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ORIGINAL ARTICLE **OPEN ACCESS**

Antioxidant scavenging assay of the leaf extracts of *Pseuderanthemum bicolor* (Schrank) Radlk. Ex Lindau

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ABSTRACT

Pseuderanthemum bicolor, commonly known as "limang-sugat, belongs to the family Acanthaceae, an important ornamental undershrub. The antioxidant activities of three different leaf extracts of P. bicolor (Methanol, chloroform and Ethyl acetate) were estimated by DPPH, ABTS, and Hydroxyl radical scavenging assay. All the three extracts showed mild antioxidant activity than the standards such as Quercetin and Catechin.The DPPH assay of the tested samples showed significant dose dependent level of scavenging activity.ABTS scavenging activity of all the three leaf extracts and standard increased with the increase in the concentration. The present study confirms the existence of less antioxidant potential in P. bicolor.
Keywords: P. bicolor, Antioxidant, DPPH, ABTS, Quercetin and Catechin.

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INTRODUCTION

Plants can synthesize a large variety of chemical compounds that are of physiological importance not only to the plants but also for human beings. Medicinal plants are the richest bio-resource of drugs of both traditional& modern systems of medicines. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases and responsible for the production of free radicals. Plants are the rich source of antioxidants that help to encounter the detrimental effects of oxygen free radicals which arise during oxidation process in cells of our body. Free radicals are unstable atoms that carries an unpaired electron, due to the insufficient stable number of electrons inouter most shell, these atoms are constantly searching for other molecules to become stable. In a living system free radicals are produced during an uncontrolled mitochondrial respiration or as byproducts of other metabolic processes. These arerecognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis[1],[2]. Reactive oxygen species (ROS) and Reactive nitrogen species (NOS)are mainly synthesized in cell through mitochondrial oxidative phosphorylation.Antioxidants are the substances that delays or inhibits oxidative damage to a target molecule[3].The main characteristic of an antioxidant is its ability to trap free radicals [4]. Many plants contain antioxidant compounds and these compounds protect cells against the free radicals. *Pseuderanthemum bicolor,* commonly known as "limang-sugat, belongs to the family Acanthaceae, an important ornamental undershrub.Traditionally, the decoction of aerial parts of the plant used for aphthae, also used as a cicatrizing for wounds and ulcers.Apart from this antimicrobial, antipyretic, anti-inflammatory, hepatoprotective, CNS depressant, anticonvulsant properties were also recorded in the plant.Some of the substances identified in the plant possess strong antioxidant properties [5]. However, antioxidant activity has not been done on the plant. Hence during the present investigation methanol, chloroform, and ethyl acetate extracts obtained from leaf of *P. bicolor* to screen the antioxidant potential in detail.

MATERIAL AND METHODS

Pseuderanthemum bicolor plants were collected from Gudemaranahalli,Magaditaluk, Ramanagaram district (13.051951°N, 77.263177°E). The plant was authenticated by late Dr. K. GopalakrishnaBhat, Retd. Professor, Taxonomy Research Centre, Department of Botany, Poornaprajna College, Udupi, Karnataka.Voucher specimen was deposited in the Herbarium of Department of Botany, Bangalore University, Bengaluru.Fresh leaves were collected and washed under the running tap water and shade dried for 8-10 days. The dried leaves were ground into fine powder using mixer grinder. 15 g of powder

was mixed with 90 ml of organic solvents viz., methanol, chloroform and ethyl acetate for 4 h in water bath at 50˚ C. The contents were filtered through the Whatman No.1 filter paper. The extract obtained was allowed to evaporate. The condensed extracts were stored in microcentrifuge vials at 4˚ C till further use [6].

Determination of antioxidant scavenging assay

DPPH Radical scavenging assay

TheDPPH (2,2,– diphenyl-picrylhydrazyl) radical scavenging assay of *P. bicolor* leaf extracts was determined by using the method followed by Rajkumar*etal.* (1994). Various concentration of extracts (6.25, 12.50, 25, 50, 100, 200µg/ml) in methanol were mixed with 75µl of DPPH and incubated at25°C for 15 min in dark.After incubation the absorbance was measured at 590nm using semi-autoanalyzer against control. DPPH with methanol without the extract in the reaction mixture served as blank and Quercetin (0.35, 0.61, 1.25, 2.50, 5, 10 µg/ml) used as reference standard [7].

