Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

ANTIOXIDANT STUDY OF DIFFERENT EXTRACTS OF SCOPARIA DULCIS

PRATAP KUMAR PATRA*[1], JITENDRA DEBATA[2], E SRAVANTHI REDDY[2] AND HIMANSU BHUSAN SAMAL[2]

[*1]Sree Dattha Institute of Pharmacy, Ibrahimpatnam, Hyderabad 501510, [2]Guru Nanak Institutions Technical Campus-School of Pharmacy, Hyderabad 501506, *Email: pratappatra83@gmail.com

Received: 29 Oct 2013, Revised and Accepted: 20 Nov 2013

ABSTRACT

Objective: The objective of the present investigation is to study the antioxidant power of different extracts of *Scoparia dulcis. Scoparia dulcis* Linn belongs to the family Scrophulariaceae and have speculated medicinal properties.

Materials and Methods: The different extract of this plant were obtained by successive extraction with petroleum ether, chloroform and ethanol. These extracts (PEESD, CESD and EESD) were then taken for phytochemical screening using different chemical reagents. An in-vitro antioxidant study is carried out by using various antioxidant screening methods like estimation of total Phenolic compounds, reducing power, nitric oxide scavenging and superoxide ion scavenging activity. The antioxidant activity of the extracts was related to their phytochemical composition in terms of polyphenol and carotenoid contents.

Results: The chloroform extract was found rich in phytochemical constituents and had the highest antioxidant activity in the different antioxidant systems.

Conclusion: Result shows PEESD, CESD and EESD have significant antioxidant activity. The antioxidant potential may be attributed to the presence of polyphenolic compound.

Keywords: *Scoparia dulcis* Linn, Antioxidants, Estimation of total Phenolic compound, Reducing Power, Nitric oxide scavenging method, Superoxide ion Scavenging Activity.

INTRODUCTION

Free radicals, the partially reduced metabolites of oxygen, are highly toxic, mutagenic and reactive. A free radical is a molecule with one or more unpaired electrons in its outer orbital. Many of these molecular species are oxygen (and sometimes nitrogen) centered. These highly unstable molecules tend to react rapidly with adjacent molecules, donating, abstracting, or even sharing their outer orbital electron. This reaction not only changes the adjacent target molecule, sometimes in profound ways, but often passes the unpaired electron along to the target, generating a second free radical or other reactive oxygen species (ROS), which can then go on to react with a new target. In fact, much of high reactivity of ROS is due to the generation of such molecular chain reactions; effectively amplifying their deleterious effects many fold [1]. The later include various diseases like cancer ischemia, atherosclerosis, diabetes, Alzheimer's disease etc.

An antioxidant is a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate [2-3] Antioxidan**t** gets their name because they combat oxidation. Some antioxidant produces endogenously, to protect the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation. During this reaction the antioxidant sacrifice itself by becoming oxidized. However endogenous antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical. Therefore, there is a constant need to replenish antioxidant resources, either endogenously or through supplementation [4**].**

Many antioxidant defense systems in the body such as superoxide dismutase (in mitochondria & cytosol), catalase (in peroxisome), glutathione peroxidase, α-tocopherol (in membrane & lipoproteins) etc. limit the levels and the damage caused by the free radicals[5]. When this multiple defense system fails due to increased production of reactive oxygen species or decreased level of cellular antioxidants, the net result is (i) DNA damage leading to mutations and cancer; (ii) oxidative inactivation and accumulation of metabolic enzymes and an increase in the level of oxidatively modified proteins resulting in aging; (iii) induction and inhibition of lipid peroxidation and oxidative modification of low density lipoproteins leading to deleterious cardiovascular effects.

Due to the advance presented pathological implication of ROS, it is important to find an antioxidant, which may scavenge multiple ROS so that it can be used in multiple disease states and also to maintain a healthy status. The need to identified antioxidants, which can scavenge several free radicals and prevent multiple diseases, can be achieved by simple in vitro test systems. Further the in vitro results can conform to *ex-vivo* and *in-vivo* systems.

