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ANTIOXIDANT POTENTIAL OF *GARCINIA* SPECIES FROM SONITPUR DISTRICT, ASSAM, NORTH EAST INDIA

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ABSTRACT

The fruits of *Garcinia pedunculata* Roxb. ex Buch.-Ham., *G. xanthochymus* Hook. f. and *G. morella* (Gaertn.) Desr. collected from Sonitpur district of Assam, India were evaluated for the antioxidant potential and total phenolic content. The methanolic extract of these fruits were subjected to DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay for determining the free radical inhibition property and Folin Ciocalteu's method for quantification of total phenolic content (TPC). *G. pedunculata* Roxb. ex Buch.-Ham. showed highest antioxidant potential with IC_{50} value 47.03±13.48 µg/ml. All species contains low phenolic content ranging between 1.83±0.62 to 88.2±2.19 mg GAE/g dry extract. No significant correlation between total phenolic content and antioxidant potential was found.

INTRODUCTION: Fruits and vegetables are major sources of dietary antioxidant [1] which help in cellular defenses and prevent cellular components against oxidative damage [2]. Fruits of most *Garcinia* species are reported as edible and provide a food supplementary among the tribal peoples.

Garcinia species are evergreen trees and shrubs of the family Clusiaceae which are native to Asia, Australia, Africa and Polynesia. Some prominent species are known to have good medicinal value and are used in traditional medicines for treatment of various diseases [3, 4].

(-)-Hydroxycitric acid (HCA) has been found in the fruits of certain species of *Garcinia*. HCA is known to curb appetite, suppress food intake, increase the rates of hepatic glycogen synthesis, reduce fatty acid synthesis and lipogenesis, and decrease body-weight gain. HCA is a good dietary supplement to any weight management program [5].

G. pedunculata Roxb. ex Buch.-Ham. (*Bor-thekera* in Assamese) is a large evergreen tree with fluted trunk and short spreading branches. Fruit is globose, 8-12 cm diameter with fleshy edible aril **(Figure 1)**. The mature fruit is eaten raw or cooked with pulses. *G. xanthochymus* Hook. f. (*Tepor-tenga* in Assamese) is a small middle sized evergreen tree distributed mostly near riverbank. The ripe fruit is golden yellow colour and 4-6 cm in diameter **(Figure 2)**. The ripe fruit can be eaten raw having sour test or cooked with other vegetables. *G. morella* (Gaertn.) Desr. (*Kuji-thekera* in Assamese) is a small evergreen tree with ripe fruit about 2-3 cm in diameter, globose and yellow colour **(Figure 3)**.

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Raw fruits are preferred for pickles and sundried fruits are used as spices in foods. The present study was undertaken to determine antioxidant potential and total phenolic content of methanolic extract of fruits of *Garcinia* species collected from Sonitpur district of Assam, North East India.

FIGURE 1: *GARCINIA PEDUNCULATA*- FRUIT

FIGURE 2: *GARCINIA XANTHOCHYMUS*- FRUIT

FIGURE 3: *GARCINIA MORELLA*- FRUIT

MATERIALS AND METHODS:

Chemicals and Solvents: Folin-Ciocalteu's phenol reagent and sodium carbonate were obtained from *Merck*, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and L-Ascorbic acid from *SIGMA* and gallic acid from *BDH*.

Preparation of Crude Extract: Fresh ripen fruits of *G. pedunculata, G. xanthochymus* and *G. morella* were collected from Tezpur market in Sonitpur district of Assam, North East India. Fruit pulp were dried and pulverized into fine pieces. 100 g sample pieces were soaked in 500 ml methanol for 24 hours and filtered through Whatman paper No. 41. The residue was re-extracted twice with 500 ml methanol each. The total filtrate was concentrated by rotary evaporator at 45°C under reduced pressure and stored at -4°C until further use.

Determination of Antioxidant Activity Using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Method: The DPPH assay was done by the method given by Brand-Williams *et. al.* [6] with some modifications. Test samples were prepared by adding 750µl of 0.1mM methanolic DPPH solution in 750µl methanolic plant extract solution of varying concentrations (5, 10, 50, 100, 250, 500 and 1000 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1, 2.5, 5, 10, 25, 50 and 100 µg/ml) were used as reference standard. Mixer of 750µl methanol and 750µl DPPH solution was used as control. The decrease in absorbance (*A*) was measured at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer (SPECORD-250 Analytic Jena, AG, Germany). The inhibition % was calculated using the following formula:

$$\text{Inhibition \%} = \frac{A_{(\text{Control})} - A_{(\text{Test Sample})}}{A_{(\text{Control})}} \times 100$$

