extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were monitored at 517 nm. Results were compared with the activity of rutin, quercetin, BHA and BHT. The per cent DPPH· discolouration of the samples was calculated using the following formula:

DPPH radical scavenging activity (%) = [(A_{517} of control - A_{517} of sample) /A_{517} of control] × 100.

Antioxidant activities of the extracts were expressed as $IC_{50,}$ (the microgram of extract to scavenge 50% of the DPPH radicals) and were obtained by interpolation from linear regression analysis. A lower IC_{50} value indicates greater antioxidant activity.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method suggested by Sreejayan and Rao[7]. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions, which can be estimated using the Griess reagent. Scavengers of nitric-oxide act against oxygen, leading to reduced production of nitrite ions. In brief, 3.0 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with different concentrations of the extract and incubated at 25°C for 150 min. 0.5 mL of the incubated solution was removed and diluted with 0.5 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylenediamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standards. IC_{50}, an inhibitory concentration was estimated from the % inhibition plot.

Hydroxyl radical scavenging activity

The scavenging activity for the sample extracts on hydroxyl radical was measured according to the method of Klein *et al*.[8]. 20 µg concentration of the extract was added with 1.0 mL of iron – EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) sequentially. The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90ºC for 15 min in a water bath. The reaction was terminated by the addition of 1.0 mL of ice – cold TCA (17.5% w/v). Then, 3.0 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2.0 mL of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at laboratory temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectrophotometrically at 412 nm against reagent blank. Results were compared with the activity of standard antioxidants viz., rutin, quercetin, BHA and BHT. The % hydroxyl radical scavenging activity (HRSA) was calculated using the following formula:

$$HRSA\ (\%) = [A_{control} - A_{sample} / A_{control}] \times 100.$$

Chelating ability for ferrous ions

The ferrous chelating potential of the extracts were assessed according to the method suggested by Yamaguchi *et al*. [9]. The reaction was initiated with the sequential addition of 250 µg of sample extract, 0.25 mL of 1 mM $FeSO_4$ solution, 1.0 mL of 0.2 M Tris–HCl buffer (pH 7.4), 1.0 mL of 2, 2' bipyridyl solution, 0.4 mL of 10% hydroxylamine hydrochloride and 2.0 mL of ethanol. The final volume was made up to 5.0 mL with deionized water and the absorbance was determined at 522 nm. EDTA was used to benchmark the chelating abilities. Lower absorbance of the reaction mixture indicated higher ferrous ion chelating ability. Results were expressed as mg EDTA equivalent/ g sample extracts.

Trolox equivalent antioxidant capacity (TEAC) assay

Antioxidant activity was performed using an improved $ABTS^{\cdot+}$ method proposed by Siddhuraju and Manian[10]. The ABTS radical cation ($ABTS^{\cdot+}$) was generated by a reaction of 7 mmol/ L ABTS and 2.45 mmol/ L potassium persulfate after incubation for 16 h at laboratory temperature in dark. Blue – green $ABTS^{\cdot+}$ was formed at the end of this period. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to obtain an absorbance of 0.700 ± 0.02 at 734 nm, the wavelength of maximum absorbance in the visible region. The stock solution of the sample extracts in ethanol was diluted such that, after introduction of a 10 µL aliquot of each dilution into the assay, they produced between 20- 80% inhibition of the blank absorbance. After the addition of 1.0 mL of diluted $ABTS^{\cdot+}$solution to 10µLof sample extracts or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was recorded at 30ºC, exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicates were maintained for the experiments and the per cent inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration[11]. The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as µmol/ g sample extracts on dry weight basis.

