

Health and Wellness Product from Mangosteen (*Garcinia mangostana* L.) Rind: Bioactive Potentials

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Abstract: Mangosteen rind (MSR) (*Garcinia mangostana* L.) is a predominant component of the fruit contributing to 62% of the whole fruit. However, utilization of the same for the preparation of health products was not explored due to its sensorially less acceptable parameters. Differential extraction in different polarity solvents of MSR was done and evaluated their acceptability for product preparation. Current study thus is a detailed investigation on bioactivity profiling of MSR fraction and utilization of the same for health product preparation. Among various extracts, 70% ethanol (70%AE) yielded the maximum (15g/100g). Xanthone:Phenolic ratio was 1: 2.8, in 70%AE as opposed to hot water extract – HWE and 50% AE, which contained Xanthone:Phenolic ratio of 1:1.4/5. Higher the phenolic content obviously reduces the bitterness of Xanthenes. 70% AE contained phenolics 60.08± 0.213 mg/g and xanthenes 22.56± 0.317 mg/g. HPLC analysis revealed a spectrum of phenolic acids such as gallic, chlorogenic, caffeic, epicatechin, catechin and ferulic acids at various levels. Potent Free Radical Scavenging (FRS) activity, cytoprotectivity, DNA protectivity, H⁺K⁺ATPase inhibitory (PPAI) activities were observed in 70% AE. Gallic/tannic acid appear to contribute to antioxidant activity; while ferulic acid was responsible for PPAI activity in 70%AE. Among xanthenes, although α-mangostin was the dominating component, gartanin, 8 deoxygartanin and 3-isomangostin contributed to FRS activity. The products were prepared from 70%AE which are sensorially acceptable. Data thus for the first time delineate the specific health beneficial role of both phenolic and xanthone constituents in MSR particularly with higher abundance of phenolics than xanthenes.

Keywords: Phenolic, Xanthone, *Garcinia mangostana* L., Free Radical Scavenging, Cyto/DNA.

INTRODUCTION

Garcinia mangostana L., commonly known as mangosteen, is a tropical fruit belonging to the Clusiaceae or Guttiferae family [1]. It is widely cultivated in Thailand, India, Malaysia, Srilanka, Myanmar, Phillipines, China and Indonesia. Worldwide production of *G. mangostana* is about 1, 50,000 tons per annum [2,3]. Mangosteen species are of economic and commercial interest, with tremendous demand for the fruit in domestic and export markets. Thailand is responsible for approximately 85% of the total production of 1,50,000 tons per year. Malaysia and Indonesia are also major commercial producing countries. In the United States alone, mangosteen juice sales for 2007 were estimated at \$98 million [2].

G. mangostana is a climacteric fruit, named as “the queen of fruit” and mostly eaten fresh. Depending on the growth cycle, a single tree may produce 500 to 800 fruits in one year. The fruit is round, 2.5 to 7.5 cm in diameter, and weighs about 75 to 150 g. The rind is smooth and 0.6 to 1 cm thick. The exterior is pale green when immature and dark purple when fully ripened.

The inner pulp contains 4 to 8 juicy white segments that are sweet and faintly aromatic. The fruits may or may not contain seeds [4,5]. The edible aril is white, soft and juicy with a sweet, slightly acid taste and pleasant aroma [6]. The pericarp / rind of the fruit is about two-thirds of the whole fruit by weight, bright purple in color, usually not eaten, and represents an agricultural waste. In fact, the non-edible pericarps have been used for treating diarrhoea, wounds and skin infection in traditional medicine [7]. The rind is rich in anthocyanins [8,9]. The anthocyanins in the rind are primarily cyanidin -3-sophoroside with smaller amounts of cyanidin [10]. The polyphenols in the pericarp of *G. mangostana* could have evolved as a defensive mechanism against herbivores and believed to contribute potentially to health beneficial effects.

Peel / rind / pericarp are often the waste part of various fruits. These wastes have not received much attention with a view to being used or recycled rather than discharged [11]. This might be due to lack of their commercial application [12]. Interestingly, the peel and seed fractions of some fruits have higher anti-oxidant activity than the pulp fractions [13]. Illustrated examples like pomegranate peel [14], apple fruit peel [15], and grape seed [16] have higher antioxidant activities than pulp.

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The rind of *G. mangostana* is rich in bio-active compounds with potential applications as therapeutic agents or as functional food additives [8]. In the United States, *G. mangostana* products are now widely available and are highly popular because of their perceived role in enhancing human health [17]. *G. mangostana* (MS) fruit juice has become a major dietary supplement, and was ranked as one of the top selling "botanicals" on the market in 2005 [18]. Xanthone derivatives as the major secondary metabolites of *G. mangostana* fruits [19-21], exhibit antibacterial [22,23], antifungal [24], anti-inflammatory [25], antioxidant [26,6], antiplasmodial [20] and cytotoxic activities [27, 28]. The most abundant xanthone from *G. mangostana*, α -mangostin was found to inhibit alveolar duct formation in mouse mammary organ culture model and to suppress the carcinogen induced formation of aberrant crypt foci in a short-term colon carcinogenesis model [26,29]. As a result of these studies, the possible cancer chemo-preventive activity of *G. mangostana* has been strongly suggested.

