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Comparative evaluation of antioxidant properties of extracts of fruit rinds of *Garcinia* species by *in vitro* assays

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ABSTRACT

Fruit rind extracts of eight *Garcinia* species, namely *G. cambogia, G. cowa, G. indica, G. loniceroides, G. mangostana, G. morella, G. pedunculata and G. xanthochymus* were prepared using six solvents of varying polarity, namely hexane, chloroform, ethyl acetate, methanol, water and water: methanol (hydroalcoholic; 80:20). The total phenolic content and antioxidant properties of these extracts were determined. Results of present study suggested that different extracts of fruit rinds of *G. cambogia, G. cowa, G. indica, G. loniceroides, G. mangostana, G. morella, G. pedunculata* and *G. xanthochymus* have different antioxidant properties. In general, hexane extract of *G. cowa*, *G. loniceroides* and *G. xathochymus*, ethyl acetate extract of *G. cowa* showed higher antioxidant activity in DPPH, ABTS and reducing power model of *in vitro* assay.

Key words: *Garcinia*, antioxidants, free radical, reactive oxygen species.

INTRODUCTION

Garcinia is the largest genus of the *Clusiaceae* family comprising over 250 species. In India, 43 species and 5 varieties of *Garcinia* are reported, of which 37 species and 4 varieties occur in wild naturally (Anu *et al.*, 1). Commonly, plants in this genus are called Kokum, Saptrees, Mangosteens, Garciniasor and ambiguously "Monkey fruit" (Chandran, 2). The fruit is brownish or purple about the size of an orange, marbled with yellow, and is crowned by the 4-parted stalk less stigma. The fruit pulp is juicy, white and delicious in taste and odour, consists 6-8 seeds (Watt, 13). The fruit of many species are edible and serve as a substitute for tamarinds in curries. Many species produce a yellow resin which is used in making varnishes and treating wounds. Some species have shown to exhibit significant antimicrobial and pharmacological activities (Valdir *et al.*, 11).

Dietary antioxidants from natural sources such as vegetables, fruits and beverages may help to reduce the risk of mortality from coronary heart disease and incidence of myocardial infraction (Waltenberger *et al.*, 12). Antioxidant deficiency correlates with many diseases. Antioxidant is capable of slowing or even preventing the oxidation of other molecules. The continuing growth of the market of antioxidants reflects the hope to cure the wide range of diseases that are believed to be caused or promoted by 'oxidative stress' (Halliwell and Gutteridge, 3). It has been well known that plant phenolics possess high antioxidant activity. Different solvents could sequester varying percentage of total phenolics present in the plant samples because of their solubility in a selected solvent.

In the present study, total phenolic content (TPC) and antioxidant properties of *G. cambogia, G. cowa, G. indica, G. loniceroides, G. mangostana*, *G. morella, G. pedunculata* and *G. xanthochymus* fruit rind extracts were evaluated by using DPPH free radical, ABTS radical cation scavenging ability and ferric reducing antioxidant power methods.

MATERIALS AND METHODS

Mature fresh fruits of *Garcinia* species were collected from the different geographical locations of India. *G. cambogia* from Indian Institute of Spices Research (IISR), Kerala in August-2017, *G. cowa* from Assam Agricultural University, Jorhat, Assam in March-2017, *G. indica* from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (BSKV), Dapoli, Ratnagiri, Maharashtra in June-2017, *G. loniceroides* from Pengeri, Tinsukia, Assam in August-2017, *G. mangostana* from Vengurla, Maharashtra in May-2017, *G. morella* from Bamakhepa, Barpeta, Assam in August-2017. *G. pedunculata* and *G. xanthochymus* were collected from Ulubari, Kamrup, Assam in March 2017.