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

ABTS Radical scavenging assay

The free radical scavenging activity of*P. bicolor* extracts for ABTS (2, 2'– azinobis – 3 ethyl– benzothiazoline – 6 – sulphonic acid) assay was performed as per Auddy*et al.*(2003). 10 ml of ABTS (7Mm) and 10 ml of Ammonium per sulfate (2.45 mM) solutions were mixed and allowed to incubate at room temperature in dark for 16 hrs. The solution thus obtained is further diluted with Phosphate buffered saline to give an absorbance of 1.000 at 734. Fresh ABTS solution was prepared for each assay. Various concentration of extracts(6.25, 12.50, 25, 50, 100, 200 µg/ml) in methanol were allowed to react with 1ml of the ABTS solution and the reaction mixture was allowed to incubation for 10 min in dark, later absorbance was taken at 734 nm using UV–spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of Quercetin standard (0.35, 0.61, 1.25, 2.50, 5, 10 µg/ml) and control reaction was carried out without the test sample. The percentage inhibition of ABTS radical scavenging assay was calculated by using the following formula and IC_{50} value is calculated by Graph pad prism software [8].

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

Hydroxyl Radical Scavenging Assay

As per the modified method of Halliwell*et al*. the scavenging ability for hydroxyl radical was estimated.2.5 mM phosphate buffer solution with pH 7.4 was prepared using dilute de-ionized water and stored at room temperature. Stock solutions of0.1% EDTA, 0.1% $FeCl_3$,1% H_2O_2, 0.1% Ascorbic acid,1% Deoxyribose, 1% Catechin (Reference standard) and plant extracts were prepared using 2.5 mM phosphate buffer solution.The reaction mixture contains 5.6 mMdeoxyribose, 2.8 mM H_2O_2, 40 µM $FeCl_3$, 100 µM EDTAand varying concentrations of plant samples (10, 20, 40, 80, 160, 320 µg/ml) in 2.5 mM phosphate buffer. Initiation of assay was carried out by adding 0.1 mM Ascorbic acid to the reaction mixture.Catechin (0.3, 0.6, 1.2, 2.5, 5, 10 µg/ml) was used as standard and control reaction was carried out without the test sample Then the mixture was incubated at 37° C for 90 mins. After incubation,1ml ofThiobarbituric acid-TBA (0.7 % of TBA in 0.05 N KOH solution) and 1ml of 2.5 % Trichloroacetic acid-TCA (2.5 g of TCA is dissolved in 100 ml of de-ionized water) were added to the reaction volume. The reaction mixture was heated at 100° C for 8 mins, cooled to develop the pink chromogen which was measured at 532 nm. The hydroxyl radical scavenging action of the extract is reported as % inhibition of deoxyribose. Percentage inhibition of hydroxyl radical was calculated using the formula as shown below and $IC_{50}$is calculated by Log-Probit analysis [9].

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

RESULTS AND DISCUSSION

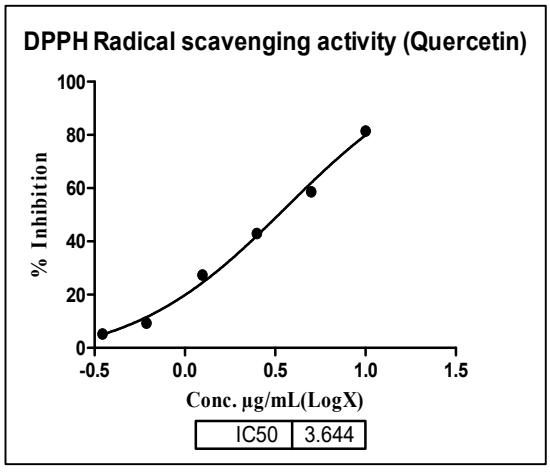
The antioxidant activities of three different leaf extracts of *P. bicolor* (Methanol, chloroform and Ethyl acetate) were estimated by DPPH, ABTS, and Hydroxyl radical scavenging assay. All the different extracts used for screening the antioxidant activityshowed mild antioxidant activity with their respective IC_{50}values than the standard. The DPPH assay of the tested samples showed significant dose dependent level of scavenging activity. Among the three different leaf extracts, the Methanolic extract exhibited