It has to be emphasized here that *G. mangostana* rind (MSR) being the predominant component of the fruit contributing to ~ 62% as opposed to 19,13 and 6% of pulp, seeds and stalk respectively, utilization of the same for the preparation of health products was not explored due to its sensorially less acceptable parameters. An attempt has been made from our laboratory for fractionation of MSR and evaluated their acceptability for product preparation [30]. In the current study, therefore, a detailed investigation has been undertaken to evaluate the bioactivity profiling in addition to the identification of phenolic and xanthone constituents. Outcome of the study reveals the spectrum of bioactivity in 70% AE of MSR and utilization of the same for the product preparation.

MATERIALS AND METHODS

Chemicals

Phenolic acid standards such as gallic acid, tannic acid, caffeic acid, coumaric acid, ferulic acid, gentisic acid, protocatechuic acid, syringic acid, vanillic acid and cinnamic acid, and synthetic antioxidant namely butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., (St. Louis, MO, USA), Other chemicals and reagents such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-Thiobarbituric acid (TBA), Folin ciocalteu reagent, Ferrous sulphate, Ascorbic

acid, Agarose and HPLC grade solvents used in the experiments were purchased from M/s Sisco Research Laboratory Pvt Ltd. (Mumbai, India), Sperm DNA from fish and Brain Heart Infusion Broth were purchased from M/s HiMedia (Mumbai, India). The HPLC column (Shimpak C₁₈) was obtained from Shimadzu Corp., Tokyo, Japan.

Samples

Mature, ripe *G. mangostana* fruits were procured from the orchards of Ooty (Nilgiris, Tamil Nadu, India). The fruits were harvested along with peduncle. Fruits were cleaned; cut into two halves; pulp was removed and kept for further use in preparing beverages. The rind left after removal of edible pulp was sliced and dried in cabinet hot air drier (Jaydeep Engineering works, Ambalacantt, Harayana, India) (~55°C). The dried rind slices were subjected to Apex mill (M/s Cadmach Machinery Co., Ltd. Germany) to obtain a powder (~ 1mm particle size). The dried powder (with residual moisture of ~ 5%) was stored in polypropylene bottles in the refrigerator (5°C) till use.

Extraction

The dried powder (100 g) was soaked and extracted in 1000 mL of hot water (~60°C), 50% (v/v) and 70% ethanol (v/v) separately. Extracts were flash evaporated at 40°C and a reduced volume was dried in hot air oven (~50°C), weighed and stored in a refrigerator (5°C) until use. They were designated as Hot Water (HWE), 50% Alcohol (50%AE) and 70% Alcohol Extracts (70%AE) respectively. The yield of extraction was expressed as % on dry weight basis. 50 mg of *G. mangostana* rind powders (MSR - HW, 50%AE, 70%AE) were resuspended in 1 mL of respective solvents. All the extracts were stored at 4°C till the completion of the experiment.

Estimation of Total Phenolics

The total phenolic content of HWE, 50%AE and 70%AE was determined spectrophotometrically using the Folin-phenol reagent [31]. Sample aliquot of 100 μ L was added to 900 μ L of water, 1 mL of Folin-Ciocalteu reagent, and 2 mL of 10% sodium carbonate solution, mixed in a cyclomixer, and incubated for 1 h at room temperature. The absorbance was measured at 765 nm with a Shimadzu UV-visible spectrophotometer. The standard curve was drawn using 10-100 μ g of gallic acid. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of sample [32].

Determination H⁺, K⁺-ATPase Inhibition (PPAI)

Parietal cells were collected from Fresh sheep stomach, homogenized in 0.2 M Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 6000g for 10 min. The supernatant (enzyme extract) was used for the assay [33]. The enzyme extract (5900 µg/mL) was incubated with different fractions of *G. mangostana* rind extracts, in a reaction mixture containing 0.2 M Tris buffer (pH 6.5), and the reaction was initiated by adding substrate 2 mM ATP, in addition to 2 mM MgCl₂ and 2 mM KCl. After 30 min of incubation at 37 °C, the reaction was stopped by the addition of the assay mixture containing 3.6% ammonium molybdate and 20% perchloric acid. Inorganic phosphate formed was measured spectrophotometrically at 400 nm. Enzyme activity was calculated as micromoles of Pi released per hour at various doses (10-30µL) of MSRE water and methanol extracts. Results were compared with the known antiulcer proton potassium ATPase inhibitor drug - Lansoprazole.

