Original Article

Quantification of phytochemicals and *in vitro* antioxidant potential of various solvent extracts of certain species of Acanthaceae

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Background: Scientific evaluation of traditional knowledge is essential to obtain effective drugs for commercial purposes. The present study is guide to the invention of new antioxidant sources from the Acanthaceae members. **Aim:** Quantification of certain secondary metabolites and *in vitro* antioxidant activities of alcoholic and aqueous leaf extracts of *Nilgirianthus heyneanus* and *Peristrophe bicalyculata*. **Materials and Methods:** Both the leaf extracts were tested for various phytochemical constituents such as phenols, tannins, condensed tannins, flavonoids, vitamin C and saponin contents. The extracts were further screened for different antioxidant assays such as reducing power, $DPPH^{\bullet}$, nitric oxide, metal chelating, $ABTS^{\bullet+}$ scavenging activity, lipid peroxidation preventive property and anti-haemolytic activities. **Results:** Acetone fractions of *Nilgirianthus heyneanus* leaf registered significantly high amount of secondary metabolites and also it effectively scavenged the free radicals in a concentration dependent manner than *Peristrophe bicalyculata* leaf extracts. Results were compared with standard antioxidants such as rutin, quercetin, BHA and BHT. **Conclusions:** This study further confirmed the traditional use of *N. heyneanus* and *P. bicalyculata* as a natural source of antioxidants and prevents free radical-induced life-threatening diseases.

Key words: *In vitro* antioxidant activity, *Nilgirianthus heyneanus*, *Peristrophe bicalyculata*, quantitative estimations

INTRODUCTION

Antioxidants as the name suggests are capable of stabilising free radicals before they cause damage. Antioxidants are present in fresh fruits, greens and vegetables as well as whole grains. Vitamin A, C and E, β-carotene, lycopene and selenium are among the most well known antioxidants.[1] They help the body to neutralise free radicals. There are over 4000 bioactive components present in food that have antioxidant properties. In recent years, the use of natural antioxidants in the food processing and pharmaceutical industry has gained interest due to their presumed safety, nutritional and therapeutic values.

The family, Acanthaceae contains several species with a rich diversity of ethnobotanical uses. One of the important species in this family is *Nilgirianthus heyneanus* L., mostly found in the south west regions of India, commonly called as Karun kurinji. Ayurvedic drugs prepared from this species is useful in all Vata Vikaras. They are commonly used in rheumatic complaints, sprain of the angle, hernia etc.[2] Similarly, *Peristrophe bicalyculata* L. is found almost throughout India. The leaves are used in the treatment of analgesic, antipyretic, antiinflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhoea. Traditional healers used this plant for curing many skin related problems, as an antidote for snake poison and as an insect repellent.[3] Similarly, certain Acanthaceae members are reported to contain effective antioxidant activity.[4,5] Based on the traditional knowledge the present study was undertaken to evaluate the above selected medicinal plants for major phytoconstituents and their relationship with the antioxidant properties.

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MATERIALS AND METHODS

Chemicals and Reagents

2,2′-azinobis (3- ethylbenzothiozoline- 6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), 2,2′-bipyridyl, 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine tetra acetic acid (EDTA) disodium salt, ferric ammonium sulphate, ferric chloride ($FeCl_3$), ferrous ammonium sulphate, Folin-Ciocalteu reagent, gallic acid, linoleic acid, polyvinyl polypyrrolidone (PVPP), potassium ferricyanide ($K_3Fe(CN)_6$), potassium persulphate,

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quercetin, rutin, sodium carbonate, sodium nitroprusside, trichloroacetic acid (TCA), vitamin C (L-ascorbic acid) and β-carotene were obtained from Himedia, Merck or Sigma - Aldrich. All other reagents and solvents used were of analytical grade.

Plant Materials

Fresh leaves of the study species, *Nilgirianthus heyneanus* and *Peristrophe bicalyculata* were collected from the forest margins of Nilgiri district, Tamil Nadu, India. They were cleaned by washing with distilled water, shade dried and coarsely powdered in a Willy Mill to 60 mesh size for extraction.

Preparation of Crude Plant Extracts

Fifty grams of coarsely powdered plant samples were extracted with acetone and ethanol using soxhlet apparatus. Further, the air dried residues were subjected to cold maceration with water. The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator to remove traces of water molecules and the lyophilised powder were stored at 20°C until used directly for the assessment of various *in vitro* antioxidant activities.