Inhibition of β – carotene bleaching

The antioxidant capacity of the extract was evaluated using β – carotene-linoleate model system [12]. 1.0 mg of β – carotene was dissolved in 10 mL of chloroform and mixed with 20 mg of linoleic acid and 200 mg of Tween – 40 emulsifier mixture. Chloroform was completely removed at 45°C under vacuum using a rotary vacuum evaporator. 50 mL of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5.0 mL aliquot of the emulsion was dispensed into tubes containing 100 µg/ mL of the sample extract. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Subsequent absorbance readings were recorded at 15 min intervals by keeping the sample tubes in a water bath at 50°C until the visual colour of β – carotene in the control sample disappeared (about 120 min). A blank, devoid of β – carotene was prepared for background subtraction. Rutin, quercetin, BHA and BHT were used as standards. All determinations were performed in triplicate and averaged.

The antioxidant activity (AA) was measured in terms of reduction in β – carotene bleaching activity using the following formula:

$$AA\ (\%) = [1- (A_s^0 - A_s[12]^0) / (A_c^0 - A_c[12]^0)] \times 100$$

Where, $A_s^{\ 0}$ is the absorbance of sample at 0 min, A_s $[12]^0$is the absorbance of sample at 120 min,

$A_c^{\ 0}$ is the absorbance of control sample at 0 min and A_c $[12]^0$is the absorbance of control sample at 120 min.

Antihemolytic activity

The preparation of erythrocyte membrane ghost and the subsequent determination of the antioxidant activity of the extracts on the chemically induced lipid peroxidation were performed according to the method set forth by Naim *et al.* [13]. The erythrocytes from cow blood were separated by centrifugation (2000 rpm for 10 min) and washed with saline phosphate buffer (0.9 g of sodium chloride dissolved in 100 mL of 0.2 M phosphate buffer of pH 7.4) until the supernatant becomes colourless. The erythrocytes were then diluted with saline phosphate buffer to give 4% (v/v) suspension. 500 µg of extract/ mL of saline phosphate buffer was added to 2.0 mL of erythrocyte suspension and the volume was made up to 5.0 mL with saline phosphate buffer. This mixture was pre-incubated for 5 min and then 0.5 mL of H_2O_2 solution of appropriate concentration in saline buffer was added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring about 90% hemolysis of blood cells after 240 min. After the incubation time, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extend of hemolysis was determined by measurement of the absorbance (at 540 nm) corresponding to haemoglobin liberation. Natural and synthetic standards at the same concentration as sample extract were used for comparison.

The percent hemolysis inhibition was calculated using the formula:

Inhibition percentage = [$A_{control} - A_{Sample} / A_{control}$] × 100.

Statistical analysis

For *in vitro* antioxidant activity of the extracts, the results were recorded as mean ± standard deviation (SD) (n = 3) and subjected to one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS (version 9, SPSS Inc., Chicago, USA). $P < 0.05$ was chosen as the criterion for statistical significance.

RESULTS

Reducing Power

Fig 1 shows the dose- response curves of *B. malabarica* leaf and stem bark extracts. Their reductive abilities displayed an apparent linear relationship with concentration. The activity increases exponentially with the increase of concentration of the test drug. At the same concentration (20-100 µg/ mL) tested, acetone extracts of stem bark provided higher reductive power values which even surpassed the efficiencies of all the natural and synthetic standard antioxidants tested. Similarly, the leaf acetone extracts also manifested stronger activity.

DPPH radical scavenging activity

DPPH, a relatively stable organic radical with a characteristic strong absorption band at 517 nm in visible spectroscopy (deep violet colour) was used to evaluate the free radical scavenging ability of the investigated samples (Table 1). The best known natural and synthetic antioxidant standards, viz., rutin, quercetin, BHA and BHT were used as positive control for comparison. Among the plant parts examined, the crude acetone extracts of *B. malabarica* stem bark (7 µg/ mL) demonstrated effective DPPH radical quenching capacity with their IC_{50} values lesser than 10 µg/ mL. Interestingly these values were significantly lower than the standard antioxidants tested. Additionally, the acetone extracts of *B. malabarica* leaf also contributed fairly outstanding antiradical capacity.