Measurement of Antioxidant Activity

Antioxidant activity of *G. mangostana* rind extracts was determined as free radical scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical [32].

G. mangostana rind extracts both water and methanol extracts at various concentrations (5 – 30 µg GAE/mL) were added to 1 mL of 0.004% methanol solution of DPPH. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517nm. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{Free Radical Scavenging effect (\%)} = \left\{ \frac{\text{Absorbance of control at 517 nm} - \text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}} \right\} \times 100$$

Measurement of Cytoprotective Ability

Effect on Red Blood Cells (RBC)

To 1 mL of 10% suspension of erythrocytes in phosphate buffer saline (20 mM PBS, pH 7.4) was added, 150 µL of 50 mM ascorbic acid and 15 µL of 80 mM FeSO₄ in presence and absence of 10 – 30 µg GAE of MSRHW. After incubating the sample at 37 °C for 30 min, the tubes were centrifuged at 3000 g for

10 min and to 800 µL of the supernatant, PBS was added to the final volume of 2 mL and absorbance was read at A₄₁₀ nm. Oxidative damage resulted in oxidation of Red Blood Cells (RBC) leading to RBC lysis and release of Haemoglobin content from them. Taking water as a lysing agent, ensured >90-100% lysis of RBC in the oxidized controls. Percent inhibition of RBC lysis was considered as protective effect. Concentration required to inhibit 50 % of RBC lysis is considered in the experiment and expressed as IC₅₀ at µg GAE/mL [34].

Effect on Buccal Cells (BC)

Buccal cells (1×10⁴ cells/well) were exposed to UV radiation and a carcinogen- N-nitroso-N-methyl urea (10 µg/mL for 1 X 10⁴ cells /well), in presence and absence of 10 µg GAE of samples for 1h at 37° C. Twenty five microliters of cell suspension of both treated and untreated cells were mixed with 1 µL of dye mix containing 100 µg/mL each acridine orange and ethidium bromide and observed under the microscope at 40X. Viable cells stained green with acridine orange whereas damaged cells stained more orange due to ethidium bromide staining of nuclear components. Staining pattern between untreated and UV treated cells were compared and cytoprotective ability of MSREs was determined.

Measurement of DNA Protective Ability

The DNA protective effect of extracts was determined electrophoretically (Submarine Electrophoresis System, Bangalore Genei, Bangalore, India) using calf thymus DNA (1mg/mL) [35]. The DNA was subjected to oxidation by Fenton's reagent (100 µM ascorbic acid and 10µM FeSO₄) followed by gel electrophoresis. Relative difference in the migration between the native and the oxidized DNA was ensured on 1% agarose gel electrophoresis after staining with ethidium bromide. Gels were documented and the intensity of the bands was determined. Protection to DNA was calculated based on the DNA band intensity comparing with that of the native in the presence and absence of extracts.

HPLC of Phenolics

Phenolic acids in the three extracts were analyzed according to the method of Wulf & Nagel [36], on a reversed phase Shimpak C₁₈ column (4.6 x 150 mm), using a diode array detector (operating at 280 nm). The Shimpak C₁₈ HPLC column was obtained from Shimadzu Corp. A solvent system consisting of water / acetic acid / methanol (80:5:15) (v/v/v) was used as

mobile phase at a flow rate of 1 mL/min. Phenolic acids such as gallic acid, tannic acid, caffeic acid, coumaric acid, ferulic acid, gentisic acid, protocatechuic acid, syringic acid, vanillic acid and cinnamic acid were used for the identification of phenolic acids present in the three extracts. Quantitation of phenolic acids was achieved by the absorbance recorded in the chromatograms related to external standards at 280 nm.

HPLC Analysis of Xanthenes

Dried, ground *G. mangostana* rind was extracted in acetone-water mixture (80:20 v/v) for 30 min at room temperature. The sample was filtered through a 0.45 µm filter and 10 µL of the sample was injected to HPLC (Shimadzu LC10 AVP chromatography system, Tokyo, Japan) that was conducted using C₋₁₈ column. The mobile phase consisted of A: 0.1% formic acid in water and B: methanol; optimal separations were achieved using a gradient of 65 to 90% B over 0-30 min at the flow rate of 1mL/min. UV spectra were collected across the range of 200-400 nm, extracting 254 nm for chromatograms [37].