Scoparia dulcis Linn commonly known as *Mithi Patti* (Hindi) is distributed in the tropical region of India. It grows as a wasteland herb. The traditional healers have developed its many promising traditional uses. The leaves of this plant used traditionally for abortion, menstrual irregularities and used as female contraceptive. It also used against stomach aches, injuries, wounds, bronchitis, coughs, diarrhoea, eye infection, fever, and kidney failure and liver diseases. This has been used in case of infections such as gonorrhoea, skin infections and warts [6-9].

MATERIALS AND METHODS

Plant collection and Preparation of extract

The plant *Scoparia dulcis* Linn was collected from Brahmapur, District- Ganjam, Odisha and was authenticated by Prof (Dr.) S. K. Dash, Head of the Department, P.G. Bioscience, College of Pharmaceutical Sciences, Mohuda, Berhampur (Ganjam), Odisha.

After collection of plant, was cleaned, shade dried and reduces into coarse powder in an electrical blender. The powdered material was then subjected to Soxhlet extraction with petroleum ether (60-80°C) then extracted with chloroform and then reflux with dehydrated ethanol. The solvent was removed under reduced pressure. The dried extract was used for experiment and converted to the solution as per the procedure.

Preliminary Phytochemical Screening

Different extracts obtained from the above extraction process were analyzed for different phytoconstituents present in these by the method of qualitative phytochemical analysis. The desired chemical tests were carried out by using specific test methods. Petroleum ether extracts rich in alkaloids, tannins-Phenolic compound, proteins- amino acids, steroids-sterols and fat. Chloroform extract was containing alkaloids, glycosides (Cardiac glycosides) tannins-

Phenolic compounds, flavones-Flavonoids, Steroids & Sterols. Ethanol extract was found to contain alkaloids, carbohydrates, glycosides (Cardiac glycoside), tannins-Phenolic compounds, proteins–amino acids, flavones-Flavonoids and Saponins [10-11].

Estimation of total Phenolic compounds

The method of Naczk, M. and Shahidi, F. [12] was followed. 0.1ml of 10mg/ml aqueous solution of the extract was diluted with 46 ml of distilled water in an Erlen Meyer flask. Afterwards, 1 ml of Folin-Ciocalteu Reactive (FCR) was added into this mixture followed by addition of Na_2CO_3 (2%) after 3 min. Subsequently, mixture was shaken on a shaker for 2 hr at room temperature and then absorbance was measured at 760 nm. The concentration of total Phenolic compounds was determined as micrograms of pyrocatechol equivalent by using the equation that was obtained from the standard pyrocatechol graph.

Reducing Power

The reducing power of the extracts was determined according to the previously carried method [13]. Accurately weighted 10 mg of the extract in 1 ml of distilled water was mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. Following incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

Nitric oxide scavenging method

The method of Sreejayan et al., 1997 was followed [14]. For the experiment, sodium nitropruside (10mM) in phosphate buffered saline was mixed with different concentrations of the extract dissolved in methanol and incubated at room temperature for 2 ½ hour. The same reaction without the sample but equivalent amount of methanol served as control. After incubation period, 0.5 ml of Griess reagent was added. Absorbance of the chronopher formed was measured at 546nm. Ascorbic acid was used as positive control.

The procedure is based on the principle that, Sodium nitropruside solution spontaneously generates nitric oxides, which react with oxygen to produce nitric ions that can be estimated using Griess reagent. Scavengers of nitric oxides compete with oxygen leading to reduce production of nitric ions.

Assay for Superoxide ion Scavenging Activity

The assay for superoxide ion scavenging activity was performed as per standard procedure Beuchamp C et al., 1997 [15]. The reaction mixture contained 50mM phosphate buffer (pH 7.6), 20 µg riboflavin, 12mM EDTA and 0.1 mg/ml of NBT (nitro blue tetrazolium), all added in chronological sequence. Reaction was started by illuminating the reaction mixture containing different concentration of sample extract for 90 sec and then measuring the absorbance at 590nm. Ascorbic acid was taken as the positive standard.

