J Food Sci Technol (August 2014) 51(8):1626–1631
DOI 10.1007/s13197-014-1381-z

SHORT COMMUNICATION

Antioxidant and antiplatlet aggregation properties of bark extracts *of Garcinia pedunculata* and *Garcinia cowa*

Anushi Sharma · G. S. Joseph · R. P. Singh

Revised: 3 April 2014 / Accepted: 15 April 2014 / Published online: 24 April 2014

Abstract The bark extracts of *Garcinia pedunculata* and *Garcinia cowa,* which are abundant in the Northeastern regions of India, were screened for their antioxidant and in vitro antiplatelet aggregating activities. By β-carotene linoleate model for antioxidant assay, acetone extract of *G. pedunculata* and hexane extracts of *G. cowa* exhibited higher antioxidant activity (86.47 and 66.94 % respectively, at 25 ppm) than other extracts. Similar pattern was observed for superoxide radical scavenging method for antioxidant assay. The ethyl acetate extract of *G. pedunculata* and hexane extract of *G. cowa* exhibited higher antiplatelet aggregation capacity towards ADP induced platelet aggregation (IC_{50} 0.16 and 0.43 ug, respectively) than other extracts.

Keywords *Garcinia peduculata* · *Garcinia cowa* · Antioxidant · Superoxide · Antiplatelet aggregation

Introduction

The changing pattern of substitution of synthetic food antioxidants by natural ones and the health implications of antioxidants as nutraceuticals, has led to active research on fruit and vegetable sources and the screening of raw materials for identifying potent sources of bioactive materials. These beneficial effects of plant materials are due to the combinations of secondary metabolites.

Garcinia pedunculata is an evergreen tree, found in North Eastern region of India. Its fruit is used as an antiscorbutic, astringent, cooling, cardiotonic, emollient. Cold water infusion of dry pericarp is taken as antidiarrhoeic, antidysentric, in dyspepsia and inflatulence (Kagyung et al. 2010). Fruit rinds of *Garcinia pedunculata* are rich in (−)-hydroxycitric acid (HCA), which is a proven natural antiobesity agent. Studies have been carried out to determine antioxidant activities of *G. pedunculata* rind extracts by in vitro methods (Jayaprakasha et al. 2006; Mudoi et al. 2012).

Garcinia cowa grows in humid mixed forests on hills or valleys and is found in East Indies, South and West Yunnan, East Bangladesh, India, Indochina and Malaysia. Bark is dark brown in colour. *G. cowa* rind extracts have been evaluated for their antioxidant and antimutagenic properties (Joseph et al. 2005; Negi et al. 2010).

In the present paper, in vitro antioxidant and other properties of the bark extracts of *G. pedunculata* and *G. Cowa* have been studied.

Materials and methods

Materials

All solvents/chemicals used were of analytical grade and obtained from Merck, Mumbai, India. β-Carotene, linoleic acid and butylated hydroxyanisole (BHA) were obtained from Sigma Chemical Co., (St. Louis, MO, USA). Phenazine methosulphate (PMS), NADH and ADP were procured from S.D. Fine Chem Limited. Nitroblue tetrazolium salt (NBT) was procured from Merck, Mumbai, India. Yeast extract was procured from Hi Media Laboratories. Spectrophotometric analyses were done using Spectronic 20-D spectrophotometer and Genesys–5 UV-visible spectrophotometer.

Raw material

The bark of *G. pedunculata* and *G. cowa* were procured from Jallah (Dist. Barpeta) of Assam (India).

A. Sharma · G. S. Joseph · R. P. Singh (✉)
Human Resource Development Department, CSIR-Central Food Technological Research Institute, Mysore 570 020, India
e-mail: singhrp317@gmail.com

Extraction

The extracts of bark samples were made after drying under sun and subjected to size reduction using kitchen blender to get 40-mesh size powder . The bark powder was extracted as described by Jayaprakasha et al. (2001). Fifty grams of bark powder of both *G. pedunculata* and *G. cowa* were serially extracted with 150 ml of hexane, ethyl acetate, acetone, methanol and methanol–water (90:10) in a soxhlet extractor for 6 h at 65–70 °C. The extracts were filtered through Whatman No. 41 filter paper to obtain particle free extract and were concentrated and dried under vacuum (Jena et al. 2002) and the dried extracts were used to explore their various properties.