Preparation of the Health Products from the *G. mangostana* Rind Extract

Health products like beverage and jelly were prepared using 70% Alcohol Extract (70%AE) fraction of *G. mangostana* rind. 250 mg of 70% AE in 100 mL water, yielded pleasant colored beverage after adjusting the acidity with citric acid to 0.3% and addition of sucrose to reach 15°B. After filling the glass bottles 180-200mL, followed by crown corking, they were pasteurized at 85°C for 20 min, cooled, wiped and stored at room temperature. Jelly was prepared by heating the juice obtained by dissolving the 70% AE of *G. mangostana* rind at 250mg / 100 mL, after adjusting the acidity to obtain pH of ~ 3.3, along with 55 parts of sugar to every 45 parts of juice followed by addition of 1.4 % pectin and boiling to reach 65°B. The mass is allowed to set after pouring hot, into glass bottles (500mL). Sodium benzoate (236 ppm) was used as

preservative. Sensory and microbial analyses were performed to understand the acceptability and microbial load respectively [38, 39]

Statistical Analysis

All the experiments were carried out in triplicates and the results are expressed as mean ± standard deviation (SD). Correlation between the activity and phenolic and xanthone composition was calculated as coefficient of determination -R² using linear regression model to understand the strong, moderate or weak linear trend employing the statistical programme SPSS for Windows; Version 10.0. P value was calculated by the Mann-whitney test. P value <0.05 is considered as significant.

RESULTS AND DISCUSSION

G. mangostana fruits processed with 100 Kg yielded a rind of about 62% in addition to pulp (19%), seeds (13%) and the stalk (6%). Dried rind powder (100 g) after extraction yielded 4.5 g, 6.0 g and 15.0 g of residues. Different levels of phenolics and xanthenes were present in these fractions. The total phenolic content in three extracts is depicted in Table 1. The phenolic content was almost 2 folds higher in 50% (72.76±0.345 mg GAE/g) and 70% (65.08±0.213 mg GAE/g) alcoholic extracts (AE) compared to that of HWE (32±0.261 mg GAE/g). Xanthone was also in significant quantities in all the three extracts. Due to high abundance of total yield 70% AE predominated with xanthenes similar to that of phenolics (Table 1). The ratio between Xanthenes: Phenolics were found to 1:1.5, 1:1.4 and 1:2.8 in HWE, 50%AE and 70% AE respectively (Table 1).

Bioactivity in MSRE

H⁺K⁺-ATPase Inhibition and Free Radical Scavenging Activity

H⁺,K⁺-ATPase is an enzyme responsible for the release of H⁺ into the lumen of the stomach leading to

Table 1: Yield, Total Phenolic/Xanthone Content and their Ratio in *G. mangostana* Rind Extract

Sample	Yield of extract (%)	Total phenolic Content (mgGAE/g)	Total xanthenes content (mg/g)	Xanthone: Phenolic
HWE	4.5± 0.43	32.00± 0.261	20.8 ± 0.86	1:1.5
50% AE	6.0± 0.55	72.76±0.345	52.23± 0.301	1:1.4
70% AE	15.0± 1.15	65.08±0.213	22.56± 0.370	1:2.8

Total weight was determined after extracting with Hot water (HW), 50% alcohol (50%A) and 70% alcohol (70%A), followed by powdering. Total phenolic and xanthone contents were determined as described under materials and methods. Values are expressed as mean of three replicates ± standard deviation.

Table 2: IC₅₀ of Various Bioactivities of *G. mangostana* Rind Extract

Samples *	IC ₅₀ of different activities in µg/mL				
	Total phenolic content (mg GAE/g)	FRS activity	RBC protection	DNA protection	PPAI
HWE	32.00±0.26	82.48±0.28 (1)	84.6±0.32(1.1)	8.36±0.16(1.4)	7.6±0.15 (16)
50% AE	72.76±0.34	88.35±0.15 (1.2)	101.5±0.26(1.2)	15.87±0.54(1)	164.58±0.32(1)
70 % AE	65.08±0.21	122.02±0.35(2.25)	316±0.18 (1)	11±0.32(3.6)	19.96±0.36 (20)
*Gallic acid *Ascorbic acid *BHA* Lansoprazole		1.2±0.27	13.9±0.67	0.625±0.25	19.31±2.3

*indicates standards.

Results in the parenthesis indicates relative fold difference in the activity.

Hot water (HWE), 50% alcohol (50%AE) and 70% alcohol (70%AE) extracts were examined for free radical scavenging (FRS), Red blood cell protectivity, DNA protectivity and Inhibition of H⁺K⁺-ATPase activity (PPAI). Results are expressed as concentration required in µg/mL to inhibit 50% of activity (IC₅₀). Respective standards were also included in the experiment. Values provided are mean of three replicates ± standard deviation. Considering the IC₅₀ Values provided in Table 2 for various bioactivity and the total abundance of phenolics in Table 1, total activity was calculated. Minimum activity is taken as 1 fold and relative fold differences were expressed for each activity in different fraction.

hyperacidity and gastric ulcerations. Inhibitors of H⁺,K⁺-ATPase enzyme therefore are gastroprotective [33]. Significant inhibition of H⁺, K⁺-ATPase was observed with IC₅₀ of 7.60±0.15, 164.58±0.32, 19.96±0.36 µg/mL in HWE, 50%AE and 70%AE fractions respectively, when compared to IC₅₀ of 19.31 ±2.3 µg/mL of lansoprazole (Table 2), a known proton pump inhibitor. The extracts showed free radical scavenging activity with IC₅₀ of 82.48±0.28, 88.35±0.15 and 122.02±0.35 in HWE, 50%AE and 70% AE respectively (Table 2).

