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Anti-glycation and antioxidant activities of Polysaccharides from *Muntingia Calabura* roots

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ABSTRACT

Muntingia is a genus of plants in the family Muntingiaceae, comprising only one species (*Muntingia calabura*). It is native to the neotropics, from Mexico south to Bolivia. The parts like fruits, leaves, barks, and roots are used as folk medicine. The decoction of root has been used to confer health benefits in a number of inflammatory diseases. In present study, antioxidant, metal chelating and ferric ion reducing and anti-glycation property was checked for the polysaccharide isolated from *Muntingia calabura* plant root extract. At 10 µg/mL sugar concentration, it gave 67 % and 65 % inhibition in DPPH radical scavenging assay and Antiglycation assay respectively. The extract also shows ferrous ion chelating and ferric ion reducing activity. It also inhibited fructosamine formation by 71% after 3 days of incubation. The above studies suggested that the inhibition of glycation exhibited by extract was not only due to its free radical scavenging property but also due to the modification in the amino or carbonyl groups in the Millard reaction, which resulted in the inhibition of fructosamine formation. The sugars present in the extract exhibiting the antioxidant activity by donating hydrogen atom. So polysaccharide isolated from *Muntingia calabura* root extract can be used in preventing many diseases resulting by free radicals and as the extract has anti-glycating activity, can be used in preventing complications of diabetes.

Keywords: Muntingia calabura, Antioxidant, Antiglycation, Diabetes and Free radicals

INTRODUCTION

Reactive oxygen species (ROS) plays a vital role in many diseases such as Cancer, Alzheimer disease, Parkinson disease, Diabetes, Cardio vascular diseases, Inflammation, Viral infections, Autoimmune pathologies, etc. [1,2] ROS can induce peroxidation of lipids, which turns promotes glycation of protein and plays a role in immune deficient diseases.[3,4]

A non-enzymatic reaction like glycation takes place between free amino groups of proteins and reducing sugars.[5,6] This is associated with the pathogenesis of age and diabetes related complications. This process represents post translational modification of proteins, which can impair their functions in living organisms. If the oxidative step is involved in glycation process, then it is called as glycoxidation. During the course of glycooxidations free radicals, products of the autooxidation of the glycating sugar, and a heterogeneous group of substances called advanced glycation end products (AGEs) are formed.[7,8] AGEs acts, either by modifying structural intra- and extracellular proteins or by binding to their receptors that belong to the immunoglobulin family.[9,10] They also generate ROS which modifies cellular function and may induce inflammatory processes.[11,12]

Therefore, search for antiglycative and antioxidant agents from various plant sources is gaining lot of importance.[11,13] There are several reports, which mentions about the identification of antiglycative and antioxidant agents from plant species. It was reported

that, presence of chlorogenic acid, caffeic acid in the mate tea (Ilex paraguaiensis are responsible for the anti-glycation effect.[14,15] The presence of phenolic acids, gallic acid and ellagic acid, and punicalagin A and punicalagin B, two polyphenolics are responsible for the anti diabetic effect of Pomogranate (Punica granatum) fruits.[16,17] It is reported that, leaves of Custard apple leaves showing promising antiglycation and antioxidant properties.[18] Studies on guava leaf extracts show that they are potent antiglycation agents, which can be of great value in the preventive glycation-associated complications in diabetes.[19,20] There are several reports on plant polysaccharides being studied for anti-glycation activity. *Muntingia calabura* is a shrub like plant, though it is not native of India but can find throughout. The parts like leaves, fruits, flowers, roots of the plant showing excellent anti-inflammatory, antioxidant and anti diabetic properties.[21, 22] In this regard, no reports have been found on the anti-glycation and antioxidant properties of polysaccharide fractions from *Muntingia calabura* root extract. Hence, the investigation is focused on polysaccharide fractions with anti-glycative and its antioxidant properties.

