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In vitro antioxidant and free radical scavenging potential of *Cyanotis fasciculata* var. *fasciculata*

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ABSTRACT

Being encouraged by the results of qualitative analysis followed by quantification studies of antioxidants found in various extracts of *Cyanotis fasciculata*, the present study was designed to assess the free radical scavenging activity of 70% hydro alcoholic (AE) and methanolic extracts (ME) of this plant through DPPH and hydroxyl radical scavenging activities, in addition to ferric reducing assay were performed and IC_{50} values were calculated. Our results enunciated promising antioxidant potential of both the extracts.

Key words: *Cyanotis fasciculata*, DPPH radical, hydroxyl radical, reductive ability, IC_{50}

INTRODUCTION

Free radicals, especially Reactive Oxygen Species (ROS) are undesirable but inevitable emitants of aerobic metabolic pathways. These being highly reactive can initiate & propagate many disastrous chain of interactions such as lipid peroxidation, denaturation of polypetides, fragmentation of nucleic acid strands etc., leading to extensive cellular damage that culminates in to quite a lot of denegenerative diseases ranging from inflammation to ischemic heart disease and cancer[1]. It is well documented that plant phenolics, tannins and flavonoids can efficiently quench these larcenous free radicals and retards the process of oxidative damage to tissues and organs[2]. Hence, this has become impetus for search of herbal phytoconstituents with antioxidative and organ protective actions[3].

Cyanotis fasciculate var., fasciculata (Commelinaceae) is a small, terrestrial, annual herb of 4-10 inches long at branches, commonly found on dry grass lands and rocks. Flowers blue, purple or pink in colour and are present in auxiliary or terminal position with 3 petals united into a tube below. Stems are slender, slight pinkish, with cottony cob webby appearance. Leaves are broadly ovate to narrowly linear usually obtuse, juicy, woolly Cob-Webby[4, 5, 6].

The juice from succulent leaves used to treat skin fungus disease and mouth sores[7,8]. The hydro alcoholic extract of entire plant is reported to be useful in lymphatic leukemia, possess diuretic and antiviral properties[9,10].

In the earlier phase of our research work various extracts of this plant were subjected to qualitative analysis for phytoconstituents and quantitative estimation of Total Phenolics, Total Flavonoids & Total Antioxidant Activity etc., in which studies AE & ME appeared superior to all extracts in contest with antioxidant activity. Hence in the present study an attempt is made to establish their free radical scavenging activity in some *in vitro* models.

Material and methods

Plant Material

The plants of *Cyanotis fasciculata* were collected from Fort-hill top of Bellary, Karnataka in the month of September and were authenticated by Dr. Kotresh, Department of Botony, Karnataka University, Dharwad, Karnataka. The voucher specimens of these plants were preserved in the herbarium of the pharmacognosy department of this institution.

Extraction

The plants were air-dried in shade and were pulverized in a mechanical grinder to cottony lumps. The powder was exhaustively extracted with 70% hydroalcohol(AE) and methanol (ME) individually by soxhlation; extracts were dried in rotary vacuum evaporation and relevant yields were calculated and stored in airtight containers at 4^0C. The different concentrations of extracts were prepared using corresponding solvents for free radical scavenging activities.

EXPERIMENTAL

DPPH radical scavenging activity

The free radical scavenging activity of ME and AE were measured by 1, 1 – diphenyl di-picryl-hydrazil (DPPH) using the method of Singh et al[11] (0.1mM solution of DPPH in methanol was prepared and absorbance was measured at 517nm using Shimadza model 150-02 double beam spectrophotometer, referred as absorbance of control reaction). DPPH reagent radily forms free radicals in solution. An antioxidant i.e. free radical scavenger reduces DPPH radical and the extent of violet colour reduction of DPPH is directly proportional to the free radical scavenging activity.