Cyto/DNA Protectivity

Red Blood Cells (RBC's) were used as a model to study the cytoprotective ability of MSRE. Free radicals generated by Fenton's reagent by its thermal decomposition attacked the erythrocytes to induce a chain oxidation of lipid and protein, disturbing the membrane organization and eventually leading to hemolysis [34]. 50 mM and 80 mM concentration of ascorbic acid and ferrous sulphate could induce 100% hemolysis in human erythrocytes. A protective effect was evident as we could see the inhibition of hemolysis as measured at 535 nm. Results indicated that HWE had more potent cytoprotective ability than other samples with IC₅₀ of 84.6±0.32 µg/mL. 50%AE and 70%AE showed an IC₅₀ of 101.5±0.26 and 316±0.18 µg/mL respectively against IC₅₀ of the standard RBC protectant - Ascorbic acid which showed effective inhibition at 13.9±0.67 µg/mL (Table 2).

The efficiency of phenolic acid extracts of mangosteen rind extracts in preventing oxidative damage of DNA was also evaluated. The hydroxyl radical generated by Fenton's reagent caused DNA

fragmentation. This fragmentation was recovered by the treatment of different phenolic acid extracts in addition to BHA. The intensity of bands (Figure 1A) was measured by densitometry and percentage of protection of DNA was calculated. IC₅₀ of the extracts is as follows - 8.36±0.16, 15.87±0.54 and 11±0.32 µg/mL was observed in HWE, 50%AE and 70%AE fractions respectively (Table 2).

Due to limitations in the sample, only 70% AE was examined for its ability to protect mammalian buccal cells against UV and MNU induced cellular damage. Control cells were symmetrical, flattened with cytoplasm and the nucleus. UV and MNU treatment damaged the cell as evidenced by the alterations in their structural integrity. However, cells treated prior with 70%AE showed complete protection, even better than those that were pretreated with a potent antioxidant – gallic acid (Figure 1B).

Determination of Constituents in MSRE

Phenolic Composition by HPLC

Phenolic acids present in these three extracts have been analyzed by HPLC to quantitatively determine their composition and the abundance based on the respective standard. Figure 2a provides the HPLC profile of standard phenolic acids and phenolic acids present in MSRE fractions. Data compiled in Table 3, expresses the phenolic acid content in mg/g on dry weight basis of these fractions. HWE contained gallic, chlorogenic and caffeic acid; while, increased levels of gallic and chlorogenic acids were observed in 50 %and 70% alcohol fractions. In 70% alcohol fraction,