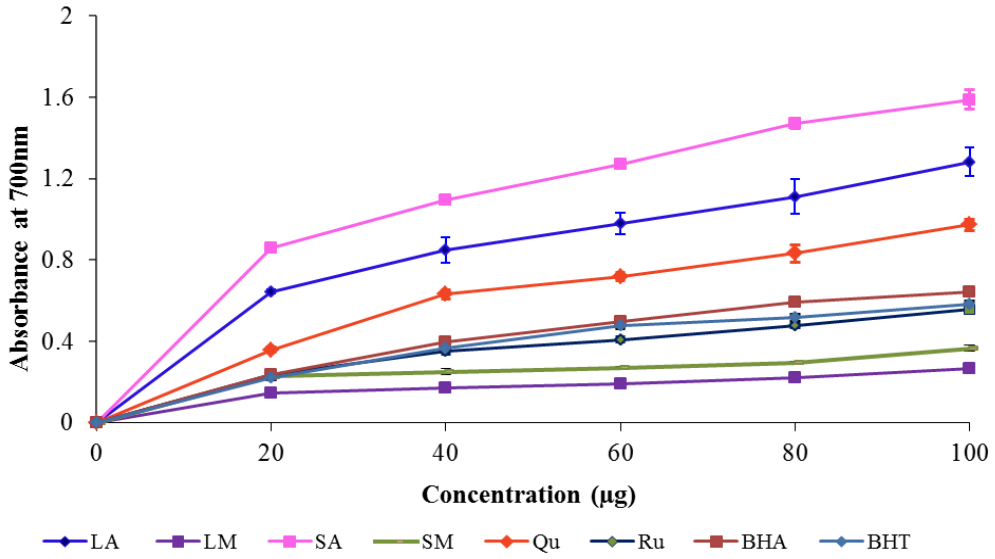

Fig. 1: Reductive capability of different parts of *B. malabarica* compared with standard antioxidants

LA – Leaf Acetone, LM – Leaf Methanol, SA- Stem bark Acetone, SM – Stem bark Methanol, Qu – Quercetin, Ru – Rutin.

Values are presented as the mean ± standard deviation of three independent experiments.

Nitric oxide scavenging activity

The calculated IC_{50} values obtained from the plots of concentration-dependent inhibition of NO were presented in Table 1. The extracts registered excellent to moderate antioxidant activity with their IC_{50} values ranging between 20.7 and 92.5 µg/ mL, respectively. The highest measurable activity was found in the acetone extracts of stem bark (20.7 µg/ mL) and leaf (29.1µg/ mL). Comparably, it surpassed the performance of all the standard antioxidants tested.

Hydroxyl radical scavenging activity

At the concentration of 20 µg/ mL, both the samples of *B. malabarica* arrayed fairly moderate scavenging activities (17.8 to 36.3%) towards the hydroxyl radicals generated in the reaction mixture (Table 1). Interestingly, the acetone extracts of stem bark (36.3%) showed the highest efficiency of scavenging hydroxyl radicals and it is comparable to that of the standards used viz., BHT (45.6%) and BHA (35.5%).

Chelating ability for ferrous ions

The Fe^{2+} chelating ability of different solvent extracts of *B. malabarica* were examined and the results were presented in Table 1. Among them, it was observed that the acetone extracts of *B. malabarica* stem bark showed markedly higher metal chelating activities than the other said samples.

Trolox equivalent antioxidant capacity (TEAC) assay

In the evaluation of antioxidant capacity by $ABTS^{\bullet+}$method, all the sample extracts of *B. malabarica* were able to quench $ABTS^{\bullet+}$ radical more efficiently with their TEAC values ranging between 1518.7 and 6098.6 µ mol Trolox equivalent/ g extract (Table 1). Among the samples investigated, the crude acetone extracts of *B. malabarica* stem bark and leaf were characterized by their relatively elevated TEAC values such as 6098.6 µmol Trolox equivalent/ g and 3945 µmol Trolox equivalent/ g, respectively. However, the methanolic extracts of *B. malabarica* stem bark registered markedly very low $ABTS^{\bullet+}$ radical scavenging activity (1518.7 µmol Trolox equivalent/ g).

