

FREE RADICAL SCAVENGING ACTIVITY OF *AESCULUS INDICA* LEAVES

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ABSTRACT : Antioxidant activity of chloroform extract of *Aesculus indica* leaves was investigated for its free radical scavenging activity by determining the nitric oxide and superoxide radical scavenging activity. Maximum scavenging of nitric oxide and superoxide radical found were 27.62% and 48.65% respectively at 250 µg/ml concentration.

Keywords: *Aesculus indica*, antioxidant, free radical, nitric oxide

INTRODUCTION

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases¹. Thus the present investigation was aimed at evaluating the antioxidant activity of *Aesculus indica* (Linn.) leaves.

Aesculus indica (Linn.) belonging to the family Sapindaceae is an herbaceous, soft perennial plant abundantly found in Northern Western Himalayas. Generally it is called as Bankhor. It is generally known for its medicinal properties. Seeds are used as astringent, nutritious, while the oil is used in the treatment of skin disease and rheumatism². Fruit are used in colic disorders³; roots are used in leucorrhoea treatment⁴. As a medicine, it is especially used for the complaints of the veins, such as phlebitis, haemorrhoids, varicose veins; in ulcers; to prevent thrombosis; in some cases of migraine, effusions of blood; for limb complaints and frost bite. The plant is reported to contain a mixture of saponins, one of which is described as aescine, which easily crystallizes. In addition it also contains flavonoid glycosides, aesculine, albumin and fatty oils. Aesculin is used medically and extracts from the seeds are used industrially. The hydroxycoumarin glycoside aesculin from the bark of the branch absorbs ultra-violet rays and is an ingredient for suntan oil⁵.

EXPERIMENTAL MATERIALS AND METHODS

PLANT MATERIAL: Fresh leaves of the plant *Aesculus indica* (Linn.) were obtained and identified from authentic sources. A voucher specimen has been identified and deposited at the Department of Pharmacognosy, School of Pharmacy and Technology Management, Shirpur. The collected leaves were dried in shade, crushed to coarse powder and used for further studies.

PREPARATION OF EXTRACT: The dried plant material leaves (500 gm) were subjected to continuous hot extraction with chloroform for 48 h in a Soxhlet apparatus. The chloroform extract was filtered and partitioned by using petroleum ether to remove the fixed oils, fats and other non polar constituents present in it. The solvent was evaporated under reduced pressure and dried in a vacuum desiccator and was used when required. The dried extract (CEAI) thus obtained (280 gm) was used for the assessment of antioxidant activity. 36 hours⁶.

The extracts were subjected to preliminary qualitative tests to identify the various phytoconstituents present in leaves⁷. 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. All these tests gave positive results when they were compared with Rutin and Quercetin, standard drugs of the class.

NITRIC OXIDE RADICAL INHIBITION ASSAY

Nitric oxide radical inhibition was estimated by the use of Griess Illosvov reaction⁸. In this investigation, Griess Illosvov reagent was generally modified by using

naphthyl ethylene diamine dihydrochloride (0.1 % w/v) instead of the use of 1- naphthylamine (5 %). The reaction 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) was incubated at 25°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 dihydrochloride was added, mixed and was allowed to stand for 30 min at 25°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: % inhibition = $[(A_{\text{cont}} - A_{\text{test}})/A_{\text{cont}}] \times 100 \dots\dots(1)$. Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of samples with the extracts.

SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Superoxide radical scavenging activity is generally based on the anion radical which is associated with PMS-NADH system. The measurement of superoxide scavenging activity of the is based on method as described by Liu⁹. They are generated within PMS-NADH systems by the oxidation of NADH and are assayed by the reduction of nitroblue tetrazolium (NBT). Tris HCl buffer (3 ml, 16 mM, pH 8.0) containing 1 ml NBT (50 µM) solution, 1 ml NADH (78 µM) solution and a sample solution of extract (20-250 µg/ml) in water were mixed. The reaction was started when 1 ml of phenazine methosulfate (PMS) solution (10 µM) was added to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance was read at 560 nm against the corresponding blank samples. Quercetin was used as a reference drug. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition was calculated by using the same formula (1). Chloroform extract was dried under reduced vacuum pressure and finally it was made soluble in the corresponding solvents

(phosphate-buffer). The tests were performed with the filtrate soluble portion.