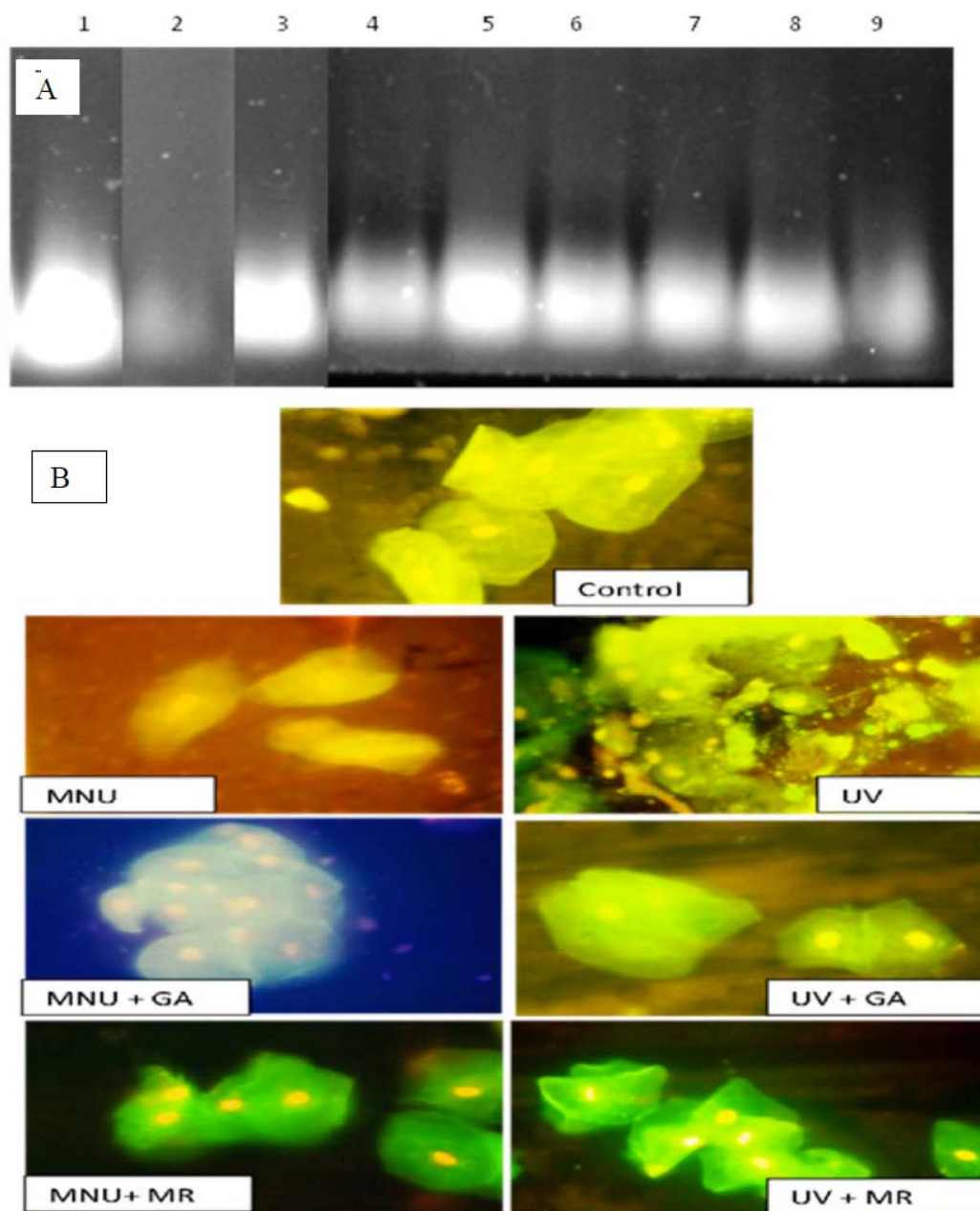


Figure 1: A. Electrophoresis analysis of DNA protection by *G. mangostana* extracts. Lanes 1: Native DNA; 2: Oxidized DNA; 3: BHA + Oxidants treated DNA; 4: HWE (5µg GAE) +oxidants treated DNA; 5: HWE (10µg GAE) + oxidant treated DNA; Lane 6: 50%AE (5µg GAE) + oxidants treated DNA; Lane 7: 50%AE (10µg GAE) +oxidant treated DNA; Lane 8: 70%AE (5µg GAE) + oxidants treated DNA; Lane 9: 70%AE (10µg GAE) treated DNA and Induction of apoptosis. **B.** Buccal cell protection by *G. mangostana* extracts. MNU- N-nitroso -N-methyl urea, UV-Ultra Violet radiation, GA-Gallic acid, MR- Mangosteen Rind extract.

significant levels of gallic/tannic acid (~ 7 folds higher than HWE and 50% AE), chlorogenic acid (about 10 folds higher), epicatechin (about 10 folds higher) and ferulic acid (about 4 folds higher) and catechin similar to that found in HWE and 50% AE were observed. Based on the IC_{50} value, we calculated the total activity expressed in each fraction in terms of total phenolic content. Relative fold activity was also calculated taking the least activity expressed as 1 fold in each of the activity. As indicated in Table 2, about 2-20 folds higher

activity was observed in 70%AE. Further, since we had established previously the precise contribution of each of these phenolic acids in antioxidant and PPAI activity [33], we determined the effective phenolic acids contributing to free radical scavenging and PPAI activity; gallic/tannic and ferulic acids found to contribute to the activity.

Similarly we attempted to determine the contribution of various xanthenes present in the fraction to

