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Research Article

In Vitro Preliminary Phytochemical Screening and Free Radical Scavenging Ability of *Drosera indica* L.

K R Asha[1], S Hemmalakshmi[1], S Priyanga[1], K Devaki[1,2*]

[1]*Department of Biochemistry, Karpagam University, Coimbatore- 641 021*
[2]*Department of Bioinformatics, Karpagam University, Coimbatore- 641 021*

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ABSTRACT

Aim: The present study is carried out to explore the preliminary phytochemical screening and free radical scavenging activity of the whole plant *Drosera indica* L. Methods: a) Phytochemical screening - The qualitative analysis of secondary metabolites is carried out by the standard qualitative methods. b) *In vitro* free radical scavenging activity of the ethanolic and aqueous extract of the whole plant *Drosera indica* L is used for the analysis .Various concentrations (100 – 500mcg/ml) of the ethanol and aqueous extracts of *Drosera indica* L. are used in the various antioxidant assay methods such as reducing power, ferric reducing antioxidant power assay (FRAP), nitric oxide (NO) radical,2,2' azinobis-3 ethylbenzothiozoline-6 sulfonic acid (ABTS+) radical, hydroxyl radical (OH.), 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical , super oxide radical and hydrogen peroxide (H_2O_2) is carried out with the standard protocols. In all the assays ascorbic acid is used as the standard antioxidant. Results: Phytochemical screening of the plants reveal the presence of numerous chemicals including flavanoids, tannins, polyphenols, cardiac glycosides and saponins. The ethanolic extract of *Drosera indica* L. shows better ability to scavenge ,1,1-diphenyl-2-picryl hydroxyl(DPPH)radical, hydroxyl radical, hydrogen peroxide, nitric oxide radical and superoxide radical. FRAP and the reducing power abilities of the ethanolic extract is increased with the increase in concentration of the plant extract. Conclusion: The ethanolic extract of *Drosera indica* L. shows better ability to scavenge the free radicals than the aqueous extract. From this study, a conclusion is drawn that *Drosera indica* L. can have more beneficial effects with respect to the presence of many active secondary metabolites which may likely to combat with the oxidative stress diseases like diabetes, cancer, cardio-vascular diseases and in general boost the immune system.

Keywords: *Drosera indica* L., Free radicals, Phytochemicals and Antioxidants.

INTRODUCTION

Medicinal plant plays an essential role in the management of various diseases[1]. Plants form the basis of sophisticated traditional medicine systems that are in existence for thousands of years. It possesses many effective means and also ensures health care. Inspite of the spectacular advances in the synthetic drugs in the recent years, some of the drugs of the plant origin still retain their importance. It is believed that herbal drugs are relatively safe and exhibit a remarkable efficacy in the treatment of chronic ailments[2]. Free radicals are highly reactive molecules having unpaired electrons and produced by radiation or by-products of the metabolic processes. Humans are exposed to free radicals in the environment through the radiation and pollution. Free radicals are released during the oxidative stress, possess the major endogenous damage in the biological system. This type of damage is often associated with the various degenerative diseases and disorders like cancer, cardiovascular disease, immune function decline and aging. To gain stability, free radicals capture the electrons quickly from the other compounds and the attacked compound becomes a free radical, which continues to attack other compounds and leads to a chain reaction. These results in the disintegration of cell membranes and cell compounds, including lipid, protein and nucleic acids[3]. Antioxidants scavenges the free radicals which enable the cells to rejuvenate or stabilize for the process of life[4]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases like cancer, heart diseases and stroke[5].

Drosera indica L. isaherbaceous, insectivorous plant native of the tropical countries in the world and consists of approximately 170 species. In India, *Drosera indica* L., *Drosera burmannii Vahl* and *Drosera peltata* are mostly seen. These species are used as vital components in the Ayurvedic preparation 'Swarnabhasma' (Golden ash). This is used in the several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness and incidence of early aging. It is also used for the treatment of diseases like bronchial asthma, rheumatoid arthritis and nervous disorders[6]. The secondary metabolites like phenolics and flavonoids from plants are reported to be potent free radical scavengers. Various species of *Drosera indica* L. are reported for the presence of flavonoids in them[7]. So,the aim of the study

**Author for Correspondence: dr.devaki.bc@gmail.com*

Table 1: Extraction yield of *Drosera indica* L.

