Pharmacophore 2010, Vol. 1 (2), 77-81. ISSN 2229 – 5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

EVALUATION OF NITRIC OXIDE AND HYDROGEN PEROXIDE SCAVENGING ACTIVITY *DALBERGIA SISSOO* ROOTS

Pooja[1]*, Priyanka Sharma[1], K.C.Samanta[1], Vikas Garg[2]

[1]* Department of Pharmacology,

Suresh Gyan Vihar University,

Jaipur,Rajasthan, India

[2]Department of Pharmaceutical Chemistry,

Manipal University, Manipal, India

ABSTRACT

Antioxidant activity of methanolic extract of *Dalbergia sissoo* root was investigated for its free radical scavenging activity by determining the nitric oxide and hydrogen peroxide scavenging activity. Maximum scavenging of nitric oxide and hydrogen peroxide found were 26.66% and 50.68% respectively at 250 µg/ml concentration. The results were compared with rutin as a standard. These results clearly indicate that *Dalbergia sissoo* is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

Keywords: *Dalbergia sissoo*, antioxidant, free radical, nitric oxide.

INTRODUCTION

Plants are the one of the important source of medicines. It has been known since ancient time for its medicinal use.

Dalbergia sissoo Roxb. (Synonyms-Shisham or Sisam),a large deciduous tree found through India, has been reported in folk medicines and is used mainly as aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic. It is also used in conditions like emesis, ulcer, leucoderma, dysentery, stomach troubles and skin diseases.[1-3]

MATERIAL AND METHOD

The roots of the plant *Dalbergia Sissoo* were collected from Haridwar and authenticated by Rajasthan university.The roots were dried under shade , coarsely powdered and 50g root powder was extracted with 400ml of methanol for 18h by hot continuous extraction method. The methanolic extract was filtered and partitioned by using petroleum ether to remove impurities .The solvent was evaporated under reduced pressure and dried in vacuum. The dried extract of *Dalbergia Sissoo* thus obtained was used for the assessment of antioxidant activity.

The extracts were subjected to preliminary qualitative tests[4,5] to identify the various phytoconstituents present in leaves. The qualitative chemical tests performed were Shinoda test, ammonia fuming test, lead acetate, boric acid for flavonoid containing compounds and ferric chloride test, nitric acid test, ammonia hydroxide, potassium ferricyanide test, lead acetate test for the presence of tannins. This entire test gave positive results when they were compared with Rutin, standard drugs of the class.

NITRIC OXIDE RADICAL INHIBITION ASSAY

Nitric oxide radical inhibition was estimated by the use of Griess Illosvoy reaction.[6, 7] In this investigation, Griess Illosvovy reagent was generally modified by using naphthyl ethylene diamine dihydrochloride (0.1%w/v) instead of the use of 1- naphthylamine (5%). There action mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and the extract (20-250 µg/ml) standard solution (rutin, 0.5 ml) were incubated at 25^{o}C for 150 minutes. A control test compound equivalent amount of methanol was taken. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotization. Further, 1 ml of the naphthyl ethylene diamine dihydrochoride was added, mixed and was allowed to stand for 30 min at 25^{o}C. The concentration of nitrite was assayed at 540 nm and was calculated with the reference to the absorbance of the standard nitrite solutions. Rutin was taken as a standard. The percent inhibition was calculated using the formula[8]:

% inhibition = [(Acont- Atest)/Acont] X 100 …… (1).

Where Acont is the absorbance of the control reaction and A test is the absorbance in the presence of samples with the extracts.

SCAVENGING OF HYDROGEN PEROXIDE

The ability of the *Dalbergia Sissoo* to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng and Klaunig.[9] A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). *Dalbergia Sissoo* (16–250µg /ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction.

The percentage scavenging activity of hydrogen peroxide by *Dalbergia Sissoo* was calculated using the following formula,

% scavenging activity [H_2O_2] = [Abs (control) – Abs (standard) / Abs (control)] × 100.

Where, Abs (control): Absorbance of the control and Abs (standard): Absorbance of the extract/standard.