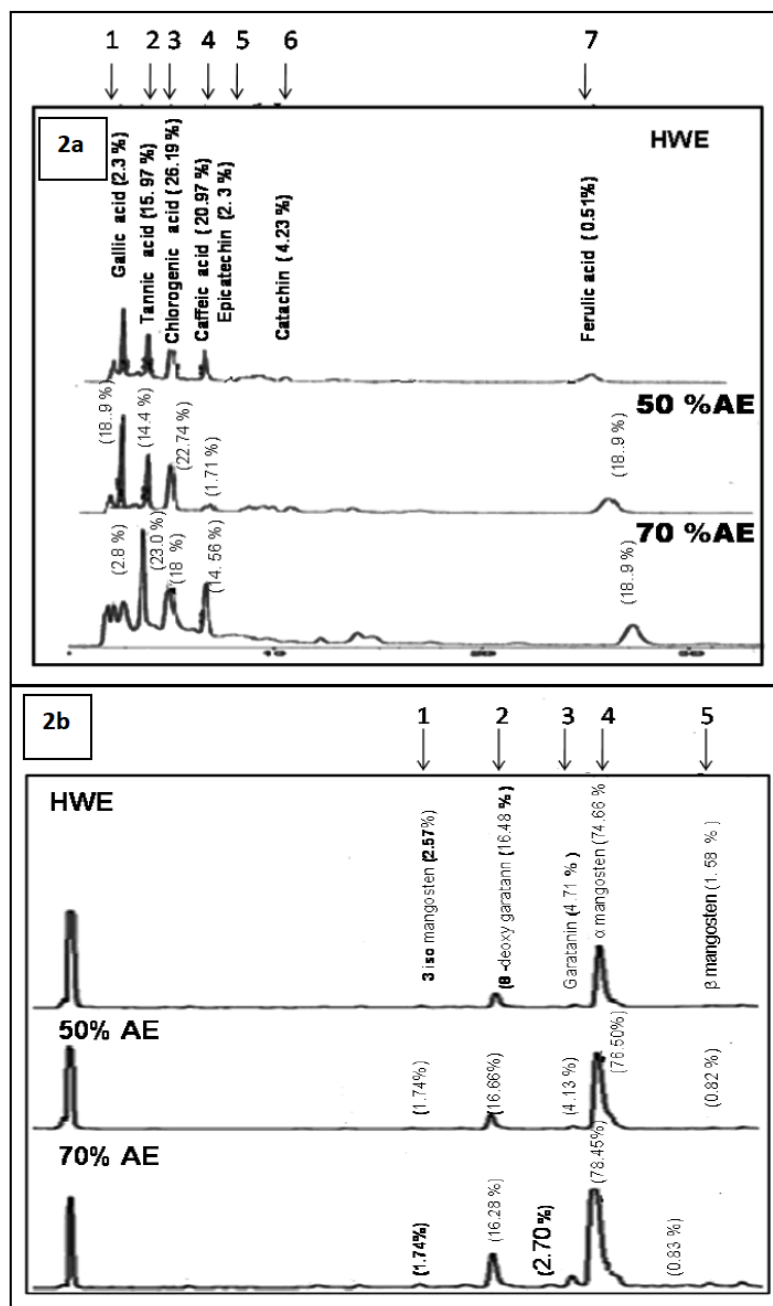


Figure 2: HPLC analysis of phenolic acid constituents and xanthenes in *G. mangostana* rind extract.

Phenolic acids in each fraction were identified by comparison of their retention time with known standards. Retention of standards are indicated by arrows. (2a) 1-gallic acid, 2-tannic acid, 3-chlorogenic acid, 4-caffeic acid, 5-epicatechin, 6-syringic acid, 7-catechin, 8-ferulic acid. (2b) 1. α -Mangosten, 2. Gartanin, 3. 8-Desoxygartanin, 4. β -Mangosten, 5. 3-Isomangosten.

bioactivities examined (Table 3). Xanthenes were resolved as α -mangosten, gartanin, 8-desoxygartanin, β -mangosten and 3-isomangosten (Figure 2b). α -mangosten and 8-desoxygartanin were found as major components in all the extracts. However, differences in the ratios of α -mangosten, gartanin, β -mangosten and 3-isomangosten were observed. Since this being the first paper to report the comparative efficacy of different xanthenes on various bioactivity, correlation coefficient

– R2 between the levels of xanthenes and bioactivity was determined with level of significance of P value < 0.05, using the statistical program SPSS for windows, version 10.0. Free radical scavenging (FRS) and RBC protection (RP) activity correlated with the levels of gartanin with R2 value of 0.995 and 0.949 for FRS and RP activity with P value < 0.05. However, DNA protection, and H^+K^+ -ATPase inhibition correlated with β -mangosten with R2 value of >0.9 with P value <0.05.

Table 3: Phenolics and Xanthenes Profile in the Extracts of *G. mangostana* Rind

Constituents	HWE	50% AE	70% AE
Phenolics			
Gallic/Tannic	1.52±0.038	2.93±0.058	15.05±0.57
Chlorogenic	0.74±0.011	0.9±0.009	9.0±0.085
Caffeic	0.54±0.011	-	0.31±0.004
Epicatechin	--	0.71±0.009	7.27±0.174
Catechin	0.8±0.012	0.95±0.015	0.91±0.010
Ferulic	5.8±0.10	2.06±0.04	8.50 ±0.153
Xanthenes			
α-Mangostin	15.5± 0.307	39.9±0.650	17.6±0.152
Gartanin	0.98±0.045	2.16±0.080	0.607±0.007
8-Desoxygartanin	3.42±0.243	8.6±0.102	3.66±0.132
β-Mangostin	0.32±0.050	0.42±0.020	0.186±0.003
3-Isomangostin	0.53±0.065	0.99±0.025	0.39±0.036

Various extracts of *G. mangostana* rind were subjected to HPLC analysis along with phenolic acid and xanthone standards. Based on the concentration of the standard and the peak area, concentration of phenolic acids and xanthenes were calculated and expressed as mg/g. Values provided are mean of three replicates ± standard deviation.