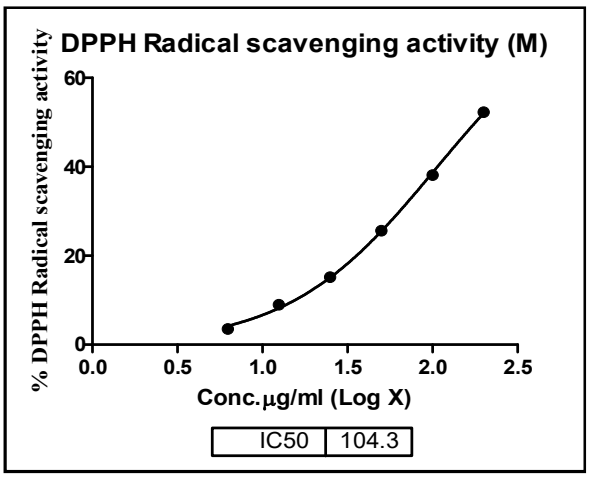
highest DPPH radical scavenging activity with IC_{50} value of 104.30 µg/ml was proved to be the best antioxidant potential than the standard quercetin (Figs:1-4&Table: 1). The presence of antioxidant activity in aqueous leaf extract of *P. palatiferum* having IC_{50} value of 221.14 µg/ml compare to the standard Trolox [10]. Similar observations were made during the present investigation which confirmed with IC_{50} value of 104.30 µg/ml. The antioxidant activity of ethanol leaf extract of *P. palatiferum* with an IC_{50} value of 58.13 µg/ml [11]. The DPPH scavenging activity of methanol leaf extract of *P. palatiferum* along with other Vietnamese medicinal plants was evaluated [12]. The IC_{50} value observed was 9.974 ± 0.602 mg/ml. Methanol leaf extract of *P. malabaricum* revealed highest DPPH free radical scavenging activity with an IC_{50} value of 311.51 µg/ml [13]. The IC_{50} value for DPPH radical scavenging capacity of the methanol leaf extract of *P. reticulatum* was found to be 6.94 µg/ml [14].

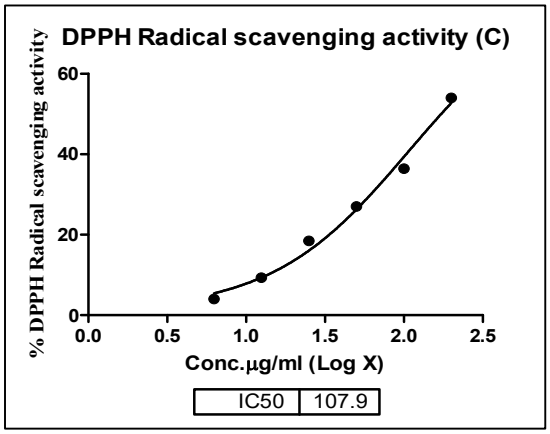
ABTS scavenging activity of all the three leaf extracts and standard increased with the increase in the concentration were shown in Figs: 5-8 & Table: 2.The percentage inhibition of leaf extracts showed highest activity at 200 µg/ml concentration. Ethyl acetate leaf extract demonstrated maximum scavenging activity with IC_{50} value of 79.06 µg/ml while, the IC_{50} values of methanol and chloroform leaf extracts were found to be 116.40 µg/ml and 111.70 µg/ml respectively. In the present study ethyl acetate extract is highly potent in neutralizing ABTS cation radicals and has significant scavenging effect with IC_{50} value of 79.06 µg/ml. Similar observation was recorded in *Asystasiatravancorica* with IC_{50} value of 17.94 µg/ml in ethyl acetate and 24.97 µg/ml in methanol extract [15]. The different solvent leaf extracts of certain species of Acanthaceae like *Peristophebibicalyculata* and *Nilgirianthusheyneanus* showed their respective antioxidant activity with an IC_{50} values of 4178 µg/ml and 438.7 µg/ml respectively [16]. In *Rungiarepens*, the methanolic leaf extract revealed the ABTS radical scavenging activity with an IC_{50} value of 69.26 µg/ml. But in *P. bicolor* the ABTS radical scavenging activity is minimum as compare to standard quercetin [17].

