



IN VITRO* ANTI-OXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF THE PLANT *UTRICULARIA RETICULATA

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Abstract

Utricularia reticulata (Family: Lentibulariaceae) commonly known as kaakka poovu in Malayalam and kaage kannu in kannada, is a sub-scandent glabrous herb commonly found on wet rocky areas or in the paddy fields during the month of September to November. The present work was aimed to evaluate the antioxidant activity of the ethanolic extract of the whole plant of *Utricularia reticulata* by using *In vitro* methods such as DPPH assay, Superoxide scavenging assay, Nitric oxide scavenging assay, Hydrogen peroxide scavenging assay, and Total antioxidant activity. The preliminary phytochemical studies indicated the presence of steroids and flavonoids. The ethanolic extract of the plant gave positive results for anti-oxidant activity. The extracts were evaluated from the concentration range of 20µg/ml – 100µg/ml. The extract showed the concentration dependent activity. At the dose of 100µg/ml it showed maximum activity.

Key word: *Utricularia reticulata*, *in vitro* antioxidant studies, *in vitro* anti-inflammatory studies.

Introduction

Free radicals or reactive oxygen species (ROS) are the molecules containing unpaired electrons in their atomic orbital, mainly produced by the normal metabolic process of the body (Lobo V. 2010). The superoxide anion is formed as the side product of various stages in electron transport chain (Aruoma O.I. 1998). There are different types of reactive oxygen species like hydrogen peroxide, (H₂O₂), hypochlorous acid (HClO) and free radicals such as the hydroxyl radical (.OH) and the superoxide anion (O₂⁻). Among them hydroxyl radical (.OH) is highly reactive (Devasagayam T P A 2004). These radicals may react with some of the important biomolecules like carbohydrates, DNA, lipids and proteins which may lead to cell damage and homeostatic disruption. DNA damage may cause mutations and in some cases cancer, if the reaction is not reversed by DNA repair mechanism. On the other hand on reacting with proteins causes denaturation and protein degradation (Lamson, D.W. 1999).

Antioxidants are the form which is the defensive mechanism of our body against the free radicals. There are a huge variety of naturally occurring antioxidants in

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nature which are having different compositions, different physical and chemical properties, different mechanism and site of action. Few of them are enzymes (SOD, etc.), high molecular weight compounds (proteins like albumin), low molecular weight compounds (tocopherol, ascorbic acid, etc.), minerals and vitamins. The equilibrium between the generated free radical as well as the antioxidants are always maintained in the body. If any disturbances occur in this equilibrium, it leads to oxidative stress. This leads to a number of pathological diseases such as aging, cancer, diabetes, atherosclerosis and other neurodegenerative disorder (Geronikaki A.A. 2006).

Utricularia reticulata (Family: Lentibulariaceae) commonly known as kaakka poovu in Malayalam and kaage kannu in Kannada, is a sub-scandent glabrous herb commonly found on wet rocky areas or in the paddy fields during the month of September to November. The herbs have been used traditionally in the treatment of urinary tract infections, as astringent, diuretic, and are also known to be used as poultice on wounds (Gossell-Williams 2006). The present study was designed to evaluate the antioxidant activity of the plant using *In vitro* methods like DPPH assay, Nitric oxide assay, Hydrogen peroxide assay, Superoxide assay and Total anti-oxidant activity.

Materials and Methods

Collection of plant material

The plants are collected from in and around Madaayipara, Kannur (dist), Kerala; in the month of July and August 2016. The authentication of plant was done by Dr. Nagalakshamma, Dept. of Botany, Aloysius College, Mangalore. The cleaned plant materials were dried in the shade for about 2 weeks and then it was ground to coarse powder.

Preparation of extracts

The powdered plants were subjected to cold maceration extraction using ethanol as the solvent. Cold maceration was done in 3 parts of the plant powder for 9 days (Devasagayam T P A 2004). After the extraction, the remaining solution was cooled in room temperature and was filtered using a muslin cloth. The ethanolic extract thus obtained was concentrated and evaporated on a water bath. Thus obtained dried extract has been kept in a desiccator.

Preliminary phytochemical analysis

The preliminary qualitative phytochemical investigation of the ethanolic extract of *Utricularia reticulata* was carried out to detect the active constituents existing in the plant as per standard procedure (Kokate CK 1995).