Quantification of Non-enzymic Antioxidants

Major non-enzymic antioxidants of the plant extracts were determined by using standard quantitative methods. The total phenolic and tannin contents were estimated and expressed as gallic acid equivalents (GAE) mg/g extract according to the method described by Siddhuraju and Becker.[6] Condensed tannins in the extracts were estimated using leucocyanidin as a standard by the method of Porter *et al*.[7] The total flavonoids content was determined spectrophotometrically using a standard curve rutin as per the method of Zhishen *et al*.[8] Content of ascorbic acid was calculated on the basis of calibration curve of authentic L-ascorbic acid and the results were expressed as mg of ascorbic acid (AA) equivalent/100 g of extract, proposed by Klein and Perry.[9] Total saponin content was determined by the method described by Makkar *et al*.[10] with some modifications. The values were expressed as diosgenin equivalents (DE) derived from a standard curve.

Determination of *In Vitro* Antioxidant Activity

Reducing Power Assay

The Fe^{3+} reducing power of the extract was determined according to the method suggested by Oyaizu.[11] The plant extracts (100-500 μg/mL) were mixed with 5.0 mL of 0.2 M phosphate buffer of pH 6.6 and 5.0 mL of 1% $K_3Fe(CN)_6$ and the mixtures were incubated at 50°C for 20 min. The reaction was terminated by adding 5.0 mL of 10% TCA (w/v), and the mixture was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5.0 mL) was mixed with 5.0 mL of distilled water and 1.0 mL of 0.1% (w/v) $FeCl_3$ and the absorbance was read at 700 nm. Rutin, quercetin, BHA and BHT served as the reference material. Increased absorbance indicates increased reductive capability.

DPPH Radical Scavenging Activity

The hydrogen donating capacity was assessed using the stable $DPPH^{\bullet}$ method.[12] Briefly, a solution of 0.1mM $DPPH^{\bullet}$ was prepared using methanol. The samples (50-250 μg/mL) were mixed with 5.0 mL of $DPPH^{\bullet}$ solution. Reaction mixture was shaken, incubated at 27°C for 20 min and the absorbance was measured at 517 nm. Results were compared with the activity of rutin, quercetin, BHA and BHT. Per cent $DPPH^{\bullet}$ discolouration of the samples was calculated using the formula:

DPPH radical scavenging activity (%) = [(Control OD – Sample OD)/Control OD] ×100.

Antioxidant activities of the extracts were expressed as $IC_{50,}$ these values were calculated from the linear regression of the percentage antioxidant activity versus concentration of the extracts.[13] A lower IC_{50} value indicates greater antioxidant activity.

$NO^{\bullet}$ Scavenging Activity

Nitric oxide generated from sodium nitroprusside was estimated using Griess reaction.[14] 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. From the incubated solution 0.5 mL was taken and 0.5 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride) was further added. Absorbance was measured at 546 nm and the percentage scavenging activities of the samples were measured. IC_{50} an inhibitory concentration was estimated from the % inhibition plot.

Chelating Ability for Ferrous Ions

The ferrous chelating potential of the extracts were assessed according to the method suggested by Yamaguchi *et al*.[15] 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 deionised 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.

Trolox Equivalent Antioxidant Capacity Assay

Antioxidant activity was performed using an improved $ABTS^{\bullet+}$ method proposed by Siddhuraju and Manian.[16]

The ABTS radical cation ($ABTS^{•+}$) was generated by a reaction of 7 mM $ABTS^{•+}$ and 2.45 mM potassium persulphate and the mixture was incubated for 12-16 h at room temperature in dark. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to obtain an absorbance of 0.700 ± 0.02 at 734 nm. 10 µL/mL of sample was added to 1.0 mL of diluted $ABTS^{•+}$ solution. After 30 min of incubation, absorbance was read at 734 nm. Trolox was used as a reference material.

Inhibition of β – Carotene Bleaching

The antioxidant capacity of the extract was evaluated using β–carotene-linoleate model system.[17] One milligram of β – carotene was dissolved in 10 mL of chloroform and mixed with 20 µL of linoleic acid and 200 mg of Tween – 40 emulsifier mixture. Chloroform was completely evaporated using rotary vacuum evaporator at 45°C. Fifty millilitres of oxygenated distilled water was added to the flask with vigorous shaking, to form an emulsion. Five millilitres of emulsion was added to 100 µL of sample from each tube, the zero-time absorbance was measured at 470 nm. Subsequent absorbance readings were recorded at 15 min intervals by keeping the sample tubes in a water bath at 50°C until the colour of 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. β – carotene bleaching activity was calculated as:

$$AA (\%) = [1- (A_s^0 – A_s^{120})/(A_c^0 – A_c^{120})] \times 100$$

Where, A_s^0 - absorbance of sample at 0 min, A_s^{120} - absorbance of sample at 120 min, A_c^0 -absorbance of control sample at 0 min and A_c^{120} - absorbance of control sample at 120 min.

