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NUTRACEUTICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF *GARCINIA KYDIA* ROXB., FRUIT FOUND IN ASSAM, INDIA

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ABSTRACT
Since past fruits are integral part of human food, nutrition and health, particularly as a source of vitamin C, folic acid, minerals and dietary fibres. Some components of fruits have strong antioxidants and could modify the metabolic activation and detoxify the carcinogens and may even influence processes that may change the course of the tumor cell. The present study was considered to evaluate the nutraceutical properties and antioxidant activity of the fruits of *Garcinia kydia* Roxb. *Garcinia kydia* (Vern: *Kuji-thekera*) is a fruits bearing tree with narrow crown found in Assam. The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl(DPPH)free radicals and hydrogen peroxide scavenging activity and IC_{50} value of methanolic extract of *G. kydia* are found to be 197.52µg/ml and 88.39µg/ml by DPPH method and Hydrogen peroxide method respectively. Among the nutraceutical properties carbohydrate (68.05 ± 0.32 µg/mg) protein (75.62± 0.015µg/mg), crude fibre (15.64 % ±0.64), total phenolic content (110.50± 1.5µgGAE/g) and ascorbic acid (62.93 ± 0.35 mg/100g) were recorded. The result indicates that *G. kydia* have a high nutraceutical value. The study also revealed that the fruit of *G. kydia* has sufficient dietary supplement as well as therapeutic agent as they would exert several beneficial effects by virtue of their antioxidant property.

KEYWORDS: *Garicinia kydia*, antioxidant property, DPPH, hydrogen peroxide, total phenolic content, Ascorbic acid, Gallic acid.

INTRODUCTION
Dietary habits are the habitual decisions an individual or culture makes when choosing what foods to eat. Proper nutrition requires ingestion and absorption of vitamins, minerals, and food energy in the form of carbohydrates, proteins, and fats. Dietary habits and choices play a significant role in the quality of life, health and longevity. Free radicals are highly reactive chemicals that have the potential to harm cells. Free radicals are formed naturally in the body and play an important role in many normal cellular processes .At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and many diseases such as rheumatoid arthritis and atherosclerosis. Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers."

The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants.

It has been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds.

MATERIALS AND METHOD
The Fruits of *Garcinia kydia* were collected from Forest. The collected fruits were washed with water and were shade dried and grinded to coarse powder. Powdered plant materials were soaked in methanol for 24 hrs. Extract was filtered and concentrated by evaporation under reduced pressure using rotary vacuum evaporator, dried and kept in an air tight container. The percentage yield was noted.

Methods of analysis
Chemical analysis was done on moisture free basis. Analysis was carried out to estimate the macro nutrient

components *viz*. total protein, total carbohydrates and ascorbic acid and phenolic compounds of the samples.

Total Protein Estimation

The total protein content of the samples was estimated by following the method developed by Lowry *et al.*, 1951. Extraction was carried out with Phosphate Buffer (0.1 M, p^H -7.6). 500 mg of sample was ground in pastel mortar with 20 ml of Phosphate buffer. These samples were kept overnight for complete extraction of protein. These were centrifuged at 10000 rpm for 20 minutes. The supernatant was used for protein analysis. The reading was taken in a UV-Vis Spectrophotometer at 660nm and the amount of protein present was calculated by plotting the value in a standard curve of Bovine Serum Albumin (BSA).

Total Carbohydrate Estimation

The total carbohydrate content of the samples is estimated by Anthrone method (Hedge, and Hofreiter, 1962). 100 mg dried samples were hydrolyzed with 2.5N HCl for about 3 hours in a boiling water bath. Sodium carbonate was added to neutralize the extracts. Subsequently the extracts were centrifuged and supernatant were collected. The residue was washed thrice with distilled water and all the supernatants were pooled and final volume was adjusted to 100ml. From this, 0.5 ml of the extracts was taken and volume made up to 1ml distilled water. 4ml of Anthrone reagent was added to the above solution. Absorbance was taken in a UV-Vis spectrophotometer at 630 nm and the amount of carbohydrate present was calculated by plotting the value in a standard curve of Standard Glucose solution.