Fruits were compressed to remove the juice and seeds were removed from pulp manually. Left over fruit rinds were dried in shade followed by oven drying (50°C, 10 hour). Dried fruit rinds were made

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into powder using an electric grinder. The powdered rinds of *Garcinia* fruits were further dried in oven (50°C, 2 hour) in order to remove residual moisture and extracted with following solvents: hexane, chloroform, ethyl acetate, methanol, water and water : methanol (hydroalcoholic, 80:20, v/v) individually by reflux method. The extraction was carried out for 6-7 hours on a water bath. After cooling the flasks, the extracts were filtered through Whatman filter paper no. 1 and concentrated under vacuum to get crude viscous extracts. Further, the extracts were dried in vacuum desiccators for complete removal of solvents. The solvent free extracts were used in the present study.

All extraction solvents used were of analytical grade (SRL, Mumbai). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Sigma-Aldrich, Mumbai, India), Folin-Ciocalteu reagent (Sigma-Aldrich, Mumbai, India), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma-Aldrich, Mumbai, India), gallic acid (SRL, Mumbai, India), Trolox (Sigma-Aldrich, Mumbai, India) were used. Analytical grade potassium ferricyanide, trichloroacetic acid and ferric chloride (SRL, Mumbai) were used.

TPC in the extracts were determined by a colorimetric method using Folin–Ciocalteau reagent (Singleton *et al.*, 10). Briefly, the dried extracts were dissolved in distilled water (1 mg/ml). The extract (0.5 ml), Folin–Ciocalteau reagent (0.5 ml) and distilled water (7.5 ml) were mixed in a test tube and further mixed vigorously by using a Vortex mixer. Test tubes were kept at room temperature for 10 min and thereafter sodium carbonate (20%, 1.5 ml) was added to test tube mixture. The resultant mixture was allowed to incubate in a water bath at 40°C for 20 minute. The intensity of the blue colour developed was measured by recording the absorbance at 755 nm using a UV–visible spectrophotometer (Electronic Corporation of India). The reagent blank was also prepared using distilled water. For quantification of TPC in the extracts, a standard calibration curve was prepared using gallic acid. TPC of the extract samples was expressed as gallic acid equivalent (GAE) milligram per gram of the extract.

The radical scavenging activity of extracts of *Garcinia* species was evaluated using DPPH free radical scavenging assay. Different concentrations of the extracts were taken in test tubes. The total volume was adjusted to 8.5 ml by the addition of methanol. 5.0 ml of methanolic solution of DPPH (0.1 mM) was added to these tubes and mixed thoroughly using a Vortex mixer. Thereafter, tubes were kept at room temperature for 20 min. The blank was prepared in the same as described above but without the extract and methanol was used for the baseline correction. Changes in the absorbance of the extract samples were measured at 517 nm using UV–visible spectrophotometer.

Radical scavenging activity (RSA) was expressed as the inhibition percentage and was calculated using the following formula:

Radical scavenging activity (%) = $[(A_{blank}-A_{sample})/A_{blank}] \times 100$

Where, A_{blank} = Absorbance of blank and A_{sample} = Absorbance of test sample

Free radical scavenging activity was determined by ABTS radical cation decolorization assay (Re *et al.*, 8). ABTS was dissolved in water to get 7 mM concentration and radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS solution with 2.45 mM potassium persulphate at room temperature in dark (12–16 h) before use. For assay, $ABTS^{\cdot+}$ solution was diluted with water to an absorbance value of 0.700 ± 0.02 at 734 nm. After addition of 3 ml of diluted $ABTS^{\cdot+}$ solution to 100 µl of extracts solutions, absorbance was recorded after 6 min.

The total reducing power of standard antioxidants and extracts were determined as described by Oyaizu (6). Different concentrations of extracts were mixed with distilled water (2.5 ml), phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The resulting mixture was incubated at 50 ℃ for 20 min in a water bath. After cooling, trichloroacetic acid (2.5 ml, 10 %) was added to the mixture. The upper layer of solution (2.5 ml) was taken and mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). The absorbance was recorded using a UV-visible spectrophotometer at 700 nm. The increasing absorbance value was interpreted as increased reducing activity (Wong and Kitts, 14).

The extract concentration corresponding to 50 percent inhibition (IC_{50}) was calculated from the curve prepared from RSA percentage against extract concentration. Trolox and gallic acid were used as standards. Each sample was assayed in triplicate for each concentration. All experiments were performed in triplicate and the results were expressed as mean of three replicates.

