

ANTIOXIDANT ACTIVITY AND ESTIMATION OF TOTAL PHENOLIC CONTENT OF MUNTINGIA CALABURA BY COLORIMETRY

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Abstract: The antioxidant potential of the 99% methanolic extract of leaves of *Muntingia calabura* was assessed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and total phenolic content was measured by Folin-Ciocalteu (FC) by Singleton and Rossi using Gallic acid and Tannic acid as the calibration standard. Moreover *Muntingia calabura* leaf extract showed strong reducing power and significant antioxidant activity. In the DPPH radical scavenging assay, the IC₅₀ value of the extract was found to be 22µg/ml. The total phenolic content was measured by Folin-Ciocalteu was found to be 0.903 for Gallic acid when compare to 2.900 for tannic acid.

Key words: *Muntingia calabura*, Antioxidant, phenolic content, UV-visible spectrophotometer.

Introduction

Muntingia calabura., also known as ‘gasagase hanninamara’ in kannada, is belonging to the family Elaeocarpaceae [1]. This is most common road side trees in Karnataka after its initial introduction to the Philippines [2]. *Muntingia calabura*, the sole species in the genus *Muntingia*, is a flowering plant native to southern Mexico, the Caribbean, Central America, Western South America south to Peru, Bolivia, India. Commonly known as Jamaican cherry, Panama berry, Singapore cherry, Strawberry tree. An infusion of the flowers is valued as an antispasmodic. The fruits sometimes eaten fresh and often cooked and made into jam, while the leaf infusion is drunk as a tea like beverage [1]. According to traditional use the flowers are said to possess antiseptic properties and also antispasmodic properties. It is also used to relieve headache and the first symptoms of cold [2,3]. According to Peruvian folklore, its leaves can either be boiled or steeped in water to provide relief from gastric ulcers or reduce swelling of prostate gland respectively[1]. The leaves possess antinociceptive, anti-inflammatory and antipyretic activities [4]. There are not many scientific articles published on this plant extract, thereby indicating lack of exploration into its

pharmaceutical benefits. The present study was carried out to investigate the antioxidant activity and total phenolic content of methanolic extract of leaves of *Muntingia calabura*.

Reactive oxygen species [ROS] have been implicated in many diseases like cancer, diabetes, atherosclerosis and heart disease [5]. ROS can be classified into free radicals [superoxide ion] (O²⁻), hydroxyl radicals (OH[•]) and non free radicals (hydrogen peroxide) (H₂O₂) [6, 7]. Free radical, generated *in vivo* due to various biochemical reactions occurring in the living tissues, is chemical species that have tendency to rob the electrons from other molecules in the intermediate surroundings in order to replace their own losses. This process will lead to the damage of important biomolecules including cell membrane, mitochondria, DNA etc. The mechanism of antioxidant is to protect against the oxidative damages, and various types of enzymes responsible for the removal or repair of the damaged molecules [8]. Thus natural mechanisms can be ineffective and therefore, dietary intake of antioxidant is essential [9, 10]. For this reason, synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole, commonly used in

processed foods, possessed some side effects that have limited their use as antioxidant agents [11, 12].

The aim of the present study was to carry out the antioxidant activity and its total phenolic content in the methanolic extract of *Muntingia calabura* leaves. Antioxidant and free radical scavenging activities of the extracts of *Muntingia calabura* leaves were studied.

2. Experiment

2.1. Plant material

The leaves of *Muntingia calabura* were collected from Bangalore, Karnataka in March 2009 and shade dried.

2.2. Extraction

Dried ground leaves 100gm were soxhlet extracted with 99% methanol (500ml). The extract was concentrated by evaporation to yield a gummy concentrate of greenish color extract.

2.3. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals. Ascorbic acid, Gallic acid, tannic acid, methanol, Folin-Ciocalteu (FC) reagent, sodium carbonate was purchased from S.D.Fine chemicals. All the chemicals used were analytical grade.

2.4. The DPPH radical scavenging assay

To a set of clean and dried test tubes 3ml of methanol and 150 μ l of 0.1% DPPH reagent was added and mixed thoroughly. Allowed the solution to stand for 30 minutes. The initial absorbance of each test tube was measured at 517nm. To these test tubes 1ml of aqueous solution of extracts were added in increasing concentration of 5-25 μ g/ml. The solution of ascorbic acid (5-25 μ g/ml) was taken as the standard. The solution were mixed and allowed to stand for half an hour at room temperature and final absorbance was measured at 517nm using a Spectrophotometer (UV-visible 1700 Pharma Spec) The experiment was performed in triplicate. The percentage reduction in absorbance was calculated from initial and final absorbance at each level. Concentration of the substance required for 50% reduction in absorbance (IC₅₀) was calculated from the calibration curve (concentration of extract in μ g/ml Vs Percentage reduction in absorbance). The results were tabulated in Table.1 and Figure.1.