Different concentration (10µg to 500µg) of extracts and BHA as standard in 100 µl methanol were taken and added with 5mL of 0.1mM DPPH in methanol. Shaken vigorously and allowed to stand at 27^0C for 20 minutes. Later the absorbance was measured at 517 nm in spectrophotometer using DPPH solution as blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Scavenging activity is expressed as the inhibition percentage calculated using the equation: % Antioxidant activity = {(control Abs-Samble Abs)/control Abs} × 100. Each experiment was carried out in triplicate and results averaged, expressed as mean % antiradical activity ± SD.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of ME and AE were studied by using method of Singh et al[11]. To various concentrations of (10 to 25µg) of extracts and BHA made upto 250 µl with 0.1m phosphate were added with 1mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA (0.018%) and 1mL of Dimethyl sulphoxide (0.85% v/v in 0.1m phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL ascorbic acid (0.22%) and reaction mixtures were incubated at room temperature for 15 minutes. Later the reaction was terminated by adding 1ml of ice cold TCA (Tricholroacetic acid 17.5% w/v). To all reaction mixtures 3mL of Nash reagent (150 g of ammonium acetate, 5ml of glacial acetic acid and 2ml of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15 minutes for yellow colour development, the intensity of which was measured spectrophotometrically at 412 nm against reagent blank. The percentage of hydroxyl scavenging activity was calculated by using the equation: 1 – [difference in Abs. of sample / difference in Abs. of blank] × 100. Each experiment was carried out in triplicate and results averaged expressed as mean ± SD.

Reducing ability

Reducing power of ME and AE were determined according to the method of Barreira et al[12] using BHA as standard compound. Various concentration of extracts (10 µg – 500 µg) were mixed with phosphate buffer (2.5 mL, 0.2m, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50^0C for 20 minutes. A portion (2.5 ml) of trichloro acetic acid (10% w/v) was added to the mixture which was then contrifused at 3000 rpm for 10 minutes. The upper layer of the solution (5 mL) was mixed with distilled water (5 mL) and $Fecl_3$ solution (1mL, 0.1%) and the absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of reaction mixture indicated increasing reducing power. All the analyses were performed in

triplicate and the results were averaged, expressed as mean reducing ability ± SD.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH is unstable nitrogen centered free radical that accepts an electron or hydrogen radical from suitable antioxidants and gets reduced to stable dia-magnetic molecule along with stiochiometric loss of colour. This phenomenon has been widely used by researchers as a quick and reliable parameter to assess the in vitro antioxidant activity of crude extracts[13]. From the radical scavenging potency of AE and ME shown in Table 1, it is clear that AE exhibited a dose dependent activity and the IC_{50} values of AE and ME : 208.62 µg/ml and 313.45 µg/ml, claims that AE is more efficient is scavenging DPPH radical than ME.

Hydroxyl radical scavenging effect

It is a most common, extremely reactive, highly damaging reactive oxygen species generated in our body that can adversely affect almost all components of cells. It is evident from results of hydroxyl scavenging activity that both extracts exhibited almost equipotent scavenging activity. However, AE had slightly better IC50;194 µg/ml than ME i.e., 230.40 µg/ml.

Reductive ability

The antioxidant activity has been reported to be concomitant with reducing power of extracts in their ability to donate hydrogen to reduce Fe^{3+} to Fe^{2+}. In contrast to results of earlier methods, in this antioxidant activity ME with IC_{50} 260 µ g/ml appeared better than AE (IC_{50} 520 µg/ml). This may be attributed to the ability of methanol to draw more reductones , which are associated with reducing ability.

Table 1: Free radical scavenging activity of AE and ME in some *in vitro* methods

Conc. in µg	% DPPH radical scavenging		% Hydroxyl scavenging radical		% Reducing ability	
	AE	ME	AE	ME	AE	ME
12.25	2.01 ± 0.24	2.21 ± 0.89	12.20 ± 1.21	8.81 ± 1.89	10.01 ± 0.66	11.85 ± 1.11
25.00	4.46 ± 1.02	5.46 ± 1.11	18.33 ± 0.89	22.84 ± 1.67	-	-
50.00	14.79 ± 0.73	15.78 ± 0.39	22.14 ± 0.91	33.18 ± 0.83	16 ± 0.89	18 ± 1.25
100.00	28.31 ± 1.36	25.67 ± 2.25	34.12± 1.39	39.42 ± 2.01	21 ± 0.75	34 ± 1.45
200.00	49.01 ± 0.46	37.54 ± 1.91	50.96 ± 1.77	49.04 ± 2.31	28 ± 0.68	43 ± 1.55
400.00	-	-	-	-	39 ± 0.90	64 ± 1.60
IC_{50}	208.62	313.45	194	230.40	520	260

CONCLUSION

By the virtue of appreciable quantities of antioxidants, both extracts exhibited good free radical scavenging activities in the *in vitro* models employed. Hence, this has offered enough scope to subject AE and ME to Biological screening for its organ protective role in various *in vivo* models involving free radical mediated pathogenesis.

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