RESULTS AND DISCUSSION

TPC (mg/g GAE) of hexane, chloroform, ethyl acetate, methanol, water and hydroalcoholic extracts of selected 8 *Garcinia* species was estimated by colorimetric method using Folin–Ciocalteau reagent (Table 1). For *G. cambogia*, TPC varied in the following order: chloroform (46.03) > hydroalcoholic (45.35) > water (37.24) > hexane (36.86) > ethyl acetate (32.39) > methanol (20.65). TPC was highest

Table 1. Total phenolic content* (mg/g GAE) of various extracts of *Garcinia* fruit rinds.

Extract	GC	GCo	GI	GL	GM	GMo	GP	GX
Hexane	36.86	65.20	38.38	35.12	41.56	48.68	10.20	165.84
Chloroform	46.03	18.15	47.02	37.70	35.58	29.74	8.53	146.75
Ethyl acetate	32.39	11.41	40.35	37.47	143.30	32.62	5.05	190.54
Methanol	20.65	40.73	39.89	17.62	27.09	10.80	13.83	99.93
Hydroalcoholic	45.35	36.48	47.39	19.29	21.56	26.94	18.45	47.17
Water	37.24	32.92	44.14	12.55	25.35	22.77	14.52	44.29

*Mean of three replications
GC = *G. cambogia*, GCo = *G. cowa*, GI = *G. indica*, GL = *G. loniceroides*, GM = *G. mangostana*, GMo = *G. morella*, GP = *G. pedunculata*, GX = *G. xanthochymus*

in hexane (65.20) followed by methanol (40.73), hydroalcoholic (36.48), water (32.92), chloroform (18.15) and ethyl acetate (11.41) extract of *G. cowa*. In case of *G. indica*, it varied in the following order: hydroalcoholic (47.39) > chloroform (47.02) > water (44.14) > ethyl acetate (40.35) > methanol (39.89) > hexane (38.38). For *G. loniceroides*, TPC varied in the following order: chloroform (37.70) > ethyl acetate (37.47) > hexane (35.12) > hydroalcoholic (19.29) > methanol (17.62) > water (12.55). TPC was highest in ethyl acetate extract of *G. mangostana* (143.30) followed by hexane (41.56) > chloroform (35.58) > methanol (27.09) > water (25.35) > hydroalcoholic (21.56). For *G. morella*, TPC was in the following order: hexane (48.68) > ethyl acetate (32.62) > chloroform (29.74) > hydroalcoholic (26.94) > water (22.77) > methanol (10.80). TPC varied in the following order in extracts of *G. pedunculata*: hydroalcoholic (18.45) > water (14.52) > methanol (13.83) > hexane (10.20) > chloroform (8.53) > ethyl acetate (5.05). In case of *G. xanthochymus* extracts, the trend was in the following order: ethyl acetate (190.54) > hexane (165.84) > chloroform (146.75) > methanol (99.93) > hydroalcoholic (47.17) > water (44.29). Based on the above observations, it could be inferred that non polar (hexane) or semi polar (chloroform and ethyl acetate) solvent was more effective in extracting the phenolics from the fruit rinds of *Garcinia*.

The free radical scavenging activity of hexane, chloroform, ethyl acetate, methanol, water and hydroalcoholic extracts of *G. cambogia, G. cowa, G. indica, G. loniceroides, G. mangostana*, *G. morella, G. pedunculata* and *G. xanthochymus* were tested through DPPH method. Trolox and gallic acid were used as control. The free radical scavenging activity of extracts increased with increase in concentration of extracts. The percentage of DPPH scavenged was plotted against the concentration of extracts of *Garcinia* species and concentration (µg/ml) of extracts required to 50 % inhibition (IC_{50}) was calculated. Lower is the IC_{50}, stronger is the antioxidant activity. For hexane extracts, IC_{50} value of *G. cambogia* (5.41), *G. cowa* (4.82), *G. loniceroides* (5.23) and *G. xanthochymus* (4.28) were comparatively low. For chloroform extracts, IC_{50} value of only G*. loniceroides* (2.63) and *G. cambogia* (7.01) were low. In case of ethyl acetate extracts, only *G. cowa* (5.13) extract showed low IC_{50} value. All methanol extracts of all *Garcinia* species exhibited high IC_{50} value (Table 2).

