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Research article

# Antioxidant Activity: Root, Leaves and Fruits Aqueous Extracts of *MuntingiaCalabura*

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## Abstract

**Aim:** To determine the antioxidant activity of the water extract of root, leaves and fruit of *MuntingiaCalabura* were evaluated for their antioxidant activity. **Materials& Methods:** The phytochemical analysis antioxidant activity of the extract was studies using plant's root, leaves and fruits. **Results & Discussion:** extracts showed that, each extract contains rich with proteins, carbohydrates, polyphenols, flavonoids, ascorbic acid, chlorophyll and negligible amount of α-tocopherol. The antioxidant activity of the above was evaluated by DPPH (1,1-diphenyl-2-picryhydrazyl) radical scavenging activity. Standard antioxidants like BHA, Curcumin and α-tocopherol were used as positive control. The leaf extract (52%) showed more antioxidant activity in comparison to fruit extract (25%) and the root extract (43%) showed less. **Conclusion:** The above antioxidant activity of the extracts may due to the presence of phytochemicals like polyphenols, proteins, flavonoids, ascorbic acid and α-tocopherol.

**Key words:** *MuntingiaCalabura*, Leaves, Root, Fruits, Antioxidant, DPPH, Phytochemical analysis

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## 1. Introduction

Oxidation is essential for the production of energy in biological system. During the normal course of producing energy, free radicals are generated. Though free radicals, radical derivatives and non radical reactive species are useful during oxidation but hazardous to living organisms at high concentration and damage all major cellular constituents in our body [1]. In this scenario, the medicinal properties of plants have been investigated in several countries with a focus on identifying key phytochemicals with potential therapeutic effects. The

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important active phytochemical founding plant are alkaloids, tannins, flavonoids, phenols, proteins and carbohydrates [2] [3]. Phytochemicals are widely used to treat many oxidative stress induced diseases such as cancer, diabetes etc., which are associated with ROS that damage cellular biomolecules. Medicinal plants, spices are often rich in phenols, flavonoids, proteins are known to have potent antioxidant properties that can prevent oxidative stress and provide health benefits [4] [5] [6]. *Muntingia calabura*is an evergreen tree originally distributed in tropical America [7]. Literature shows that, the fruits, leaves and roots are used as medicine or part of food. The fruits can be processed into jams and the leaves can be used for making tea. Earlier studies have revealed flavones, flavanones, flavans, and biflavans to be the major constituents of this species, some of which have displayed anti-platelet aggregation and cytotoxic activities [8].

## 2. Materials and Methods

Curcumin, 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.

### Sample collection

The plant *MuntingiaCalabura's* root, leaf and fruits were collected from authentic source, Karnataka, India. The collected roots, leaves and fruits were cleaned thoroughly with double distilled water and dried under the shade. Once the drying process is complete, the dried roots, leaves and fruits were ground to powder, stored for further studies.

### Aqueous extract preparation

Aqueous extract was prepared by dissolving 5g of powdered *Muntingia calabura* root, leaf and fruits in 100ml of double distilled water, vortexed for 4 hours, centrifuged at 10000 rpm, supernatant collected, lyophilized and stored at -10°C.

### Phytochemical analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures.

### Protein estimation

The protein estimation was carried according to Bradford's method [9] using BSA as standard and hexane extracts of different plant materials into a series of test tubes. Volume was made up to 100µl with distilled water and 900µl of Bradford's reagent was added to each tube. Absorbance was read at 535nm. Concentration of protein was calculated accordingly using standard graph.

### α-tocopherol estimation

α-tocopherol estimation was carried out according to Kivcak and Mer T [10]. 20µl-100µlof standard α-tocopherol solution and 20 and 40 µl of the hexane extracts was used for the estimation. Volume was made up to 3ml using chloroform, 1 ml of 2, 2-dipyridyl, and 1 ml of FeCl3 solution, Incubated at $37^0$C for 15 minutes, and the absorbance of the reaction mixture was read at 520nm, concentration was calculated accordingly by using the standard graph.

