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Research Article

SCRUTINY OF VARIOUS LEAF EXTRACTS OF *CARALLUM FIMBRIATA* FOR ANTIOXIDANT ACTIVITY IN WISTAR RATS

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ABSTRACT

Caralluma fimbriata is an edible succulent cactus that belongs to *Asclepiadaceae* family which is called as famine food. Native Indian diets have comprised these edible wild succulent cacti over many centuries. It has found to be useful for remedy of diabetes, obesity and heart problems. Antioxidant activity of aqueous and ethanol extracts of *Caralluma fimbriata* was evaluated by reckoning of lipid peroxide level in brain and liver homogenate of rats tissues and appraising of hydroxyl radical scavenging through deoxy ribose assay. The results obtained from assessment manifested leaves of *Caralluma fimbriata* possessed substantial antioxidant activity by declining in level of lipid peroxidation and hydroxyl radical activity. Fifty percentage of inhibition concentration was perceived to be 630 µg/ml and 487 µg/ml for aqueous and ethanol extracts of *Caralluma fimbriata* respectively for lipid peroxidation in brain homogenates. IC_{50} values of aqueous and ethanol extracts of *Caralluma fimbriata* was found to be 800 µg/ml and 781 µg/ml respectively for lipid peroxidation assay in liver homogenates. IC_{50} values of aqueous and ethanol extracts of *Caralluma fimbriata* was found to be 746 µg/ml and 662 µg/ml respectively for hydroxyl radical scavenging activity. The current research on *Caralluma fimbriata* extracts exhibits prospective antioxidant activity so it can be considerable beneficial for remedy of Alzheimer's disease, Parkinson disease and cancer as nutritional supplements.

Keywords: lipid peroxidation, hydroxyl radical scavenging, nutritional supplements.

INTRODUCTON

Oxidative stress reflects an imbalance between the systemic manifestation of reactive nitrogen species or oxygen species and a biological system's ability (antioxidants) to readily detoxify the reactive intermediates or to repair the resulting damage. The mechanism of action of many synthetic drugs includes free radical scavenging, which defends against oxidative damage but it has also adverse effects[1]. The consumption of plant products are worthwhile due to have reactive compounds especially phenolic substances. The antioxidant activity of these phytoconstituents can initiate the stimulation of cellular defence system and biological system against oxidative damage[2]. Ergo, current investigation was designed to estimate the antioxidant activity of aqueous and ethanol extracts of leaves of *Caralluma fimbriata* by assessing of lipid peroxide level in liver and brain homogenate of rats tissues and evaluating of hydroxyl radical scavenging through deoxy ribose assay.

MATERIALS AND METHODS

Plant material and Preparation of extracts

The leaves of *Caralluma fimbriata* were collected from Chennai, Tamil Nadu, India and authenticated by Green Chem of India, Doddinduvadi district, Bangalore, Karnataka, India. The fresh leaves of *Caralluma fimbriata* were washed with tap water and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The leaves powder was macerated in the solvents including ethanol 95% (v/v) and water at room temperature, undergoing mechanical shaking for 4 hours followed by filtration. The extracts acquired were concentrated in a rotary evaporator at 40°C and the residue was extracted twice again analogously, there by obtaining the crude solvent extracts.

Chemicals

All chemicals were analytical grade and obtained from local store of Visveswarapura Institute of Pharmaceutical Sciences.

Animals

Female Albino Wistar rats (180-220gm) were obtained from the NIMHANS animal house, Bengaluru and were housed at room temperature in a well-ventilated animal house under 12 hrs light / dark cycle in polypropylene cages (29"x22"x14") with stainless steel grill top, bedded with paddy husk. The animals were maintained under standard conditions in an animal house as per the guidelines of "Committee for the Purpose of Control and Supervision on Experiments on Animals" (CPCSEA) for at least one week prior to use. The rats had free access to standard rat chow and water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. (Registration No: 152/1999, renewed in 2012).

Estimation of lipid peroxide level in brain and liver homogenate of rat's tissues

Appraisal of lipid peroxidation formed by Fe2+- ascorbate system in rat brain and liver homogenate was performed by

TBA reaction method. The rat brain/liver homogenate 0.1 ml (25% w/v) was mixed with potassium chloride (30 mM), Tris–HCL buffer (20 mM, pH 7.0), ascorbate (0.06 mM), ferrous ammonium sulphate (0.16 mM), and various concentrations of aqueous and ethanol extracts of *Caralluma fimbriata* (50, 100, 200, 400, 800, 1000 µg/ml) in a final volume of 0.5 ml. Incubated for 1 h at 37 ^{o}C. Then a portion (0.4 ml) of mixture was removed and combined with 1.5 ml glacial acetic acid (20%, pH 3.5), 0.2 ml sodium dodecyl sulphate (SDS) (8.1%) and 1.5ml TBA (0.8 %). The Total volume was made up to 4 ml with distilled water and then kept in a water bath at 95-100^{o} C for 1 h. Added 0.5 ml of n- butanol, pyridine mixture (15: 1, v/v) and 1.0 ml of distilled water and mixture was shaken vigorously and was centrifuged at 4,000 g for 10 minutes. The organic layer was removed and measured absorbance at 532 nm. The % inhibition and IC_{50} was calculated[3].