RESULTS

Determination of total Phenolic compounds

The three extract PEESD, CESD and EESD were investigated to have 51.03, 41.36 and 45.7 µg pyrocatechol equivalent of phenols respectively (Table no.1). The Phenolic compounds may contribute directly to the antioxidative action [16]. Thus, the antioxidative properties of *Scoparia dulcis* may be possible attributed to the Phenolic compounds present.

PEESD: Petroleum extract of *Scoparia dulcis*

CESD: Chloroform extract of *Scoparia dulcis*

EESD: Ethanol extract of *Scoparia dulcis*

Table 1: Total Phenolic compound content of *Scoparia dulcis*

Sample	Pyrocatechol equivalent
PEESD	51.03±3.05
CESD	41.36±3.78
EESD	45.7±3.00

Results are Mean ± SD,

Absorbance = 0.001XPyrocatechol (µg) + 0.0033

Reducing power

These three samples were having reducing power in concentration dependant manner, i.e., increase in concentration reducing power increases. The results were given in table no. 2.

Table 2: Reducing power of *Scoparia dulcis*

	Absorbance of sample			
Concentration	PEESD	CESD	EESD	Ascorbic acid
15	0.010	0.045	0.033	0.156
30	0.033	0.088	0.079	0.230
45	0.073	0.133	0.115	0.379
60	0.110	0.185	0.176	0.666
100	0.162	0.241	0.223	0.967

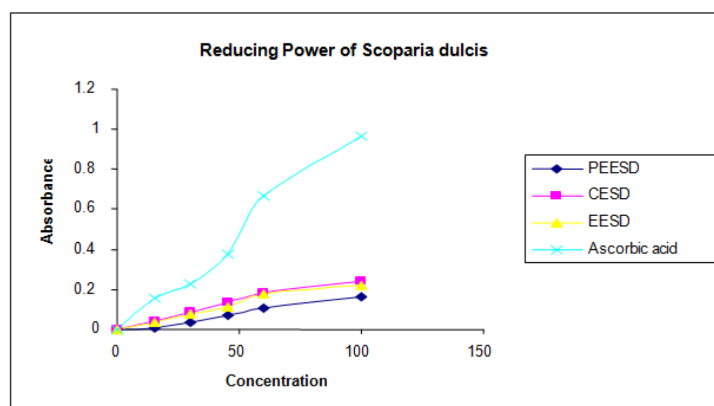

Fig. 1: Reducing power of Scoparia dulcis Linn.

Nitric oxide radical scavenging activity

In present study, the PEESD, CESD and EESD were investigated for their inhibitory effects on nitric oxide production. The percent inhibition of nitric oxide generation by these three extract was shown in table no. 3. It was observed that chloroform extract (CESD) showed more actively inhibit the production of nitric oxide radicals than PEESD and EESD.

Super oxide anion radical scavenging activity

These entire tested samples were found to be possessing scavenging effects on super oxide anions at concentration depending manner. Chloroform extract (CSD) in the dose of 100µg/ml inhibited the production of superoxide anion radical by 41.16±1.77% scavenging activity (Table no. 4).

Table 3: Nitric oxide radical scavenging activity of *Scoparia dulcis*

	% of inhibition		
Concentration	**PEESD**	**CESD**	**EESD**
20	7.34 ± 1.15	17.48 ± 0.92	15.41 ± 1.29
40	13.44 ± 1.36	21.32 ± 1.72	18.82 ± 1.30
60	18.97 ± 1.51	28.14 ± 1.73	24.49 ± 1.94
80	20.65 ± 1.22	37.65 ± 1.59	29.97 ± 1.42
100	24.63 ± 1.51	40.82 ± 2.01	38.90 ± 1.74
r*	0.9805	0.9715	0.9771