Methods

Antioxidant assay using β–carotene linoleate model system (β CLAMS)

The antioxidant activity of bark extracts of *G. pedunculata* and *G. Cowa* was evaluated using β-carotene-linoleate model system, as described by Jayaprakasha et al. (2001). 0.2 mg of β-carotene in 0.2 ml of chloroform, 20 mg of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed. Chloroform was removed under vacuum and the mixture was diluted with 10 ml water and mixed. 40 mL of oxygenated water was added to this emulsion. Four ml aliquots of the emulsion were taken into different test tubes containing different concentration of extracts and BHA in final volume of 0.2 ml. BHA was used for comparative purposes. A control with 0.2 ml of ethanol and 4 ml of the above emulsion was also prepared. The tubes were placed in a water bath at 50 °C and the absorbance was taken at 470 nm at zero time (t=0). Measurement of absorbance was continued till the color of β-carotene disappeared in the control tubes (t=120 min) at an interval of 15 min. A mixture prepared as above without β-carotene served as blank. All determinations were carried out in triplicate. The antioxidant activity (AA) of the extracts was evaluated as bleaching of the β-carotene using the following formula,

$$AA = 100\left[1-(Ao-At)/\left(Ao^0-At^0\right)\right]$$

where Ao and Ao^0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively. At and At^0 are the absorbance measured in the test sample and control, respectively, after incubation for 120 min. The results were expressed in % basis of preventing bleaching of β-carotene.

Superoxide radical scavenging assay

The assay was performed using the method of Oktay et al. (2003). Superoxide radical was generated in the reaction mixture containing NADH, NBT and PMS. It gave blue colour on formation of superoxide radical. The rate of formation of superoxide radical was monitored in presence and absence of various extracts. The lower rate of free radical formation indicated the higher scavenging effect of the extract and vice versa. To the test tubes containing extracts in different concentrations, 0.1 ml each of 1 mM NBT and 1.5 mM NADH were added. The final volume was adjusted to 0.9 ml with 0.016 mM Tris buffer (pH 8.0) and kept at room temperature for 2 min. To this reaction mixture, 0.1 ml of 0.3 mM PMS was added and the reaction rate was monitored at 560 nm for 3 min. The reaction rate was expressed as absorbance/minute. The superoxide radical scavenging capacity was calculated by the formula -

$$\frac{\text{Rate of reaction in control}-\text{Rate of reaction in test}}{\text{Rate of reaction in control}} \times 100$$

Total polyphenol content

The method of Heinonen et al. (1998) was followed for polyphenols estimation. Polyphenols when react with Folin-Ciocalteu reagent give blue colour chromogen in alkaline media, which can be measured at 760 nm. The concentration of polyphenols in extracts was calculated by using standard curve prepared with tannic acid. Various aliquots of tannic acid solution (1 mg/ml) and extracts were taken in test tubes and volume was adjusted to 1 ml with distilled water. 9 ml of distilled water was added to all tubes and mixed. 0.5 ml of Folin reagent (1:1 diluted) was added to all tubes, allowed to stand for 10 min. This was followed by addition of 1 ml of saturated sodium carbonate solution. Absorbance was read against reagent blank at 760 nm after 30 min. The concentration of polyphenols in extracts was calculated using standard curve and expressed as % (w/w) concentration.

Platelet aggregation analysis

The effect of extracts on human platelet aggregation was studied by the method of Gerrard (1982). One gram of each extract was separately suspended in 10 ml water and heated on boiling water bath for 6 h. It was allowed to cool and filtered to remove all particulate matter. A known volume of the solution was dried in a pre-weighed petriplate at 105 °C for 6 h and the weight of the residue was determined to get the concentration of extract in solution. Blood from healthy fasting individuals was collected in 3.8 % trisodiumcitrate solution (1:9 v/v;

citrate to blood) and centrifuged at 1,100 rpm for 20 min at room temperature to separate platelet rich plasma (PRP). The remaining blood was centrifuged at 2,000 rpm for 20 min to obtain platelet poor plasma (PPP). Both PRP and PPP were kept at 37 °C. An aliquot of 0.45 ml of PRP was taken in aggregometer cuvette with the same amount of PPP as blank in other cuvette. The aggregation was initiated with 2.5 mM ADP (8 μl) in a dual channel Chronolog platelet optical aggregometer (Chrono-log, model 490-2D, Havertown, PA, USA). The temperature was maintained at 37 °C with constant stirring at 1,200 rpm. The analysis was carried out within 2 h of collection of blood.

Inhibition of aggregation by different extracts was evaluated by incubating the PRP with different concentrations of the extracts for 1 min before adding ADP. The IC_{50} value (concentration of extract at which 50 % of platelet aggregation was inhibited) was calculated from the chart obtained from the aggregometer and was expressed in mg.

