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ANTIOXIDANT EFFECT OF *SCOPARIA DULCIS* LINN. ETHANOL EXTRACT (*IN VITRO STUDY*)

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ABSTRACT *Scoparia dulcis,* Linn., an indigenous herbal plant used traditionally in Ayurvedic medicine, in India, for its possible anti ulcer, urolithiasis[1], antidiabetic and antimicrobial activities. An aqueous extract of the plant showed marked antioxidative activity in vitro, which supports the therapeutic effects claimed by traditional practitioners for its antidiabetic activity. The annual herb is belongs to the family Scrophulariaceae and distributed throughout the tropics. The present study is aimed to investigate the anti oxidant activity of ethanol extract of dried whole plant, *Scoparia dulcis* collected from south region of Tamil Nadu state, India. The plant is here and after referred as *S.dulcis* and the ethanol extract as EESD. A stock solution of the extract 1mg/ml was prepared. Then aliquate dilutions of 125, 250, 500 and 1000 and 2000 µg/ ml were prepared from the stock for studying the in vitro anti oxidant activity.

KEYWORDS : *dulcis*, anti oxidant, sodium nitro prusside.

1. INTRODUCTIONS[2,3,4]

Scoparia dulcis Linn. (Commonly known as sweet broomweed) is an annual herb widely distributed in tropical and subtropical regions, belongs to the family Scrophulariaceae, is growing up to 2 m in height. The fresh or dried *S. dulcis* plants have been traditionally used as remedies for stomach trouble, hypertension, diabetes, bronchitis and as analgesic and antipyretic agents. A number of different phytoconstituents, namely scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin have been shown to contribute to the observed medicinal effect of the plant.

2. MATERIALS AND METHODS[5]

a) Collection of plant material

The plant species were collected from nagercoil district, the southern region of Tamil Nadu state, India, with the help of local people. The plant material was identified at the field using standard keys and descriptions.

b) Preparation of plant extract

The selected materials were washed under running tap water for cleaning to remove dust and other unwanted debris attached. The samples were then dried by placed under sunshade for two weeks. The dried material was cut in to small pieces with a sharp knife and stored in polythene bags. The ethanolic extract was prepared by using maceration technique. Placed and soaked the sample in 95 % ethyl alcohol for a period of one week with intermittent agitation to replace concentrated menstrum with fresh part for effective extraction. The extract was concentrated by effective evaporation and a greenish black thick sticky mass was obtained.

c) Reagents, Chemicals and Standard drugs

All the reagents, chemicals and drug standards were purchased from approved and certified laboratories and used as supplied for the study.

d. Determination of Antioxidant Activity

The antioxidant effect of EESD was studied by the following methods.

1) DPPH Radical scavenging Assay.
2) Hydrogen peroxide scavenging Assay.
3) Nitric oxide scavenging Assay.
4) Reducing power Assay.

1) DPPH Radical scavenging Assay [6]

DPPH (1, 1-diphenyl-2-picryl hydrazyl)), is a stable pink coloured free radical. When the free radical is scavenged, the colour turns to yellow.

The reaction between (DPPH) and an antioxidant (H-A) is as
DPPH + [H-A] → DPPH-H + (A)

Antioxidants will reduce DPPH to DPPH-H. The resulting colour of the compound changes pink to yellow and thereby a decrease in the percentage absorbance obtained. The degree of discoloration indicates the scavenging potential of the antioxidant compounds present in the EESD in terms of hydrogen donating ability.

METHOD:

Different concentrations (125, 250, 500, 1000 and 2000 µg /ml) of EESD were made up to a final volume of 20ml with DMSO and 1.5ml DPPH (0.1mM) solution was added to each dilutions. The reaction mixture incubated in dark condition at room temperature for 20 min. Simultaneously a reference standard drug, ascorbic acid solutions were also prepared and the control as DMSO and DPPH solution. The solutions were incubated in the same way as done for samples. After 20 min, absorbance of the samples was measured at 517nm.

