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ANTIOXIDANT ACTIVITY OF CURCULIGO ORCHIOIDES USING DIFFERENT METHODS - AN OERVIEW

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ABSTRACT

Free radicals are produced in normal or pathological cell metabolism. Oxidation is essential to living organism for the production of energy to fuel for biological processes. Uncontrolled production of oxygen from free radicals leads to many diseases like cancer, atherosclerosis, cirrhosis and also aging. An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate. The aim of this study is to evaluate the antioxidant activity of the rhizomes of *Curculigo orchioides* belonging to family Amaryllidacae.

KEYWORDS: Antioxidant, *Curculigo orchioides*, DPPH assay, Atherosclerosis, Aging, Cirrhosis

INTRODUCTION

An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate.[1] Antioxidants have been traditionally divided into two classes, primary or chain breaking antioxidants and secondary or preventative antioxidants.[2] A number of chemical and physical phenomena can initiate oxidation which proceeds continuously in the presence of a suitable substrate(s) until a blocking defense mechanism occurs. Target substances include oxygen, polyunsaturated fatty acids, phospholipids, cholesterol and DNA.[3] The plant derived compounds have always been an important source of medicine for various diseases.

Curculigo orchioides Gaertn (Black musli or Golden eye grass) is one of the highly useful plant in indigenous system of medicine, belongs to Amaryillaceae family. It is known as talamuli in Sanskrit, kalimusli in Hindi and nilappana in Malayalam. It was first introduce in "Chark samhita of agnivesha", the epic treatise of the medicine school of thought of the Hindu system of medicine and narrated as an ingredient of a cigar to alleviate cough. Curculigo orchioides gaertn is a small herb, up to 30cm high with tuberous root stock, occurring wildly in sub tropical Himalayas and almost all parts of India. Drug is collected from two year old plant. The active compounds that have been reported are flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites.[4] The rhizomes of this plant are sweet, cooling, diuretic, aphrodisiac, viriligenic and tonic which can be used against hemorrhoids, leucorrhoea, pruritis, skin diseases, asthma, bronchitis and jaundice. It is also used as antioxidant, spermatogenic, hepatoprotective, immunostimulant, anticancer, antibacterial, antiosteoporotic and hypoglycaemic.[5]

Antioxidant activity

The antioxidant activity of methanolic extract of rhizomes of Curculigo orchioides (MEC) using carbon tetrachloridet intoxicated rat liver as the experimental model .the hepatotoxic rats were administered MEC for 90 days(daily orally at dose of 70 mg/kg body weight).[6] Lipid peroxidation (LPO) in CCl4- intoxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS) and diene conjugates(CD) and also adistinct diminution in glutathione (GSH) content in the liver . in CCl4 +MEC- treated rats these biochemical parameters attained an almost normal level. The decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalyse (CAT), glutathione peroxidase (GPX) and glutathione reductase (GRD) in CCl4-intoxicated rats and its retrivel towards near normalcy in CCl4+MEC-administered rats revealed the efficacy of MEC in combating oxidative stress due to hepatic damage.

A study using methanol extract of *C.orchioides* rhizomes having doses ranging 40 - 120 mg/ kg body weight revealed the extract with dose 70 mg/ kg body weight offering the maximum hepatoprotection with respect to different liver marker enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyltranspeptidase (GGT). The body has an effective mechanism to prevent and neutralize the free radical – induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, GPX and GRD etc. When the balance between

ROS production and antioxidant defenses is lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to various pathological conditions[7] Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

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The elevated level of TBARS and CD observed in CCl4- treated rats indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. TBARS produced as byproducts of LPO that occurs in hydrophobic core of bio-membranes[8] The significant decline in the concentration of these constituents in the liver tissue of CCl4 +MEC administered rats indicates anti-lipid peroxidative effect of *C.orchioides.* The antioxidant activity of hydroalcoholic extract of *Curculigo orchioides* gaertn plant in different *in vitro* models. The following models are performed for antioxidant activity.[9]

DPPH Assay (1, 1 diphenyl 2, picryl hydrazyl)[10] 0.3 ml solution of DPPH in 100% ethanol was prepared.5 ml of this solution + 1 ml of the fraction dissolved in ethanol at different concentrations (50- 250 µg/ml), mixture was shaken and allowed to stand at room temperature for 30 min Absorbance was measured at 517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and compared with that of Butylated hydroxy toluene, which was used as the standard.

