Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2009; 1(2): 121-123

Short Communication

ISSN 0975-248X

Antioxidant Activity of the Successive Extracts of *Aesculus indica* Leaves

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ABSTRACT

Plants are the best source of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like Analgesic, Anti-inflammatory, Antioxidant, hypoglycemic agents and many more. The successive extracts of *Aesculus indica* leaves were screened for *in vitro* antioxidant properties using the standard procedures. The successive extracts such as petroleum ether, ethyl acetate, methanol and water and 50% crude methanol extracts exhibited IC 50 values of respectively in DPPH and respectively in nitric oxide radical inhibition assays. The values are comparable with the standards such as ascorbic acid and quercetin. The *Aesculus indica* leaves are showing significant antioxidant activity**.**

Keywords: *Aesculus indica*, Antioxidant, DPPH, Nitric Oxided, Peroxidation, Free radical scavenging.

INTRODUCTION

Aesculus indica (Linn.) (Sapindaceae) is a herbaceous, soft perennial plant abundantly found in Northern Western Himalayas. [1] Generally it is called as Bankhor. It is generally known for its medicinal properties. Seeds are used as astringent, nutritious [2], while the oil is used in the treatment of skin disease and rheumatism. [1] The whole plant is used in the treatment of diabetes. Fruit are used in colic disorders [3]; roots are used in leucorrhoea treatment. [4] As medicine, it is especially used for the complaints of the veins, such as phlebitis, haemorrhoids, vari-cose veins; in ulcers; to prevent thrombosis; in some cases of migraine, effusions of blood; for limb complaints and forst bite. The plant is reported to contain a mixture of saponins, one of which is described as aescine, which easily crystallizes. In additional it also contains flavonoid glycosides, aesculine, albumin and fatty oils. Aesculin is used medically and extracts from the seeds are used industrially. The hydrosycoumarin glycoside aesculin from the bark of the branch absorbs ultra-violet rays and is an ingredient for suntan oil. [5]

From the literature cited very few works has been carried out in this plant. Thus it was thought worthwhile to explore this plant for its therapeutic activity. Lipid peroxidation is the outmost important biochemical assay which is involved in pathogenesis of many diseases like diabetes mellitus, atherosclerosis, tumor, myocardial infraction and also in the process of ageing. Free radicals generally called as reactive oxygen species (ROS) are synthesized *in vivo* from a various biochemical reactions and tends to form a chain in the system. [6-7] These free radicals are the major points in lipid peroxidation. [8-9] Plants containing Flavonoids have been reported to possess strong oxidant properties. Thus in the present investigation the successive extraction of *Aesculus indica* leaves was screened for *in vitro* antioxidant properties using standard operating procedures.

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MATERIAL AND METHODS

Chemicals

Chemicals used in this study were 1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, sodium nitrite, trichloroacetic acid, Folin-Ciocalteu reagent, butylated hydroxyl anisole (BHA), ascorbic acid (Merck [India]), Gallic acid, linoleic acid (Sigma).All reagents used for the experiments were of analytical grade (AR).

Collection of plant material: The plant was collected from the wild sources of Shirpur forest, Maharashtra, India in the month of May 2008. The plant was identified and authenticated from standard resources.

Preparation of extracts and Standards: The successive extracts of the shade dried powdered leaves (500 g) of

Aesculus indica was prepared with different solvents as per the order of their polarity in Soxhlet apparatus. The solvents were evaporated with the help of rotary evaporated to get a solid residue (12-gram). The solid residue was placed in a vacuum desicator and was further used for the experiments. [10-12] The *in vitro* experiments, a weighed quantity (5-gram) of the extract was dissolved in Dimethyl Sulphoxide (DMSO) or methanol and used. Solution of ascorbic acid and quercetin were used as standards for *in vitro* studies were prepared in distilled DMSO.

Estimation of total phenolics: The total phenolic contents of ethanol extract was determined with Folin-Ciocalteu reagent according to Slinard & Singleton [13] and slightly modified. The stock solution of extract 1mg/ml in water was prepared. From the stock solution, 5 ml was transferred to a 25 ml volumetric flask and made up with distilled water. Out of this 5 ml of sample and 2 ml of standard was taken in 25 ml volumetric flask, to this 10 ml of distilled water, and 2 ml of phenol reagent (20 % v/v) was added, and then the volume was made up with 29 % sodium bicarbonate. The mixture was kept in the dark for 20 min. and the absorbance was read at 760 nm. The total phenolic content was calculated as gallic acid and expressed as percent of gallic acid detected. Standard used was gallic acid.