MATERIALS AND METHOD

Ethanol, phenol, sulphuric acid, Folin-Ciocalteu reagent, sodium azide, nitro blue tetrazolium (NBT), sodium dodecyl sulphate, ferrous sulphate, Potassium ferricyanide and L-ascorbate, all other chemicals and reagents used were of analytical grade and were purchased from Sisco Research Laboratory and Himedia, India.

Preparation of *Muntingia calabura* root extract

The *Muntingia calabura* plant roots were collected from locally. The roots were washed thoroughly with current of water, followed with 0.1% KMnO4 solution and again with double distilled water. The root pieces are crushed using known amount of water, squeezed and filtered using glass wool. The extract was kept at 4^0C overnight to precipitate sugars. Extract was centrifuged at 10,000 rpm at 4^0C for 20 min, and then the precipitated sugar pellet were collected and was re-dissolved in minimum amount of distilled water, filtered through 0.22 micron filter and stored at -20^0C for further use. The extract obtained was called as *Muntingia calabura* plant root extract (MCPRE or MCRE). Protein (Bradford) and Sugar estimation (Dubois's 1976) was done for the *Muntingia calabura* plant root extract.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of MCRE

DPPH radical scavenging activity study was done according to the method of Shimada et al. with minor modifications [22], at a dose dependent concentration of 0 to 10 µg each was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured spectrophotometrically at 517 nm. Ascorbic acid and α-tocopherol was used as positive control under the same assay conditions. Negative control was without any inhibitor or MCRE. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of extracts of *Muntingia calabura* root extract was calculated from the decrease in absorbance at 517 nm in comparison with negative control.

Ferric ion reducing power of MCRE

100µl of 4mM potassium ferricyanide solution was mixed with 200µL of 20mM phosphate buffer pH 6.5 in the presence or absence of *Muntingia calabura* plant extract and polysaccharide fraction. A similar assay was done with Ascorbic acid at 12µg concentration. The contents were incubated at 50^0C for 20min. 200µL of 10% TCA was added to the reaction mixture and centrifuged at 5000rpm for 10min at room temperature. The resulting supernatant was taken and mixed with 100µL of 2mM ferric chloride solution and final volume was made up to 1mL with distilled water and then incubated at 37^0C for 10min. The absorbance was recorded at 700nm. Absorbance increases with increase in reducing power.[23]

Ferrous ion chelating ability of MCRE

Ferrous ion chelating activity was measured for the fruit extract. The reaction solution contained ferrous chloride (200µM) and potassium ferricyanide (400µM) with or without *Muntingia calabura* plant root extract and polysaccharide fraction. A similar assay was done with Ethylene Diamine Tetra Acetic acid (EDTA) at 20µg concentration. The components in the reaction mixture were added in final volume of 1 mL distilled water and mixed. The reaction mixture

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was incubated at 20°C for 10min. Formation of the potassium hexacyanoferrate complex was measured at 700nm. The assay was carried out at 20°C to prevent Fe^{2+} oxidation. Lower absorbance indicated higher iron chelating capacity. [24]

In vitro non enzymatic glycation of bovine serum albumin

Bovine serum albumin (BSA, 20 mg/mL) incubated in glucose (500 mM) and sodium azide (0.02%) with or without CuSO4 (100µM) in 0.2 M phosphate buffer (pH 7.4). The test compound was added to the reaction mixture, and the reaction mixture was incubated for 3 days at 37°C. After incubating, the fluorescent reaction products were assayed in a fluorescence spectrophotometer with an excitation wavelength of 350 nm and an emission wavelength of 450nm. Results were expressed as percentage inhibition of formation of the glycated protein.[25]

Spectrophotometric analysis of fructosamine

The procedure of fructosamine assay followed the method of Baker, et al 1994 with slight modifications. The reaction mixture which contained 0.2 mL glycated material and 0.8 mL nitro blue tetrozolium (NBT) reagent (300µM) in sodium carbonate buffer (100 mM, pH 10.35) was incubated at ambient temperature for 15 min, and the absorbance was read at 530 nm against a blank.[26]

Statistical analysis

Statistical analysis was done using students t-test. All the values represent mean of triplicates and are expressed as Mean ± SD. $p<0.05$ was considered as significant.