**Total phenolics**

Total phenolics were determined according to the method of FolinCiocalteu reaction [11] with minor modifications using gallic acid as a standard (0-100µg). Various concentrations of hexane extracts ranging from 0-100µg were taken in series of test tubes & the volume was made up to 500µl with distilled water. 500µl of the Folin-ciocalteu reagent was added to each tube, the mixture was allowed to stand for 10 minutes followed by addition of l.0ml of 20% Sodium carbonate, incubated at 10 minutes at 37°C. Absorbance was read at 750nm and the concentration was calculated using the standard graph accordingly.

**Ascorbic estimation**

Ascorbic estimation was carried out according to Sadasivam S., Manickam [12]. Different concentrations (0-100µg) of hexane extracts were taken along with standard ascorbic acid. A drop of thiourea solution and 1ml of 2,4dinitrophenyl hydrazine reagent was added to each tube and the volume as made up to 100µl with 4% oxalic acid and incubated at 37°C for 3 hours. Then tubes cooled on ice water and 5ml of 85% sulphuric acid was added to each tube. Mix the reaction mixtures thoroughly. The orange color developed was read against a reagent blank at 540nm. The concentration was calculated on the basis of the standard curve.

**Total sugar estimation**

Sugar estimation was done according to Dubois method [13]. 10 - 100 µg of the working standard solution was pipetted into a series of test tubes 200µl of the extracted sample was pipetted into two separate test tubes. The volume in each tube was made up to 1000µl with double distilled water. 1ml of 5% phenol was added to each tube followed by 5ml of 96% sulphuric acid, intensity of the colour was read at 520 nm. The amount of total sugar present in the given unknown sample solution was calculated using the standard calibration curve.

**Flavonoid estimation**

Flavonoid estimation was done according to Cheon et al [14] by using Quercitin as a standard. Various concentrations (0-100µg) of hexane extracts were taken in test tubes. Made up the volume to 1.5 ml with 95% ethanol. Then 100 µl of 10% of aluminium chloride, 100µl of 0.1M of potassium acetate was added to each tube. The total volume was made up to 2.8ml of by using distilled water. Optical Density (O.D) was measured at 415 nm and the concentration was calculated accordingly.

**Estimation of Total chlorophyll**

Chlorophyll estimation was determined according to the method of Sadasivam and Manickam[12]with minor modifications. In brief, 0.5ml of the hexane extract was mixed with the 20ml of 80% acetone. Centrifuged at 5000 rpm for 5 minutes and supernatant was collected. The process was repeated for several times till the clear supernatant was obtained. All the supernatants were combined and volume was made up to 1 mL with 80% acetone. The absorbance of the solution was read at 645 and 663 nm. The amount of the total chlorophyll present in the extract, mg chlorophyll/gram extract was calculated accordingly using the calibration curve.

**Antioxidant activity**

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

DPPH radical scavenging activity was assessed according to the method of Shimada et al. with minor modifications [15] [16]. The Root, leaves and fruit extracts of *MuntingiaCalabura* at a concentration of 25µg 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 spectrophoto metrically at 517 nm. BHA and Ascorbic acid (400 µM) was used as positive control under the same assay conditions. Negative control was without any inhibitor or Root, leaves and fruit extracts of *MuntingiaCalabura*. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of extracts of *MuntingiaCalabura* was calculated from the decrease in absorbance at 517 nm in comparison with negative control.