Inhibition of β – carotene bleaching

B. malabarica leaf and stem bark extracts were examined for their β-carotene/linoleic acid bleaching assay (Fig 2). At the concentration of 100 µg/ mL in the final reaction mixture, all the extracts inhibited peroxidation of linoleic acid and subsequent bleaching of β-carotene to various degrees. Apparently, the most effective were the crude acetone extracts of *B. malabarica* stem bark and leaf which recorded the highest antioxidative power of 90.4% and 89.4%, respectively. Furthermore, these values were comparably higher than to those of the natural and synthetic antioxidants used.

Table 1: DPPH scavenging, nitric oxide scavenging, hydroxyl scavenging, total antioxidant activity (TAA), and ferrous ion chelating ability of different parts of *B. malabarica*

Plant parts	IC_{50} values (µg/mL)		Hydroxyl scavenging activity (%)	Metal chelating activity#	$ABTS^{\bullet+}$ scavenging activity*
	$DPPH^{\bullet}$	$NO^{\bullet}$			
LA	12 ± 1.3[b]	29.1± 6.2[b]	24.9 ± 5.2[e]	216.5 ± 1.0[b]	3945 ± 8.9[b]
LM	15.5 ± 0.7[c]	92.5 ± 1.4[h]	17.8 ± 1.6[g]	103.5 ± 0.4[d]	1658.1 ± 24.5[c]
SA	7.0 ± 0.1[a]	20.7±1.5[a]	36.3 ± 0.8[b]	336.6 ± 1.1[a]	6098.6 ± 5.8[a]
SM	16.2 ± 1.4[d]	35.8 ± 3.1[c]	18.5 ± 1.8[f]	168.8 ± 0.2[c]	1518.7± 52.9[d]
Qu	20.7 ± 0.05[e]	50.8± 4[f]	34.9± 3.5[d]	-	-
Ru	15.8 ± 0.01[c]	42.1± 0.3[e]	15.7± 0.8[h]	-	-
BHA	21.4 ± 0.1[f]	52.7±8[g]	35.5± 1.9[c]	-	-
BHT	34.7 ± 0.3[g]	38.5±1[d]	45.6 ± 0.9[a]	-	-

LA – Leaf Acetone, LM – Leaf Methanol, SA- Stem bark Acetone, SM –Stem bark Methanol, Qu – Quercetin, Ru – Rutin.

#Values expressed in mg EDTA / g extract.

* Values expressed as TEAC (Trolox equivalent antioxidant capacity) in µ mol/g extract.

Values are presented as the mean ± standard deviation of three independent experiments.

Mean values not sharing a common letter in a column were significantly different (P< 0.05).