Antihaemolytic Activity

Antihaemolytic activity was performed according to the method set forth by Naim *et al*.[18] The erythrocytes from cow blood were separated by centrifugation (2000 rpm for 10 min) and washed with saline phosphate buffer (pH 7.4) until the supernatant become 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 were added to 2.0 mL of erythrocytes suspension and 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% haemolysis of blood cells after 240 min. After the incubation time, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of haemolysis 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 haemolysis inhibition was calculated using the formula:

$$\text{Inhibition percentage} = [A_{control} – A_{sample}/A_{control}] \times 100.$$

Statistical Analysis

All the values were expressed as mean ± standard deviation (SD) of three determinations 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

In the present study, it was determined that the percentage yield of leaves of the two studied species, *N. heyneanus* and *P. bicalyculata* extracts varied widely from 0.2 to 0.6% [Table 1]. Among the two species, the highest yield (0.6%) was determined in the ethanol extracts of the species *P. bicalyculata*.

Quantitative Estimations

In quantitative estimations, it was noted that *N. heyneanus* leaf had significantly higher content of total phenolics, flavonoids, tannins and condensed tannins than *P. bicalyculata.* Comparing the amount of phytochemical constituents extracted in the various extracts of *N. heyneanus,* plant exhibited the following

Table 1: Extractive yield, total phenolics, tannins, total flavonoids, vitamin C, saponin and condensed tannin contents of different solvent extracts of leaf of *Nilgirianthus heyneanus* and *Peristrophe bicalyculata* extracts

Sample*	Solvent	% yield (w/w)	Total phenolics#	Tannins#	Total flavonoids@	Vitamin C£	Saponin**	Condensed tannin$
NL	Acetone	0.4	1.61±0.01a	1.47±0.01a	19.1±0.1a	2.1±0.1k	18.8±0.02b	
	Ethanol	0.3	0.76±0.01e	0.43±0.01d	17.43±0.1c	51.9±0.26c	17.4±0.02c	0.41±0.01a
	Water	0.4	0.92±0.01d	0.61±0.01c	15.48±0.1d	16.9±0.5j	13.6±0.01f	
PL	Acetone	0.4	0.24±0.01h	0.09±0.01h	3.9±0.1i	73.2±0.02b	11.4±0.01h	
	Ethanol	0.6	0.22±0.01h	0.01±0.01i	1.1±0.1k	49.2±0.1d	11.5±0.01h	0.09±0.01b
	Water	0.2	1.39±0.01b	0.62±0.01c	13.02±0.1e	74.3±0.1a	19.8±0.02a	

Values are mean±standard deviation (SD) of three independent experiments. Values not sharing a common letter in a column are significantly different (P<0.05). *NL – *Nilgirianthus heyneanus* leaf; PL – *Peristrophe bicalyculata* leaf. #Values expressed as mg GAE/g extract; @Values expressed as mg RE/g extract; £Values expressed as mg ascorbic acid/100 g extract; **Values expressed as g DE/100g extract; $Values expressed in mg LE/g dry samples order acetone >water >ethanol and in *P. bicalyculata* found different order water >acetone >ethanol [Table 1].

In Vitro Antioxidant Activities

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Figure 1a shows the dose response curves of two plant species studied. Their reductive abilities displayed an apparent linear relationship with concentration. The activity increases exponentially with the increase in concentration of drug. The ethanol extracts of the species, *P. bicalyculata* leaf provided higher reductive power values. Interestingly, these values surpassed the efficiency of standard antioxidants *viz.*, BHA, quercetin and rutin [Figure 1b].

DPPH, a stable organic radical, widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. It was visually noticeable by a colour change from purple to yellow. The IC_{50} values were ranging between 121.0 and 501.2 µg/mL as shown in Table 2.

