

Research Article**Antioxidant and free radical scavenging activities of root extracts of *Muntingia Calabura*****Mohamed Azmathulla Khan Y¹, Dinesha Ramadas^{2*}, Subhas Chandrappa Mundasada³, Santosh Kumar N⁴, Chikkanna D⁵**¹Associate Professor, Dept. of Physiology, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur-572106, Karnataka, India²AIMS- Central Research Laboratory, Adichunchanagiri Biotechnology & Cancer Research Institute, B.G. Nagara-571448, Karnataka, India³Professor, Dept. of Biochemistry, Basaveshwara Medical College, Chitradurga, Karnataka, India⁴Dept. of Biochemistry, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur-572106, Karnataka, India⁵Associate Professor, Dept. of Biochemistry, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Karnataka, India***Corresponding author**

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Abstract: The in vitro antioxidant properties of different extracts (water, alcohol, alcohol: water, hexane or chloroform extract) of *Muntingia Calabura* roots were evaluated using various assays. The water and alcohol: water (1:1) extracts of *Muntingia Calabura* roots were rich with carbohydrates (65.3mg to 138 mg), proteins (1.12 to 2.63mg), polyphenols (7.32 to 14.06mg), flavonoids (6.91 to 14.34mg) and Ascorbic acid when compared to other extracts. The antioxidant and free radical scavenging activity (4.31 to 9.13mg). In addition, the *Muntingia Calabura* root water extract at a dosage of 80µg showed more hydroxyl radical scavenging activity and at a dosage of 300µg, the water extract showed more superoxide radical scavenging activity. These results establish the antioxidant potential of *Muntingia Calabura* root extract could be used as natural antioxidant source.**Keywords:** *Muntingia Calabura*, Root, Antioxidant, Superoxide radical, Hydroxyl radical, Phytochemicals.

INTRODUCTION

Oxidative stress is a natural phenomenon in the human body. During the above, reactive oxygen species (ROS) are generated. The intracellular levels of ROS are maintained at low levels by various enzyme systems for a short life span [1, 2]. Appropriate amount of ROS has a significant impact on health and disease. ROS includes oxygen ions, free radicals (superoxide and hydroxyl radicals), and peroxides (hydrogen peroxide) and are the products of normal oxygen consuming metabolic process in the body. Medicinal plants and spices are defined as any part of a plant that is used in the diet for their medicinal, aromatic properties and nutritional value [3]. In this direction, the medicinal plants have been investigated in different parts of the world towards identifying the right phytochemicals having potential therapeutic effects. *Muntingia Calabura* is a medicinal plant traditionally used to relieve headache and cold, pain associated with gastric ulcers or to reduce the prostate gland swelling. Following the recent establishment of anti nociceptive activity of *M. Calabura* leaf, the present study was performed to further elucidate on the possible

mechanisms of anti nociception involved. It is reported that, the extracts of leaf, root and fruits shown good antioxidant activity [4]. Earlier, it was reported that, the preliminary studies on the root, leaves and fruit aqueous extracts of *Muntingia Calabura* showed good antioxidant activity and hence, the root of the plant was chosen for further studies [1].

MATERIALS AND METHODS

BHA, Ascorbic acid, DPPH, α -tocopherol were purchased from the Sigma Aldrich co. USA, Quercetin, A-tocopherol, Gallic acid, BSA were purchased from the Himedia Co. All the other chemicals and reagents were of Analar grade were purchased from the Merck Co, and S.d. fine chem., Mumbai, India. Reagents were distilled before use.

The plant *Muntingia Calabura* root were collected from authentic source, Karnataka, India. The collected roots were cleaned thoroughly with water, rinsed in 1% KMnO₄ to remove microbes, cleaned in double distilled water and dried under the shade. The

dried roots were ground to powder, sieved in 100 mesh British Pharmacopeia and stored.

5g of powdered *Muntingia Calabura* root powder was added individually to 100ml of water, alcohol, alcohol: water, hexane or chloroform extract, and vortexed for 4 hours, distilled using glass wool. The supernatant collected was allowed to evaporate using dry freezer for water extract and flash evaporator for solvent extracts and minimized sample stored at -10°C for further studies.

Phytochemical analysis

The extracts of roots of *Muntingia Calabura* were subjected to phytochemical analysis of the bioactive compounds by using standard protocols.

The protein estimation was carried according to Bradford's method [5] using BSA as standard. Absorbance was read at 535nm. Concentration of protein was calculated accordingly using standard graph. Total phenolics was determined according to the method of Folin Ciocalteu reaction [6] using gallic acid as a standard. Absorbance was read at 750 nm and the concentration was calculated using the standard graph accordingly. Ascorbic estimation was carried out according to Sadasivam S, Manickam [7]. The absorbance was read against a reagent blank at 540nm. The concentration was calculated on the basis of the standard curve. Sugar estimation was done according to Dubois method [8]. The absorbance was read at 520 nm. The amount of total sugar present in the given unknown sample solution was calculated using the standard calibration curve. Flavonoids estimation was done according to Cheon *et al.*; [9] by using Quercetin as a standard. The absorbance was measured at 415 nm and the concentration was calculated accordingly.

