Research Article

Antioxidant *(Invitro)* studies on the flowers of Urticularia *reticulata*

PL. Rajagopal[1*], K. Premaletha[2], SS. Kiron[3], KR. Sreejith[4], Robin Jose[4] and PN. Sajith Kumar[1]

[1]Depatment of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala, India.
[2]Department of Pharmaceutics, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala, India.
[3]Depatment of Pharmacy Practice, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala, India.
[4]Department of Pharmaceutical Chemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala, India.

ABSTRACT
Antioxidant studies were carried out for methanolic extract of the flowers of *Urticularia reticulata*. The methanolic extract of the flower was anlysed for antioxidant activity by nitric oxide radical scavenging and DPPH methods at different concentrations. Throughout the studies flower extract showed potent antioxidant activity.. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of high flavanoid content in the flowers of *Urticulariareticulata*
Keywords: *Urticularia reticulata,* Antioxidant activity, Nitricoxide scavenging, DPPH.

INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number of antioxidants detected no benefit and even suggested that excess supplementation with certain putative antioxidants may be harmful[7].

MATERIALS AND METHODS

Source of plant

Flowers of *Utricularia reticulata* were collected from the hilly areas of Madaippara, Pazhayangadi, Kannur District, Kerala State, south India in the month of October-November 2012 . It was then shade dried and its botanical identity was confirmed.

Preparation of the Extract

The shade dried flowers were finely powdered and extracted with 80% aqueous methanol using Soxhlet apparatus at 55^{o}C. The soluble part was concentrated over water bath maintained below 60^{o}C and dried in a vacuum oven. The crude flower extract thusobtained is used for anti oxidant screening

Evaluation of Antioxidant property

Reduction of 1, 1- Diphenyl- 2- Picryl Hydrazyl (DPPH) free radical

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- diphenyl-2-picryl hydrazine. The ability to scavenge the free radical, DPPH was measured in the absorbance at 517 nm.[1]

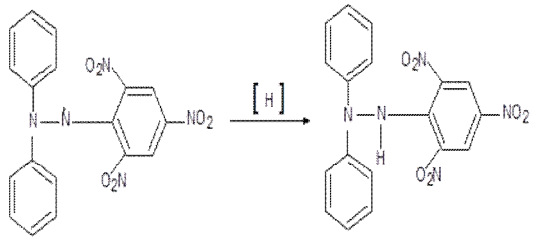

Fig.1: Reduction of DPPH free radical

To the 1ml of various concentrations of methanolic extract in a test tube, 1ml of solution of DPPH 0.1 mM (0.39 mg in 10ml methanol) was added to the test tube. An equal amount of ethanol and DPPH were added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate.

Nitric Oxide Scavenging Activity.

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates NO_2, N_2O_4 and N_3O_4. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, which is measured at 546 nm.[2]

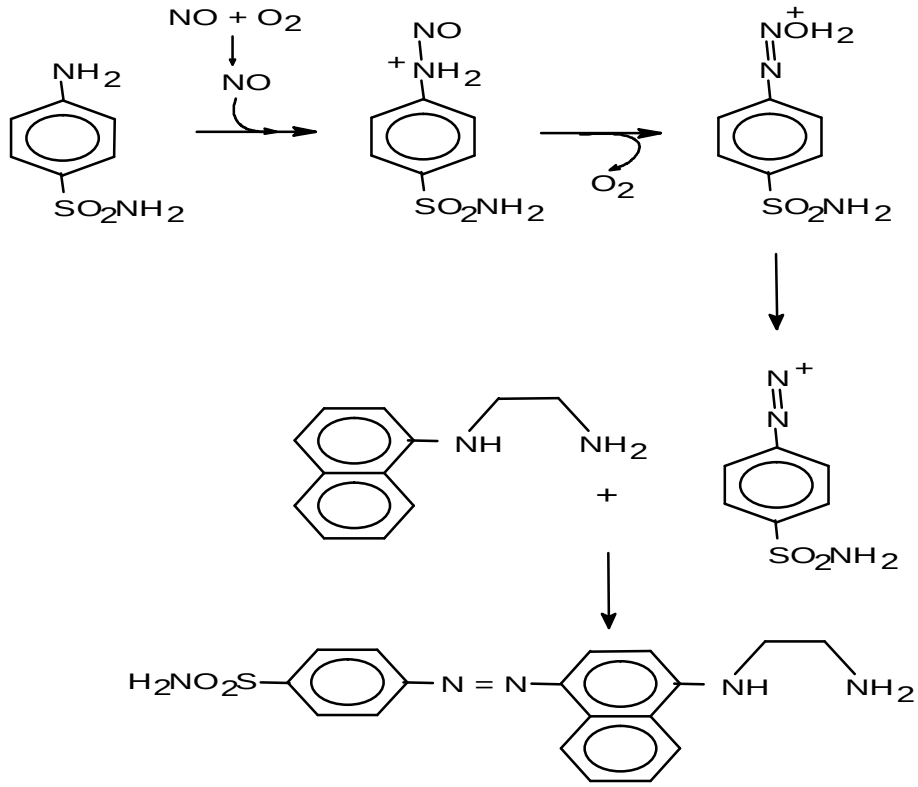

Fig. 2: Griess reaction

Sodium nitroprusside 5mM (0.0373g in 25 ml) was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of the extract, 0.3 ml of sodium nitroprusside was added in the test tubes. The test tubes were incubated at 25°C for 5hr. After 5hrs, 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Table 1: Effect of alcoholic extract of *Utricularia reticulata* flowers on DPPH scavenging

S.No.	Conc. µg/ml	Alcoholic extract		Ascorbic acid	
		Abs	% Sca.	Abs	% Sca.
1	5	0.889	1.49	0.803	9.65
2	10	0.831	7.72	0.635	28.33
3	15	0.785	13.25	0.424	52.15
4	25	0.611	32.59	0.089	89.93
5	50	0.469	48.17	0.058	93.42
6	100	0.238	73.72	0.036	95.89
7	250	0.132	85.52	0.034	96.09
8	500	0.114	87.51	0.036	95.89
9	1000	0.102	88.83	0.029	96.65
10	Control	0.905		0.887	

Table 2: Effect of alcoholic of *Utricularia reticulata* flowers on NO scavenging

S. No.	Conc. µg/ml	Alcoholic extract		Ascorbic acid	
		Abs	% Sca.	Abs	% Sca.
1	5	0.726	2.68	0.629	9.32
2	10	0.689	7.64	0.608	12.29
3	15	0.626	16.08	0.515	25.75
4	25	0.598	19.83	0.317	54.31
5	50	0.528	29.22	0.216	68.77
6	100	0.452	39.41	0.121	82.56
7	250	0.328	56.03	0.084	87.87
8	500	0.286	61.66	0.062	91.03
9	1000	0.254	65.95	0.009	98.67
10	Control	0.746		0.694	

The DPPH system is a stable radical generating procedure[3]. DPPH is a potent scavenger for any other radicals , due to the easiness in following the procedure – violet colour of DPPH faints into the yellow colour of its reduced congener , with a high shift in the visible spectra (from 520 nm to 330nm)[4].

Sodium nitroprusside serves as a chief source of free radicals. Scavengers of nitric oxide compete with oxygen leading to reduced formation of Nitric Oxide. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as the marker for NO scavenging activity[5]. The chromophore formation was not complete in the presence of MECA, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the MECA extract increases in a dose dependent manner[6].

Utricularia reticulata is widely used in number of pharmacological actions with high content of flavonoids seems to have a high potential for antioxidant activity.

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