Table 1: Antioxidant activity of chloroform extract of *Dalbergia Sissoo Roxb.* Root Nitric oxide inhibition Assay

Concentration (µg/ml)	Nitric oxide Radical Scavenging Activity (% inhibition)		Hydrogen Peroxide Scavenging Activity (% inhibition)	
	Rutin (std.)	DSME	Rutin (std.)	DSME
16	25.7 ± 0.44	7.95 ± 0.53	31.2 ± 0.37	18.7 ± 1.03
32	41.2 ± 1.6	12.05 ± 1.4	51.78± 0.67	25.1 ± 1.09
63	49.4 ± 0.61	16.62 ± 2.10	55.6 ± 1.14	36.4 ± 1.5
125	61.2 ± 1.60	30.31 ± 1.65	62.4 ± 1.9	43.7 ± 2.80
250	71.6 ± 0.49	25.54 ± 1.55	61.5 ± 1.7	49.8 ± 2.50
IC_{50}	63.2	510.89	31.5	252.5

Data are presented as the mean ± SD (n=3)

DSME-*Dalbergia sissoo* methanolic extract; Std.-Standard

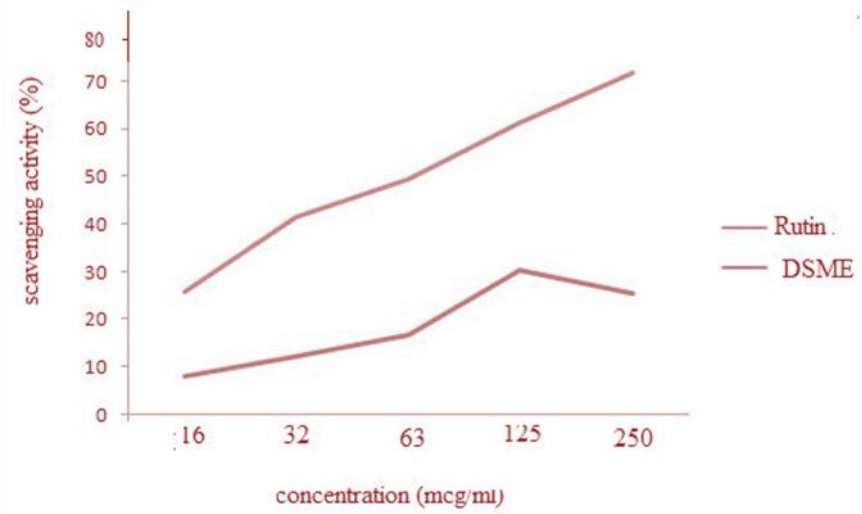

Figure1: Nitric oxide radical scavenging activity

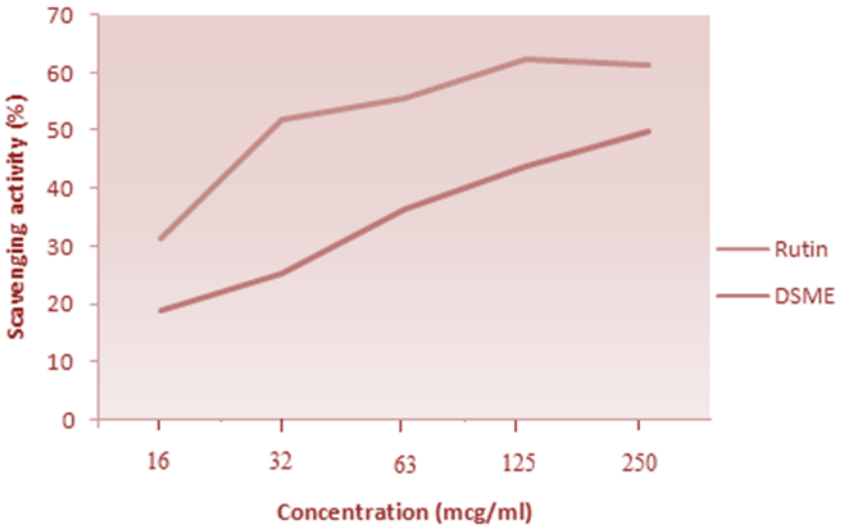

Figure2: Hydrogen peroxide scavenging activity

RESULTS

The preliminary qualitative tests indicated the presence of flavonids, phenols and tannins. Several concentrations ranging from 16-250 µg/ml of the *Dalbergia Sissoo* were tested for antioxidant activity in different *in vitro* models. Table 1 show that the percentage inhibition of nitric oxide and hydrogen peroxide by DSME. The percentage inhibition for the hydrogen peroxide was found to be moderate and nitric oxide radical is significant when compared to the reference standard. (Fig 1 and Fig 2). Values are expressed as mean ± SEM of three measurements.

