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Evaluation of Anti-Oxidant and *in vitro* Antioxidant Activity of Various Extracts of *Scoparia dulcis* L

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Abstract
The present study was undertaken to evaluate the concentration of total phenolics and flavanoids present in various extracts prepared from *Scoparia dulcis* L. The hydroalcohol extract showed a maximum concentration of total phenolics and flavanoids up to 121.65% and 323.19%. Antioxidant activity of these extracts had been determined by estimation of DPPH radical and nitric oxide radical scavenging activity. From our observations, it was confirmed that the hydroalcohol extract of *Scoparia dulcis* L showed a significant antioxidant activity than other extracts which reduces the Nitric oxide radical and DPPH radical up to an extent of 0.124 and 0.11 at a concentration of 500µg/ml of the extract.Also increase in concentration decrease the radical formation.
Keywords: *Scoparia dulcis L,* Extracts, Total phenolic compounds, Total flavonoids, NO radical, DPPH radical.

INTRODUCTION

Oxidative stress is continuously discovered to be involved in various age related diseases leading to rise in the importance of studies on the antioxidant properties of specific plants and their active constituents[1]. Antioxidant properties from natural sources enormous scope in correcting various ailments including hepatotoxicity resulting from free radicals[2]. *Scoparia dulcis* L (fam: Scrophulariaceae) leaves have been reported to contain triterpenoids[3] and to possess significant hepatoprotective activity. Traditionally it is claimed to be useful in ulcers, G.I disorders, dysentery, jaundice and tooth ache[4]. In our literature survey no report was available on evaluation of this plant for its possible antioxidant activity. So the present study was undertaken to evaluate antioxidant activity of various extracts of whole plant of *Scoparia dulcis* L by *in vitro*.

MATERIALS AND METHODS

Collection of plant materials

Scoparia Dulcis L was collected from the Acharya N.G.Ranga Agricultural University, Muthukur Road, Nellore. The plant was authenticated by Dr.S.Md.Khasim, Head, Department of Botany, Acharya Nagarjuna University, Guntur. A plant specimen was also planted in the medicinal garden of Narayana Pharmacy college and a voucher specimen (BN/SD/009) was also submitted to the Head of the Institution.

Chemicals

All the Chemical reagents used for the entire experiment work are procured from S.D.Fine Chemicals and BDH Fine Chemicals in Mumbai, which are analytical grade. DPPH was procured from Sigma Aldrich Chemicals India (Pvt) Ltd.

Experimental Activity

After the whole plant was collected including the roots, they were washed with fresh water to remove the soily and adhered matters. Then they were dried under shade at room temperature and fumigated. Then they were powdered by using a pulveriser and sieved with 40 mesh size. About 1kg of powdered drug was weighed and subjected to successive soxhlete extractions with petroleum ether (60-70°C) and Benzene for a period of 48 hours[5]. Then the dried marc was further subjected to cold maceration[6] by using acetone and hydro alcohol (1:1) for 3 consecutive days respectively. Finally the obtained extracts were filtered through a muslin cloth. Then they were concentrated under reduced pressure and dried in vacuum condition to get a semisolid mass whose yields were chartered in table -1.The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents[7-11] present in them.

EXPERIMENTAL PROCEDURES

Determination of total phenolic contents

Total phenolic content in the extracts were determined by using Folin-Ciocalteau reagent according to the method of Slinkard and Singleton[12] using Tannic acid as a standard phenolic compound. 1.0ml of extract solution containing 1.0g of extract in a volumetric flask was diluted with 46ml of distilled water. 1.0ml of Folin Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3 minutes later, 3.0ml of 2% sodium

carbonate was added and the mixture aws allowed to stand for 2 hours with intermittent shaking. The absorbance of the blue colour developed was read at 760nm with a Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Japan). The concentration of total phenolic in each extract was determined as µg of Tannic acid equivalent using the equation after absorbance:

Percentage (%) concentration of total Phenolics in each extract = $(A_{test} – A_{cont} \div A_{std}) \times 100$

The values are furnished in table 1.

Determination of Total flavonoid content

Total soluble flavonoids in the extracts were determined by Dowd method[13]. About0.5ml of the extracts were separately mixed with 1.5ml methanol, 0.1ml of 10% of aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415nm with a Shimadzu UV-160A spectrophotometer (Shimadzu Corporation, Japan). The concentration of total flavonoids in each extract was determined as µg of Tannic acid equivalent using the equation after absorbance:

Percentage (%) concentration of total flavonoids in each extract = $(A_{test} – A_{cont} \div A_{std}) \times 100$

The values are furnished in table 2.

Determination of nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generatesnitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent[14]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffered saline (PBS) was mixed with 3.0ml of different concentrations (10 - 320µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150minutes.The samples from the above were reacted with Griess reagent (1%sulphanilamide, 2% Phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was read at 546nm with a Shimadzu UV-160A spectrophotometer and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess Reagent.

Nitric Oxide Scavenged (%)= $[(A_{cont} – A_{test}) / A_{cont}] \times 100$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance of the test reaction.

The results are furnished in Table 3 and graphical bar diagram.