Nitric oxide inhibitors have been shown to have beneficial effects on inflammation and tissue damage seen in inflammatory diseases. Leaf extracts of *N. heyneanus* and *P. bicalyculata* registered excellent to moderate antioxidant activity with their IC_{50} values ranging between 46.2 and 381.1 µg/mL, respectively. The highest measurable activity was found in the acetone extracts of *N. heyneanus* leaf (46.2 µg/mL). Comparably, it surpassed the performance of standard antioxidants such as quercetin (50.8 µg/mL) and BHA (52.7 µg/mL). Noticeably, the water extract of *P. bicalyculata* leaf contributed very low potential for radical scavenging with the IC_{50} value of 381.1 µg/mL [Table 2].

The Fe^{2+} chelating ability of different solvent extracts of both the leaf extracts were examined and the results were presented in Table 2. Among the samples investigated, the water extracts of *N. heyneanus* leaf showed markedly higher metal chelating activity (174.1 mg EDTA/g extract) than the other said samples. While, the acetone extracts of *N. heyneanus* leaf exhibited correspondingly very low ability for iron binding (1.3 mg EDTA/g extract).

Table 2: Comparison of radical scavenging ability of different solvent extracts of leaf of *Nilgirianthus heyneanus* and *Peristrophe bicalyculata* extracts

Sample*	Solvent	(IC_{50} µg/mL)		Metal chelating activity£	ABTS$^{•+}$ scavenging activity@
		DPPH$^{•}$ Scavenging activity	NO$^{•}$ scavenging activity		
NL	Acetone	342.6±1.5^{m}	46.2±0.1^{c}	1.3±0.4^{l}	438.7±25.5^{k}
	Ethanol	247.8±1.2^{l}	237.2±0.6^{h}	11.3±0.7^{j}	556.9±20.2^{j}
	Water	121.0±0.2^{h}	281.2±0.5^{m}	174.1±0.4^{a}	1508.6±36.5^{i}
PL	Acetone	144.7±1.6^{i}	262.1±0.9^{k}	19.2±0.9^{i}	3810.4±21.1^{f}
	Ethanol	501.2±0.5^{o}	221.0±0.2^{g}	31.7±0.7^{h}	2403.0±40.9^{h}
	Water	471±0.07^{n}	381.1±0.1^{n}	97.6±0.4^{c}	4178.2±40.9^{c}
Standards	Rutin	15.8±0.01^{a}	42.1±0.03^{b}	-	-
	Quercetin	20.7±0.05^{b}	50.8±0.1^{d}	-	-
	BHA	21.4±0.1^{b}	52.7±0.1^{e}	-	-
	BHT	34.7±0.3^{e}	38.5±1.0^{a}	-	-

Values are mean±standard deviation (SD) of three independent experiments. Values not sharing a common letter in a column are significantly different (P<0.05). *NL – *Nilgirianthus heyneanus* leaf; PL – *Peristrophe bicalyculata* leaf. £Values expressed in mg EDTA/g extract; @Values expressed as TEAC (Trolox equivalent antioxidant capacity) in µmol/g extract; IC_{50} – Half maximal inhibitory concentration

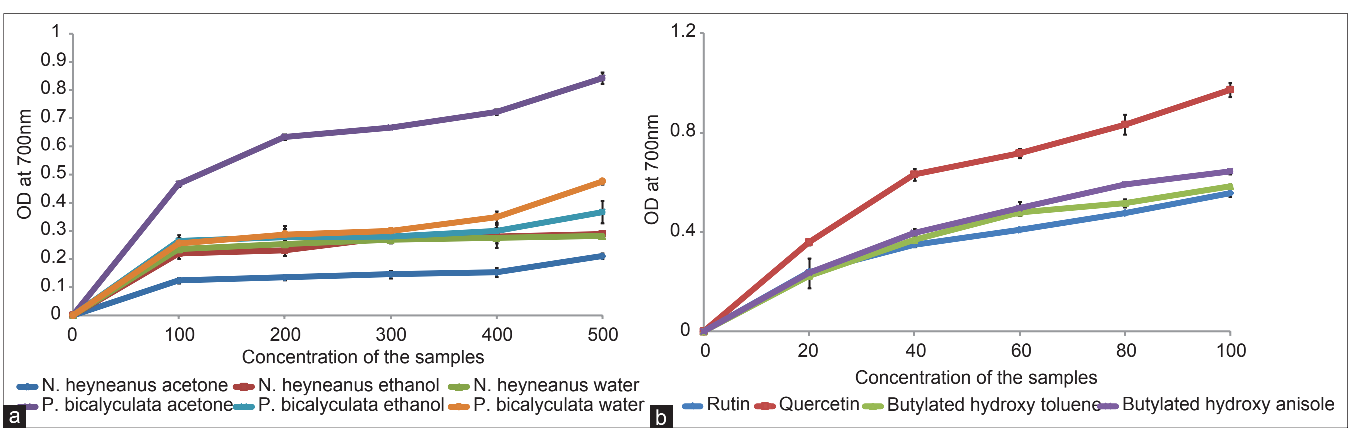

Figure 1: (a) Reductive capability of different solvent extracts of leaf of *Nilgirianthus heyneanus* and *Peristrophe bicalyculata*. (Values are represented as mean ± SD (n = 3, P < 0.05)); (b) Reductive capability of standard antioxidants (values are represented as mean ± SD (n = 3, P < 0.05))