Determination of reducing power.[11] 2.5 ml of solution of different concentrations of extract (50, 100, 150, 200, 250 µg/ml). 2.5ml PO4 buffer solution (pH 6.6). 2.5 ml potassium ferricyanide solution (1 %w/v). Mixture placed in water bath/Incubate at 500C for 20 min. After incubation resulting solution cooled & mixed with 2.5 ml 10% Trichloro acetic acid to each test tube. The mixture was centrifuged at 650 rpm for 10 min.2.5 ml upper solution layer was mixed with 5 ml of deionised water & 0.5 ml ferric chloride (1%w/v). Absorbance was measured at 700 nm.

Hydrogen peroxide scavenging activity**:** Hydrogen peroxide 2mm/L solution prepared with standard (PO4 buffer pH- 7.4).Different concentrations of extract (50, 100, 150, 200, 250 µg/ml) prepared in distilled water.1ml of solution of different concentrations of extract (50, 100, 150, 200, 250 µg/ml). 0.6 ml hydrogen peroxide solution. After 10 min Absorbance was measured at 230 nm against blank solution containing PO4 Buffer without hydrogen peroxide.

The results revealed that the extract shows potent scavenging activity when compared with standard Butylated hydroxy toluene.. These active constituents may be responsible for the observed antioxidant activity. Further study on the active components may provide a better understanding about plant with a goal of elucidating their active potential compounds.

The antioxidant activity of ethanolic root extract of *Curculigo orchioides* which is commonly called as Golden eye grass). The Antioxidant potential of *Curculigo orchioides* was investigated by three different established in vitro methods DPPH, Reducing Power and Phosphomolybdenum assay. Gallic acid was used as reference standard.[12]

a) DPPH (2,2-diphenyl 1-picryl hydrazyl) Free Radical Scavenging Activity[13]

The free radical scavenging activity was followed by DPPH method.0.1mM solution of DPPH in methanol was prepared.Gallic acid was taken as reference standard.Different concentrations of extract (50.0,100.0,300.0, 500.0µg/ml) and standard (1.0,2.5,5.µg/ml) were prepared using methanol .1.0 mL of 0.1mM DPPH solution was added to 3.0 Ml of all concentrations of extract and standard seperately.0.1mM DPPH and methanol were used as blank.These mixtures were kept in dark for about 30min and the absorbances were measured at 517nm. Finally the % inhibition was calculated by usingthe formula.

DPPH Scavenged: $\{(A_0-A_1)/A_1\}*100$

Where A_0 is the absorbance of the blank (containing all reagents except the sample extract), and A1 is the absorbance of sample extract. The anti oxidant activity ofethanolic root extract of *Curculigo orchioides* was expressedas IC_{50}. The IC_{50} value is defined as concentration in (g/ml) of extract that scavenges DPPH radical by 50%.

b) Reducing Power Assay[14]

Different concentrations of extract (50.0, 100.0, 300.0, 500.0µg/ml) and standard Gallic Acid (1.0,2.5,5.0 µg/ml) were prepared using distilled water. 1.0% Potassium ferricyanide, 10.0%Trichloroaceticacid, 0.1%Ferricchloride, 0.2M Phosphate buffer (PH6.6) were prepared using distilled water. Then 1.0 mL of each concentration of standard & extract were taken separately and to this 1.0 mL of phosphate buffer, 1.0mL of potassium ferricyanide were added.Then these samples were incubated at 50^{o}Cfor 20min.Then 2.5mL of 10.0% Trichloro acetic acid was added,which was then centrifuged at 3000rpm for 10min.Then upper layer (2.5mL) was separated and then add 2.5mL of distilledwater,0.5mL of freshly prepared ferric chloride was added and then absorbances were measured at 700nm.

c) PhosphoMolybdenum Assay[15]

Different concentrations of extract (50.0,100.0,300.0, 500.0µg/ml) and standard Gallic acid(1.0,2.5,5.0 µg/ml) were prepared using distilled water. 0.3 mL of each concentration of extract and standard were combined with 3.0mL of reagent(0.6 M sulfuric acid, 28m M sodiumphosphate and 4 mM ammonium molybdate).The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The results obtained indicates the significant antioxidant activity in all the three methods and the results were compared with standard reference drug Gallic acid.

