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RESEARCH ARTICLE

PHYTOCONSTITUENTS ESSAY

Quantitative Analysis of Garcinol, HCA, HCA Lactone, Other Organic Acids, minerals and Antioxidant Properties in Fruits of Eight *Garcinia* Species Prevalent in Assam

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Abstract

Fruits of plants under genus *Garcinia* are valued highly in preparation of culinary, ayurvedic, and ethnomedicinal products. Fruits of different differ in content of beneficial bioactive constituents and minerals and selection of superior type is desirable. Eight species of this genus prevalent in Assam, India were evaluated for content of bioactive constituents, minerals and antioxidant activity. Garcinol, HCA, HCA lactone were found to occur in higher concentration. The garcinol content of the two species, namely *G. xanthocymus* (286.37 mg/g) and *G. sopsopia* (195.980 mg/g) was found to be more than those in other species fruits. *G. lancifolia*, *G. pedunculata* and *G. cuspida*had rich content of HCA and HCA lactone and HCA ranged from 445.85 to 539.13 mg/g and HCA lactone 131.95 to 239.25 mg/*G.* In comparison, other organic acid except tartaric acid were low and varied widely (below detection limit to 85.54 mg/g). Considerable antioxidant activity was found in *G. xanthocymus* and *G. sopsopia* which also contained phenol and flavonoids in more concentration. None of the fruit contained heavy metals and were found to be rich source of Calcium (Ca), Magnesium (Mg), Phosphorus (P), Iron (Fe), and Zinc (Zn).

Keywords: *Garcinia, Ethonomedicine, Nutraceutical, Culinary, Ayurvedic product*

1 Introduction

The genus *Garcinia*(Family: Clusiaceae) comprises nearly 250 species distributed in tropical Asia, including occurrence of 43 species and 6 varieties in India, of which 37 species and 4 varieties still confined in wild (1; 2). North East India hosts 17 species and 5 varieties, of which 2 species and 1 variety are endemic to the region(3). Plants in this genus have been used for culinary, ayurvedic, and ethnomedicinal purpose. Various products have been developed from fruits by pharmaceutical and other industries. The beneficial effects of the fruits attributed to bioactive molecules such as hydroxycitric acid (HCA) and its derivatives. Flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives such as garcinol, isogarcinol, xanthochymol and guttiferone isoforms have also been isolated from *Garcinia* genus plants and shown to have beneficial effect on health(4; 5). In recent years, hydroxycitric acid (HCA) and its lactone (HCA lactone) have received considerable attention as a potential metabolic regulator and inhibitor of lipogenesis. It was first reported from *G. sopsopia* (6) and in recent past it has also been reported from other species of Garcinia(4; 5). In various animal studies, HCA and its lactone were found to be effective in reducing appetite, lipogenesis and bodyweight gain and increasing hepatic glycogen synthesis(7; 8). These findings led to use of HCA and its lactone in various nutraceutical and pharmaceutical preparations in combination with other ingredients. Garcinol and other polyisoprenylated benzophenone and xanthone derivatives are reported to exhibit antioxidant, apoptotic, anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, antiulcer, anti-protozoal, and HAT inhibiting properties(4). However, content of bioactive constituents in fruits may vary depending upon the species and varieties and systematic study to compare different constituents in fruits of *Garcinia* is scanty. Report of occurrence of total of eight (8) different species of *Garcinia* is available and it is commonly

known as 'thekera' by Assamese people. *Garcinia* has rich traditional uses in this region. The sundried slice fruits of *G. pedunculata* and *G. morella* are commonly used for various gastrointestinal problems and culinary preparations by different communities of Assam. In the present study, we have carried out a comparative analysis of the HCA, HCA lactone, garcinol along with few other organic acids contents in fruits of all the eight (8) available species under *Garcinia* genus. Their antioxidant activity, phenolic as well as flavonoid content was also determined. In addition elemental composition and heavy metal residues in the *Garcinia* fruits were also estimated.