Crude Fibre Content

Crude fibre in the samples was determined by the method described by Maynard (1970). Defatted sample (2g) was placed in a glass crucible and attached to the extraction unit. 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 600°C in a muffle furnace (for 2 h, cooled in a desiccator and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:

% crude fiber in ground sample
=Loss in weight on ignition (W2 -W1)/ Weight of the sample x 100

Ascorbic Acid Content Estimation

The amount of ascorbic acid present in the sample was calculated by extracting the sample in 4% oxalic acid and titration of the extract against the 2, 6- dichlorophenol indophenols dye until the end point where pink colour to be appeared that persist for a few minutes (Sadasivam and Balasubraminan, 1987). The amount of dye consumed was equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution was used as the reference and the calculation was done by the following formula:

$$\text{Amount of ascorbic acid (mg/100gm) sample} = \frac{0.5\text{mg} \times V2 \times 100}{V_1 \text{ ml} \times 5 \text{ ml} \times \text{Wt. of the sample}} \times 100$$

Where, V_1= Volume of oxalic acid, V_2= Volume of the sample

Total Phenol Content Estimation

Total Phenolic content was determined by using spectrophotometer following the method described by Folin –Ciocalteu's (Lachman et al., 1989) with slight modification. Briefly, 0.5 mL of the sample was pipetted into a 10 mL volumetric flask containing 0.5 mL of Folin–Ciocalteu's reagent, 5 mL of distilled water and 1.5 mL of Na_2CO_3 solution (w = 20 %), and the volume was made up with distilled water. During the oxidation of phenolic compounds, phosphomolybdic and phosphotungstic acid, contained in the Folin– Ciocalteu's reagent, were reduced to blue-colored molybdenum and tungsten oxides. After two hours, the absorbance of blue coloration was measured at λ = 765 nm against a blank sample. The measurements were compared to a standard curve of prepared gallic acid solutions (20, 40, 60, 80, 100 mg L–1) and expressed as milligrams of gallic acid equivalents per 100 g ± SD. All measurements were performed in triplicate

Determination of total flavonoids

Aluminum chloride method was used for flavonoid determination (Olajire, 2011). In this method Quercetin was used as standard and flavonoid contents were measured as quercetin equivalent. For this purpose, the calibration curve of quercetin was drawn .1ml of standard or extract solution (0.1, 0.5, 1, 2.5,5 mg/ml) was taken into 10ml volumetric flask, containing 5 ml of distilled water and 0.3ml of 5% $NaNO_2$. After 5min, 0.3ml 10% $AlCl_3$ was added to the mixture. At the 6th min 2ml of 1M NaOH was added sequentially and the volume made up to 10ml with distilled water. The test solution was vigorously shaken. The absorbance was noted at 510 nm using UV-Visible spectrophotometer after 10mints of incubation.

