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In Vitro Antioxidant and free Radical Scavenging activity of the Ethanolic extract of *Aesculus hippocastanum*

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Abstract: The aim of the present study was to evaluate the antioxidant and free radical scavenging activity of the ethanolic extract of *Aesculus hippocastanum* (Horse chest nut). Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidants play an important role in protecting cellular damage caused by reactive oxygen species. Plants containing phenolic compounds have been reported to possess strong antioxidant properties. Antioxidant potential of the ethanolic extract of *Aesculus hippocastanum* was studied using different *in vitro* free radical scavenging models like DPPH and Hydrogen Peroxide. The DPPH results have been compared with the standard Ascorbic acid .The extract showed good dose dependent free radical scavenging property in both the models used in this study.

Keywords: Horse chest nut, Free Radical Scavenging, Antioxidant Activity, DPPH, Hydrogen Peroxide, Plant Extract.

Introduction

During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS), exert oxidative stress towards the cells of human body. The oxidative damage caused by free radical is related to pathogenesis of many chronic degenerative diseases like cancer, diabetes, neurodegenerative disease, atherosclerosis, cirrhosis, malaria and AIDS.[1] Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS) [2].Although the human body produces antioxidant enzymes to neutralize free radicals, a diet rich in edible antioxidants is recommended to assist the human body to protect itself.

Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity. *In vitro* experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species.[3]

Aesculus hippocastanum (family Hippocastanacae) is commonly known as Horse chestnut is native to Western Asia. It is widely cultivated as an ornamental tree, especially in northern Europe and North America. The seeds have been used as an analgesic, antipyretic,

narcotic, tonic, and vasoconstrictor.[4] They have been used to treat backache, sunburn, neuralgia, rheumatism, whooping cough and hemorrhoids. The bark has been used as a tonic, narcotic, antipyretic and to induce sneezing.[5] The extracts of Horse chestnut have been traditionally employed both in the West and East for the treatment of peripheral vascular disorders including haemorrhoids, varicose veins, leg ulcers and bruises. It is used in the treatment for chronic venous insufficiency and peripheral edema.[6] It has antilipemic, expectorant, diuretic properties and antimicrobial activity. It is also used for the prevention of gastric ulcers, reduction of cerebral edema, reduction of cellulite, as adrenal stimulant, hypoglycemic agent, antithrombotic, anti-inflammatory, and also for reduction of hematomas and inflammation from trauma or surgery.[7] Active chemical constituents of horse chest nut are coumarin derivatives like aesculin, fraxin, scopolin; flavonoids like quercetin, kaempferol, astragalin, isoquercetrin, rutin, leucocyanidine and essential oils like oleic acid, linoleic acid.[8] Other constituents include amino acids (adenosine, adenine, guanine), allantoin, argyrin, carotin, choline, citric acid, epicatechin, leucodelphinidin, phyosterol, resin, scopoletin, tannin, and uric acid. [9] The present study was to evaluate the antioxidant and free radical scavenging activity of the ethanolic extract of *Aesculus hippocastanum* (Horse chest nut).

Materials and Methods

Plant Material

The ethanolic and aqueous extracts of (Horse chestnut) were obtained from Green Chem Herbal Extract & Formulations, Bangalore.

DPPH Radical scavenging test:

The free radical scavenging activity of the ethanolic extract of *Aesculus hippocastanum* was determined by using 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) using UV-Spectrometry at 517nm. The DPPH solution was prepared in 95% methanol. The stock solution of the extract was also prepared in 95% methanol (10mg/100ml). From the stock solution 2 ml,4 ml, 6 ml, 8 ml and 10 ml were taken in five test tubes and diluted with same solvent to get a final concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing 1 ml of the test extract and after 30min, the absorbance was taken at 517nm using spectrophotometer. A similar procedure was repeated with distilled water instead of extract which serves as control. Ascorbic acid was used as standard. 95% methanol was used as blank. All the tests were performed in triplicate to avoid test error. % scavenging of the DPPH free radical was measured using following equation.[10,11]