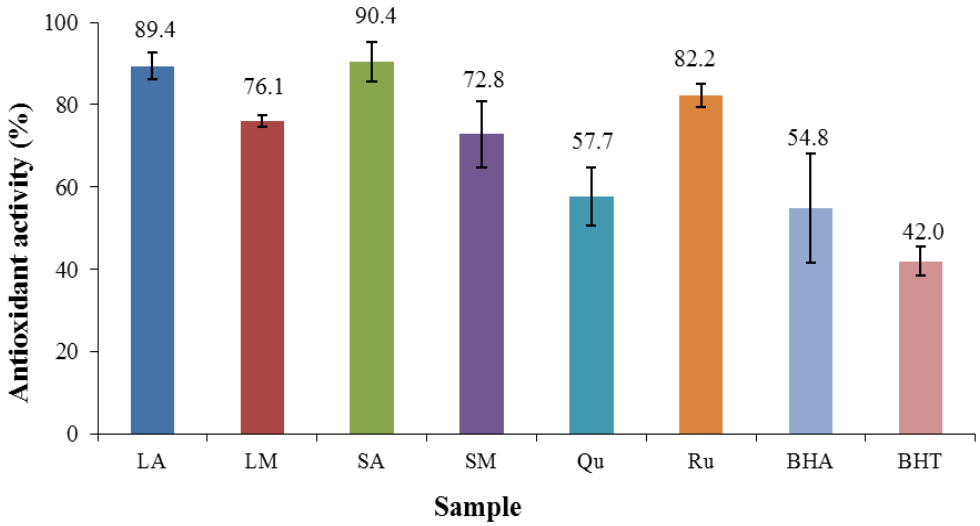

Fig. 2: Lipid peroxidation preventive property of different plant parts of *B. malabarica* in β- carotene-linoleic acid system

LA – Leaf Acetone, LM – Leaf Methanol, SA- Stem bark Acetone, SM – Stem bark Methanol, RA – Root Acetone, RM- Root Methanol, Qu – Quercetin, Ru – Rutin.

Values were presented as the mean ± standard deviation of three independent experiments. Vertical bars labeled with different letters are significantly different ($P < 0.05$).

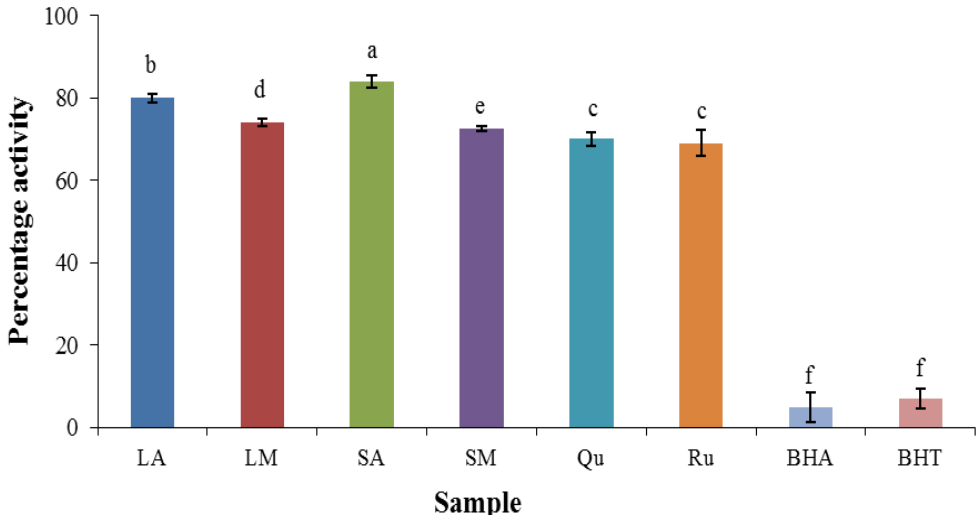

Fig. 3: Antihemolytic property of different plant parts of *B. malabarica*

LA – Leaf Acetone, LM – Leaf Methanol, SA- Stem bark Acetone, SM – Stem bark Methanol, RA – Root Acetone, RM- Root Methanol, Qu – Quercetin, Ru – Rutin.

Values were presented as the mean ± standard deviation of three independent experiments. Vertical bars labeled with different letters are significantly different ($P < 0.05$).

Antihemolytic activity

The protective effect of *B. malabarica* leaf and stem bark extracts against H_2O_2 mediated hemolysis were investigated and it fluctuated between plant parts and among solvent types (Fig 3). In general, all the sample extracts contributed satisfactory antihemolytic activity in terms of percentage inhibiting activity ranging from 72.6 to 84%. Apparently, the inhibition of hemolysis of all the extracts was observed to be higher than those of the standard antioxidants tested. However, the acetone extracts of *B. malabarica* stem bark out performed all other extracts in offering protection against erythrocyte hemolysis.