Statistical analysis

Results are expressed as Mean ± SD of three experiments. Data analyzed by non-parametric one-way ANOVA followed by post hoc Tukey's test ($P<0.05$).

Results and discussion

Antioxidant assay of bark extracts by β-carotene Linoleic acid model system

In this method, β-carotene undergoes rapid discoloration in the control via a free radical mediated mechanism, resulting from the hydroperoxide formed from linoleic acid. The linoleic acid free radicals formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attack the highly unsaturated β-carotene molecules which loose their double bonds by oxidation and their chromophore and characteristic orange colour, which can be monitored spectrophotometrically. Based on the composition of their constituent molecules, different extracts can slow down the extent of discolouration by neutralising the linoleate free radicals and other free radicals formed in the system.

The antioxidant activity of *G. pedunculata* and *G. cowa* bark extracts as measured by bleaching of β-carotene, is presented in Table 1. The bark extracts prepared by different solvents exhibited varying degree of antioxidant activity at different concentrations. No measurable β-CLAMS activity was found for hexane extract of *G. pedunculata* and 90 % MeOH extract of *G. cowa.*

The acetone extract of *G. pedunculata,* showed higher antioxidant activity, exhibiting 86.23 % at 25 ppm concentration, than other extracts. In case of *G. cowa,* the hexane and methanol extract exhibited higher activity than others. At 50 ppm concentration, hexane extract showed 82.60 % activity, while methanol extract showed 83.74 % activity. The other extracts showed lower antioxidant activity than hexane and methanol extracts. EtOAc and MeOH extracts showed of *G. cowa* showed significantly higher activity than *G. pedunculata* extracts, while acetone extracts of both exhibited reverse pattern.

Antioxidant activity of bark extracts by superoxide radical scavenging method

The free radical scavenging potential of *G. pedunculata* and *G. cowa* bark extracts was evaluated by studying their effect on generation of superoxide radicals in the reaction between NADH, NBT and PMS. The generation of superoxide radicals leads to development of blue colour, which can be measured spectrophotometrically as absorbance/minute. In presence of various extracts, the radical production is retarded and the rate of reduction in the development of colour gives the extent of radical scavenging by different extracts. The inhibition of the rate of radical production was expressed as % radical scavenging capacity and is shown in Table 2.

In case of *G. pedunculata,* the acetone extract showed higher radical scavenging activity as compared to other extracts. At 100 ppm concentration, acetone extract showed 90.03 % activity while ethyl acetate extract and methanol extract exhibited only 45.56 % and 34.63 % activity, respectively. The methanol:water (9:1) extract at 100 ppm showed only 9.5 % activity.

In case of *G. cowa,* the hexane and acetone extract exhibited higher activity compared to other extracts. At 50 ppm concentration, hexane extract showed 32.32 % activity and acetone extract showed 29.90 % activity, while methanol and ethyl acetate extracts showed only 15.68 % and 10.29 % activity respectively. Comparatively, EtOAc and MeOH extracts of *G. pedunculata* exhibit significantly higher superoxide radical scavenging activities than *G. cowa* extracts.

Polyphenolic content of bark extracts

The polyphenolic content (% w/w with respect to extract) of different extracts was estimated by Folin-ciocalteau method and is shown in Table 3. Among the extracts of *G. pedunculata,* the acetone extract has highest polyphenol content than other extracts. This is also in accordance with the results obtained from β clams and superoxide radical scavenging method where acetone extract showed highest antioxidant capacity by both the methods. However, in case of G. *cowa,* the polyphenol contents of various extract does not differ widely and unlike *G. pedunculata* extracts, the polyphenol content of *G. cowa* extracts are not in accordance with the antioxidant capacity, as determined by β clams and superoxide radical scavenging method. Here the ethyl acetate and the acetone extract show higher polyphenol content than other

Table 1 Antioxidant activity of bark extract of by β-CLAMS

Extract	*G. pedunculata*		*G. cowa*	
	Conc. (ppm)	% antioxidant activity	Conc. (ppm)	% antioxidant activity
Hexane	–	–	25	66.94±1.02
			50	82.60±1.14
			100	88.20±0.86
Ethyl acetate	25	44.47±1.88	25	61.43±2.6*
	50	52.89±1.9	50	68.54±2.6*
	100	64.55±3.68	100	79.70±2.0*
Acetone	25	86.23±0.64	25	61.61±2.77*
	50	87.75±3.12	50	76.57±2.56*
	100	88.53±0.95	100	81.58±2.03*
Methanol	25	54.82±0.95	25	74.50±1.01*
	50	70.80±1.79	50	83.74±0.40*
	100	86.71±0.74	100	89.02±0.93
90 % methanol	25	50.92±2.82	–	–
	50	74.43±2.74		
	100	81.67±0.49		
BHA	50	94.35		

The values are mean ± SD of three experiments. * indicates significance at ($P<0.05$)

extracts while hexane extract exhibits highest antioxidant capacity. The polyphenol content of EtOAc and MeOH extracts of *G. peduculata* were significantly higher than *G. cowa* extracts, in contrast to acetone extract. The differential distribution of phenolics in various extracts may reflect the type of compounds present, which may be dependent on the characteristics (eg. polarity) of the solvent/solvent system used for the extraction.