RESULTS:

The *Muntingia calabura* root extract was rich polysaccharides with negligible amount of proteins in it.

Table-1: "DPPH radical scavenging & Anti-glycation activity of polysaccharide isolated from *Muntingia calabura* plant root extract"

Treatment	% Inhibition of DPPH radicals *scavenging activity*	% Inhibition of Protein glycation activity
Muntingia Calabura plant root extract (10µg/ml)	67 %	65 %
Ascorbic acid (10µg/ml)	55%	77 %
Vitamin – E (10µg/ml)	69%	71 %

Results are expressed as means ± SD of triplicates from three independent experiments.

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Figure 1: Ferric ion reducing activity of Muntingia calabura Plant root extract (MCPE).

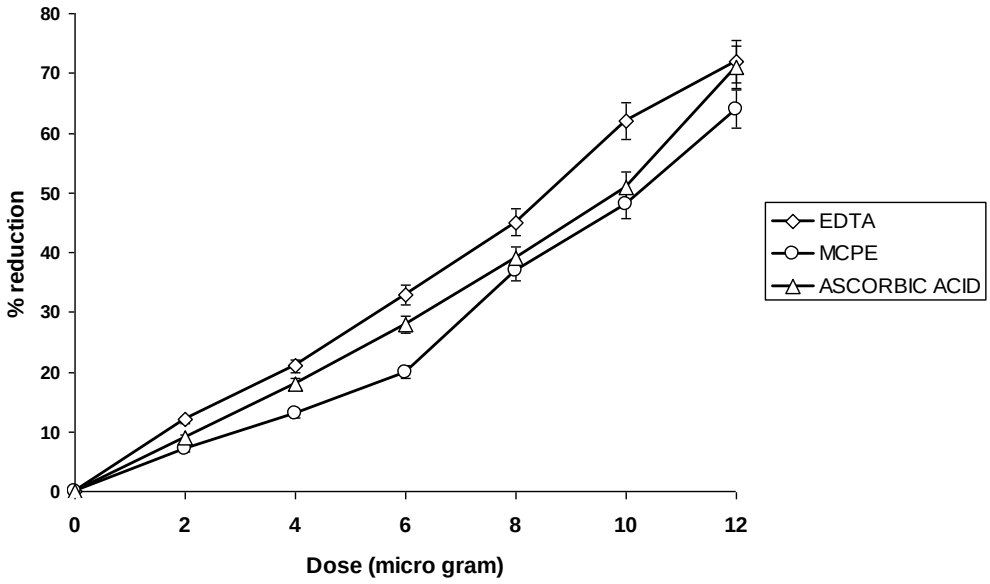
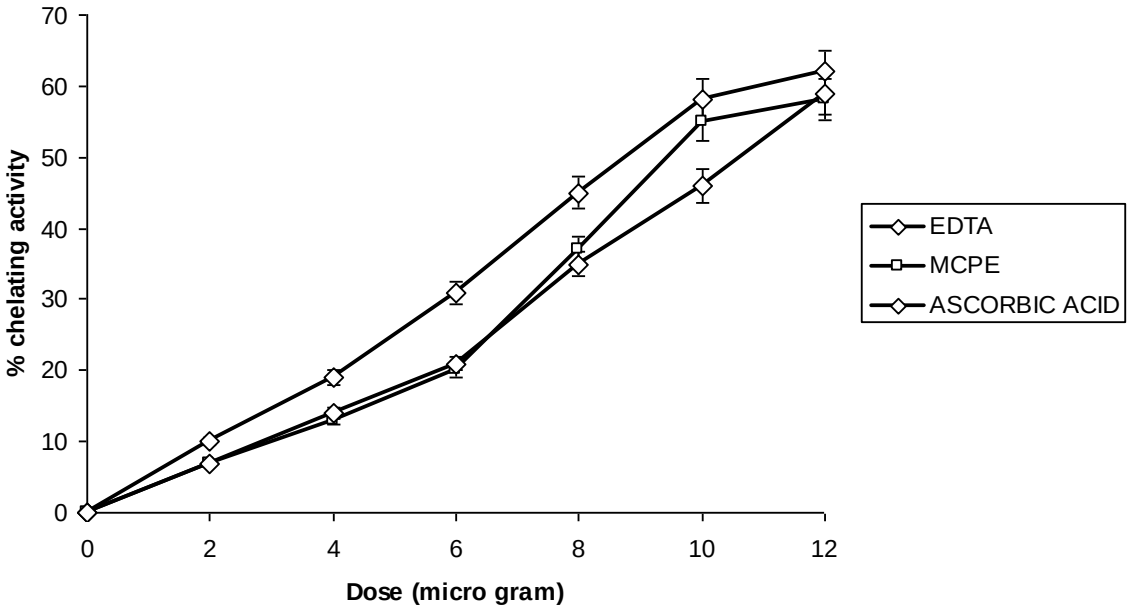