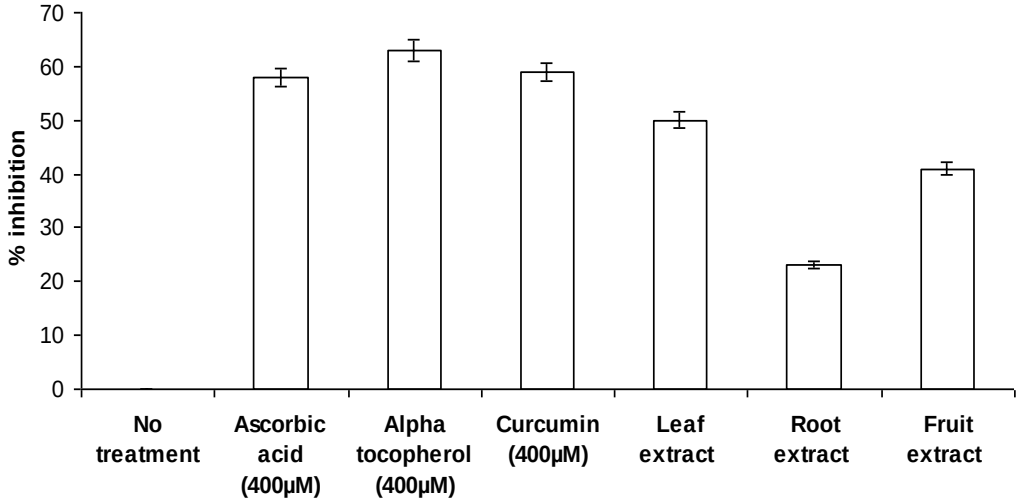
## 3. Results and Discussion

As shown in Table 1, the water extract of root, leaf and fruits of *Muntigiacalabura* plant was done and its phytochemical analysis was done. The leaves extract was rich with Carbohydrates (194mg/g), proteins (6.21mg/g), Flavonoids (42.61mg/g), chlorophyll (79.12mg/g) and Ascorbic acid (11.21mg/g) when compared to root and fruits. Where as fruits extract rich with polyphenols (29.03mg/g) when compared to root and leaves extract. The root extract rich with α-tocopherol when compared to leaves and fruits extract. When these extracts are subjected to antioxidant analysis using DPPH method (Figure 1), where Curcumin, α-tocopherol and Ascorbic acid are used as positive control at a dosage of 400µM concentration. The extracts of *Muntigacalabura* used at a dosage of 25µg. The positive controls Curcumin (61%), α-tocopherol (63%), Ascorbic acid (58%) inhibited the DPPH radicals. The leaf extract (52%), fruit extract (25%) and root extract (43%) inhibited the DPPH radicals. The above results indicated that, all the three extract of *Muntigiacalabura* plant showed good antioxidant activity when compared to positive controls.

**Table 1. Phytochemical analysis of *Muntingiacalabura* aqueous extract**

| | Root (mg/g) | Leaves (mg/g) | Fruits (mg/g) |
|---|---|---|---|
| Carbohydrates | 138.0± 1.36 | 194.0±1.44 | 65.33±1.62 |
| Protein | 2.63±0.05 | 6.21±0.11 | 2.11±0.09 |
| Polyphenols | 14.06±0.35 | 23.06±1.55 | 29.03±1.25 |
| Flavonoids | 14.34±0.01 | 42.61±1.02 | 21.71±0.31 |
| Ascorbic acid | 09.13±0.04 | 11.21±0.12 | 13.03±0.03 |
| α-tocopherol | 0.63±0.02 | 0.41±0.01 | 0.13±0.01 |
| Chlorophyll | 0.82±0.01 | 79.12±0.02 | 0.11±0.01 |

**Figure 1. DPPH radical scavenging activity by *Muntingiacalabura* aqueous extract of leaf, root and fruit**




DPPH (0.5mM) + with or without extract of *Muntingiacalabura* (25µg) /α-tocopherol / Ascorbic acid / Curcumin (400 µM). Mixture incubated at 37°C for 30 min and the absorbance read at 517 nm using spectrophotometer.
Values are means ± SD of triplicates.

## Conclusion

These results indicated that, the antioxidant activity of the *Muntigiacalabura* plant is may due to the presence of proteins, flavonoids, Ascorbic acid, polyphenols and α-tocopherol present in it.

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