STATISTCAL ANALYSIS

Statistical analysis was performed by the student t-Test and by ANOVA. IC_{50} values for all the above experiments were determined by linear regression analysis. The activity is increasing with the concentration and difference were statistically significant ($p<0.01$).

DISCUSSION

Antioxidant compounds act by several mechanisms such as, inhibition of generation and scavenging activity against reactive oxygen species (ROS); reducing power; metal chelation; activity as antioxidative enzymes; inhibition of oxidative enzymes. Oxidative damage caused by ROS will lead to among others to DNA lesions, loss of functions of enzymes, increased cell permeability, disturbed signaling over the cell and eventually necrotic cell death or apoptosis.[10, 11]

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons etc and is involved in the regulation of various physiological processes.[12]

Nitric oxide radical generated from the sodium nitropruside and measured by the Greiss reduction. Sodium nitropruside at physiological pH spontaneously generates nitric oxide, which thereby interacts with

oxygen to produce nitrate ions that can be estimated by use of Greiss reagents.

Hydrogen peroxide is a non-radical form of ROS that is formed in living organisms by superoxide dismutase. Hydrogen peroxide is not by itself very active but it can cross biological membranes and generates hydroxyl radicals which are toxic to cells and can damage a number of biomoleucles.[13] Thus, removing of H_2O_2 is very important for protection of living organism. Hydrogen peroxide is highly diffusible and can cross the plasma membrane.

REFERENCES

1.Kirtikar, KR and Basu, BD (1933), ***Indian Medicinal Plants***, 2nd Ed., Vol.1, Lalit Mohan Basu, 49-Leader Road, Allahabad,818.

2.Nadkarni, KM (1954), ***Indian Materia Medica***, 3rd Ed., Vol.1, Bombay Popular Book Depot, 432.

3.Chopra, RN; Nayer, SL and Chopra, IC (1956), ***Glossary of Indian Medicinal Plants***, CSIR, New Delhi, 90-91.

4.Hyoung, Lee S (1992), "Antioxidant activity of browning reaction products isolated from storage aged orange juice", ***J Agric Food Chem***, 40(4), 550-552.

5.Khandelwal, KR (2000), ***Practical Pharmacogonosy, Techniques and Experiments***, 2nd Ed., Nirali Prakashan, Pune, 45.

6.Garrat, DC (1964), ***The quantitative analysis of Drugs***, Vol.3, Chapman and Hall Ltd.Japan.

7.Green, LC; Wagner, DA; Glogowski, J *et al*. (1982), "Analysis of nitrate, nitrite and 15 N in Biological fluids", ***Anal Biochem***, 126, 131-136.

8.SK, Surendra and GK,Vivek (2007), "Free Radical Scavenging Activity of *Ficus racemosa* Roots", ***Indian J Pharm.Educ.Res***, 41(4).

9.Ruch, RJ;Cheng, SJ and Klaunig, JE (1989), "Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from chinese green tea", ***Carcinogenesis***, 10,1003-1008.

10.Lata, H and Ahuja, G K(2003), "Role of free radicals in health and disease", ***Ind J Physio & Allied Sci***, 57, 124.

11.Halliwell, B and Gutteridge, JM (1984), "Oxygen toxicity, oxygen radicals, transition metals and disease", ***Biochem. J.***, 219,1–14.

12.Halliwell, B (1989), "***Free radicals in biology and medicine***", 2nd Ed, Clavendon press, Oxford New York, 236.

13.Kerr, ME; Bender, CM and Monti, EJ (1991), "An introduction to oxygen free radicals", ***Heart and Lung***, 25 (3), 200-209.

14.Chakraborthy, GS (2009), "Free radical scavenging activity of *aesculus indica* leaves", ***IJPTR, Vol.1***, (3), 524-526.