% of DPPH radical scavenging =

$$\frac{\text{(Absorbance of control – Absorbance of test Sample) x 100}}{\text{(Absorbance of control)}}$$

Table 1: DPPH radicals scavenging activity of Ascorbic acid and Ethanolic extract of *Aesculus hippocastanum*

Concentration of the Extract (µg)	Scavenging activity of Ascorbic Acid (%)	Scavenging activity of the Extract (%)
20	40.32	21.24
40	55.26	29.52
60	76.10	71.05
80	89.18	83.35
100	97.35	92.72

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Hydrogen Peroxide Scavenging Activity:

The ability of plant extract to scavenge hydrogen peroxide is determined by taking 0.5ml of hydrogen peroxide (1ml of 30% of hydrogen peroxide was made up to 45ml with distilled water), 1 ml of Sodium phosphate buffer pH 7.4, 0.01m, w/v (mixing 30ml of solution A-156mg of sodium dihydrogen phosphate was dissolved in 100ml of distilled water; with 70ml of solution B-178mg of disodium hydrogen phosphate was dissolved in 100 ml of distilled water) and 0.4ml water. 0.1ml of the sample was added to initiate the reaction. 2ml dichromate acetic acid reagent (Dichromate acetic acid -5% potassium dichromate with glacial acetic acid in ratio 1:3) was added after 15, 30, 45 and 60 sec to arrest reaction to the control tubes. The tubes were then heated for 10 minutes allowed to cool and the green colour developed was read at 240 nm using spectrophotometer. Extract (20 -100 µg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide. [12,13]

The percentage of hydrogen peroxide scavenging is calculated as follows.

% Scavenged (H_2O_2) = ($A_0 – A_1$ / A_0) X 100

Where; A_0 is the absorbance of control and A_1 is the absorbance of test

Table 2: Hydrogen Peroxide Scavenging Activity of the Ethanolic Extract of *Aesculus hippocastanum*

Concentration of the Extract (µg)	Scavenging activity of the Extract (%)
20	35.20
40	47.85
60	69.25
80	77.60
100	86.47

RESULT AND DISCUSSION

The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. DPPH, a protonated radical, has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants. When DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517nm. In this analysis, the scavenging activity of ethanolic extract was similar to that of Ascorbic acid. The DPPH radical scavenging activity of ascorbic acid and ethanolic extract, increased in a dose-dependent manner. At a concentration of 100µg/ml ethanolic extract of *Aesculus hippocastanum* and standard Ascorbic acid showed 92.72 % and 97.35 % antioxidant activity respectively by DPPH radicals scavenging assay.

Hydrogen peroxide (H_2O_2) is a byproduct of respiration and is made in all living cells. It is harmful and must be removed as soon as it is produced in the cell. The generation of even low levels of H_2O_2 in biological systems may be important. Cells make the enzyme *catalase* to remove hydrogen peroxide. Different plant materials show different amounts of *catalase* activity. Hydrogen peroxide scavenging activity depends upon the phenolic content of the extract, which can donate electrons to H_2O_2 and thereby neutralizing it in to water. The ethanolic extract of the *Aesculus hippocastanum* was

capable of scavenging H_2O_2 in a dose dependent manner. At a concentration of 100 µg, the test extract showed 86.47 % scavenging activity. Thus, the present study demonstrated the significant antioxidant activity of the extract tested.

Conclusion

The use of natural antioxidants as a potential preventive for free-radical mediated diseases has become a very important issue for improving the quality of life. This study demonstrates the significant antioxidant activity of the ethanolic extract of *Aesculus hippocastanum* in both the models utilized for the free radical scavenging activity. The antioxidant activities observed can be attributed to the presence of different phenolic compounds and to the synergistic effects of other compounds present in the extract.[14] Further investigation of the compounds responsible for the antioxidant activity, its isolation and *in vivo* studies are needed.

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