Solvent system for extraction	% of yield (g/100g)
Petroleum ether	1.7
Chloroform	1.9
Ethyl acetate	0.8
Ethanol	4.2
Aqueous	3.0

is to explore the phytochemical screening and the free radical scavenging activity of the whole plant *Drosera indica* L.

MATERIALS AND METHODS

Collection of plant material

The plant specimen (whole plant) for the proposed study are collected from Wagamonhills, Idukki district, Kerala, India. The plant is taxonomically authenticated from Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram district, Kerala, India, with the voucher number (JNTBGRI/PS/215/2015,No-76853).

Sample extraction

The plant sample, *Drosera indica* L. is cleaned, dried and coarsely powdered and subjected to the solvent extraction for 16 hours with the solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate, ethanol and water. 50 g of dried plant powder is extracted in 250ml of each solvents and kept in a shaker for 72 hrs. Extraction is repeated with the same solvent till the clear colourless solvent is obtained. Each time before extracting, with the next solvent, the residue is dried thoroughly to remove the used solvent. Finally the extract is evaporated and stored at 0-4˚C in an air tight container. After the extraction, the percentage of yield is calculated as follows.

Extraction yield

The percentage of yield is calculated by using the formula:

$$\text{Percentage of Yield (\%)} = \frac{\text{Amount of extract yield (g)}}{\text{Amount of dried plants used (g)}} \times 100$$

Phytochemical screening

The qualitative analysis of the secondary metabolites is carried out by following the methods of Trease and Evans[8] (1996) and Harborne (1987)[9].

In vitro free radical scavenging activity of the ethanolic and aqueous extract of the whole plant Drosera indica.

DPPH radical scavenging assay is estimated by Murthy *et al.*,[10], reducing power of the whole plant extract is quantified according to the method of Oyaizu[11], Nitric oxide (NO) scavenging activity of the extract is determined by the method of Garrat[12], Hydrogen peroxide radical scavenging assay is determined by Zhang[13] ,2,2'-azinobis- (3-ethylbenzothiazoline-6- sulfonic acid) (ABTS+) radical activity is estimated by the method of Re, *et* al.,[14], Hydroxyl radical scavenging activity is measured according to the method of Klein, *et* al.,[15] measurement of the superoxide radical scavenging activity is done using the standard method of Liu, *et* al.,[16] and the total antioxidant potential of the sample is determined using the ferric reducing antioxidant power (FRAP) by the method of Benzie and Strain[17].

Statistical analysis

All the experiments are done in the triplicate and the results are expressed as Mean ± Standard Error of Mean.

RESULTS AND DISCUSSION

The free radicals cause severe damage to cells, which can lead to degenerative diseases as well as premature ageing[18]. Antioxidants scavenges the free radicals which enables the cells to rejuvenate or stabilize for the process of life[19]. *Drosera indica* L. possesses an effective antioxidant and anticancer activities. This is due to the presence of phytochemicals like naphthoquinones and quercetinetc[20].Solvent extraction studies show (Table 1) that the ethanolic extract of *Drosera indica* L. has the highest yield (4.2 %), whereas ethylacetate extract has the lowest yield percentage (0.08 %).

Phytochemical Screening of the plant reveals the presence of the numerous chemicals including flavanoids, cardiac glycosides, saponins, tannins and polyphenols.

The study shows that ethanolic and aqueous extract contain maximum number of phytoconstituents than the other extracts. So, these two extracts were selected for the free radical scavenging studies.

Free Radical Scavenging Assays

DPPH Radical Scavenging Assay

DPPH is a common abbreviation for an organic chemical compound 1,1-diphenyl-2-picryl hydroxyl. Scavenging of DPPH free radical is the basis of a common antioxidant assay. In the assay DPPH radical is converted to the corresponding hydrazine, and the colour of the solution is changed from violet to yellow which indicates the

Table 2: Phytochemical Screening of *Drosera indica* L.