Hydroxyl radical scavenging activity ofall the three leaf extracts and standard is represented in Figs: 9-10 & Table: 3-4. Among the solvents tested, the percentage inhibition ofleaf extracts showed 54.94% and is maximum at 320 µg/ml concentration. Methanol, Ethyl acetate and Chloroform extracts showed IC_{50} value of 267.8 µg/ml and 305.7 µg/ml and 285.0 µg/ml while standard catechin showed 8.927 µg/ml(Figs: 9-10 & Table: 3-4).Hydroxyl radical scavenging activity can be measured using the deoxyribose assay, a Fenton type reaction system which is considered one of the best-known models for determining the hydroxyl radical scavenging activity. Methanol leaf extract of *P. bicolor* exhibited substantial activity with particular IC_{50} value. Comparable remark was viewed in *Asystasiatravancorica* with an IC_{50} value of 18.06 µg/ml from methanol leaf extract [15].

Fig. (1-4): DPPH scavenging activity of different solvent extracts of *P. bicolor*







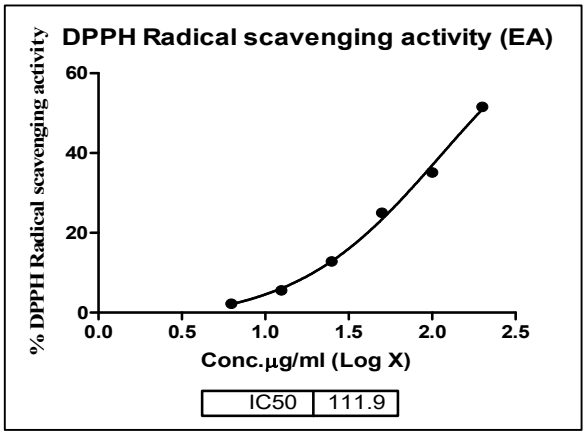
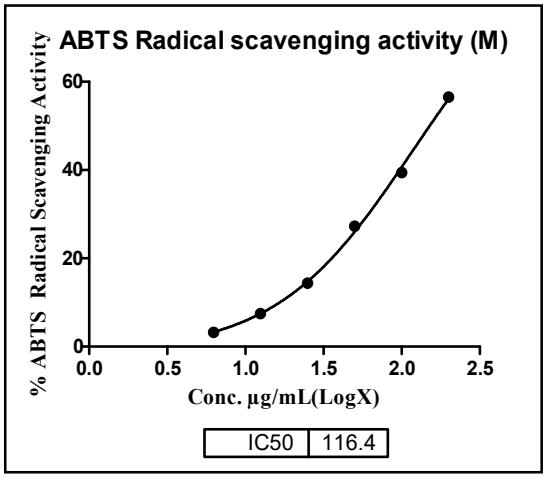
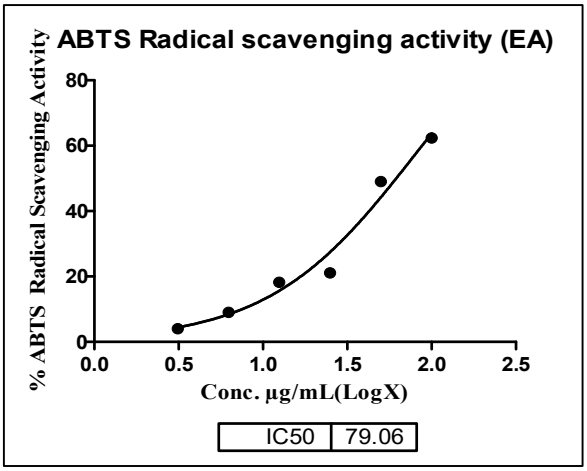
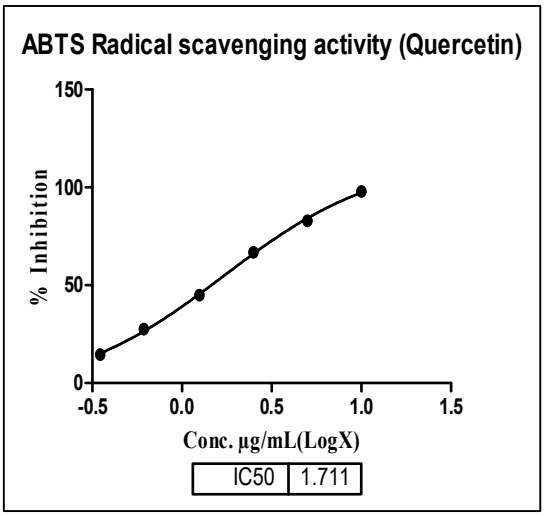