3-Isomangostin may also contribute to the DNA protectivity, since it also showed an R² of >.9 (P<0.05). A derivative of gartanin, 8- deoxy gartanin although showed good Correlation with R² of .95 (P<0.05), did not show good correlation with other assays (Table 4). Data thus suggest that the observed activity in MSRE is mainly from a minor components such as Gartanin, β-mangostin and 8-desoxy gartanin indicating that differential interaction of different components of MSRE with specified chemical structural components may be responsible for exhibiting bioactivity. Data is supported by another study where they also indicated that gartanin than α-mangostin may execute the activity [40]. *In vivo* efficacy of these components needs to be analyzed in order to understand the biotransformation of the compounds and attributing of bioactivity to the human system. Such studies are underway in the laboratory in order to find the potential bioutilization of the major ingredients of MSRE. The products prepared from the 70%AE (both beverage and jelly) tasted sweet with pleasant aroma, and color. Microbiological and

sensory examination shows the suitability of the product for human consumption. The *G. mangostana* jelly product has been patented (2934/DEL /2011 Dated 22-9-2011). Detailed invention on the product was covered in the above mentioned patent.

The current study is important in that it delineates the bioactive potentials and bioactive components in *G. mangostana*, which can throw light on the precise molecule(s) involved in varieties of health beneficial properties. Over past decades the “Queen of tropical fruits”- *G. mangostana* has been the subject for utilization of naturals for human health and wellness. Different parts of *G. mangostana*, mostly fruit hull, bark and roots have been used for hundreds of years in Southeast Asia as a medicine for a great variety of medical conditions. In India, Thailand, China and other parts of Asia dried and powdered fruit hull is used as antimicrobial agents and for the antiparasitic treatments in dysentery [21,41,42]. Mangosteen leaves and barks have shown anti-inflammatory properties [6]. *G*

Table 4: Correlation Co-Efficient between Different Bioactivity and Various Xanthone Components of *G. mangostana* Rind Extracts

	α-Mangostin	Gartanin	8-Deoxygartanin	β- Mangostin	3-Isomangostin
FRS	0.6822 (p<0.31)	0.995(p<0.0049)	0.95 (p<0.04)	0.89 (p< 0.104)	0.97 (p<0.02)
RBC	0.369 (p<0.60)	0.95 (p<0.048)	0.755 (p<0.24)	0.858 (p<0.14)	0.878 (p<0.121)
DNA	0.574(p<0.475)	0.871 (p<0.128)	0.889 (p<0.11)	0.963 (p<0.036)	0.957 (p<0.04)
PPAI	0.04 (p<0.95)	0.625 (p<0.374)	0.515 (p<0.48)	0.889 (p<0.116)	0.732 (p<0.207)

Correlation coefficient – R² between the levels of xanthenes and various bioactivities such as free radical scavenging, Red Blood Cell protection, DNA protection and PPAI was determined using the statistical programme SPSS for windows, version 10.0, with level of significance of P value < 0.05.

.mangostana extracts were also used as tonic for various ailments [26,43] particularly as antimicrobial agent [22,23], antidiabetic, gastrointestinal health [4] etc. Based on these reports some products were also developed and sold in the market with a claim of health beneficial properties [1]. However as reviewed by Obolskiy [1], despite the generation of product of *G. mangostana* as juice, dried powder, decoction in the market, their claims were purely on the traditional knowledge only. As a result of this some of the global market issues concerning the *G. mangostana* food products existed. It is therefore pointed out by the reviewer of Obolskiy and his group [1] that it is unfortunate to witness the marketing strategies of mangosteen that reached levels of dishonest advertisement and misleading claims about the products. In this context it is imperative for food scientists to study and evaluate the bioactive potentials of *G. mangostana*. Current paper adds scientific values to *G. mangostana* product (s) that has potentials in the global market. Study highlights the precise of role of phenolics and xanthenes in health and wellness properties.

CONCLUSION

G. mangostana rind being the major fruit portion constituting 62% of the whole fruit has been underutilized. The results of the study proved that the rind extracts which are rich in phenolics, flavonoids and xanthenes possessed varied degree of bioactive potentials. The extracts can be incorporated into products preparation which is of health importance.

ACKNOWLEDGEMENT

The authors thank the Director, CSIR-Central Food Technological Research Institute, Mysore, India, for his constant encouragement throughout the course of the study.

ABBREVIATIONS

DPPH	=	1,1- diphenyl-2-picrylhydrazyl
BHA	=	Butylated hydroxyanisole
HWE	=	Hot water extract
50% AE	=	50% Alcoholic extract
70% AE	=	70% Alcoholic extract
BHA	=	Butylated hydroxyl anisole

MNU = N-nitroso – N-methylurea

FRS = Free Radical Scavenging

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Received on 10-11-2014

Accepted on 10-12-2014

Published on 06-01-2015

DOI: <http://dx.doi.org/10.6000/1927-3037.2014.03.04.1>© 2014 Nanjarajurs *et al.*; Licensee Lifescience Global.

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