RESULTS AND DISCUSSION

The preliminary qualitative tests indicated the presence of flavonoids, phenols and tannins. Table 1 and Table 2 shows that the percentage inhibition of nitric oxide and superoxide radical by CSCE. The percentage inhibition for the superoxide radical was found to be moderate and nitric oxide radical is less significant when compared to the reference standard.

Values are expressed as mean \pm SEM of five measurements. Statistical analysis was performed by Dunnett's Test 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 ($p < 0.01$). After 250µg/ml there was decrease in the activity with a negative effect.

DISCUSSION

Oxidative stress in large quantities of reactive oxygen species (ROS) are generated is one of the earliest responses to stress. The antioxidant system protects the pathogens against the ROS-induced oxidative damage. Nitric oxide radical generated from the sodium nitroprusside and measured by the Greiss reduction. Sodium nitroprusside 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. Thus the scavengers of nitric oxide compete with the oxygen, leading to reduced production of nitric oxide. In the PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Thus, CSCE fraction, possessed good antioxidant property so, work should be carried out with more antioxidant models. The results were positive at the laboratory level and further work can be carried out to find out the exact constituent responsible for these activities by the process of modern analytical tools. Thus the plant can be a potential source for antioxidant property.

Table 1 Antioxidant activity of chloroform extract of *Aesculus indica costus speciosus* Linn. leaves
Nitric oxide inhibition Assay

Concentration (µg/ml)	Rutin (Std. %)	AICE (%)
20	31.0 \pm 0.43	6.75 \pm 0.21
40	45.2 \pm 1.4	11.0 \pm 1.00
100	54.4 \pm 0.78	19.44 \pm 1.13
125	69.8 \pm 1.16	21.43 \pm 2.22
250	77.3 \pm 0.42	27.62 \pm 1.67
IC ₅₀ (µg/ml)	67.8	513.84

Values are presented as the mean \pm SEM (n=10)

AICE- *Aesculus indica* chloroform extract; Std.- Standard.

**Table2.Antioxidant activity of chloroform extract of *Aesculus indica* Linn. leaves
Superoxide Scavenging Assay**

Concentration ($\mu\text{g/ml}$)	Quercetin (Std. %)	AICE (%)
20	35.0 \pm 0.32	16.75 \pm 0.44
40	51.2 \pm 0.51	29.0 \pm 1.02
100	57.3 \pm 0.86	36.5 \pm 1.12
125	59.5 \pm 1.16	43.7 \pm 2.64
250	61.3 \pm 1.12	48.65 \pm 1.71
IC ₅₀ ($\mu\text{g/ml}$)	62.5	235.4

* Values are presented as the mean \pm SEM (n=10)

AICE- *Aesculus indica* chloroform extract;Std.- Standard.

REFERENCES

1. Perianayagam J.B., Sharma S. K., Joseph A. and Christiana A. J. M., Evaluation of Antidiarrheal Potential of *Embllica officinalis* ,J Ethnopharmacol.,2004, 95, 1, 85-87.
2. Badoni A.K., An Ethnobotanical Survey of Pinswari Community – A Preliminary Survey, Bul Bota Survey India, 2000, 32, 110-115.
3. Rana T. S. and Datt B., Ethnobotanical Observation Among Jaunsaries of Jaunsar-Bawar, Dehradun (U.P.) India, Intern J Pharmaco, 1997 35, 371-374.
4. Shah N. C. and Joshi M. C., An Ethnobotanical Survey of the Kumaon Region of India, Econo Bot, 1971,25, 414-422.
5. Singh B., Agarwal P. K., and Thakur R. S., Aesculuside A- A new triterpene glycoside from *Aesculus indica*., Planta Medica, 1986,52, 5, 409-410
6. Kokate C. K., Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 2005, 110-111.
7. Harbone J. B., Phytochemical Methods- A Guide To Modern Techniques of Plant Analysis , Chapman and Hall London, 1998, 42, 129, 189, 203.
8. Hyung Lee S., Antioxidant activity of browning reaction products isolated from storage-aged orange juice., J Agric Food Chem, 1992; 40, 4, 550-552.
9. Liu F., Ooi VEC., and Chang S. T., Free radical scavenging activity of mushroom polysaccharide extracts., Life Science, 1997, 60, 763-771..