2 Materials and Methods

2.1 Chemicals and Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), ascorbic acid, hydroxy citric acid, hydroxy citric acid lactone, oxalic acid, citric acid, tartaric acid and succinic acid were obtained from Sigma Aldrich Inc. Garcinol was obtained from Santa Cruz Biotechnology. All other reagents, chemicals and solvents are of analytical grade and were procured from Himedia Laboratories, Acros organics and Milli-Q quality water was used.

2.2 Plant Materials

Fresh ripen fruits of eight different species of *Garcinia* L, i.e. *Garcinia morella* (Gaertn.) Desr., *Garcinia sopsopia* (Buch.-Ham.) Mabb., *Garcinia pedunculata* Roxb. ex Buch.-Ham, *Garcinia lanceifolia* Roxb., *Garcinia cowa* Roxb. ex Choisy, *Garcinia kydia* Roxb., Garcini *morella* var. *cuspida* Ajima *et al.* and *Garcinia xanthochymus* Hook.f. ex T.Anderson were collected from different areas of Assam, India. Plant samples were identified by expert taxonomist and herbarium specimens were deposited at IASST, Guwahati.

2.3 Extraction

Fruits of the different species of *Garcinia* were washed properly with tap water followed by milli-q quality water to remove any contamination. The fruit samples were deseeded, cut into small pieces and kept for drying under shade at room temperature and appropriate aeration was given by rotating the samples at different time intervals to avoid any microbial growth. The dried samples were powdered using a mixture grinder and 50 g of each of the samples were macerated with 80% ethanol. After 24 hours, the filtrate through Whatman No. 1 filter paper was collected and the marc was immersed in fresh solvent. The process was repeated until the colour of the filtrates faded. Filtrates obtained were concentrated under reduced pressure using a rotary evaporator (Buchi Labortechnik AG, Switzarland) at below 50°C. Concentrated extracts were dried completely using a lyophilizer (Labconco, USA) at 50 °C.

2.4 HPLC Quantification of Garcinol, Hydroxy Citric Acid, Hydroxy Citric Acid Lactone and Other Organic Acids

Quantification of garcinol, hydroxy citric acid, hydroxy citric acid lactone and other organic acids in the different *Garcinia* species was carried out by reverse phase HPLC using Waters 1525 HPLC system coupled with a UV–Vis diode array detector. Analysis was carried out using Spherisorb ODS2 C18 column (5 µm pore size, 4.6 × 250 mm). In case of garcinol, an isocratic solvent system consisting of 0.1% TFA (trifluoroacetic acid) in water (solvent A) and acetonitrile (solvent B) was used as mobile phase in the isocratic ratio of 20:80 (A: B v/v) with a flow rate of 1 ml/min. For, HCA, HCA lactone and other organic acids (oxalic, citric, tartaric and succinic acid), isocratic solvent system consisting of Solvent A (30% H3PO4 diluted to 1:9 ratio in water with pH maintained to 2.89) and methanol as solvent B was used. The flow rate of 0.4 mL was used for HCA and HCA lactone while for the remaining organic acids it was 0.8 mL. The injection volume was 20 µl and detection wavelength was 254 nm for garcinol and 210 nm for the hydroxy citric acid, hydroxy citric acid lactone and remaining organic acids. Extracts and the standards were filtered through a 0.2 µm syringe filter prior to HPLC injection and column temperature was maintained at 30°C throughout the analysis. Quantification of all the standards was done by comparing with standard using the retention time and absorbance spectrum profile.

2.5 DPPH Radical Scavenging Assay

The free radial scavenging activity of extracts was measured in terms of hydrogen donating ability using DPPH radical as described by the method(9). Ascorbic acid was used as positive control. The free radical scavenging activity was calculated by using the formula below:

$$\%\ \text{radical scavenging activity} = \frac{(Abs_{control} - Abs_{sample})}{(Abs_{ControlOD})} \times 100 \quad (1)$$

2.6 ABTS Radical Scavenging Assay

The ABTS free radical scavenging activity of the plant extracts was determined by following the method described by(10) with some minor modifications. The ABTS free radical scavenging activity was calculated by using Equation 1.