In the evaluation of antioxidant capacity by ABTS•+ method, all the sample extracts were able to quench ABTS•+ radical more effectively and the values ranged between 438.7 and 4178.2 μmol Trolox equivalent/g extract [Table 2]. Among the samples investigated, water extracts of *P. bicalyculata* (4178.2 μmol/g extract) showed the maximum value. In similar lines, the acetone extracts of *N. heyneanus* leaf registered markedly very low ABTS•+ radical scavenging activity (438.7 μmol/g extract).

Both the leaf extracts and positive standards were examined for their β-carotene/linoleic acid bleaching assay [Figure 2]. All the extracts inhibited peroxidation of linoleic acid and subsequent bleaching to β-carotene in various degrees. Apparently, the most effective were the acetone extracts of *Nilgirianthus heyneanus* leaf (78.3%). Furthermore, these values were comparably higher than those of the natural and synthetic antioxidants tested.

The protective effect of leaves of *N. heyneanus* and *P. bicalyculata* plant extracts and positive standards against H_2O_2 mediated haemolysis were investigated and it fluctuated between plant samples and among solvent types. In general, all the sample extracts contributed satisfactory antihaemolytic activity ranging between 11 and 48% [Figure 3].

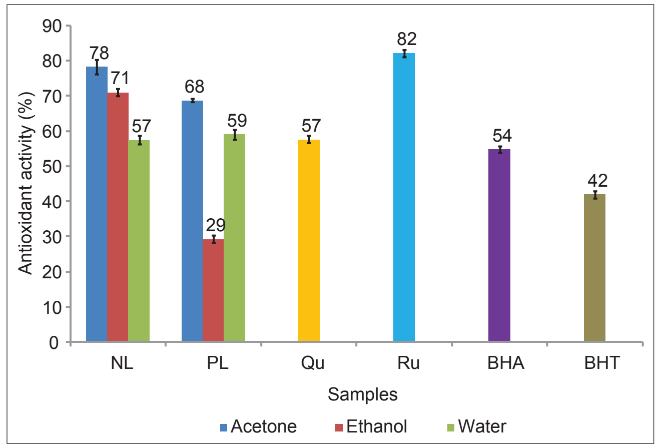

Figure 2: Lipid peroxidation preventive property of different solvent extracts of leaf of *Nilgirianthus heyneanus* and *Peristrophe bicalyculata* extracts. (Values are represented as mean ± SD (n = 3, P < 0.05))

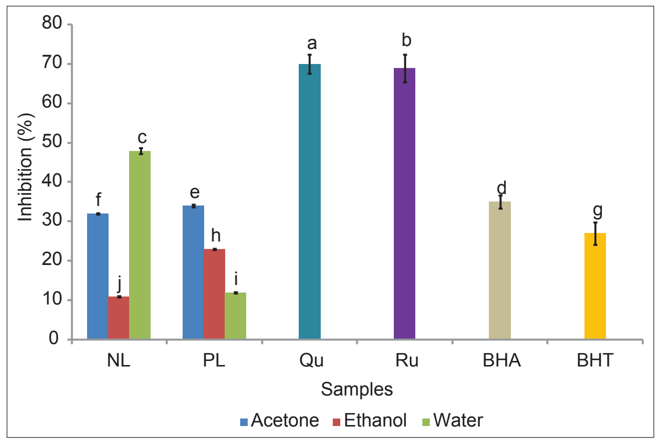

Figure 3: Antihaemolytic property of different solvent extracts of leaf of *Nilgirianthus heyneanus* and *Peristrophe bicalyculata* extracts. (Values are represented as mean ± SD (n = 3, P < 0.05))