The antioxidant activity can be calculated as $\%\ \text{inhibition} = \frac{control - test}{control} X100$

b) Hydrogen peroxide scavenging Assay[12]

Method: Hydrogen peroxide solution, H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of EESD (125, 250, 500, 1000 & 2000 µg /ml) were prepared and added to 0.6 ml of H_2O_2 solution. Mixed well and after 10 min the absorbance was read at 230nm. Simultaneously a control (H_2O_2 solution) and the reference standard drug ascorbic acid solutions, same concentrations as in the case of EESD were also prepared in water. Mixed well and after 10 min the absorbance was read at 230nm.

The antioxidant activity can be calculated as $\%\ \text{inhibition} = \frac{control - test}{control} X100$

c) Nitric oxide scavenging Assay.[13]

The oxidation reaction of nitric oxide has been reported to be involved in many biological functions including antimicrobial, antitumor, neurotransmission and in vascular homeostasis. The peroxy nitrite anion, a potential oxidative agent which is produced by the reaction between nitric oxide and superoxide that can be decomposed to produce OH and NO.

The procedure is based on the principle that, sodium nitro prusside in aqueous solution at physiological pH, spontaneously produces nitric oxide, which reacts with oxygen to produce nitrite ions. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. This can be estimated by using Griess reagent and the nitric oxide scavenging activity was measured spectrophotometrically.

METHOD:

Sodium nitro prusside solution (5mmol/L) was prepared in phosphate buffered saline pH 7.4. Different concentrations of EESD and reference standard gallic acid were prepared in methanol. Mixed with sodium nitro prusside solution and incubated at 25°C for 30min. A control without the test compound, but was also carried out, with an equivalent amount of methanol. After the stated period of incubation 1.5ml of the incubated solution was taken and diluted with 1.5ml of Griess reagent (1% sulphanilamide, 2 % phosphoric acid and 0.1% N-1-naphthyl ethylene diaminedihydrochloride). Absorbance of the

chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphathyl ethylene diaminedihydrochloride was measured at 546nm. The percentage scavenging activity was calculated and compared to the reference standard gallic acid.

The antioxidant activity can be calculated as $\% \text{ inhibition} = \frac{control-test}{control} X100$

d) Reducing power Activity.[15]

The reducing power effect of EESD was determined by the method of YEN and DUH (1993).

Method: Different concentrations of EESD (125, 250,500, 1000 and 2000µg /µl) were mixed with 2.5ml of phosphate buffer (200mM)of pH 6.6 and 2.5ml of 1% potassium ferric cyanide was added and boiled for 20 min at 50°C. After incubation, 2.5 ml of 10% Trichloro acetic acid was added to the samples followed by centrifugation for 10 min. 5ml of the supernatant layer was mixed with 5ml of distilled water. Added 1ml of 0.1% ferric chloride and the absorbance was read at 700nm. Simultaneously a standard drug Quercetin was prepared and treated in the same manners as for samples studied. Finally the absorbance was measured at 700nm.

RESULTS AND DISCUSSION

DPPH radical scavenging assay method carried out for the study of antioxidant effect of EESD, a change in the pink colour of DPPH to yellow was observed. This observation describes the antioxidant effect of samples as a result of radical scavenging activity. Also the effect was dependent on concentration. The study was compared with a standard drug and the result was also concentration dependent. The absorbance and percentage of inhibition for standards as well as sample data are tabulated. The IC_{50} value of the sample is calculated as 912µg/ml.

Table 1: DPPH Radical scavenging effect of Ascorbic acid and EESD

(Absorbance measured at 517nm, Control: 1.7983)

Si. No.	Concentration (µg/ ml)	Absorbance		% of inhibition	
		Ascorbic Acid	EESD	Ascorbic Acid	EESD
1	125	1.4044	1.6093	21.90	10.51
2	250	1.0782	1.3781	40.04	23.36
3	500	0.7121	1.1164	60.40	37.92
4	1000	0.2921	0.8792	83.75	51.11
5	2000	0.0692	0.5985	96.15	66.72

In the Hydrogen peroxide scavenging assay method, both standard drug and EESD showed scavenging effect. It is observed that the activity was increased as the concentration of the samples increases. The absorbance and percentage of inhibition for standard as well as sample data are tabulated. The IC_{50} value for the sample is calculated as 926µg/ml.