Determination of DPPH Free radical scavenging activity

Radical scavenging activity was determined with DPPH (2,2-diphenyl-1-picryl-hydrazil)[15] as a free radical by using various concentration (10-500 µg/ml) of the sample prepared in spectrum alcohol. The absorbance of each extract was determined at 517nm after 30 min at room temperature using with a Shimadzu UV-160A spectrophotometer. Ascorbic acid used as standard. Antiradical activity is defined as the amount of inhibitor (phenolic compound) necessary to decrease the initial DPPH radical concentration by 20% (EC20).

DPPH Scavenged (%)= $[(A_{cont} – A_{test}) / A_{cont}] \times 100$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance of the test reaction.

The results are furnished in Table 4 and graphical bar diagram.

Table :1 Total Phenolic contents of various extracts of *Scoparia dulcis* L

Name of the sample	Absorbance	Concentration (%)
Control	0.064	-
Standard (Tannic acid)	0.79	-
Pet-ether extract	0.814	94.94
Benzene extract	0.606	68.61
Acetone extract	0.349	36.08
Hydroalcohol extract	1.025	121.65

Table :2 Total Flavonoid contents of various extracts of *Scoparia dulcis* L

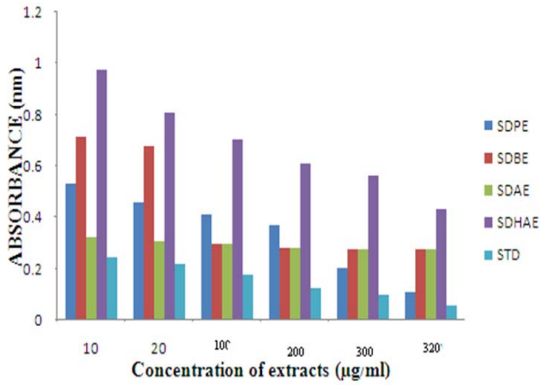
Name of the sample	Absorbance	Concentration (%)
Control	0.082	-
Standard (Tannic acid)	0.276	-
Pet-ether extract	0.532	162.68
Benzene extract	0.716	229.71
Acetone extract	0.322	86.97
Hydroalcohol extract	0.974	323.19

Table : 3 Absorbance of NO after addition of various extracts of *Scoparia dulcis* L

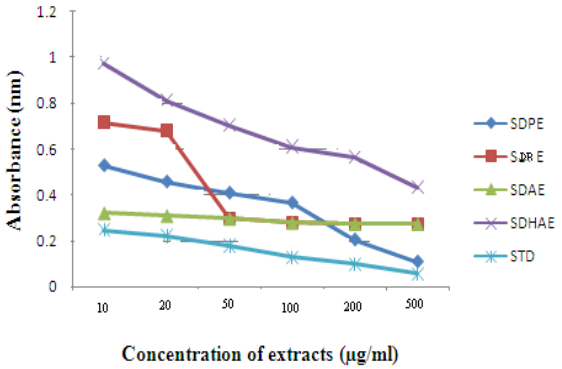
Concentration of the extract(µg/ml)	SDPE	SDPE	SDAE	SDHAE	STD
10	0.664	0.943	0.988	0.412	0.246
20	0.612	0.872	0.923	0.388	0.22
100	0.545	0.803	0.876	0.310	0.178
200	0.428	0.713	0.765	0.256	0.127
300	0.326	0.597	0.628	0.198	0.097
320	0.209	0.348	0.566	0.124	0.056

Table :4 Absorbance of DPPH after addition of various extracts of *Scoparia dulcis*

Concentration of the extract(µg/ml)	SDPE	SDBE	SDAE	SDHAE	STD
10	0.974	0.716	0.322	0.531	0.228
20	0.811	0.680	0.31	0.46	0.209
50	0.703	0.298	0.298	0.411	0.172
100	0.610	0.280	0.28	0.368	0.163
200	0.564	0.274	0.274	0.206	0.084
500	0.434	0.274	0.274	0.11	0.051



Absorbance of NO radicals after addition of extracts of *Scoparia dulcis* L



Absorbance of DPPH radicals after addition of extracts of *Scoparia dulcis* L

RESULTS AND DISCUSSIONS

From our present study it is confirmed that hydroclcoholic extract is the only extract showing more percentage concentration of both phenolics and flavonoids. Other extracts are showing the presence of both the phytoconstituents with moderate amount. Also it is also confirmed form the table and graphical representation that the hydroalcohol is superior in preventing the free radicals formed from the Nitric oxide model and DPPH model systems. The extract on the initial concentration starts to decrease the radicals and more potent in preventing the nitrite and picrazyl radical ions. Also the also extracts show increase in concentration decreases the radicals and increase in the antioxidant potential.

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is stable nitrogen centered free radical containing an odd electron in its structure that can accept an electron or hydrogen radical to become a stable diamagnetic molecule[16]. The decrease in absorbance of DPPH radical is due to the antioxidant reaction between antioxidant molecules and radical progresses[17] which visually noticeable that as a change in color from purple to yellow. Hence DPPH and NO system is usually used as a substrate to evaluate the antioxidant activity of various phenolic compounds by *in vitro* method.

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