DISCUSSION

The Fe^{3+}- Fe^{2+}transformation in the presence of different solvent extracts of *B. malabarica* leaf and stem bark were investigated (Fig 1). Most of the extracts displayed markedly higher activities and it even surpassed the efficiency of natural and synthetic antioxidant standards used. Accordingly, it can be suggested that the polyphenolic richness of the extracts might appear to function as good electron and hydrogen atom donors and therefore could terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products[14]. Furthermore, it has been reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[15]. Hence, it is presumed that the acetone extracts *B. malabarica* stem bark may have high amounts of reductones. Similar observation between the polyphenolic constituents in terms of dose dependent and reducing power activity have been reported for several plant extracts including tea [16,17].

DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity. Higher levels of DPPH activity have been correlated with the presence of biologically active biomolecules with pronounced antioxidant activity [18]. The key role of phenolic compounds as scavengers of free radical is emphasized in several reports [19]. Therefore, this test was used to evaluate the free radical scavenging ability of the plant extracts. In the present study, the antioxidant activity of *B. malabarica* leaf and stem bark extracts has been evaluated by DPPH radical-scavenging test (Table 1). All the assessed samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H, reaching 50% of reduction with IC_{50} values ranging between 7 and 16.2 µg/ mL. Not surprisingly, the aqueous acetone extracts *B. malabarica* stem bark showed appreciable antioxidant activity. This may be contributed to the fact that the hydrogen-donating compounds are more likely to be present in the polar solvents which might possibly donated hydrogen from phenolic hydroxyl groups in order to discontinue the free radical chain reaction and prevent damage from free radicals[20]. Therefore it is expected that considerable amount of phenolic compounds are probably involved in this antiradical activity.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes[21].Excess concentration of NO is associated with several diseases[22]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals[21]. In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibits generation of the anions in a concentration dependent (Table 1). Among the samples evaluated, the crude acetone extracts of *B. malabarica* stem bark exhibited more pronounced NO scavenging activity. This preferential NO scavenging potential could be attributed to the presence of water-soluble compounds with potent free radical-scavenging effects, such as phenolic compounds and flavonoids and we can speculate that these constituents might be responsible for the observed scavenging activity.

The superoxide radical and hydroxyl radical are representative reactive oxygen species generated in the body. Hydroxyl radical is the most reactive free radical and it can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions, such as copper or iron. Hydroxyl radicals can react with lipid, polypeptides, proteins and DNA, especially thiamine and guanosine. The resulting radical can undergo further reactions, such as reacting with oxygen to give peroxylradicals, or decomposing to phenoxyl-type radicals by water elimination. Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in living organisms at the second-order rate constants of 10^9-10[10] mol/s[23]. A significant difference ($P < 0.05$) in the scavenging ability of *B. malabarica* leaf and stem bark extracts against hydroxyl radicals generated in a Fenton reaction system was observed in the present study (Table 1). Seemingly, 70% acetone extracts of *B. malabarica* stem bark modestly scavenged off these free radicals and/ or inhibited the generation of free radicals and their resultant values were often comparable with the values of the standards used. The effectiveness of the above mentioned plant extracts to quench hydroxyl radicals seems to insinuate that they are good scavengers of oxygen species. It has already been discussed that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution[24].

The Fe^{2+} chelating ability of *B. malabarica* leaf and stem bark extracts were examined. All the extracts demonstrated the ability to chelate Fe^{2+} ions with their values ranging between 103.5 and 336.6 mg EDTA/ g extract (Table 1). Apparently, the highest chelating potential was exhibited by the acetone extracts of *B. malabarica* stem bark extracts. From the above Fe^{2+} chelating data, it is evident that the extracts, due to the presence of polyphenolic compounds, may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise become catalyst for Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions[25]. Hence, it is suggested that the Fe^{2+} chelating properties of the different solvent extracts of the test samples would be a positive response against the damages caused by oxidation.