2.5. Total phenolic content by Gallic acid

Stock solution 1mg/ml of Gallic acid was prepared in water. From the above stock solution 100 μ g/ml was prepared. Different concentration ranging from 1-10 μ g/ml was prepared. A volume of 1.5ml FC reagent was added in each standard flask and kept for 5mins and then 4ml of 20% sodium carbonate solution was added and made upto10ml with distilled water. The mixture was kept for 30mins and absorbance was

recorded at 738nm. Linearity was observed in the range of 1-10 μ g/ml. The data was shown in Figure.2.

2.5.1 Sample preparation and its estimation

Muntingia Calabura: 0.5 gm of methanolic extract of *Muntingia Calabura* was taken in 100ml volumetric flask and made upto100ml with distilled water. From the above solution 0.1ml was pipetted out into 10ml standard flask and added 1.5ml of FC reagent, 4ml of 20% sodium carbonate solution and made upto10ml with distilled water. After 30mins absorbance was measured at 738nm.

2.6. Total phenolic content by Tannic acid

Stock solution 1mg/ml of tannic acid was prepared in water. From the above solution 100 μ g/ml was prepared. Different concentration ranging from 2-12 μ g/ml was prepared. A volume of 1.25ml FC reagent was added to each standard flask and kept for 5mins and then 2.5ml of 20% sodium carbonate solution was added and made upto10ml with distilled water. The mixture was kept for 30mins and absorbance was recorded at 765nm. Linearity was observed in the range of 2-12 μ g/ml. The data was shown in Figure.3

2.6.1. Sample preparation and its estimation

Muntingia Calabura: 0.5 gm of methanolic extract of *Muntingia Calabura* was taken in 100ml volumetric flask and made up to 100ml with distilled water. From the above solution 0.1ml was pipetted out into 10ml standard flask and added 1.25ml of FC reagent, 2.5ml of 20% sodium carbonate solution and made upto10ml with distilled water. After 30mins absorbance was measured at 765nm.

Results and Discussion:

From the preliminary phytochemical studies, it showed high amount of phenolics, saponins, tannins and flavonoids present in the extract of *Muntingia calabura*. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *Muntingia calabura* is shown in Table.1 and Figure.1 . The DPPH antioxidant assay is best on the ability of 1-1-diphenyl-2-picrylhydrazyl, is a stable free radical to decolorize in the presence of antioxidants. The DPPH free radical contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The methanolic extract of *Muntingia calabura* leaves showed prominent IC₅₀ value of the extract was determined 22 μ g/ml was compared with ascorbic acid which showed an IC 50 value of 12 μ g/ml which is a well known antioxidant. The total phenolic content was estimated by Gallic acid and Tannic acid and it was found to be 0.903 and 2.900 respectively and shown in Figure 2 & 3.

Table.1: ANTIOXIDANT ACTIVITY OF *MUNTINGIA CALABURA*

Drug	Absorbance	Concentration ($\mu\text{g/ml}$)					IC ₅₀ $\mu\text{g/ml}$
		5	10	15	20	25	
Ascorbic acid	Initial	0.908	0.908	0.908	0.908	0.908	12
	Final	0.602	0.489	0.368	0.295	0.202	
	%Reduction in Abs.	33.70	46.00	59.50	67.50	77.70	
Muntingia Calabura	Final	0.629	0.580	0.511	0.460	0.406	22
	%Reduction in Abs.	30.73	36.12	43.70	49.30	55.30	