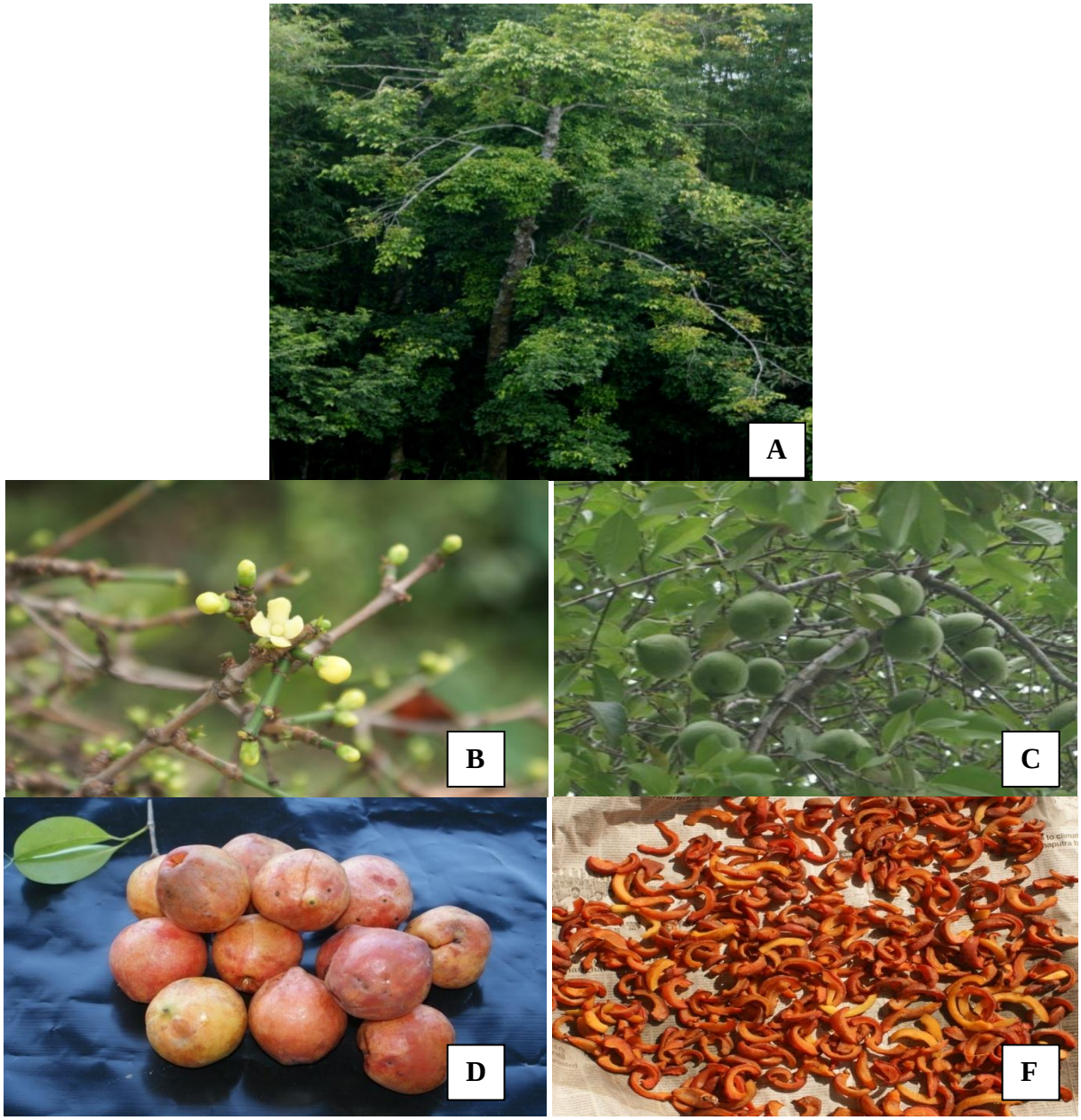

Fig. 1: *Garcinia kydia* in patch vegetation A] habitat; B] flower, C&D] fruits and F] Slices of fruits.

Antioxidant activity estimation

DPPH scavenging activity of *Garcinia kydia* fruits was estimated by using the UV spectrophotometer (Narla and Rao, 1995). DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radical reacts with various electron donating molecules (reducing agents or antioxidants). When electrons become paired off, the DPPH solution is bleached out. This caused the formation of the colourless 2, 2'-diphenyl-1-picryl hydrazine. Reduction of the DPPH radicals can be estimated quantitatively by measuring the decrease in absorbance at 517 nm.

For that, the methanolic 0.1 mM 2,2'-diphenyl-1-picrylhydrazyl (DPPH) solution was added to different concentrations of methanolic test compounds (0 – 500 mg/ml) in equal volume and mixed well and kept in dark for 20 min. The absorbance at 517 nm was measured using the UV spectrophotometer (Narla and Rao, 1995). Controls containing methanol instead of the sample solution was also made. The same procedure was repeated for the standard solution of ascorbic acid. The percentage of DPPH free radical scavenging activity was calculated from the following equation:

Scavenging effect (%) = [1- $A_{sample}517/A_{Control}517$] X 100

The IC_{50} (concentration providing 50% inhibition) values were calculated the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect.

Scavenging activity of Hydrogen peroxide (H_2O_2)

Scavenging activity of Hydrogen peroxide (H_2O_2) by the fruit extract was determined by the method described by Ruch, et al., (1989). Plant extract (4ml) prepared in distilled was at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound .The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using the following equation

Scavenging effect (%) = [1- A_{sample}230/$A_{Control}$230] X 100

Where $A_{Control}$ = absorbance of the blank control (containing all reagents except the extract solution), A_{sample}= absorbance of the test sample.