Screening of antioxidant activity by *in vitro* techniques

1, 1- Diphenyl-2- picrylhydrazyl radical scavenging activity (Premnath R 2010):

Different concentrations of test extract as well as the standard have been prepared. Ascorbic acid was taken as the standard. Incubate 3ml of different concentrations of the test extract along with 3ml of 100mM methanolic solution of DPPH taken in a test tube for 30 minutes in darkness at the ambient temperature. After incubation, the absorbance of the reaction mixture was recorded at 517nm. Following formula was used to calculate percentage inhibition:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}} / \text{Abs}_{\text{control}}) \times 100$$

Superoxide radical scavenging activity:

1 ml NBT is mixed with 1ml of NADH solution. To this solution mixture added 1ml of the sample solution and mixed well. The reaction is started after the addition of 100 μ l of PMS. This reaction mixture has been incubated at 25°C for 5 minutes. After that the absorbance is recorded at 560nm. Ascorbic acid is taken as the standard (Baskar, R 2007).

$$\% \text{ Superoxide scavenging} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}} /$$

$$\text{Abs}_{\text{control}}) \times 100$$

Nitric oxide radical scavenging activity:

1 ml of the extract solution along with 1 ml of sodium nitroprusside is taken in a test tube. This solution was incubated at 25°C for 180 minutes. To the above reaction mixture added 1 ml of Griess reagent. Finally the absorbance of the solutions is recorded at 560nm (Dsouza, N. G 2019).

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}} / \text{Abs}_{\text{control}}) \times 100$$

Hydrogen peroxide scavenging activity (Oktay, M 2003):

The capacity of the extract for scavenging hydrogen peroxide was measured and compared with that of ascorbic acid. For that to different test tubes containing 1ml of different concentrations of the extract 0.6ml of hydrogen peroxide solution was added. These test tubes were incubated in room temperature for 10 minutes. After that the absorbance reading was taken at 230nm against the blank. The percentage inhibition was calculated using the below given equation.

$$\% \text{ Scavenged H}_2\text{O}_2 = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}} / \text{Abs}_{\text{control}}) \times 100$$

Total antioxidant activity:

Spectrophotometric method is used for the determination of total antioxidant activity. Different concentrations of the test aliquot were prepared and were taken in an Eppendorf tube and added 1ml of the reagent which consists of 0.6mM sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. These tubes were incubated at 95°C for 90 minutes. After cooling to room temperature the absorbance was read at 695nm. Here ascorbic acid was used as the standard (Fernandes, V 2019).

Results and discussion

The indigenous drugs have been used for different diseases in the traditional system of medicine for years. India has one of the vast collections of medicinal plants which have been used traditionally by the Ayurvedic science. Plants are used in the production of drugs due to the capability they have to synthesize large variety of chemical compounds which plays important role to perform the specific actions. Drugs obtaining from medicinal plants has far future because half million of the plants existing on earth are yet not investigated for their therapeutic values. A huge variety of plant species are well known for their antioxidant activities. Oxidants also lead a role in the pathogenesis of disorders such as inflammation, asthma, psoriasis and contact dermatitis

action by one mechanism but that natural antioxidants use several mechanisms to exert their impact (Vadivel V. 2017). The unwanted side effects of the synthetic drugs also encourage the use of natural anti-oxidants.

In vitro anti-oxidant studies:

DPPH assay: When the ethanolic extract of the whole plant of *Utricularia reticulata* was undergone DPPH assay, the percentage inhibition of the free radical was found to be increasing with the increase in the concentration. The IC₅₀ value of the extract was found

Table 1: Effect of ethanolic extract of *Utricularia reticulata* on DPPH assay.

Sl. No.	Conc. of sample (µg/ml)	% DPPH radical scavenging activity ± SEM	
		Standard	Ethanol extract
1	20	23.2±0.020	19.9±0.011
2	40	42.4±0.012	41±0.010
3	60	60.2±0.013	58.03±0.013
4	80	75.96±0.011	74.3±0.009
5	100	88.8±0.016	83.8±0.012
	IC ₅₀ value	83.17	83.17

All values are expressed in ± SEM and are significant when compared to control P<0.05.

Table 2: Effect of ethanolic extract of *Utricularia reticulata* superoxide radical.

Sl. No.	Conc. of sample (µg/ml)	% Superoxide radical scavenging activity ± SEM	
		Standard	Ethanol extract
1	20	25.1±0.005	22.4±0.006
2	40	49.11±0.011	48±0.009
3	60	61.9±0.007	60.58±0.020
4	80	70.58±0.002	68.7±0.019
5	100	77.4±0.011	74.8±0.001
	IC ₅₀ value	104.7	114.81

All values are expressed in ± SEM and are significant when compared to control P<0.05.