Results are Mean ± SD, from three observations

% of inhibition = Control absorbance –Test absorbance /Control absorbance × 100

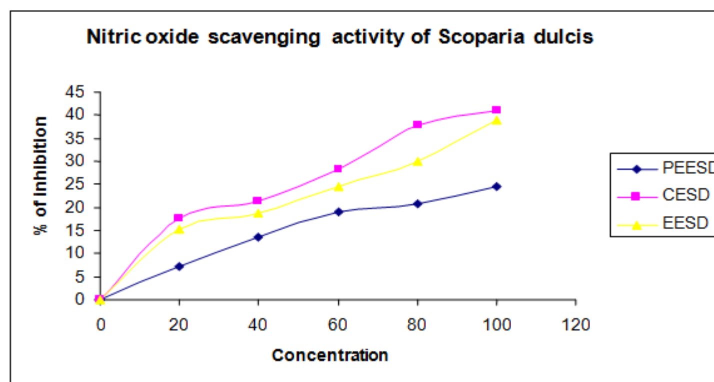

Fig. 2: Nitric oxide scavenging activity of Scoparia dulcis Linn

Table 4: Super oxide anion scavenging activity of *Scoparia dulcis*

Sample	Concentration	% of inhibition	R*
PEESD	20	6.35 ± 2.77	0.97179
	40	11.08 ± 2.66	
	60	13.52 ± 3.22	
	80	18.71 ± 2.19	
	100	30.96 ± 2.77	
CEESD	20	12.78 ± 1.71	0.99228
	40	16.33 ± 2.53	
	60	23.72 ± 2.33	
	80	33.33 ± 2.89	
	100	41.16 ± 1.77	
EESD	20	11.17 ± 3.34	0.99293
	40	15.44 ± 2.77	
	60	21.13 ± 2.44	
	80	31.18 ± 2.56	
	100	38.43 ± 2.45	

Results expressed Mean ± SD, from three observations

% of inhibition = Control absorbance –Test absorbance /Control absorbance × 100

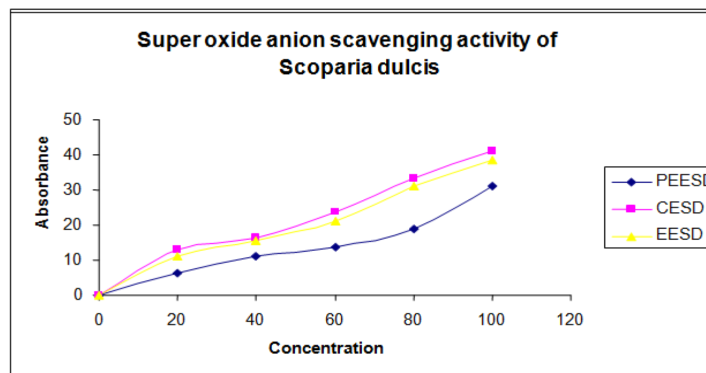

Fig. 3: Super oxide anion scavenging activity of Scoparia dulcis Linn

DISCUSSION

The entire tested sample showed antioxidant activity in different models of this study. On comparison it was found that CESD has highest antioxidant activity whereas PEESD has least activity. The antioxidant activity of these samples might be due to inactivation of free radicals or complex formation with metal ions, or combination of both.

Most of the mammals have inherent mechanism to prevent and neutralize the free radical induced the damage. In biochemical system superoxide radical and H_2O_2 reacts together to form the singlet oxygen and hydroxyl radical, these can attack and destroy almost all known biochemical [17]. Hydroxyl radical produced may cause sugar fragmentation; base loss and leakage of DNA strand [18]. The tested sample scavenges off these free radicals but also inhibits the generation of the free radicals. It was already reported that naturally occurring Phenolic compounds are effective hydrogen donors which make them antioxidant [19].

CONCLUSION

From our investigation, we concluded that all the three extracts of *Scoparia dulcis* L. (PEESD, CESD and EESD) possessed important antioxidant activity. Therefore, number of scientific reports indicated certain flavonoids, alkaloids, triterpenoids and steroids have antioxidant properties. Presence of those phytochemical in *Scoparia dulcis* L. may be responsible for the antioxidant properties.