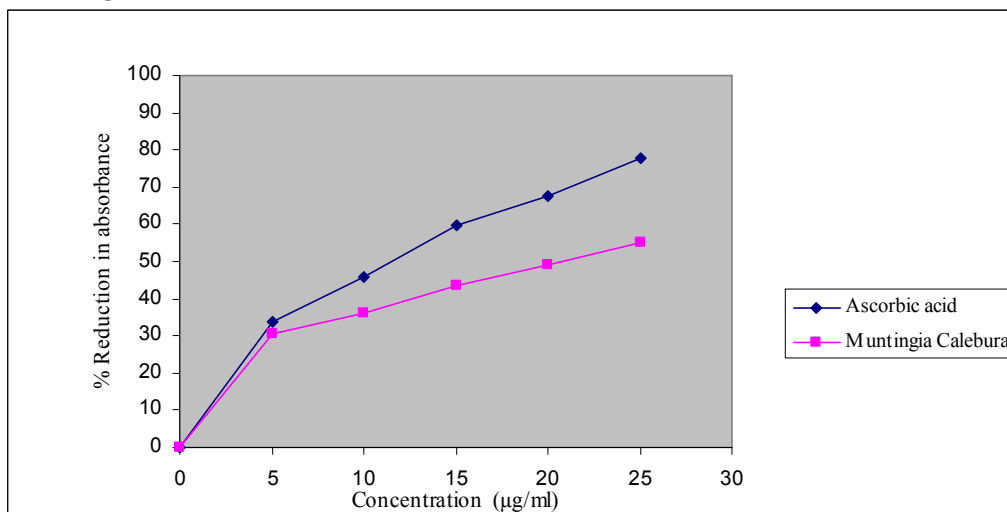
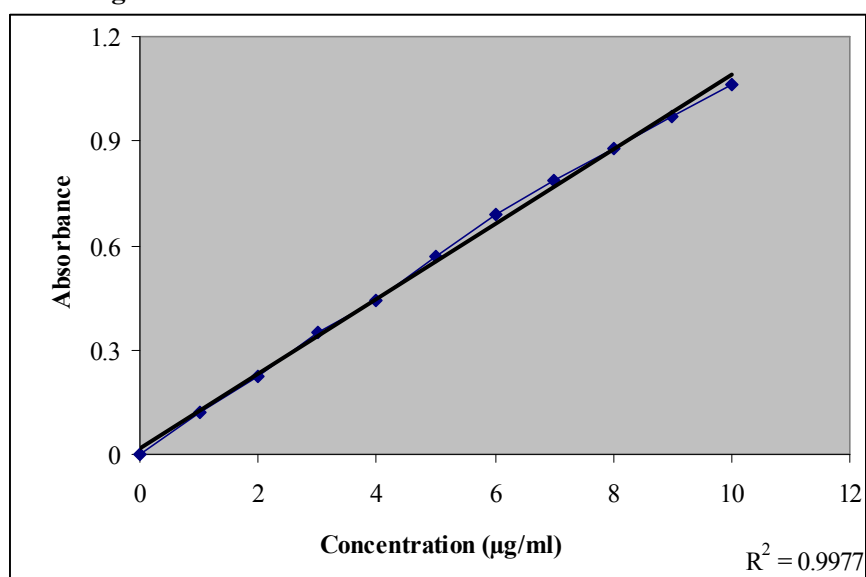
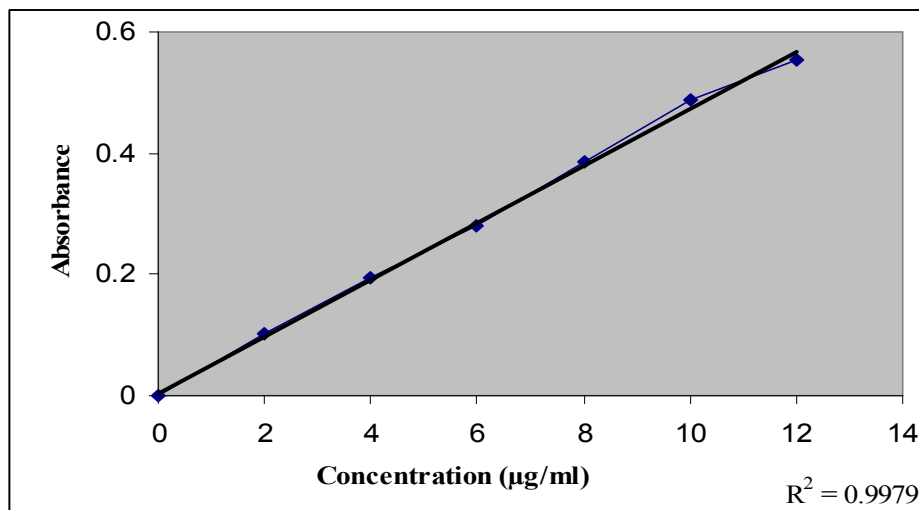
Figure.1: ANTIOXIDANT ACTIVITY OF *MUNTINGIA CALABURA***Figure.2 CALIBRATION CURVE FOR GALLIC ACID**

Figure.3 CALIBRATION CURVE FOR TANNIC ACID

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