Table 3: Effect of ethanolic extract of *Utricularia reticulata* on nitric oxide radical.

Sl. No.	Conc. of sample (µg/ml)	% Nitric oxide radical scavenging activity ± SEM	
		Standard	Ethanol extract
1	20	25.7±0.010	20.1±0.011
2	40	45.7±0.002	47.2±0.010
3	60	64.5±0.012	59.91±0.009
4	80	75.9±0.009	71.3±0.011
5	100	82.5±0.012	79.89±0.015
	IC ₅₀ value	109.6	144.54

All values are expressed in ± SEM and are significant when compared to control P<0.05.

leading to oxidative stress (Shrikumar, S. 2007). Studies have revealed that synthetic antioxidants exhibit their

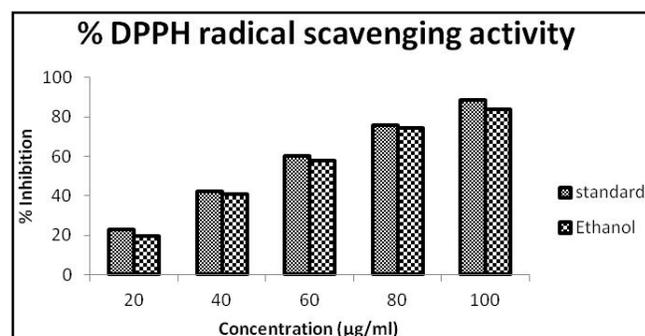


Fig. 1: Comparison of % DPPH radical scavenging activity by *Utricularia reticulata* extract (ethanolic) and standard drug.

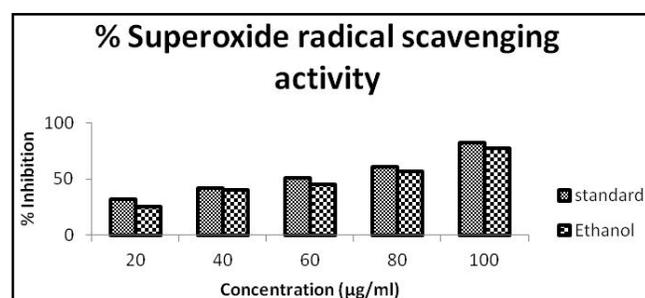


Fig. 2: Comparison of % Superoxide radical scavenging activity by *Utricularia reticulata* extract (ethanolic) and standard drug.

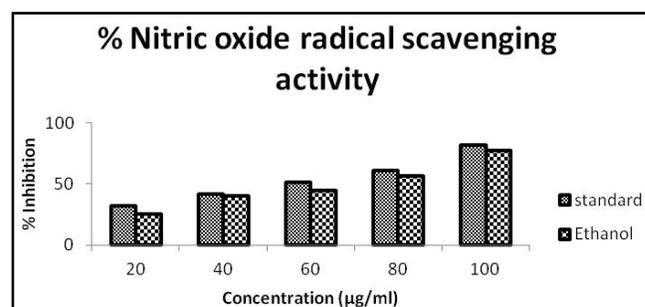


Fig. 3: Comparison of % Nitric oxide radical scavenging activity by *Utricularia reticulata* extract (ethanolic) and standard drug.

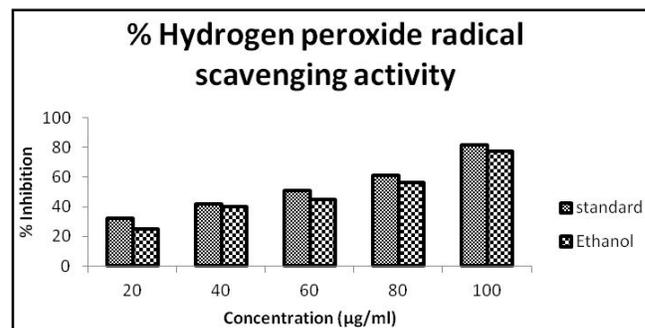


Fig. 4: Comparison of % Hydrogen peroxide radical scavenging activity by *Utricularia reticulata* extract (ethanolic) and standard drug.

to be 83.17 same as the standard. The decreased absorbance of the DPPH radical at 517nm shows the free radical scavenging activity of the ethanolic extract of *Utricularia reticulata*. From which we can confirm that the ethanolic extract of the plant have positive antioxidant activity.