Antiplatelet aggregation capacity of G. pedunculata and G. cowa extracts

A key event which leads to myocardial infarction is accumulation of platelets at the sites of atherosclerotic plaque rupture and intake of natural antioxidants may provide some protection against progression of atherogenic process (Saputri and Janatan. 2012). The evaluation of antiplatelet aggregation

Table 2 Superoxide radical scavenging activity of *G. pedunculata* and *G. cowa* bark extracts

Extract	*G. Pedunculata*		*G. cowa*	
	Conc. (ppm)	% antioxidant activity	Conc. (ppm)	% antioxidant activity
Hexane	–	–	25	13.6
			50	32.32
			75	95.95
Ethyl acetate	50	28.85	50	10.29*
	100	45.56	100	40.19*
	500	81.29	200	70.58
Acetone	5	37.05	50	29.90
	25	62.15	100	63.30
	100	90.03	200	80.88
Methanol	50	22.9	50	15.68*
	100	34.63	100	37.25*
	500	99.30	200	65.68
90 % methanol	100	9.5	–	–
	500	12.62		

The values are mean ± SD of three experiments. * indicates significance at ($P<0.05$)

Table 3 Total polyphenol content of different extracts

	Extract	% (w/w) polyphenol
G. pedunculata	Ethyl acetate	2.03±0.01
	Acetone	60.12±2.96
	Methanol	9.36±0.57
	90 % methanol	3.96±0.75
G. cowa	Hexane	22.68±0.32
	Ethyl acetate	41.31±0.19*
	Acetone	38.57±1.25*
	Methanol	30.00±0.73*

The values are mean ± SD of three experiments. * indicates significance at ($P<0.05$)

capacity of *G. pedunculata* and *G. cowa* extracts was carried by studying the inhibition of ADP induced platelet aggregation. The IC_{50} values for different extracts are shown in Table 4. These results indicate the varying degree of antiplatelet aggregation capacity of different extracts. The ethyl acetate extract of *G. pedunculata* exhibited highest antiplatelet aggregation capacity than other extracts (IC_{50} 0.16 mg) whereas in case of *G. cowa,* hexane extract exhibited higher inhibition of platelet aggregation (IC_{50} 0.43 mg). In general, the G. pedunculata extracts exhibited significantly higher inhibition of ADP induced platelet aggregation (with lower IC_{50} values than *G. cowa* extracts).

ADP is known to induce platelet aggregation by inhibiting the adenylate cyclase activity and lowering of intraplatelet cAMP levels (Hathaway et al. 1981) and/or by increasing the cytosolic levels of calcium either due to release from the internal stores or through calcium influx (Shah et al. 1999). The increased level of calcium results in the activation of enzymes, which will not be fully functional at low levels of calcium concentrations present in resting platelets (Heemskerk and Sage 1994). Various reports indicate that activation of platelets brings about changes in the cytoskeletal structure resulting in the loss of normal discoidal shape and appearance of pseudopodal projections (Castgna et al. 1982; Mukherjee et al. 1990). The extracts of *G. pedunculata* and *G. cowa* may inhibit the platelet aggregation by inhibiting adenylate cyclase and lowering of cAMP levels or calcium mobilization, which may cause the inactivation of enzymes needed for platelet aggregation. Similarly these extracts may also interact with the membranes which may cause modifications of membrane bilayer resulting in the thickening of membrane, which may in turn affect the calcium mobilization. However, the exact mechanism of the inhibition of platelet aggregation by these extracts is yet to be established.