2.7 Total Phenol Content (TPC)

TPC was determined using the Folin–Ciocalteu method(11). Quantitative estimation in the plant extracts was performed using a standard calibration curve of gallic acid. TPC was expressed in terms of gallic acid equivalents (GAE) (mg of GAE/g of plant extract).

2.8 Total Flavanoid Content (TFC)

TFC was determined by using the aluminium chloride calorimetric method(12). Quantitative estimation in the plant extracts was performed using a standard calibration curve of quercetin. TFC was expressed in terms of quercetin equivalents (QE) (mg of QE/g of plant extract).

2.9 Analysis of Minerals

The air dried fruit samples of the different species of *Garcinia* L. was oven dried at 80 °C in a clean drying chamber. One (1) g of the powdered dried sample of each of the *Garcinia* L. species was taken in porcelain crucibles and placed in a muffle furnace (Heraeus W.C. Heraeus GmbH, Hanau, Germany) at 500 °C for 2

Table 1: HPLC analysis based content of Quantitative analysis of garcinol, hydroxycitric acid, hydroxycitric acid lactone and other organic acids in the Garcinia fruits (mg/g)

Name	**Garcinol**	**Hydroxycitric acid**	**Hydroxycitric acid lactone**	**Oxalic acid**	**Citric acid**	**Tartaric acid**	**Succinic acid**
G. morella	53.27 ± 0.93^{b}	394.65 ± 82.75^{b}	88.54 ± 5.33^{b}	2.34 ± 0.06^{c}	28.05 ± 2.57^{b}	121.63 ± 12.19^{b}	BDL
G. pedunculata	43.96 ± 5.15^{b}	445.85 ± 99.49^{b}	235.95 ± 9.39^{d}	2.21 ± 0.042^{bc}	BDL	325.26 ± 19.19^{c}	BDL
G. lancifolia	3.85 ± 0.10^{a}	539.13 ± 67.19^{b}	239.25 ± 27.10^{d}	2.85 ± 0.10^{e}	1.92 ± 1.07^{a}	BDL	33.06 ± 3.18^{b}
G. kydia	BDL	396.10 ± 60.91^{b}	$101.74 \pm 5.10b^{c}$	2.04 ± 0.09^{a}	2.89 ± 1.16^{a}	153.87 ± 12.34^{b}	BDL
G. cowa	BDL	379.45 ± 50.77^{b}	89.50 ± 1.95^{b}	2.13 ± 0.04^{ab}	3.41 ± 1.47^{a}	156.85 ± 14.54^{b}	8.45 ± 2.55^{a}
G. cuspida	BDL	530.31 ± 31.97^{b}	131.95 ± 3.52^{c}	2.59 ± 0.04^{d}	BDL	BDL	12.914 ± 2.72^{a}
G. sopsopia	195.98 ± 14.27^{c}	356.81 ± 90.96^{b}	72.49 ± 7.66^{b}	2.13 ± 0.04^{ab}	5.73 ± 0.69^{a}	8.44 ± 0.97^{a}	BDL
G. xanthocymus	286.37 ± 8.55^{d}	83.30 ± 20.31^{a}	3.30 ± 0.53^{a}	BDL	85.54 ± 8.01^{c}	BDL	BDL

Values are means of triplicate ± SD; Means not sharing a common letter within each row were significantly different at $p < 0.05$; BDL: below detection limit.

Table 2: Antioxidant activity of the *Garcinia* fruits and their total phenolic and total flavonoid content