Table 2: Hydrogen peroxide scavenging effect of Ascorbic acid and EESD

(Absorbance measured at 230nm, Control: 0.9172)

Si. No.	Concentration (µg/ ml)	Absorbance		% of inhibition	
		Ascorbic Acid	EESD	Ascorbic Acid	EESD
1	125	0.7051	0.8046	23.12	12.27
2	250	0.5193	0.6912	43.38	24.64
3	500	0.3287	0.5672	64.16	38.16
4	1000	0.1654	0.4464	81.67	51.33
5	2000	0.0665	0.3073	92.75	66

The antioxidant activity determined by NO scavenging assay showed that, the effect was increased as the concentration of EESD increases. The same result was observed with gallic acid. The absorbance and percentage of inhibition for both standard and sample are tabulated (3) and figure (3) supporting the result. The IC50 value for the sample is calculated as 927µg/ml

Table 3: Nitric oxide scavenging effect of Gallic and EESD

(Absorbance measured at 546nm, Control: 0.2826)

Si. No.	Concentration (µg/ml)	Absorbance		% of inhibition	
		Gallic acid	EESD	Gallic acid	EESD
1	125	0.1962	0.2472	30.57	12.52
2	250	0.1465	0.2138	48.16	24.34
3	500	0.0972	0.1727	65.60	38.89
4	1000	0.0574	0.1329	80.89	52.97
5	2000	0.0254	0.0974	91.99	65.53

The antioxidant activity was determined by Reducing power method also explains the effect was also dependent on concentration in both standard and sample. The absorbance against varied concentration of EESD and standard is tabulated (4) and figure (4) supports the result. The IC50 value for the sample is calculated as 2633.636µg/ µl

Table 4: Reducing power effect of Quercetin and EESD

(Absorbance measured at 700nm, Control: 0.1993)

Si.No.	Concentration(µg)/ µl	Absorbance	
		Quercetin	EESD
1	125	1.036	0.2110
2	250	1.656	0.2377
3	500	2.231	0.2377
4	1000	2.381	0.3324
5	2000	2.437	0.3324

The antioxidant activity study data showed that both EESD and standard drug at the selected doses gave a significant dose dependent increase in all the tested results. Figure for the data is showed (1 to 4).

Figure:1

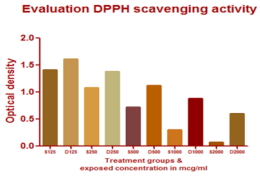


Figure:2

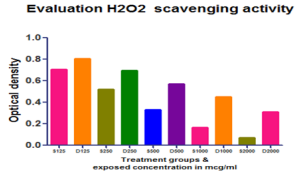


Figure:3

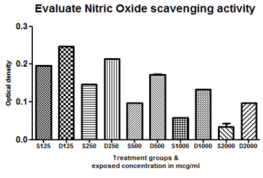
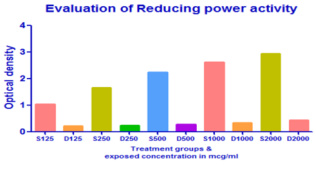


Figure:4



Statistical data showing Antioxidant effect of EESD by Fig1: DPPH Scavenging Assay,

Fig2: H_2O_2 Scavenging Assay, Fig3: NO Scavenging Assay. Fig4: Reducing Power Assay.

SUMMARY AND CONCLUSION

The plant based traditional remedies systems maintain to take part a crucial role in the health care system. The present study has shown antioxidant activity of ethanol extract of *Scoparia dulcis* Linn. Antioxidants act as radical scavenger and thereby protecting the human body from various diseases. There is a natural dynamic balance between the output of free radicals generated in the body and the antioxidant defense system that quenches or scavenges them and thereby protecting the body against pathogenesis. The study was performed by four methods and it is noticed that, the effect was correlated on concentration of the extract. To conclude, the crude plant extract proved its significant antioxidant activity and this *in vitro* antioxidant study revealed the potential antioxidant activities of the herbal plant *Scoparia dulcis* Linn.

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