The methanolic extract of curculigo orchioidesExt M exhibited substantial activity in DPPH antiradical, super oxide scavenging, nitric oxide scavenging, lipid peroxidation and protection against superoxide induced damage to erythrocytes.[16]

Antiradical activity (free radical scavenging activity) by DPPH method Antiradical activity was measured by observing decrease in absorbance at 516 nm of a methanol solution of colored DPPH (1, 1-diphenyl-2-picryl hydrazyl, a stable free radical) brought out by the test at various concentrations (20, 30, 40, 50, 60 and 70 mg/ml) as reported earlier.[17] Ascorbic acid was used as a reference standard and DPPH alone in methanol as control. The activity was expressed as an effective concentration at 50% (EC50) i.e. the concentration of the test solution required to give a 50% inhibition calculated as reported.

Superoxide radical scavenging activity

Superoxide anion radical scavenging assay was performed by monitoring the reduction of nitroblue tetrazolium (NBT) to a blue colored formazan as reported elsewhere[18], which was measured at 590 nm at regular interval of 30 sec up to 2.5 min and terminally at 4 min. The EC50 from % Inhibition of superoxide radical scavenging activity was calculated.

Erythrocyte membrane stabilizing activity

The assay was carried out according to the procedure described.[17] wherein the hemolysis of RBC was induced with superoxide radical by a riboflavin-light-NBT system. The % protection provided by test (300 mg/ml) was calculated as described previously. Hydrocortisone was taken as areference standard and control was prepared without test solution.

Lipid peroxidation

As reported earlier[19,20] in presence of hydroxyl radical and deoxyribose, lipids from liver homogenate of wistar rats, fragmentize to malonyl dialdehyde (MDA) that binds to 2-thiobarbituric acid (TBA) to form pink MDA-TBA chromogen was estimated at 532 nm. Test (6, 8, 10, 12, 14 and 16 mg/ml) and atocopherol taken as a reference standard showing antioxidant activity were expected to interfere with the above process by scavenging hydroxyl radical, evident from malondialdehyde content calculated as reported earlier. % Inhibition and EC50 was calculated as above.

Hydroxyl radical (OH˙) scavenging activity

The formaldehyde formed during the oxidation of dimethylsulphoxide (DMSO) by Fe+3 - ascorbic acid was used to detect hydroxyl radicals.[19,21] The reaction mixture with different concentrations of test sample (10, 100 and 1000 mg/ml) dissolved in phosphate buffer or 100 ml ascorbic acid (2 mM) was incubated for 30min at 37°. The reaction was stopped by adding 125ml of trichloroacetic acid (17.5% w/v).

The DPPH assay measures hydrogen atom (or) one electron donating activity and hence provides a measure of free radical scavenging antioxidant activity. DPPH is purple coloured stable free radical, it becomes reduced to the yellow coloured diphenyl picryl hydrazine. A chloroform and ethyl acetate DPPH-solution (0.15%) was mixed with serial dilutions (1 to 50 mg/ml) of extracts and shaken vigorously. The tubes were allowed to stand at27^0C for 15 min. the change in absorbance of sample was measured at 517 nm, using UV spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage.

Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5 mg/mL). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank.

% scavenging of the DPPH free radical was measured using the following equation

$$= \frac{\text{Absorbance of the control \_ absorbance of the test sample}}{\text{absorbance of the control}} \times 100$$

The inhibition curve was plotted for duplicate experiments and represented as % of Mean inhibition ± SEM. IC50 values were obtained from the graph.

RESULTS AND DISCUSSION

Phenolic compounds are the major group that contributes to the antioxidant activity of vegetables, fruits, cereals and other plant based materials. Many quercetin derivatives were found to be active in free radical scavenging property. Antioxidant activity depends on EC_{50} values.EC_{50} values were calculated by the concentration in mg/ml versus percentage inhibition. Lower the EC_{50}, higher the antioxidant activity.

CONCLUSION

From the above results, it may be concluded that the rhizomes of *curculigo orchioides* is non-toxic and is safe. As the results indicated that the extract possess significant Antioxidant activity, after carrying out a thorough study of clinical trials, the plant can be considered as a low cost, potent, herbal medicine for free radical scavenging. The findings indicated that *Curculigo orchioides* Gaertn might have a good potential as a source of antioxidants, with their potential use in different fields viz. food, cosmetics, and pharmaceuticals and also can be used in treating many diseases like cancer, cirrhosis, atherosclerosis etc.

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