Solvent Extraction	AL	FL	TP	AP	CH	CG	SA	OF	TN	ST
Petrolem Ether	-	+	-	-	-	++	+	-	-	-
Chloroform	-	++	-	++	++	++	+	-	-	-
Ethylacetate	-	+++	-	++	+	++	+	-	-	-
Ethanol	-	+++	+	++	++	++	+	-	-	-
Aqueous	-	+++	+	++	++	++	+	-	-	-

AL -Alkaloids
FL -Flavanoids
TP - Tannins &Phenolic compounds
AP -Aminoacids& Proteins
OF -Oils & Fats
CH - Carbohydrates
CG - Cardioglycosides
SA - Saponins
ST - Steroids
N - Terpenoids

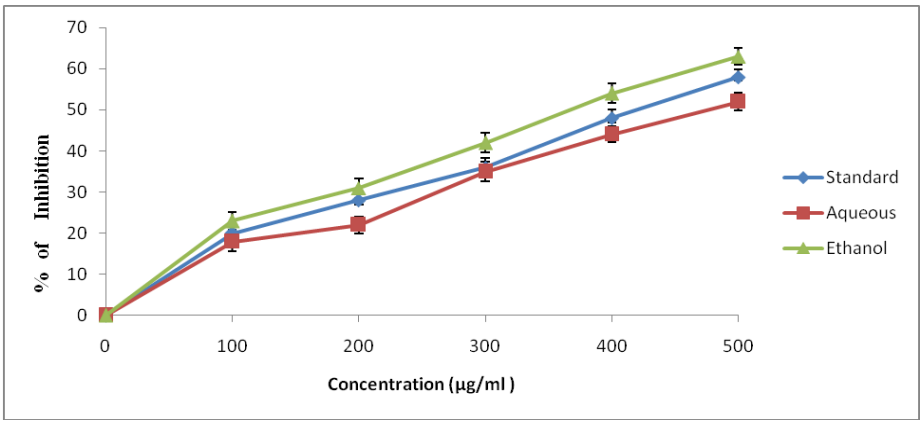

Figure 1: DPPH radical scavenging assay.
Values are expressed as Mean ± Standard Error of Mean. Standard - Ascorbic acid
IC_{50} Value standard = 416 ± 0.72 µg/ml
IC_{50} Value Aqueous = 480 ± 0.29 µg/ml
IC_{50} Value Ethanol = 368 ± 0.74µg/

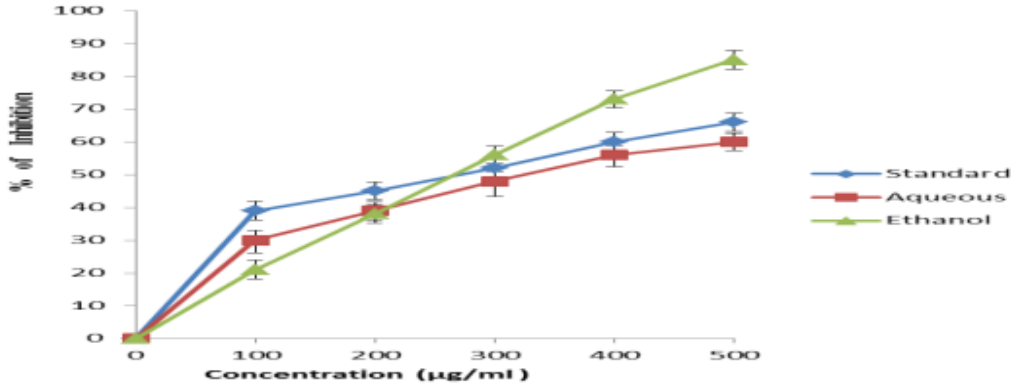

Figure 2: Hydroxyl radical scavenging assay.
Values are expressed as Mean ± Standard Error of Mean. Standard - Ascorbic acid
IC_{50} Value Standard = 290 ± 0.95 µg/ml
IC_{50} Value Aqueous = 321 ± 0.45µg/ml
IC_{50} Value Ethanol = 265 ± 0.83 µg/ml