Antioxidant activity:

Superoxide radical scavenging activity:

Superoxide radical scavenging activity was measured according to the method of Lee *et al.*; [10] with minor modifications [11] [12]. Hypoxanthine, xanthine oxidase, superoxide dismutase, disodium ethylene diamine tetra acetic acid (EDTA) and nitro blue tetrazolium were used. Different extracts of *Muntingia calabura* root, at concentrations ranging from 50 to 300 µg, were added to the reaction mixture containing 100 µl of 30 mM EDTA, 10 µl of 30 mM hypoxanthine in 50 mM NaOH and 200 µl of 1.42 mM nitro blue tetrazolium and the volume was made up to 2.9 ml with 20 mM phosphate buffer (pH 7.4). After the solution was pre-incubated at ambient temperature for 3 min, 100 µl of xanthine oxidase solution (0.5 U/ml) was added to the mixture. The solution was incubated at ambient temperature for 20 min and the absorbance of the solutions was measured spectrophotometrically at 560 nm. Superoxide dismutase (SOD) (50– 300 µg/3

ml) served as a positive control while the negative control was without any test compound or extract.

Hydroxyl radical scavenging activity

The deoxy ribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium [13]. 2-Deoxy-D ribose, FeCl₂, FeCl₃, EDTA, H₂O₂, ascorbic acid, TBA, BHA was procured from Sigma (St. Louis, USA). The reaction mixture containing FeCl₃ (100 µM), EDTA (104 µM), H₂O₂ (1 mM), 2-deoxy-D-ribose (2.8 mM) were mixed with or without various concentrations of different extracts (10–100 µg) in 1 ml final reaction volume, made with 20 mM potassium phosphate buffer, at pH 7.4 and incubated for 1 h at 37°C. The mixture was heated to 95°C in a water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5319g in a Kubota 6800 for 15 min. The absorbance was measured at 532 nm. The percent hydroxyl radical scavenging activity of extracts was determined accordingly in comparison with the negative control.

Statistical analysis

Statistical analysis was done in SPSS (Windows Version 10.0.1 Software Inc., New York) using a one-sided student's t-test. All results refer to means ± SD. P < 0.05 was considered as statistically significant when compared to relevant controls.

RESULTS AND DISCUSSION

As explained in the materials and methods, *Muntingia calabura* root extract was done in water and different solvents. The volume of supernatant of the extracts are reduced to minimum using freeze dryer for water extract and flash evaporator for solvent extracts. Later all the extracts are subjected to phytochemical analysis using standard methods. The results are as shown in Table-1. It is found that, more carbohydrates are present in water extract and in water: ethanol extract (65.3mg to 138 mg). Other phytochemicals like protein (1.12 to 2.63mg), polyphenols (7.32 to 14.06mg), flavonoids (6.91 to 14.34mg) and Ascorbic acid (4.31 to 9.13mg) present in water and Water: ethanol extract when compared to other solvent extracts like ethanol, hexane and chloroform.

Hydroxyl radical scavenging activity of various roots extracts *Muntingia calabura* of in comparison with Butylated Hydroxy Anisole (BHA). The negative control was without any antioxidant or extract. Results are shown as mean ± SD (n = 3).(Fig-1)

The superoxide scavenging activity of various root extracts of *Muntingia Calabura*. The negative control was without any antioxidant or extract. Results are shown as mean ± SD (n = 3) (Fig-2).

Table -1: Phytochemical analysis of *Muntingia calabura* root extract

	Extracts				
	Water	Ethanol	Water: Ethanol	Hexane	Chloroform
Carbohydrates (mg/g)	138.0 ± 1.36	1.21±0.02	65.3±2.1	0.87±0.03	0.42±0.03
Protein (mg/g)	2.63±0.05	0.29±0.02	1.12±1.03	0.23±0.01	0.32±0.01
Polyphenols (mg/g)	14.06±0.35	0.03±0.01	7.32±0.22	0.06±0.01	0.02±0.01
Flavonoids (mg/g)	14.34±0.01	0.04±0.02	6.91±0.03	0.02±0.02	0.03±0.02
Ascorbic acid (mg/g)	09.13±0.04	Nil	4.31±0.21	0.13±0.02	Nil