Hexane extract of *G. cowa* (5.75), *G. morella* (4.40) and *G. xanthochymus* (4.53) exhibited low IC_{50} value in ABTS assay. Chloroform extract of all *Garcinia* species exhibited high IC_{50} value. Except *G. cowa* (5.13), ethyl acetate extract of all other *Garcinia* species exhibited high IC_{50} value. Methanol, water and hydroalocoholic extracts of all *Garcinia* species exhibited high IC_{50} value. IC_{50} values for trolox and gallic acid was 6.22 µg/ml and 3.45 µg/ml respectively. In ABTS radical cation decolorization assay, hexane extracts of *G. morella* had IC_{50} value 4.40 µg/ml. The IC_{50} value of hexane extracts of *G. xanthochymus* (4.53 µg/ml), *G. cowa* (5.75 µg/ml), *G. cambogia* (8.68 µg/ml), *G. indica* (10.27 µg/ml), *G. loniceroides* (12.66 µg/ml), *G. mangostana* (25.52 µg/ml), *G. pedunculata* (1872.21 µg/ml) showed wide variation. Chloroform extracts of *G. loniceroides* (9.17 µg/ml), *G. indica* (11.81 µg/ml), *G. cambogia* (12.80µg/ml), *G. cowa* (31.67 µg/ml), *G. mangostana* (43.84 µg/ml), *G. morella* (48.34 µg/ml), *G. xanthochymus* (20.50 µg/ml) and *G. pedunculata* (1778.24 µg/ml) also showed variation in IC_{50} value. The IC_{50} value of ethyl acetate extracts of *G. cowa* (5.98 µg/ml), *G. mangostana* (34.25 µg/ml), *G. indica* (47.34 µg/ml), *G. xanthochymus* (52.49 µg/ml), *G. loniceroides* (115.00 µg/ml), *G. morella* (127.68 µg/ml), *G. cambogia* (127.82 µg/ml) and *G. pedunculata* (2610.15 µg/ml) also showed wide variation. Polar extracts such as methanol, hydroalcoholic and water exhibited high IC_{50} values in the range of 18.80-957.52 µg/ml, 33.37-140.85 µg/ml and 46.76-177.86

Table 2. IC_{50} (µg/ml) values† of extracts of *Garcinia* species estimated using DPPH assay.

Species	Hexane	Chloroform	Ethyl acetate	Methanol	Hydroalcoholic	Water
GC	5.41	7.01	95.87	65.62	58.58	72.87
GCo	4.82	31.11	5.13	15.62	66.67	73.95
GI	7.94	11.58	42.20	18.67	35.57	65.14
GL	5.23	2.63	95.77	435.70	62.96	89.28
GM	23.61	33.67	29.97	132.59	72.30	104.31
GMo	2.84	38.02	122.79	67.05	33.51	63.80
GP	1590.84	1407.19	2092.12	720.65	178.33	190.09
GX	4.28	19.34	44.03	18.06	71.68	96.30

†Mean of three replications

µg/ml, respectively for all species (Table 3). IC_{50} values for trolox and gallic acid were 6.96 µg/ml and 4.33 µg/ml, respectively.