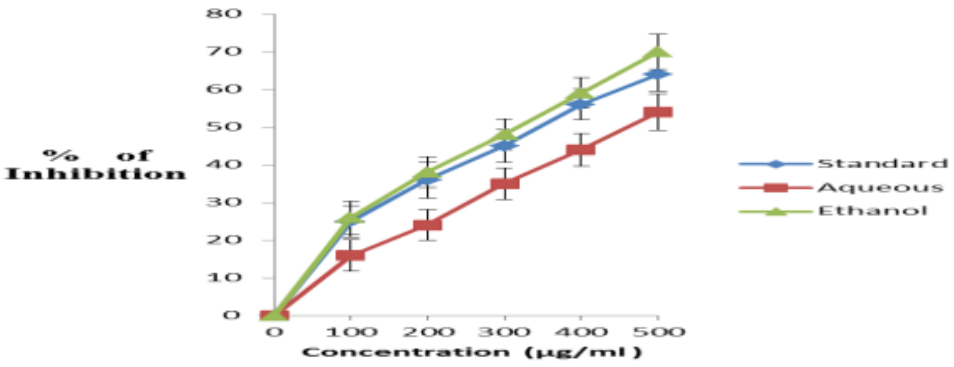

Figure 3: Nitric oxide radical scavenging assay.
Values are expressed as Mean ± Standard Error of Mean Standard - Ascorbic acid
IC_{50} Value Standard =354 ± 0.36 µg/ml
IC_{50} Value Aqueous =453 ± 0.82µg/ml
IC_{50} Value Ethanol = 324 ± 0.66 µg/ml

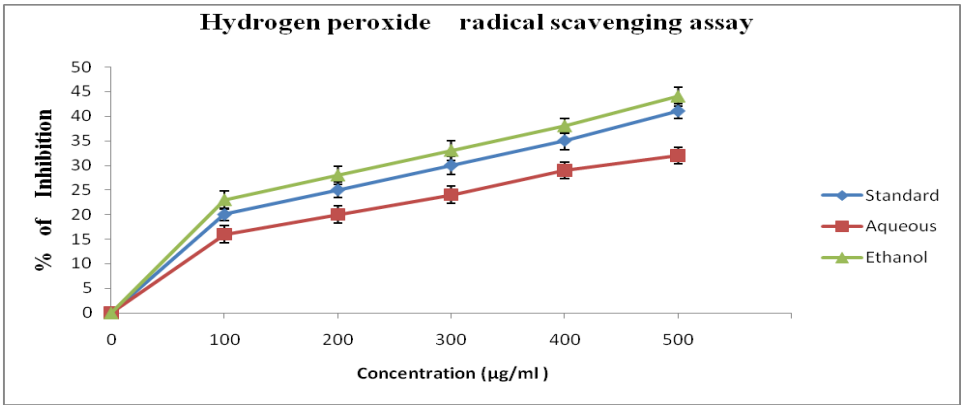

Figure 4: Hydrogen peroxide radical scavenging assay.
Values are expressed as Mean ± Standard Error of Mean Standard - Ascorbic acid
IC_{50} Value Standard = 520 ± 0.94 µg/ml
IC_{50} Value Aqueous = 580 ± 0.29µg/ml
IC_{50} Value Ethanol =510 ± 0.45 µg/ml

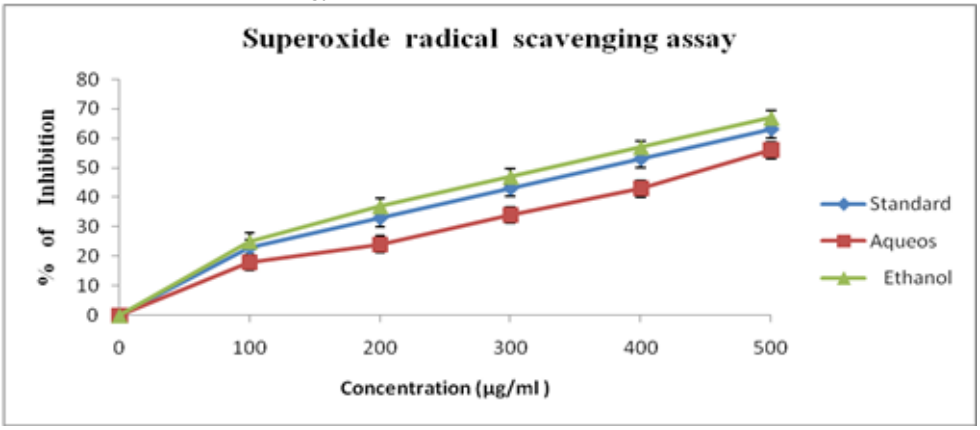

Figure 5: Super oxide radical scavenging assay.
Values are expressed as Mean ± Standard Error of Mean Standard - Ascorbic acid
IC_{50} Value Standard = 370± 0.68 µg/ml
IC_{50} Value Aqueous = 330± 0.83 µg/ml
IC_{50} Value Ethanol = 487± 0.45 µg/ml