Antioxidant Assay

Free radical scavenging activity using DPPH radical: The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. [14-15] A total of 100 µL of the methanolic extract (from 20 to 40 µg ml in DMSO solution). After the incubation period at 37°C for 50 min; the absorbance of each solution was determined at 490 nm the corresponding blank readings were also noted and the remaining DPPH was calculated and tabulated in Table 1. An IC_{50} value is the concentration of sample required to scavenging 50 % DPPH free radical.

Table 1: Antioxidant activity of *Aesculus indica* leaves extracts using DPPH method

S. No.	Test Compound	IC_{50} values ± SE *(µg/mL)
1.	Petroleum ether extract	247.12 ± 1.57
2.	Ethyl acetate extract	20.00 ± 0.57
3.	Methanol extract	28.67 ± 1.20
4.	50 % Methanol crude extract	29.34 ± 1.86
5.	Aqueous crude extract	177.82 ± 1.35
6.	Ascorbic acid	77.66 ± 1.52
7.	Quercetin	58.00 ± 0.77

* Average of 8 determination

Nitric oxide scavenging activity: Aqueous solution of Sodium nitropruside at physiological pH spontaneously released nitric oxide, which can be estimated with oxygen to produce nitrite ions, which can be estimated by the use of Griess IIosvoy reaction. [16] The scavengers of nitric oxide reduce the production of nitric oxide. The reaction mixture (3 ml) containing sodium nitropruside (10 mM, 2 ml), phosphate buffer saline (0.5) and the extract or the standard solution (0.5 ml) was incubated at 25 C for 2.5 h. After incubation, 0.5 ml of the reaction mixture containing nitric was pipette out and were mixed with 1 ml of sulphanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min. for completion diazotization. 1 ml of 1-naphthylaimne (5 %) was added, mixed and allowed standing for 30 min. a pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions and is tabulated in Table 2. An IC_{50} value is defined as the concentration of sample required to inhibit 50 % of the nitric oxide radical.

Table 2: Antioxidant activity of *Aesculus indica* leaves extracts using Nitric oxide radical inhibition assay method

S. No.	Test Compound	IC_{50} values ± SE *(µg/mL)
1.	Petroleum ether extract	28.00 ± 0.85
2.	Ethyl acetate extract	46.02 ± 0.57
3.	Methanol extract	52.09 ± 1.23
4.	50 % Methanol crude extract	70.68 ± 1.05
5.	Aqueous crude extract	153.33 ± 0.84
6.	Ascorbic acid	22.66 ± 0.98
7.	Quercetin	18.50 ± 0.88

* Average of 8 determination

RESULTS AND DISCUSSION

In vitro assay: The successive extracts of *Aesculus indica* exhibited antioxidant activity in DPPH and nitric oxide radical inhibition assay as evidence by the lowering of IC_{50} values (Table 1 and 2). The successive extracts such as petroleum ether, ethyl acetate, methanol, water and 50 % crude methanol extract exhibited IC 50 values 247.12 ± 1.57, 20.00 ± 0.57, 28.67 ± 1.20, 177.82 ± 1.35 and 29..34 ± 1.86 µg/mL espectively in DPPH and 28.00± 0.85, 46.02 ± 0.57, 52.09 ± 1.23, 153.33 ± 0.84 and 70.68 ± 1.05.98 µg/mL respectively in nitric oxide radical inhibition assay. These values were observed to be more than those which were obtained from the ascorbic acid and quercetin used as standards. Thus it can be stated that free radical oxidative stress has a major role in the pathogenesis of a wide range of clinical disorders resulting from different natural antioxidant defences. Among the five extracts of *Aesculus indica* leaves and 2 standards tested for antioxidant activity using DPPH method, the ethyl acetate successive extract showed the maximum antioxidant activity with IC_{50} values of 18.00 ± 0.57µg/ml respectively. The methanol extract showed antioxidant activity with IC_{50} values 28.67 ± 1.20µg/ml. The 50 % crude methanolic extract showed IC 50 values 29.34 ± 1.05.98µg/ml respectively. However petroleum ether extract exhibited the lowest antioxidant activity with an IC 50 value of 249.12 ± 1.57µg/ml. The standards exhibited IC 50 values 77.66 ± 1.52 and 58.00 ± 0.77 µg/ml respectively. Thus from the above investigation it can be stated that antioxidant are essential as they play an important role in the immune system and in ageing process.

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