Name	**Antioxidant activity**		**TPC**	**TFC**
	IC50 (µg/mL)		(mg GAE/g)	(mg QE/g)
	DPPH	**ABTS**		
G. morella	379.98 ± 12.78^{b}	340.63 ± 16.69^{b}	44.60 ± 3.70^{d}	19.527 ± 0.333^{d}
G. pedunculata	803.40 ± 49.39^{c}	564.71 ± 50.87^{cd}	14.54 ± 0.97^{ab}	4.52 ± 0.34^{a}
G. lancifolia	727.92 ± 14.53c	540.26 ± 42.19^{cd}	16.447 ± 0.358^{bc}	9.78 ± 0.25^{b}
G. kydia	1119.14 ± 138.88^{d}	661.70 ± 61.83^{d}	9.25 ± 0.467^{a}	4.31 ± 0.51^{a}
G. cowa	516.82 ± 11.18^{b}	479.75 ± 28.21^{c}	16.637 ± 1.719^{bc}	16.36 ± 0.89^{c}
G. cuspida	695.99 ± 45.67^{c}	523.56 ± 94.71^{c}	22.090 ± 0.629^{c}	8.26 ± 0.25^{b}
G. sopsopia	111.38 ± 3.80^{a}	77.92 ± 5.64^{a}	60.713 ± 1.448^{f}	34.08 ± 0.26^{f}
G. xanthocymus	145.62 ± 3.16^{a}	44.18 ± 4.33^{a}	53.220 ± 3.661^{e}	26.91 ± 2.28^{e}
Ascorbic acid	5.54 ± 0.28^{a}	1.81 ± 0.04^{a}	-	-

Values are means of triplicate ± SD; Means not sharing a common letter within each row were significantly different at $p < 0.05$.

Table 3: The statistical relationships between the important parameters pertaining to antioxidant activity

Parameters	**Relationship**	r^2
DPPH free radical scavenging activity and ABTS free radical scavenging activity	y = 0.637x + 45.855	0.904
DPPH free radical scavenging activity and total phenolic content	y = 0.054x + 59.772	0.847
ABTS free radical scavenging activity and total phenolic content	y = 0.084x + 63.41	0.925
DPPH free radical scavenging activity and total flavonoid content	y = 0.029x + 32.298	0.898
ABTS free radical scavenging activity and total flavonoid content	y = 0.045x + 33.621	0.908

Table 4: Elemental concentration (in ppm) of the *Garcinia* fruits

Name	**Ca**	**Mg**	**Fe**	**Zn**	**As**	**Cd**	**Pb**
G. morella	4.38 ± 0.05^{a}	1.77 ± 0.11^{b}	1.46 ± 0.07^{d}	0.09 ± 0.01^{c}	ND	ND	0.01 ± 0.001^{abc}
G. pedunculata	5.58 ± 0.03^{b}	1.369 ± 0.11^{a}	0.55 ± 0.06^{c}	0.04 ± 0.01^{b}	ND	ND	0.01 ± 0.001^{abc}
G. lancifolia	5.69 ± 0.05^{b}	1.89 ± 0.09^{b}	0.51 ± 0.02^{c}	0.09 ± 0.01^{c}	ND	ND	0.01 ± 0.00^{1c}
G. kydia	6.48 ± 0.38^{c}	2.18 ± 0.07^{c}	0.33 ± 0.03^{b}	0.01 ± 0.02^{a}	ND	ND	0.01 ± 0.001^{a}
G. cowa	10.63 ± 0.09^{e}	1.85 ± 0.10^{b}	0.57 ± 0.06c	0.09 ± 0.01^{c}	ND	ND	0.01 ± 0.001^{bc}
G. cuspida	9.28 ± 0.09^{d}	2.18 ± 0.08^{c}	0.29 ± 0.05^{ab}	0.09 ± 0.01^{c}	ND	ND	0.01 ± 0.001^{abc}
G. sopsopia	4.28 ± 0.07^{a}	1.86 ± 0.05^{b}	0.26 ± 0.04^{ab}	0.09 ± 0.01^{c}	ND	ND	0.01 ± 0.001^{ab}
G. xanthocymus	6.60 ± 0.07^{c}	2.83 ± 0.02^{d}	0.19 ± 0.04^{a}	0.18 ± 0.01^{d}	ND	ND	0.01 ± 0.001^{abc}

Values are means of triplicate ± SD; Means not sharing a common letter within each row were significantly different at $p < 0.05$; ND: Not Detected.