Values are means ± SD of triplicates

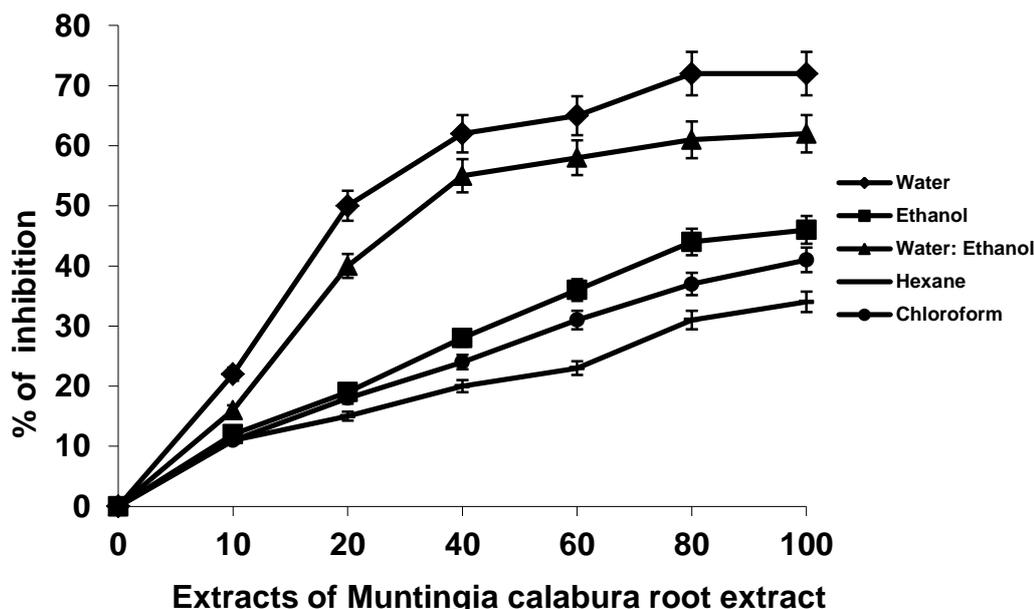


Fig-1: Hydroxyl radical scavenging activity of *Muntingia Calabura*

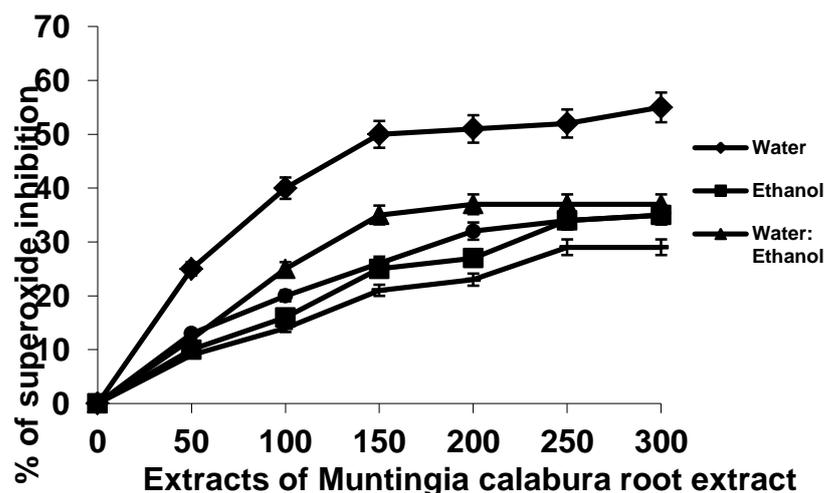


Fig-2: Superoxide radical scavenging activity of *Muntingia Calabura* root extracts

Further, the antioxidant activity of the extracts was done using different antioxidant model systems like hydroxyl radical scavenging activity and Superoxide radical scavenging activity. Hydroxyl radicals are known to be the most reactive of all the reduced forms

of dioxygen and are thought to initiate cell damage in vivo [14]. Further, the effect of curry leaf extracts on hydroxyl radicals generated by Fe³⁺ ions was measured by the extent of deoxy ribose degradation; an indicator of TBA-MDA adducts formation. In Hydroxyl radical

scavenging activity, a dose dependent study was done, where BHA (400µM) was used as standard (results not shown in graph) and other extracts are used in a range of 0 to 100µg concentration. The results are as shown in Figure 1, where the water extract showed highest activity at a dose of 80µg and the water: Ethanol mixture showed highest inhibition at 100µg dosage in comparison with other extracts.

Superoxide anions are the most common free radicals *in vivo* and are generated in a variety of biological systems and the concentration of superoxide anions increases under conditions of oxidative stress [10]. Hence, a NBT assay was carried out to test whether. The root extracts of *Muntingia Calabura* scavenge superoxide anions. A dose dependent study was done, where here also BHA (400µM) was used as standard (results not shown in graph) and other extracts are used in a range of 0 to 300µg concentration. As shown in Figure 2, the water extract showed highest activity at a dose of 300µg and the other extracts are shown negligible amount of inhibition at a highest dose of 300µg.

CONCLUSION

This work highlights the importance of *Muntingia calabura* plant which have been used in time immemorial, as a rich source of antioxidants. More specifically, the extraction of *Muntingia Calabura* root in water and Water: Ethanol, compared to other solvent systems, showed maximum antioxidant and free radical scavenging activities under *in vitro* conditions. Thus, the water extract of *Muntingia calabura* could be good lead for extraction of an effective natural nutraceutical or antioxidant drug. Further investigation into water and water: ethanol (1:1) extract of *Muntingia calabura* for its lead active compounds and *in vivo* antioxidant mechanisms is warranted.

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