DISCUSSION

Many phytochemicals are now studied extensively for their potential ability of curing diseases. Herbal preparations are effectively and extensively used for their medicinal properties and have become increasingly popular worldwide. Phytochemicals in plants have various protective and therapeutic effects which are essential to prevent and maintain a state of well being. Phenols are reported to have antitumour, antiviral, antimicrobial and antioxidant properties.[19] Tannin possess physiological astringent properties which hasten wound healing and ameliorate inflamed mucous membrane and also have haemostatic properties.[19] These compounds are responsible for the bitter taste of plants. Tannins contribute to good health, thus it revealed that *N. heyneanus* leaf is a good source of antioxidants. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. It contains hydroxyls which are responsible for the radical scavenging effect of plants.[20] These compounds are found to attribute directly to the antioxidative action, antiallergic, anti-inflammatory, antimicrobial and anticancer activity.[21] Condensed tannins are responsible for the astringent and bitter taste in plants. Vitamin C or ascorbic acid is a water soluble, non-enzymatic compound which contribute significant antioxidant activity.[22] Saponin has expectorant action which is very useful in the management of upper respiratory tract inflammation and act as a cardiotonic. Different active components in plants improve its antioxidant activity. In the present study, all the active components were excessively present in the acetone extracts [Table 1]. Different solvents have been reported to have different capacity to extract phytoconstituents according to their solubility and polarity and most of the compounds dissolve well in alcoholic solvents.[23]

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.[24] The reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The study revealed that the presence of reductones might contribute significantly to the reductive capability of the extracts.

The results of DPPH• scavenging assay indicate that the plant possess high antioxidant activity [Table 2]. It is suggested that the plant extract contain bioactive compounds that were capable of donating hydrogen ions to a free radical for removing the odd electron which is responsible for radicals reactivity. Thus, it might be speculated that the antioxidant activity of the extract may be possibly attributed to the phytochemicals present in it.

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages and neurons. Excess production of nitric oxide may cause several diseases.[25] Therefore, it can be said that the phytochemicals such as flavonoids and tannins present in these samples might play a pivotal role in scavenging of nitric oxide radicals generated. van Acker *et al.*,[26] made similar observation that flavonoids play a major role in the stabilisation of NO.

The metal chelating ability was found to be higher for the water extract of *N. heyneanus* leaf (174.1 mg EDTA/g extract). Hence it can be suggested that the metal chelating effect of the extracts would be a positive response against oxidation. It is speculated that the properly oriented functional groups of certain phenolics, tannins and flavonoids might act effectively as the antioxidant agents.[27]

$ABTS^{\bullet+}$ is a blue chromophore produced by the reaction between ABTS and potassium sulphate.[28] In the present investigation, the water extracts of *P. bicalyculata* leaf showed comparatively higher scavenging activity than the other said sample extracts. It is explained that the higher TEAC values reported in the present study revealed that the extracts might neutralise the radical ion more efficiently and also hydrogen-donating compounds which are most likely to be present in the polar solvents.[29]

In β-carotene/linoleic acid bleaching method, the degree of linoleic acid oxidation is determined by measuring oxidation products which simultaneously attack β-carotene, resulting in bleaching of its characteristic yellow colour. Lipid peroxidation is one of the causes of occurrence of cardiovascular diseases and cancer.[30] In the present study, the acetone extracts of *Nilgirianthus heyneanus* leaf showed the highest inhibition of β-carotene [Figure 2] and this may be due to the rich amount phytochemicals present in it.[31,32] Therefore, it can be considered that the highest inhibition rate of the extract obtained in the study might contribute to phenolic content of the plant. Phenolic compounds are able to donate a hydrogen atom to the free radicals thus stopping the propagation of chain reaction during lipid oxidation process.[33]

Lipid oxidation of cow blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently haemolysis. Among the plant samples investigated, water extract of *N. heyneanus* leaf has higher antihaemolytic activity of 48% [Figure 3]. Interestingly, the activity was found to be higher than the standard antioxidants, BHT and BHA tested. Dai *et al.*,[34] also recorded that flavonols and their glycosides are competent antioxidants which are capable of protecting human red blood cells against oxidative haemolysis stimulated by free radical. Therefore, it can be explained that the antihaemolytic capacity of the studied plant extracts may be attributed to the presence of phenolics and flavonoids in them.

CONCLUSIONS

The present study revealed that the antioxidant capacity of the leaf extracts of *N. heyneanus* and *P. bicalyculata* provide scientific information for the traditionally claimed health benefits of plant extracts. It was found that all the sample extracts exhibited high radical scavenging activity which put light on its scope as a nutritive and therapeutic agent and its future use for human welfare. Furthermore, isolation and characterisation of its individual active components and *in vivo* relevance of such activity awaits further comprehensive studies.

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