REFERENCES

1. Mishra MR, Behera RK, Jha S, Panda AK, Mishra A, Pradhan DK, Choudary PR. A Brief Review on Phytoconstituents and Ethnopharmacology of *Scoparia Dulcis* Linn, Int J Phytomedicine, 2011, 3, 422-438.
2. Murti K, Panchal M, Taya P, Singh R. Pharmacological properties of *Scoparia Dulcis:* Review. Pharmacologia 2012; 3(8): 344-347.
3. Orhue NE J, Nwanze EAC. *Scoparia dulcis* reduces the severity of Trypanosoma brucei induced hyperlipidaemia in the rabbit, Afr J Biotechnol, 2006, 5 (10), 883-887.
4. Trease GE, Evans MC, editors. Textbook of Pharmacognosy 14th ed. London: Bailliere Tindal, 1996, 565-566.
5. Kokate CK, Practical Pharmacognosy, 4th ed. New Delhi, Vallabh Prakashan, 1994, 107-111.
6. Naczk, M. and Shahidi, F**.** The effect of methanol ammonia water treatment on the content of phenolic acids of canola. Food Chem. 1989; 31, 159-164.
7. Oyanzu, M**.** "Studies on products of browing reaction."Antioxidative activities of products of browing reaction prepared from glucosamine, Japanese J. Nutr. 1986; 44, 307-315.
8. Sreejayan, Rao MNA**.** Nitric oxide Scavenging by Curcuminoids J. Pharm Pharmacol. 1997; 49,105-107.
9. Beuchamp, C. and Fridovich, I**.** Superoxide dismutase: Improved assays and assay applicable to acrylamide gel. Anal. Biochem. 1971; 44, 276-277.
10. T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, D. Yasuhara**.** Effects of interaction of tannins with co-existing substances VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical**.** Chemical and Pharmaceutical Bulletin. 37: 2016-2021 (1989).
11. S. Chakraborty, Naik Asha, R. Gali, R. Reddy. Phenyl hydrazine mediated degradation of bovine serum albumin and membrane proteins of human erythrocytes. Biochem et Biophys Acta. 1028:89-94(1990).
12. K. M. Ko, P. K. Yick, T. W. Chiu, T. Y. Hui, C. H. K. Cheng, Y. C. Kong. Impaired antioxidant status I in CCl4 in toxicated rats: an in vivo study. Fitotherapia. L14:539-44 (1993).
13. C. A. Rice-Evans, N. J. Miller, P. G. Bolwell, P. M. Bramley, J. B. Pridham**.** The relative antioxidant activity of plant derived polyphenolic flavonoids. *Free* Radical Research. 22: 375-83 (1995).
14. Sreejayan, Rao MNA. Nitric oxide Scavenging by Curcuminoids J. Pharm Pharmacol. 1997; 49,105-107.
15. Beuchamp, C. and Fridovich, I**.** Superoxide dismutase: Improved assays and assay applicable to acrylamide gel. Anal. Biochem. 1971; 44, 276-277.
16. T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, D. Yasuhara**.** Effects of interaction of tannins with co-existing substances VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical**.** Chemical and Pharmaceutical Bulletin. 37: 2016-2021 (1989).
17. S. Chakraborty, Naik Asha, R. Gali, R. Reddy. Phenyl hydrazine mediated degradation of bovine serum albumin and membrane proteins of human erythrocytes. Biochem et Biophys Acta. 1028:89-94(1990).
18. K. M. Ko, P. K. Yick, T. W. Chiu, T. Y. Hui, C. H. K. Cheng, Y. C. Kong**.** Impaired antioxidant status I in CCl4 in toxicated rats: an in vivo study. Fitotherapia. L14:539-44 (1993).
19. C. A. Rice-Evans, N. J. Miller, P. G. Bolwell, P. M. Bramley, J. B. Pridham**.** The relative antioxidant activity of plant derived polyphenolic flavonoids. *Free* Radical Research. 22: 375-83 (1995).