$ABTS^{\bullet+}$is a protonated radical, which has characteristic absorbance maximum at 734 nm, that decreases with the scavenging of proton radical[26]. The decolorization of $ABTS^{\bullet+}$ cation radical is an unambiguous way to measure the total equivalent antioxidant capacity of test compounds or plant samples. Since, TEAC is a measurement of the effective antioxidant activity of the extract. A higher TEAC value would imply greater antioxidant activity of the sample. This assay was calibrated with the water-soluble α-tocopherol analogue, Trolox. In the present investigation, acetone extracts of both the samples registered the highest amount of $ABTS^{\bullet+}$ radical quenching ability (Table 1) thus speculating that the activity may be contributed by the hydrogen-donating compounds are most likely to be present in the polar solvents[27]. Due to their effective $ABTS^{\bullet+}$scavenging property they can be classified as agreeable antiradical agents, equally effective as Trolox.

In β-carotene/linoleic acid bleaching method, the degree of linoleic acid oxidation is determined by measuring oxidation products (lipid hydroperoxides, conjugated dienes, and volatile by-products of linoleic acid) which simultaneously attack β-carotene, resulting in bleaching of its characteristic yellow colour in ethanolic solution. An extract capable of retarding or inhibiting the oxidation of β-carotene may be described as a free scavenger and/or primary antioxidant. In the β-carotene-bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50°C. The presence of antioxidants in the extract will minimize the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be neutralized by the antioxidants in the extracts[28]. The degradation rate of β-carotene-linoleate depends on the antioxidant activity of the extracts. There was a correlation between degradation rate and the bleaching of β-carotene; the extract with the lowest β-carotene degradation rate exhibited the highest antioxidant activity. In the present study, all the extracts had considerable antioxidant activities comparable to the natural and synthetic standards used (Fig 2). Interestingly, the aqueous acetone extracts of *B. malabarica* leaf (90.4%) and stem bark (89.4%) exhibited an appreciable antioxidant activity at100 µg/mL. These results showing more than 80% of inhibition are considered noteworthy when compared to the findings of other studies concerning medicinal plants[29]. It is believed that lipid peroxidation is one of the causes of the occurrence of cardiovascular diseases and cancer[30]. The high inhibition rate of the extracts obtained in the study might therefore contribute in a big way

to their therapeutic potential. Moreover our results indicated that high total antioxidant activity could be due to phenolic compounds present in the extracts. It has been well documented that phenolic compounds are able to donate a hydrogen atom to the free radicals thus stopping the propagation of chain reaction during lipid oxidation process[31].

H_2O_2 mediated oxidation of lipid in cow blood erythrocyte membrane induces membrane damage and subsequently it leads to hemolysis. It is a peroxyl radical initiator that generates free radicals by its thermal decomposition and will attack erythrocytes to induce the chain oxidation of lipid and protein, disturbing the membrane organization and eventually leading to hemolysis. Different solvent extracts of *B. malabarica* leaf and stem bark extracts were tested for antihemolytic activity in the cell membrane of cow blood erythrocytes (Fig 3). All the extracts displayed satisfactory antihemolytic activity in terms of percentage inhibiting activity ranging between 72.6% and 84%. Apparently, the acetone extracts of *B. malabarica* stem bark offered more protection against erythrocyte hemolysis when compared with the other studied components. Dai *et al.*[32] also recorded that flavonols and their glycosides are competent antioxidants which are capable of protecting human red blood cells against oxidative hemolysis stimulated by free radical. Therefore, it could be speculated that the antihemolytic capacity of the extracts may be attributed to the presence of phenolic and flavonoid constituents of the plant.

CONCLUSION

In conclusion, the crude acetone extracts of *B. malabarica* stem bark presented a broad range of antioxidant activities but with different efficacies. Presence of high contents of polyphenolics might be the major contributor of antioxidant capacities of these plant samples. Therefore, it must be emphasized that the promising results obtained from *B. malabarica* stem bark extract provides a preliminary pharmacological support for utilizing them in therapeutics. Furthermore, isolation and characterization of its individual active components and *in vivo* relevance of such activity awaits further comprehensive studies.

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