Fig. (5-8): ABTS scavenging activity of different solvent extracts of *P. bicolor*







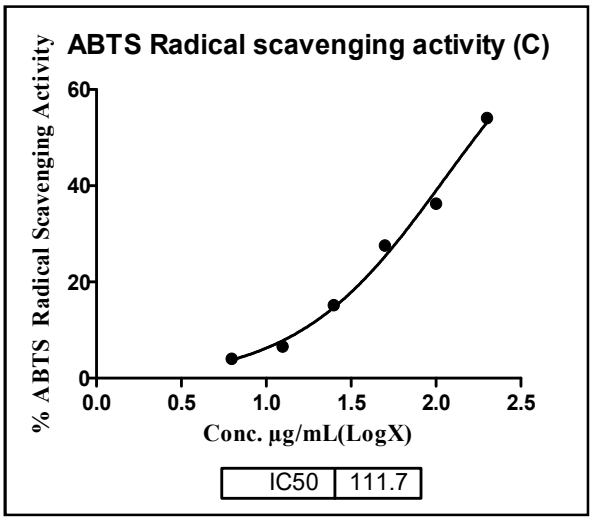


Fig. (9-10): Hydroxyl radical scavenging activity of different solvent extracts of *P. bicolor*

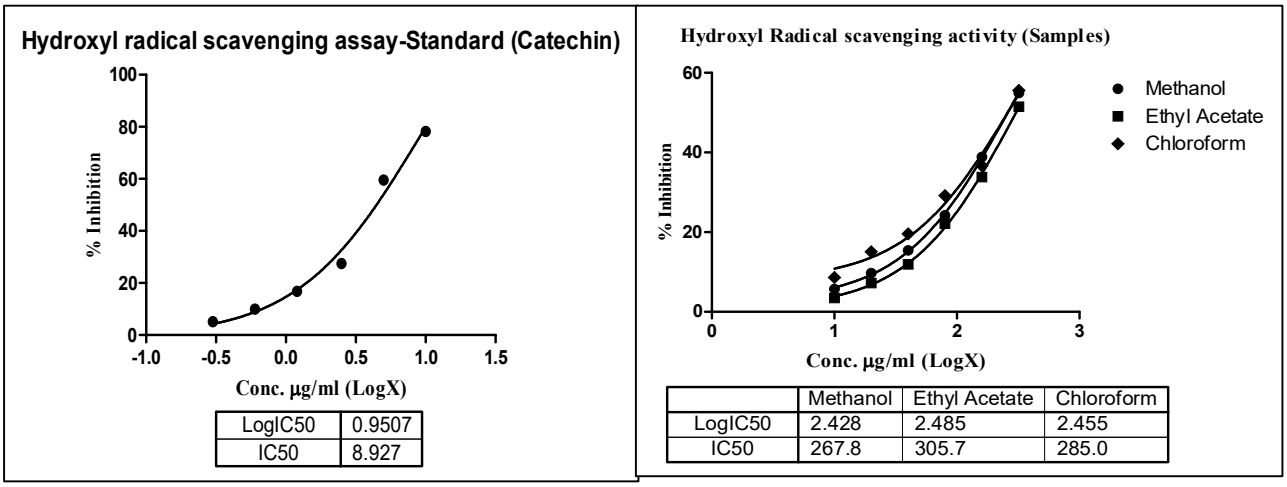


Table 1: IC_{50} values of DPPH assay of different solvent extracts of *P. bicolor*

Sample Name	Conc. (µg/ml)	Absorbance (510nm)	% Inhibition	IC50 µg/mL
Control	0.00	0.32	0.00	
Quercetin	0.35	0.31	5.24	
	0.61	0.29	9.30	
	1.25	0.23	27.41	3.64
	2.50	0.18	42.97	
	5.00	0.13	58.60	
	10.00	0.06	81.43	
M	6.25	0.31	3.50	
	12.50	0.29	8.96	
	25.00	0.27	15.19	
	50.00	0.24	25.64	104.30
	100.00	0.20	38.11	
	200.00	0.15	52.28	
C	6.25	0.31	4.03	
	12.50	0.29	9.30	
	25.00	0.26	18.48	
	50.00	0.24	27.01	107.90
	100.00	0.21	36.40	
	200.00	0.15	54.01	
EA	6.25	0.32	2.20	
	12.50	0.30	5.55	
	25.00	0.28	12.83	
	50.00	0.24	25.05	111.90
	100.00	0.21	35.13	
	200.00	0.16	51.56	

Table 2: IC_{50} values of ABTS assay of different solvent extracts of *P. bicolor*

Sample Name	Conc.(µg/ml)	Absorbance	% Inhibition	IC50 ug/mL
Control	0.00	0.75	0.00	
Quercetin	0.35	0.64	14.53	1.71
	0.61	0.54	27.57	
	1.25	0.41	44.90	
	2.50	0.25	66.81	
	5.00	0.13	82.88	