hours. The resultants samples were digested with concentrated HNO_3 and dissolved in distilled water, filtered with Whatman no. 1 filter paper, the volume was made upto 50 mL with deionised water before analysis. Estimation of the minerals Fe, Ca, Mg, K, Na, Zn, Cr, As, Cd and Pb in the *Garcinia* L. fruits was done by using Inductively Coupled Plasma-optical Emission Spectrophotometer (ICP-OES Thermo Fisher iCAP - 7600) at the Sophisticated Analytical Instrument Facility (SAIF), North-Eastern Hill University, Shillon*G.*

2.10 Statistical Analysis

For each of the samples, all parameters were evaluated in triplicate (n = 3) and the results were expressed as mean ± SD. Statistical analysis was performed using SPSS 12 and MS Excel 2013.

3 Results and Discussion

Plant bio-resources of Assam are source of wild edible plants of value in diet, nutrition and ethnomedicine. In this research, fruits of eight species of lesser used wild edible plant under *Garcinia*genus was investigated and comparative data on content of garcinol, HCA, HCA lactone and other organic acids were generated using HPLC technique. Antioxidant potential, TPC, TFC and mineral content of the fruits were also estimated. Out of the eight *Garcinia* species, five (*G. morella*, *G. pedunculata*, *G. lancifolia*, *G. sopsopia* and *G. xanthocymus*) were found to contain garcinol (Table 1). However, concentration in *G. xanthocymus* (286.37 mg/g) and *G. sopsopia* (195.98 mg/g) was significantly higher ($p < 0.05$) than those in fruits of other species. Earlier reported content of garcinol from *G. morella* and *G. pedunculata* fruits were comparable with data obtained in this study(13; 14). However, our study has produced quantitative data on content in garcinol of other six species, thereby expanding data of garcinol content in *Garcinia* species found in Assam (Table 1). Both *G. xanthocymus* and *G. sopsopia* were excellent sources of garcinol. HCA and its lactone was also detected in all the eight species and HCA content varied in the range of 83.30 to 539.13 mg/g and HCA lactone in the range of 3.30 to 239.25 mg/*G.* HCA content was found to be highest in *G. lancifolia* (539.13 mg/g) followed by *G. cuspida*, *G. pedunculata*, *G. kydia*, *G. morella*, *G. cowa* and *G. sopsopia* (Table 1). Occurrence of HCA in species of *Garcinia*genus namely *G. lancifolia*, *G. pedunculata*, *G. morella*, *G. xanthocymus* and *G. cowa* was also reported earlier(15; 16; 8; 17) and the results of the present study is comparable to that of earlier reports. Our study has also produced data on HCA content in two additional species namely *G. cuspida*and *G. sopsopia*. Based on earlier data and our study result, *G. cuspida*was found to be richest in HCA (530.31 mg/g) content (Table 1). The content of HCA lactone was found to be highest in *G. lancifolia* (239.25 mg/g) followed by *G. pedunculata* (235.95 mg/g) and content in these two species was significantly higher than those in the remaining species ($p < 0.05$) (Table 1). *G. xanthocymus* was found to contain least amount of both HCA (83.30 mg/g) and HCA lactone (3.30 mg/g). Earlier, content of HCA lactone was reported from only one species i.e. *G. morella* (Table 1). Thus our study shows that *G. lancifolia*, *G. pedunculata* and *G. cuspida*are excellent sources of both HCA and HCA lactone showing prospect of thier use in nutraceutical and pharmaceutical industries. Estimation of few other organic acids i.e. oxalic, citric, tartaric and succinic acid was also carried out (Table 1). Different studies have reported organic acids in the genus Garcinia(15; 17). Organic acids content in the fruits were found to vary widely. Oxalic acid content was very low (2.03 to 2.85 mg/kg) and in *G. xanthocymus*, it was below detection limit. Except, *G. xanthocymus* (85.536 mg/g) and *G. morella* (28.05 mg/g), the oxalic acid content in other species fruits was very low (Table 1). *G. pedunculata* was found to contain highest amount of tartaric acid (325.26 mg/g) and was significantly higher ($p < 0.05$) than its content in *G. kydia* (153.87 mg/g), *G. cowa* (156.85 mg/g), *G. morella* (121.63 mg/g) and *G. sopsopia* (8.44 mg/g) (Table 1). Similarly, succinic acid was found only in *G. lancifolia*, *G. cowa* and *G. cuspida*but the concentration was significantly higher in *G. lancifolia* (33.06 mg/g) compared to other species ($p < 0.05$) (Table 1). Oxidative stress is responsible for several chronic non-communicable diseases, such as atherosclerosis, diabetes(18) and plants containing phenolic substances are recognized as efficient natural antioxidants due to their ability to scavenge free radicals(19). The eight *Garcinia*fruit extracts was studied for their antioxidant activity (AA) and found variation in their antioxidant potentials (Table 2). Antioxidant capacity of the fruit extract was varied in a dose dependent manner in both DPPH assay and ABTS assay model. AA in terms of IC50 values were in the range of 111.38 to 1119.14 µg/mL and AA of fruit extract of *G. xanthocymus* (145.62 µg/mL) and *G. sopsopia* (111.38 µg/mL) was significantly higher than those of other fruit extract ($p < 0.05$) (Table 2). AA of *G. kydia* fruit extract was found to be lowest. AA of different fruit extracts in ABTS model was in the range of 44.18 to 661.70 µg/mL and was comparable with that of DPPH assay model (Table 2). The correlation between the two assays was established as an equation ($y = 0.637x + 45.855$) and a linear correlation of $r^2 = 0.904$ was obtained (Table 3). Phenolic compounds possess an ideal structural chemistry for free radical scavenging activity and contribute to the overall antioxidant potential of plants mainly due to their redox properties. The total phenolic content in the fruit extracts ranged from 9.25 to 60.71 mgGAE/g, while their total flavonoid content ranged from 4.31 to 34.08 mg QE/g (Table 2). *G. sopsopia*, *G. xanthocymus* and *G. morella* contained significantly higher amount ($p < 0.05$) of TPC and TFC compared to other fruit extracts. We observed that the AA of the fruit extract was linearly proportional to their phenolic as well as flavonoid contents (Table 3). Earlier few studies reported AA and phenolic content of different plants under Garcinia(4; 5), but the present study is the first report of comparative analysis of antioxidant potential of eight *Garcinia* species fruits. The correlation between TPC and TFC with DPPH and ABTS radical scavenging activity is resented in (Table 3). The significant and linear relationship of TPC and TFC with AA suggests that the phenolic compounds in the *Garcinia* fruits are major contributors to the observed AA. The minerals elements in plant sources play a major role in different biochemical functions of the body and imbalance in mineral elements in the body may lead to several pathological