For measuring the reducing power of extracts, the capability of extract to convert the ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}) was measured. In this assay, yellow colour of the solution changes to blue colour as the ferric ion (red) to ferrous ion (blue). Absorbance was measured at 700 nm. Higher absorbance indicated that the extract had more antioxidant property. In the present investigation, only hexane extract of *G. cowa* (4.45 µg/ml), *G. loniceroides* (5.86 µg/ml) and *G. xanthochymus* (5.92 µg/ml) exhibited low IC_{50} value. Other extracts exhibited high IC_{50} value. Chloroform extracts of all species exhibited high IC_{50} value ranging from 20.53 µg/ml (*G. cowa*) to 46.49 µg/ml (*G. loniceroides*). In case of ethyl acetate extracts, only *G. cowa* (5.95 µg/ml) and *G. xanthochymus* (6.39 µg/ml) exhibited low IC_{50} value. Other extracts exhibited high IC_{50} value (18.98-143.78 µg/ml). Methanolic extracts of all *Garcinia* species showed high IC_{50} value (21.71-426.78 µg/ml) (Table 4). IC_{50} values for trolox and gallic acid were found to be 5.03 µg/ml and 4.62 µg/ml, respectively.

Garcinia extracts exhibited antioxidant properties and has a potential for use as a biopreservative in food applications and neutraceuticals. Minakshi *et al.* (5) reported that TPC in hydroalcoholic extract of *G. indica* fruit rind was 63.21 mg/g GAE. Joseph *et al*. (4) studied antioxidant capacity of hexane and chloroform extracts from *G. cowa* and *G. pedunculata* by the formation of phosphomolybdenum complex concentration and reducing power by potassium ferricyanide reduction method at various concentrations. Hexane and chloroform extracts from *G. cowa* showed higher antioxidant capacity than *G. pedunculata* extracts. Similarly, both the extracts from *G. cowa* showed higher reducing power than the extracts from *G. pedunculata. Selvi et al. (9) reported* antioxidant activity of *G. indica* extract using *β*-carotene-linoleate and DPPH model systems. *G. indica* extract showed 53% and 78% antioxidant activity at 50 ppm concentration in *β*-carotene-linoleate and DPPH model systems, respectively. Phillip Jacob *et al*. (7) compared the antioxidant activity of G*. gummi-gutta* extract with ascorbic acid for determining its reducing power. From the results obtained, it was found that the *G. gummi-gutta* extract had higher antioxidant property than ascorbic acid.

Table 3. IC_{50} (µg/ml) values† of extracts of *Garcinia* species estimated using ABTS assay.

Species	Hexane	Chloroform	Ethyl acetate	Methanol	Hydroalcoholic	Water
GC	8.68	12.80	127.82	83.52	47.66	79.65
GCo	5.75	31.67	5.98	18.80	50.24	75.78
GI	10.27	11.81	47.34	20.55	33.37	55.79
GL	12.66	9.17	115.00	578.97	64.72	92.95
GM	25.52	43.84	34.25	134.09	79.15	93.62
GMo	4.40	48.34	127.68	91.35	34.56	46.76
GP	1872.21	1778.24	2610.15	957.52	140.85	177.86
GX	4.53	20.50	52.49	22.06	82.31	90.91

†Mean of three replications

Table 4. IC_{50} (µg/ml) values† of extracts of *Garcinia* species estimated using reducing power assay.

Species	Hexane	Chloroform	Ethyl acetate	Methanol	Hydroalcoholic	Water
GC	36.65	34.80	143.78	426.78	46.15	77.81
GCo	4.45	20.53	5.95	23.51	32.80	35.58
GI	32.03	37.88	39.76	86.87	75.44	95.42
GL	5.86	46.49	66.16	24.19	18.37	23.01
GM	25.41	36.95	19.38	107.84	42.74	75.92
GMo	23.09	38.35	18.98	92.61	22.01	45.43
GP	29.77	37.93	50.33	48.34	45.21	77.08
GX	5.92	31.03	6.39	21.71	32.19	57.22

†Mean of three replications

In the present investigation, except for *G. pedunculata*, extracts prepared from other seven *Garcinia* species with low polarity solvent (hexane and chloroform) exhibited strong antioxidant activity. This might be due to variation in the class and content of TPC and other bioactive principles present in the fruit rinds of different *Garcinia* species. The current findings have provided a clear overview of the antioxidant capacities of fruit rind extracts of eight *Garcinia* species which could be useful to enhance the future utilisation of these fruit wastes. Also, the findings of the present investigation may be supportive for the use of the rinds of *Garcinia* as an antioxidant botanical dietary supplement.

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