The antioxidant potential of the methanol extract of *G. kydia* was evaluated by DPPH free radical scavenging activity and Hydrogen peroxide scavenging activity. The studies were carried out taking ascorbic acid as standard the standard antioxidant. The results of the antioxidant activity by DPPH free radical scavenging activity and Hydrogen peroxide scavenging activity were expressed in terms of per cent of inhibition of generated free radicals respectively with respect to various concentrations. Concentration dependent effects were observed in each case i.e. higher concentrations were found to exhibit higher per cent of inhibition in each protocol of the antioxidant study. The graphs were constructed by taking per cent of inhibition along the Y-axis and various concentrations along the X-axis. The IC_{50} value (50% inhibition) of G. *kydia* and the standard ascorbic acid were determined in both studies. With reference to the observed IC_{50} value of *G. kydia*, the antioxidant potential was found to be higher in case of hydrogen peroxide scavenging activity.

Statistical Analysis

The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as mean ±S.D. IC_{50} value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis**.**

RESULTS AND DISCUSSION

The result of phyto-chemical analysis was done for total carbohydrate content, total protein content, crude fibre, total ascorbic acid content, total flavonoid content and total phenol content of *Garcinia kydia* fruits is presented in Table 1. The total carbohydrate content of fruit pulp was 14.05±0.32 % and the total protein content was measured for 2.67± 0.05 %. The crude fibre content of the fruit was (15.64 % ±0.64). The total phenol content of *Garcinia kydia* fruits was measured by Folin Ciocalteu's method and calculated by plotting Gallic acid standard curve (equation: y=0.166x+0.113; R2=0.991), and the amount was 78.50± 1.5µgGAE/mg, total flavonoid content 67.3mgQ/g, whereas the ascorbic acid content was 62.93 ± 0.35 mg/100g. Similar type of works had been by a few researchers in some other species of *Garcinia* (Biswas, et al., 2017; Chowdhury, 2014). Sarma et al (2014) reported total carbohydrate content 15.68 ± 0.23 %, the protein content was 2.82 ± 0.14%, the total phenol content was 74.18±0.64 µgGAE/mg whereas the ascorbic acid content was 68.03 ± 0.67mg/100 gm in fruits of *Garcinia cowa* (Fig.2). The present study reveals that in comparison to *G. cowa* these values are found to be at par in *G. kydia* fruit pulp.

Table 1: Nutritional analysis of *G. kydia* fruits pulp.

Sample	Total phenolic content (mg GAE/g)	Total flavonoids content (mg of Quercetin /g)	Carbohydrate content (%)	Protein content (%)	Ascorbic acid content (mg/100g)	Crude fibre (%)
Garcinia	78.50± 1.5	67.3	14.05±0.32	2.67± 0.05	62.93 ± 0.35	15.64 ±0.64

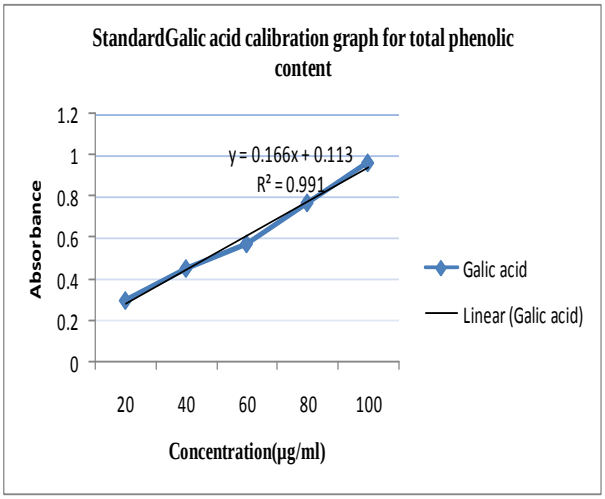

Fig. 2: Construction of Gallic acid standard curve for phenolic contents of *G. kydia* fruit pulp.

Antioxidant activity

The antioxidant activity was measured using DPPH assay and Hydrogen peroxide assay. The results of DPPH free radical scavenging activity of methanol extract of *G. kydia* fruit pulp that compared with Ascorbic acid is given in Table 2. The percent inhibition at various concentration (100-500 µg/ml) of *G. kydia* fruit as well as standard given Ascorbic acid(12.5 -100 µg/ml) were calculated and plotted in Figure 3 using Microsoft Office Excel 2007.The IC_{50} values are calculated from the graph and were found to be 37.84µg/ml (Ascorbic acid) and 197.52 µg/ml (*G. kydia*). This indicated that fruit pulp of *G. kydia* in rich for free radical and contains significantly high value of anti oxidant properties.