Hydroxyl radical scavenging

Hydroxyl radical scavenging activity was determined by using the deoxy ribose assay. A portion (20µl) of various concentration of extract sample (50, 100, 200, 400, 800, 1000 µg/ml) was mixed with 35 µl of following solution: ascorbate (1.2 mM), EDTA (1.2mM), $FeCl_3$ (300µM), H_2O_2 (33.6 mM) and phosphate buffer (120mM). Added 35µl of deoxy ribose to above mixture then centrifuged at 3200 rpm for 1 minute. Incubated at 37^{o}C for 1 minute. Cooled and again centrifuged at3200 rpm for 1 minute. The centrifuged tubes were placed in boiling water for 20 minutes and then cooled for 20 minutes. Added 1120 µl of 1-butanol and consequently centrifuged for 3200 rpm for 6 minutes. Upper layer was collected and measured absorbance at 532 nm using 1- butanol as blank. The % scavenging and IC_{50} was calculated[4].

RESULTS

Lipid peroxidation

Table 1 and 2 show the effect of aqueous and ethanol extracts of *Caralluma fimbriata* on percentage inhibition of lipid peroxidation level in brain and liver homogenates of rat tissues respectively. Lipid peroxides generated by induction of ferrous/ascorbate on rat brain/liver homogenates was found to be inhibited by aqueous and ethanol extracts of *Caralluma fimbriata*. The extracts exhibited better lipid peroxide inhibition activity in brain homogenate compared with liver therefore it indicated that it was more effective in brain. Ethanol extract of *Caralluma fimbriata* manifested superior lipid peroxide inhibition activity with IC_{50} value of 487 µg/ml and 781 µg/ml for brain and liver homogenate respectively compared to IC_{50} value of 630 µg/ml and 800 µg/ml of aqueous extract of *Caralluma fimbriata* for brain and liver homogenate respectively.

Table 1: Effect of aqueous and ethanol extracts of *Caralluma fimbriata* on percentage of lipid peroxidation level in brain homogenates of rat tissues

Sl. No.	Concentration (µg/ml)	% inhibition	
		Aqueous extract	Ethanol extract
1	50	5.97	8.41
2	100	10.39	15.09
3	200	20.09	32.87
4	400	46.79	59.45
5	800	62.13	74.62
6	1000	71.76	82.53
IC_{50}		**630 µg/ml**	**487 µg/ml**

Table 2: Effect of aqueous and ethanol extracts of *Caralluma fimbriata* on percentage of lipid peroxidation level in liver homogenates of rat tissues

Sl. No.	Concentration (µg/ml)	% inhibition	
		Aqueous extract	Ethanol extract
1	50	3.45	4.04
2	100	6.08	7.65
3	200	12.65	14.23
4	400	24.67	28.23
5	800	48.89	53.54
6	1000	64.56	62.21
IC_{50}		**800 µg/ml**	**781 µg/ml**

Table 3: Effect of aqueous and ethanol extracts of *Caralluma fimbriata* on hydroxyl radical scavenging activity

Sl. No.	Concentration (µg/ml)	% inhibition	
		Aqueous extract	Ethanol extract
1	0	0	0
2	50	6	8
3	100	10	13
4	200	19	24
5	400	40	46
6	800	53	59
7	1000	61	66
IC_{50}		**746 µg/ml**	**662 µg/ml**

Hydroxyl radical scavenging activity of aqueous and ethanol extract of *Caralluma fimbriate*

Table 3 displays the hydroxyl radical scavenging activity of aqueous and ethanol extract of *Caralluma fimbriata*. Degradation of deoxy ribose mediated by hydroxyl radicals which is generated by Fe^{3+} / ascorbtae/ EDTA/ H_2O_2 system was found to be prohibited by aqueous and ethanol extract of *Caralluma fimbriata*. Both extracts displayed hydroxyl radical scavenging activity with different concentration of 50, 100, 200, 400, 800 and 1000 µg/ml in dose dependent manner. Fifty percentage of inhibition concentration values (IC_{50}) of aqueous and ethanol extracts of *Caralluma fimbriata* was found to be 746 µg/ml and 662 µg/ml respectively for hydroxyl radical scavenging activity. Hence, ethanol extract of *Caralluma fimbriata* indicated great hydroxyl radical scavenging activity compared with aqueous extract of *Caralluma fimbriata*.

DISCUSSION

Pervious investigation revealed that major aliment of 21st century for instance neurodegenerative disease, AIDs, cancer, rheumatoid arthritis, cardiovascular disease, hepatic disorder, etc., are related with insufficient of level of antioxidant compounds in human body system. Natural antioxidant compounds in body are classified in to two group; (1) Enzymatic antioxidant such as SOD, CAT and GSH or (2) Non-enzymatic antioxidant for example vitamin C or vitamin E[5]. Phenolic compounds is major responsible bioactive constituents for antioxidant activity of plants. Phenolic compounds exhibit free radical scavenging activities by reason of having special structure which can deactivate free radical either by donating a hydrogen atom (an electron) to a free radical through the hydroxyl group or by delocalizing an unpaired electron by owing the resonance structure so free radicals cannot be available for damaging lipid, DNA and protein of cells[6]. The previous phytochemical evaluation of *Caralluma fimbriata* indicated the presence of phenolic compound such as flavonoids which could be assumed to be responsible for antioxidant activity[7].

CONCLUSION

The current scrutiny exhibited that aqueous and ethanol extracts of *Caralluma fimbriata* have antioxidant activity by reason of scavenging of hydroxyl radical and inhibiting of lipid peroxidation. Consequently, ethanol extract of *Caralluma fimbriata* displayed superiority antioxidant activity as compared to aqueous extract of *Caralluma fimbriata*.

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