Superoxide radical scavenging activity: Superoxide radical scavenging assay of the ethanolic extract of the plant shows increase in the percentage inhibition with respect to the increasing concentration. The IC₅₀ value of the plant extract was found to be 114.81 whereas that of the standard was found to be

104.7. The decreasing absorbance of the superoxide at 560nm was found to be showing the positive antioxidant activity of the ethanolic plant extract.

Nitric oxide radical scavenging activity: When the ethanolic plant extract was subjected to nitric oxide radical scavenging activity, the percentage inhibition value is found to be increasing with respect to the increasing value of the concentration. The IC₅₀ value of the ethanolic extract of *Utricularia reticulata* was found to be 144.5 whereas those that of the standard was found to be 109.6. The decreasing absorbance with respect to increase in the concentration at 546nm shows that the ethanolic plant extract shows positive antioxidant activity.

Table 4: Effect of ethanolic extract of *Utricularia reticulata* on hydrogen peroxide radical.

Sl. No.	Conc. of sample (µg/ml)	% Hydrogen radical scavenging activity ± SEM	
		Standard	Ethanol extract
1	20	28.8±0.003	22.56±0.018
2	40	48.6±0.010	36.92±0.014
3	60	53.5±0.011	51.11±0.006
4	80	68.1±0.008	65.37±0.014
5	100	85.7±0.009	80.7±0.011
	IC ₅₀ value	104.71	125.89

All values are expressed in ± SEM and are significant when compared to control P<0.05.

Table 5: Total antioxidant capacity of ethanolic extract of *Utricularia reticulata*.

Sl. No.	Conc. of sample (µg/ml)	% antioxidant capacity of extract ± SEM	
		Standard	Ethanol extract
1	20	31.7±0.008	25±0.007
2	40	41.3±0.004	39.8±0.005
3	60	50.9±0.007	44.6±0.006
4	80	60.7±0.006	56±0.010
5	100	81.6±0.012	77.1±0.009
	IC ₅₀ value	181.90	

All values are expressed in ± SEM and are significant when compared to control P<0.05.

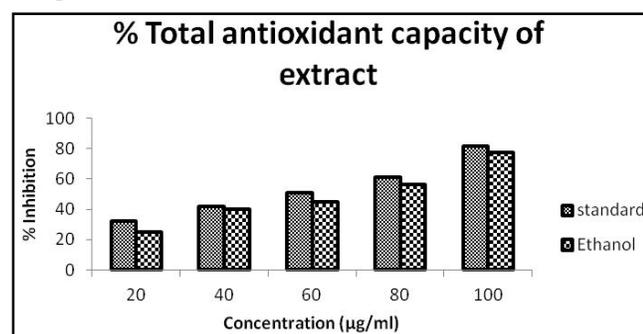


Fig. 5: Comparison of % Total antioxidant capacity by *Utricularia reticulata* extract (ethanolic) and standard drug.

Hydrogen peroxide radical scavenging activity: Hydrogen peroxide radical scavenging activity of the ethanolic extract of the whole plant of *Utricularia reticulata* shows continuously increasing percentage inhibition value with respect to the increasing concentration value. The IC₅₀ value of the extract was found to be 125.89 whereas that of the standard was found to be 104.71. The decrease in the absorbance with increase in the concentration at 270nm shows that the extract is possessing antioxidant activity.

Total antioxidant activity: The total antioxidant activity of the extract is compared with that of the standard. The percentage inhibition was found to be increasing with respect to the increasing value of concentration. The IC₅₀ value of the extract was found to be 181.97 whereas the IC₅₀ value of the standard was found to be 181.90. The decreasing absorbance of the ethanolic plant extract when taken at 695nm shows the presence of significant antioxidant activity.

Conclusion

When the ethanolic extract of the whole plant of *Utricularia reticulata* were undergone preliminary phytochemical investigation, it showed the presence of steroids as well as flavonoids. Whereas it showed negative results to carbohydrates, resins, tannins, saponins, etc. Antioxidant activity of the plant was estimated using standard methods such as DPPH assay, nitric oxide radical scavenging activity, superoxide radical scavenging activity, hydrogen peroxide radical scavenging assay and total antioxidant activity. From which all the assay gave positive result for the significant antioxidant activity in the plant of *Utricularia reticulata*. The statistical representation of the values for the antioxidant activity of the ethanolic extract of *Utricularia reticulata* was found to be significant. This activity may be contributed by the presence of active constituents like flavonoids in it.

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