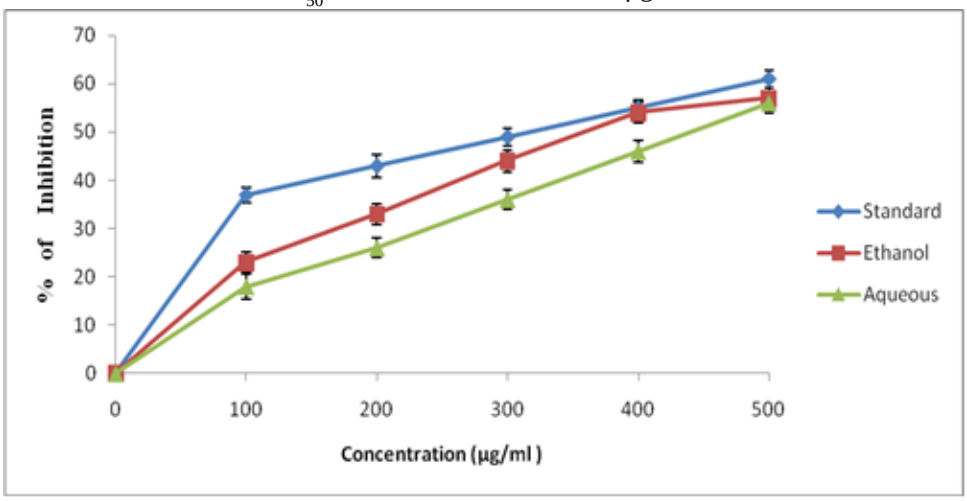

Figure 6: ABTS radical scavenging assay.
Values are expressed as Mean ± Standard Error of Mean Standard - Ascorbic acid
IC_{50} Value Standard = 310 ± 0.68 µg/ml
IC_{50} Value Aqueous = 440± 0.45µg/ml
IC_{50} Value Ethanol = 360± 0.83µg/ml

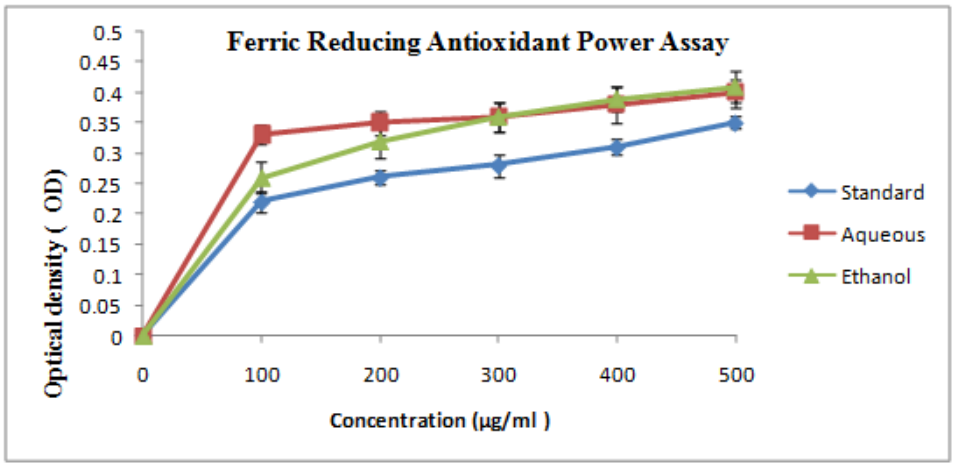

Figure 7: Ferric Reducing Antioxidant Power assay.
Values are expressed as Mean ± Standard Error of Mean Standard - Ascorbic acid