Table 2: DPPH scavenging activity of *G. kydia* fruit pulp.

Sl No.	Concentration(µg/ml)	%age of inhibition	IC_{50} value (µg/ml)
Std(Ascorbic acid)			
1	12.5	33.68±1.02	37.84
2	25	45.32± 1.01	
3	50	62.37±2.03	
4	100	81.76±1.32	
Garcinia kydia			
1	100	46.89±1.13	197.52
2	200	51.84±1.21	
3	300	57.63±2.04	
4	400	76.98±1.63	
5	500	81.64±1.09	

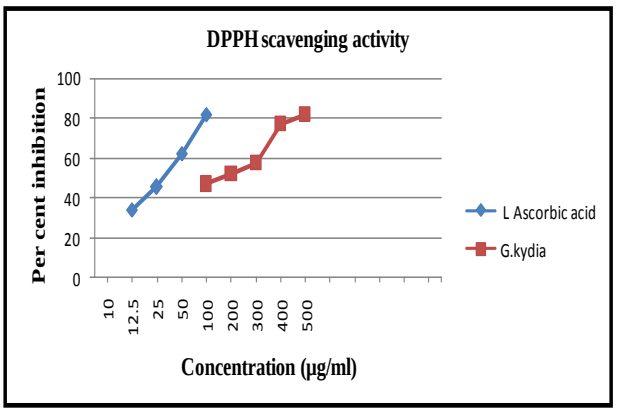

Fig. 3: DPPH scavenging activity of ascorbic acid and methanolic extract of *G. kydia*.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of methanol extract of *G. kydia* fruit pulp was evaluated and compared with Ascorbic acid and the results are given in Table 3. The per cent inhibition at various concentration (50-500µg/ml) of *G. kydia* fruit as well as standard given Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Figure 3 using Microsoft Office Excel 2007.The IC_{50} values are calculated from the graph and were found to be 41.98 µg/ml (Ascorbic acid) and 88.39µg/ml (*G. kydia*).

Table 3: Results of Hydrogen Peroxide scavenging activity of *G. kydia* fruit pulp.

Sl No.	Concentration(µg/ml)	%age of inhibition	IC_{50} value (µg/ml)
Std(Ascorbic acid)			
1	12.5	26.72±1.31	**41.98**
2	25	40.59±2.35	
3	50	76.23±1.02	
4	100	89.82±1.05	
Garcinia kydia			
1	50	40.32±2.01	**88.39**
2	100	59.14±1.21	
3	200	70.23±1.14	
4	300	81.49±1.46	
5	400	85.63±1.08	
6	500	88.38±1.07	

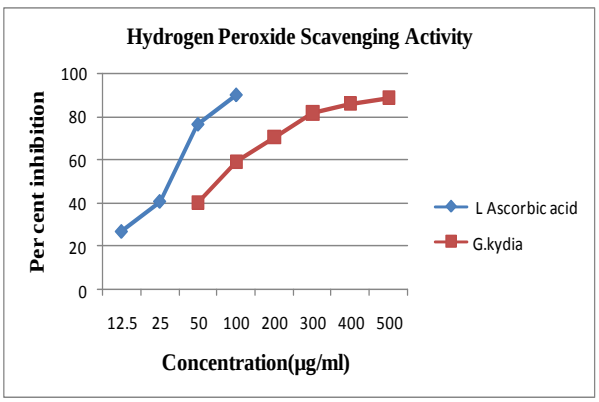

Fig. 3: Hydrogen Peroxide scavenging activity of Ascorbic acid and *G. kydia*.

CONCLUSION

The various biochemical testing were tested for nutrients components like carbohydrates, vitamins, protein, phenol content and antioxidant potential in the *G. kydia* fruit. From the present studies it can be concluded that the fruit content high value of nutrient and methanolic extract of the fruit possess antioxidant activity. The further studies on this fruit might provide the isolation of some active constituents having the antioxidant potential.

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