Figure 2. Ferrous ion chelating activities of *Muntingia calabura* plant root extract (MCPE).



DISCUSSION

The *Muntingia calabura* root extract was rich polysaccharides with negligible amount of proteins in it.

Evaluation of DPPH radical scavenging potential of MCRE

As explained in methods, a dose dependent DPPH radical scavenging activity of MCRE was done along with standards Ascorbic acid and Alpha tocopherol. MCRE showed 67%, Ascorbic acid (55%) and Alpha tocopherol (69%) at a highest dose of 10µg concentration **(Table 1)**. The above results showed MCRE is a potential DPPH scavenger in comparison with Ascorbic acid and α-tocopherol. The antioxidant property of MCRE could be due to the supply of hydrogen, which combines with radicals and thus form a stable radical.

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Measurement of reducing power and chelation property

To find the metal ion chelating and reducing capability of MCRE was further tested to find out its efficacy for ferric ion reducing activity and ferrous ion chelation properties. MCRE showed 64% reducing power in comparison to ascorbic acid (71%) at 12µg concentration **(Figure 1)**. The results obtained in the present investigation showed that the reducing power of MCRE was likely to have contributed towards observed antioxidant effect. **Figure 2,** the ferrous ion–chelating effect was studied showed MCRE (12 µg/mL) showed 58% reducing power in comparison to EDTA 62% at 12 µg/mL) and Ascorbic acid 59% (12 µg/mL).

Evaluation of anti-glycation activity:

The inhibition study for the production of AGEs was carried out for MCRE. The extract was able to inhibit the production AGEs by 65% in comparison to Ascorbic acid (77%) and Vit -E (71%) at much lower dose **(Table 1)**. The crude extract MCRE acted as a glycation inhibitor because of its free radical scavenging property. The effectiveness of MCRE in inhibiting DPPH radical formation and AGEs formation can speak about its uses for diabetic patients.

The effect of MCRE on the formation of fructosamine was studied by the reduction of NBT from the 1st day of incubation to the 3rd day. MCRE inhibited fructosamine formation by 71% after 3 days of incubation. The possible explanation for the less formation of fructosamine in MCRE treated sample was that might have the ability to modify the amino or carbonyl groups in the Millard reaction that resulted in the inhibition of fructosamine formation.

The above two studies suggested that the inhibition of glycation exhibited by MCRE was not only due to its DPPH radical scavenging property but also due to the modification in the amino or carbonyl groups in the Millard reaction, which resulted in the inhibition of fructosamine formation.

CONCLUSION

The results of the present work indicated that polysaccharides isolated from *Muntingia calabura* plant root extract (MCRE) possessed antioxidant, metal ion reducing, chelating and anti-glycation properties. However, in- vivo antioxidant activity and mechanism of action need to be further studied.

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