conditions(20). Plants contain minerals in varying concentrations. *Garcinia* fruits were found to contain varying amounts of essential elements Calcium (Ca), Magnesium (Mg), Phosphorus (P), Iron (Fe), and Zinc (Zn) (Table 4). Earlier reports on elemental composition in *Garcinia* fruits of other habitats are available in the literature(21; 22). Among the minerals, Ca was found to be in the highest concentration which was followed by Mg, Fe, and Zn. The Ca was found in the range of 4.28-10.63 ppm, while Mg, Fe, Zn and Cr was found in the range of (1.37-2.83) ppm, (0.19-1.46), (0.01-0.10) ppm, and (0.02-0.11) ppm, respectively (Table 4). We did not detect any heavy metals in the fruit extracts. Thus, HPLC based analysis has shown that among the eight *Garcinia* species, *G. xanthocymus* and *G. sopsopia* are rich sources of garcinol, while *G. lancifolia*, *G. pedunculata* and *G. cuspida*are rich in both HCA and HCA lactone content. Fruit extracts of *G. xanthocymus* and *G. sopsopia* showed higher AA which may be attributed to the high content of phenolic and flavonoid in these fruits. This comparative data on *Garcinia* fruits may be useful in selection of species for use in product development by the pharmaceutical, neutraceutical, and ayurvedic industries.

Conflict of Interest

The authors declare no conflict of interest in this reported communication.

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