Quantification of Total Phenolic Content (TPC): The total phenolic content was determined by the Folin-Ciocalteu's method [7] with reduced volumes. For the preparation of calibration curve stock solution was prepared by mixing 10 mg of gallic acid in 100 ml of distilled water. Different volumes of stock solution (100 µl - 500 µl) were mixed with 2 ml water and 0.3 ml Folin Ciocalteu's Phenol reagent in test tubes. After 5 minutes 0.8 ml 20% $NaCO_3$ was added and final volume was made to 5 ml. The absorbance was measured at 765 nm after 30 minutes using UV-Vis spectrophotometer (SPECORD-250 Analytic Jena, AG, Germany) and the calibration curve was drawn. In separate test tubes 200 µl fruit extracts (1mg/ml) were mixed with the same reagents as described above with a final volume to 5 ml and the absorbance were

measured after 30 minutes at the same wavelength for determination of TPC. Results were expressed as mg/g (Gallic acid equivalent/dry weight). Total phenolic content in methanolic fruit extracts of *Garcinia* were calculated using the formula:

$$TPC = c \times \frac{V}{m}$$

Where, '*c*' is the concentration of gallic acid in mg/ml; 'V' is the volume of plant extract in ml; and 'm' is the weight of pure plant extract in g.

Statistical Analysis: All the assays were carried out in triplicate and the experimental results obtained were expressed as mean±SD. IC_{50} value was calculated by plotting nonlinear regression curve using GraphPad Prism 5 software.

RESULTS AND DISCUSSION:

DPPH Assay: DPPH assay is one of the most common methods used to determine the antioxidant potential of various plants extract [8]. This method is based on decrease in purple/dark violet colour of alcoholic DPPH solution [9, 10] when contacted with antioxidant substances like phenolic compounds and have a strong absorption range at 517 nm [11]. Total antioxidant necessary to decrease the initial DPPH radical concentration by 50% is referred as IC_{50}, thus, a lower IC_{50} value would reflect greater antioxidant activity of the sample [12].

The inhibition rates of different *Garcinia* fruit extract are shown in **Figure 4**. Among the methanol extract of three *Garcinia* fruits, *G. pedunculata* showed highest antioxidant potential with IC_{50} value 47.03±13.48 µg/ml **(Table 1)**. The IC_{50} value of standard (L-Ascorbic acid) was found 4.98±0.24 µg/ml while the other two *Garcinia* species have low antioxidant potential.

Total Phenolic Content (TPC): Phenolic compound of plant acts as primary antioxidants or free radical scavenger [10]. Several comprehensive works have been done in the recent years and significant correlation was found between TPC and antioxidant activity [13]. TPC measured by Folin Ciocalteu's method was calculated by plotting gallic acid standard curve (equation: y=0.0833+14.5589*x; R^2=0.9944). The screening of the three *Garcinia* species revealed that there was a low phenolic content in fruit extract ranging from 1.83±0.62 to 88.2±2.19 mg GAE/g dry extract. The highest amount of phenolic content was found in *G. pedunculata* followed by *G. xanthochymus* and *G. morella* **(Table 1)**. *G. xanthochymus* with phenolic content 33.21±1.15 GAE/g showed lowest antioxidant activity.

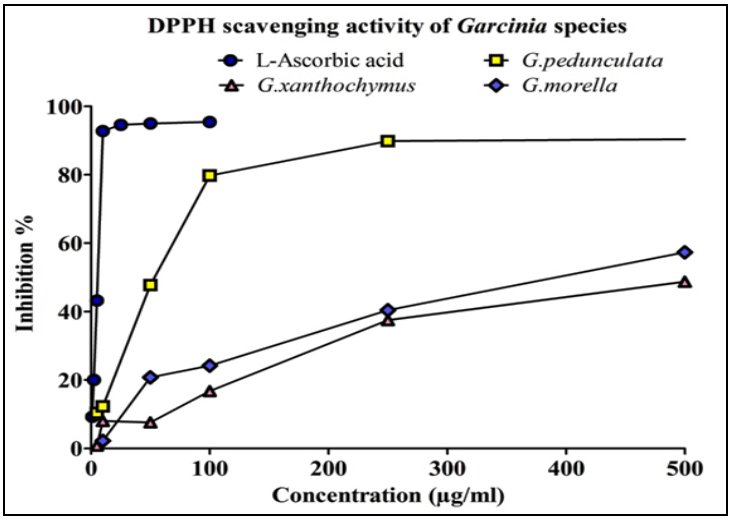

FIGURE 4: DPPH FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF *GARCINIA* SPECIES

TABLE 1. IC_{50} VALUE AND TOTAL PHENOLIC CONTENT (TPC) OF METHANOLIC EXTRACT OF *GARCINIA* SPECIES

Sample	IC_{50} (µg/ml)	TPC (mg GAE/g)
L-Ascorbic acid	4.98±0.24	-
G. pedunculata	47.03±13.48	88.2±2.19
G. xanthochymus	428.60±22.37	33.21±1.15
G. morella	303.63±5.34	1.83±0.62

CONCLUSION: The fruits of the three *Garcinia* species contain low phenolic content. However, *Garcinia pedunculata* has potent antioxidant activity. The widely available *G. pedunculata* is a health benefit fruit which might be helpful in preventing or slowing the progress of various oxidative stress related diseases and could be used as an easy accessible source of natural antioxidant. There is no significant correlation between total phenolic content and antioxidant activity (IC_{50} value) of the three species analyzed. Further investigation on identification and isolation of antioxidant compounds in *G. pedunculata* is going on.

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