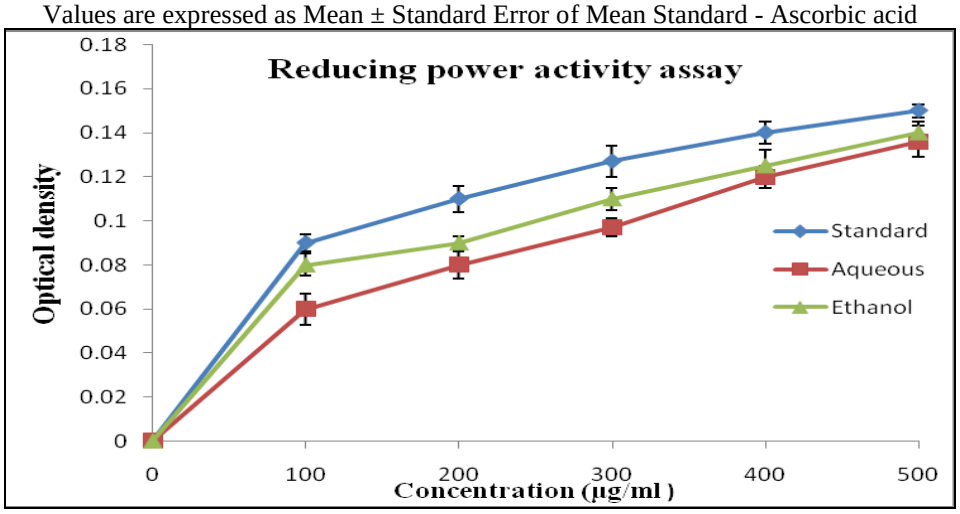

Figure 8: Reducing power activity assay.
Values are expressed as Mean ± Standard Error of Mean Standard - Ascorbic acid

scavenging behavior of the plant sample due to bioactive compounds such as phenolic compounds, flavonoids, tannins and their derivatives.

The ethanolic extract of *Drosera indica* L. shows a maximum DPPH radical scavenging activity than the standard extract. The IC_{50}values are found to be 368 ± 0.74 µg/ml and 416 ±0.72 µg/ml for the ethanolic extract and also for the reference standard ascorbic acid respectively. The aqueous extract shows least DPPH radical scavenging activity than the standard and the ethanolic extract. The IC_{50} values of the aqueous extract is found to be 480 ± 0.29 µg/ml. The results shows that the ethanolic extract of *Drosera indica* L. shows a considerable DPPH radical scavenging activity, as the activity increases as the concentration of the plant extract increases. The experiment is done in triplicate and the results is expressed as Mean ± Standard Error of Mean in the Figure (1).

Hydroxyl radical scavenging assay

The hydroxyl radical, ˙OH, is the neutral form of the hydroxide ion (OH^-). Hydroxyl radicals are highly reactive (easily becoming hydroxyl groups) and consequently short-lived. Hydroxyl radicals can occasionally be produced as a by the-product of the immune action. The ethanolic extract of *Drosera indica* L. as well as the standard, exhibits good hydroxyl radical scavenging ability in a dose-dependent manner. The IC_{50}value of the ethanolic extract is found to be 265 ± 0.83 µg/ml and the standard (ascorbic acid) is found to be 290± 0.95 µg/ml. The IC_{50} value of the aqueous extract is found to be 321 ± 0.45 µg/ml. The ethanolic extract of *Drosera indica* L. shows considerable hydroxyl radical scavenging activity, as the activity increases the concentration of the plant extract also gets increased. The results of the hydroxyl radical scavenging powers of the plant extract and ascorbic acid are depicted in the figure 2.

Nitric oxide radical scavenging assay

Nitric oxide is a highly reactive oxygen species. Nitric oxide is an important cellular signalling molecule involved in many physiological and pathological processes. The ethanolic extract of *Drosera indica* L. as well as the standard, exhibits good nitric oxide radical scavenging ability in a dose-dependent manner. The IC_{50} value of the ethanolic extract is found to 324 ± 0.66 µg/ml and the standard (ascorbic acid) is found to be

354± 0.36µg/ml. The IC_{50} value of the aqueous extract is found to be 453 ± 0.82 µg/ml. The ethanolic extract of *Drosera indica* L. show considerable nitric oxide radical scavenging activity, as the activity increases as the concentration of the plant extract increases.