Table 4 IC_{50} value for inhibition of platelet aggregation by different extracts *of G. pedunculata* and *G. cowa*

	Extract	IC_{50} (mg)
G.pedunculata	Ethyl acetate	0.16
	Acetone	0.39
	Methanol	0.57
	90 % methanol	1.26
G. Cowa	Hexane	0.43
	Ethyl acetate	1.66*
	Acetone	0.72*
	Methanol	0.92*

The values are mean ± SD of three experiments. * indicates significance at ($P<0.05$)

Conclusion

In the present scenario, the safety aspects and toxicological concerns about the use of products with synthetic ingredients have become a great concern and there is a general tendency of natural or nature like products being readily accepted by the consumer. Consequently, there is a paradigm shift with an aim to search active principles including bioactive compounds from natural sources. In the present study an attempt was made to explore the biological properties of the bark extracts of *G. pedunculata* and *G. cowa*. It may be concluded that the extracts of *G. pedunculata* and *G. cowa* are primary antioxidants, which react with the free radicals and neutralize them, thus inhibiting the free radical mediated reactions. The difference in the pattern of antioxidant capacity of various extracts of the same tree bark may be ascribed to the difference in the presence of different phenolic constituents and their total phenolic content. These extracts also inhibit ADP induced platelet aggregation to a varying dgree. The data obtained indicate that the proper selection of solvent for the extraction of biologically active compounds is very important to obtain the fraction enriched with the biological activity so that it can be utilised more efficiently at lower concentrations. Isolation, characterisation and identification of individual bioactive compounds responsible for bioactivities and possible synergy amongst them, also the possible mechanism of action of these extracts on oxidation and platelet aggregation need to be studied and established.

Acknowledgments The authors thank Director, CFTRI and Head, Human Resource Development, CFTRI, for their keen interest and constant encouragement during the course of the work.

References

Castgna DR, Takai Y, Kaibuchi K, Saw K, Kikkawe U, Nishizuka Y (1982) Direct activation of calcium activated, phospholipid dependent protein kinase by Tum8- promoting phorbol esters. J Biol Chem 257:7847–7851

Gerrard JM (1982) In: Colowick SP, Kaplan NO (eds) Methods in enzymology. Vol 86: edited by Wiliiam EMl, William LS. Chapter 75, 642–651

Hathaway DR, Eaton CR, Aderstein RS (1981) Regulation of human platelet light myosin chain kinase by the catalytic subunits of cyclic AMP dependent protein kinase. Nature 291:251–254

Heemskerk JWM, Sage O (1994) Calcium signalling in platelets and other cells. Platelets 5:295–316

Heinonen IM, Meyer AS, Frankel EN (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J Agric Food Chem 46:4107–4112

Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (*vitis vinifera*) extracts on peroxidation models *in vitro*. J Food Chem 73:285–290

Jayaprakasha GK, Negi PS, Jena BS (2006) Antioxidative and antimutagenic activities of the extracts from the rinds of *Garcinia pedunculata*, Inn. Food Sci Emerg Technol 7(3): 246–250

Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK (2002) A process for the preparation of antioxidants from *Dillenia indica*. Indian Patent 1091/DEL/02.

Joseph GS, Jayaprakasha GK, Selvi AT, Jena BS, Sakariah KK (2005) Antiaflatoxigenic and antioxidant activities of *Garcinia* extracts. Int J Food Microbiol 101:153–160

Kagyung R, Gajurel PR, Rethy P, Singh B (2010) Ethnomedicinal plants used for gastrointestinal diseases by *Adi* tribes of Dehang-Debang Biosphere Reserve in Arunachal Pradesh. Indian J Tradit Knowl 9(3):496–501

Mudoi T, Deka DC, Devi R (2012) In vitro antioxidant activity of *Garcinia pedunculata*, an indigenous fruit of North Eastern (NE) region of India. Int J Pharm Tech Res 4(1):334–342

Mukherjee G, Chatterjee GC, Banerjee D, Bhattacharya DK (1990) Differential effect of retinal acid on ADP and collagen induced platelet aggregation. Ind J Exp Biol 28:949–952

Negi PS, Jayaprakasha GK, Jena BS (2010) Evaluation of antioxidant and antimutagenic activities of the extracts from the fruit rinds of *Garcinia cowa*. Int J Food Prop 13(6):1256–1265

Oktay M, Gulcin I, Irfan Kufrevioglu O (2003) Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vuIgare*) seed extracts. Lhensem Wiss U Technol 36:263–271

Saputri FC, Janatan I (2012) Inhibitory activities of compounds from the twigs of Garcinia homobroniana Pierre on human low density lipoprotein (LDL) oxidation and platelet aggregation. Phytother Res 26:1845–1850

Shah HB, Nawaz Z, Pertani AS, Rommi A, Mahmood H, Saeed AS, Gilani HA (1999) Inhibitory effect of curcumin, a food spice from turmeric, on platelet activation factor and arachidonic acid mediated platelet aggregation through inhibition of thromboxane formation and calcium signalling. Biochem Pharmacol 58:1167–1172