	10.00	0.02	97.96	
M	6.25	0.73	3.20	116.40
	12.5	0.69	7.49	
	25	0.64	14.39	
	50	0.54	27.30	
	100	0.45	39.40	
	200	0.33	56.53	
C	6.25	0.72	4.02	111.70
	12.5	0.70	6.56	
	25	0.64	15.19	
	50	0.54	27.56	
	100	0.48	36.26	
	200	0.34	54.09	
EA	3.125	0.72	4.03	79.06
	6.25	0.68	9.05	
	12.5	0.61	18.26	
	25	0.59	21.11	
	50	0.38	49.04	
	100	0.28	62.36	

Table (3-4): IC_{50} values of Hydroxyl radical scavenging assay of different solvent extracts of *P. bicolor*

Plants Name	Conc. (µg/ml)	Absorbance 590nm	% Inhibition	IC_{50} µg/ml
Control	0.0	0.518	0.00	
Standard (Catechin*)*	0.3	0.492	5.11	8.927
	0.6	0.467	9.92	
	1.2	0.431	16.78	
	2.5	0.376	27.43	
	5	0.209	59.56	
	10	0.113	78.19	

Compound name	Concentration (µg/ml)	Absorbance 590nm	% Inhibition	IC_{50} µg/ml
Control	0	0.628	0	
Methanol	10	0.592	5.73	**267.8**
	20	0.567	9.71	
	40	0.531	15.45	
	80	0.476	24.2	
	160	0.409	34.87	
	320	0.283	54.94	
Ethyl acetate	10	0.586	3.46	**305.7**
	20	0.567	7.21	
	40	0.531	11.93	
	80	0.476	26.73	
	160	0.389	35.82	
	320	0.303	51.49	
Chloroform	10	0.574	8.60	**285.0**
	20	0.527	16.08	
	40	0.461	26.59	
	80	0.426	32.17	
	160	0.379	39.65	
	320	0.279	55.57	

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