Hydrogen peroxide radical scavenging assay

Hydrogen peroxide is a highly reactive oxygen species. Hydrogen peroxide is the simplest peroxide (a compound with an oxygen–oxygen single bond) and finds use as a strong oxidizer, bleaching agent and disinfectant. The ethanolic extract of *Drosera indica* L. as well as standard, exhibits good hydrogen peroxide radical scavenging ability in a dose-dependent manner. The IC_{50}value of the ethanolic extract of hydrogen peroxide radical is found to be 510 ± 0.45 µg/ml and the standard (ascorbic acid) is found to be 520 ± 0.94 µg/ml. The IC_{50} value of the aqueous extract is found to be 580 ± 0.29 µg/ml. The ethanolic extract of *Drosera indica* L. shows considerable hydrogen peroxide radical scavenging activity, as the activity increases as the concentration of the plant extract increases.

Super oxide radical scavenging assay

Superoxide is particularly important as the product of the one-electron reduction of dioxygen O_2, which occurs widely in nature, in mammals including humans. Super oxide radical scavenging activity increases as the concentration of the ethanolic plant extract increases. The IC_{50}value of the ethanolic extract of the plant is found to be 487 ± 0.45µg/ml and the standard (ascorbic acid) is found to be 370± 0.68µg/ml. The IC_{50}value of the aqueous extract is found to be 330 ± 0.83µg/ml. The ethanolic extract of *Drosera indica* L. as well as the standard, exhibits good super oxide radical scavenging ability in a dose-dependent manner. (Figure 5)

ABTS radical scavenging assay

In biochemistry, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS is commonly used to assess the antioxidant capacity. It is a decolorization assay. The figure 6 indicates that the standard (ascorbic acid) shows a better ABTS radical scavenging activity compared to the ethanolic and the aqueous plant extract on the ABTS radical scavenging assay. The IC_{50} value of standard is found to be 310 ± 0.68 µg/ml comparable to the ethanolic extract of 360 ± 0.83 µg/ml. The IC_{50} value of the aqueous extract is found to be 440 ± 0.45µg/ml. (Figure 6)

FRAP (Ferric Reducing Antioxidant Power) assay

The FRAP assay (Ferric Reducing Antioxidant Power), a simple test to determine the total antioxidant power, is also chosen to assess the free radical scavenging effects of *Drosera indica* L.. FRAP assay depends upon the ferric tripyridyl triazine [Fe (III)-TPTZ] complex to the ferrous tripyridyltriazine [Fe (II)-TPTZ] by a reductant at low pH. The FRAP scavenging capacity of the ethanolic extracts of *Drosera indica* L. at five different concentrations (100-500 µg/ml) exhibit an optical density like 0.26, 0.32, 0.36, 0.39 and 1.41 respectively at 595nm which is shown in the (Figure 7).

Reducing Power Assay

Reducing power of the fractions is assessed using ferric to ferrous reducing action is determined spectrophotometrically using the configuration of Perl's Prussian blue colour complex. Standard curve of the ethanolic extract of *Drosera indica* L. as well as ascorbic acid is shown in the figure 8 in which the ethanolic extract reducing ability increases with the increasing concentration (100-500 µg/ml) like the antioxidant activity of the standard curve. Ferric reducing antioxidant power (FRAP) and the reducing power abilities of the ethanolic extract increases with respect to the increase in the concentration of the plant extract (Figure 8).

CONCLUSION

This study provides evidences that different extracts of *Drosera indica* L. found to have phytoconstituents and support that the ethanolic extract of *Drosera indica* L. has more number of phytoconstituents when compared to other extracts of this plant. The ethanolic extract of the *Drosera indica* L. shows better ability to the scavenge nitric oxide radical (NO), 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical, hydroxyl radical (OH.), superoxide radical (SO_2) and hydrogen peroxide radical than the aqueous plant extract, ABTS radical (2,2' azinobis -3 ethyl benzothiozoline- 6 sulfonic acid) shows less activity compared to the standard ascorbic acid. Ferric reducing antioxidant power (FRAP) and the reducing power abilities of the ethanolic extract increased, with respect to increase in concentration of the plant extract. From this study, a conclusion is drawn that the ethanolic extract of *Drosera indica* L. can have more beneficial effects with respect to the presence of many active secondary metabolites which may likely to combat oxidative stress diseases like diabetes, cancer, cardio-vascular diseases and in general boost the immune system..

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CONFLICT OF INTEREST

We declare there is no conflict of interest.

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