LWT - Food Science and Technology 156 (2022) 112999

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Evaluation of bioactive constituents of *Garcinia indica* (kokum) as a potential source of hydroxycitric acid, anthocyanin, and phenolic compounds

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ARTICLE INFO

Keywords:
Kokum fruit
Hydroxycitric acid (HCA)
Natural colour
Flavonoids
Antioxidant activity

ABSTRACT

The composition of metabolites in the rind of ripe fruits of *Garcinia indica* was analysed using liquid chromatography-mass spectrometry (LC-MS). The fruits were particularly rich in hydroxycitric acid (15.60–22.92 g/100 g), which makes them a suitable candidate for weight-loss supplements; in anthocyanin (4.47–7.08 mg/g), a value much higher than that recorded for the majority of fruits and vegetables, which makes the fruit a good source of natural colour; and in phenolics, making it a good source of antioxidants as well (given the significant correlation between total phenolics and flavonoids and antioxidant activity). A total of 38 anthocyanin compounds were identified, most of them for the first time. Cyanidin-3-sambubioside, peonidin-3-arabinoside, and pelargonidin-3-glucoside were the major anthocyanins. Of the 30 individual phenolic compounds identified, ortho/para coumaric acid, naringenin, and apigenin were particularly abundant, which have myriad industrial applications. The first principal component (PC1) explained 93.05% of the total variability and was positively correlated to hydroxycitric acid, apigenin, and catechin. Clustering and heat map enabled the most suitable accessions for different bioactive compounds to be identified. Overall, the present study highlights *G. indica* as a rich, new, and sustainable source of bioactive substances with food, pharmaceutical, and other industrial applications.

1. Introduction

Exploring indigenous fruits and vegetables as potential natural raw materials for use in the food and pharmaceutical industries is of increasing interest to researchers (Pfukwa et al., 2020; Singh et al., 2020). *Garcinia indica*, a tall and slender tree, is one such medicinally important but underexploited tree species, native to the Western Ghats in India. The fruit of *G. indica* is globose or spherical, dark purple when ripe, and is believed to possess myriad health-promoting properties. In Ayurveda, the ancient Indian system of medicine, it is held to have antidiabetic, anthelmintic, cardiotonic, and anti-obesity properties and used in treating piles, dysentery, tumours, pain, and heart ailments (Swami, Thakor, & Patil, 2014). The dried rind of *G. indica* is traditionally used as a culinary spice to impart a sour taste to food and is also widely used in preparing soft drinks. The sour taste is mainly due to hydroxycitric acid (HCA), a potential anti-obesity agent and widely used in anti-obesity medicines (Jayaprakasha & Sakariah, 2002; Nainegali, Prasanna, & Belur, 2019; Nayak, Rastogi, & Raghavarao, 2010).

The purple colour of the rind is mainly due to anthocyanins, which are responsible for the red, blue, and purple colours of many fruits, vegetables, flowers, and even cereal grains. Anthocyanin is considered a potential replacement for synthetic colours because its brightness and water solubility make it easy to incorporate it into edible products (Pinela et al., 2019). Natural food colours including anthocyanin are in great demand as alternatives to synthetic dyes, especially because consumers increasingly prefer natural additives to synthetic additives. The global food colour market was estimated at $3.88 billion in 2018 and is projected to reach $5.12 billion by 2023 (Anon, 2017), and that for natural food colours was estimated at $1.32 billion in 2015 and is likely to increase rapidly owing to the high demand from the bakery sector (Anon, 2017).

Anthocyanins are also known to possess antioxidant, antidiabetic,

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https://doi.org/10.1016/j.lwt.2021.112999
Received 9 July 2021; Received in revised form 13 December 2021; Accepted 16 December 2021
Available online 23 December 2021

anti-cancer, and anti-tumour properties, and therefore play an important role in supporting good health and in disease prevention (Yousuf, Gul, Wani, & Singh, 2016). Apart from anthocyanin (Nayak, Srinivas, & Rastogi, 2010; Nayak, Rastogi, & Raghavarao, 2010), *G. indica* also contains other phenolic compounds (Bommayya, Kusum, & Ramachandran, 2011) such as phenolic acids and flavonoids and can serve as a natural source for these compounds as well. These bioactive phenolic compounds have a wide range of biological activity and have shown antioxidant, anti-microbial, antityrosinase, anticholinesterase, anti-inflammatory, anti-cancer, antihyperglycemic or antidiabetic, and antinociceptive activity (Jucá et al., 2020), which makes them excellent natural food preservatives (Smaoui et al., 2019) and cosmetics (Panzella & Napolitano, 2019) and useful home remedies (Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018).

The genus *Garcinia* (family Clusiaceae) includes about 200 species and is native to Asia, South America, Australia, tropical and southern Africa, and Polynesia. The fruits of most species of *Garcinia* are eaten locally. Some species have been profiled biochemically, including *G. mangostana* L. (Zadernowski, Czaplicki, & Naczk, 2009), *G. dulcis* (Deachathai, Mahabusarakam, Phongpaichit, & Taylor, 2005), *G. xanthochymus* (Hassan, Taher, & Susanti, 2018), and *G. cambogia* (Jayaprakasha & Sakariah, 1998), but *G. indica*, despite being widely consumed in Asia, is not among them—and the present study sought to fill that gap by determining the diversity in chemical profiles of six accessions using liquid chromatography and mass spectrophotometry (LC–MS/MS) to identify accessions that are rich in bioactive compounds. Such data on bioactive compounds would help in using the fruit of *G. indica* more effectively as a source of weight loss supplement, natural colorant, natural preservative, anti-browning, and therapeutic agent. These findings are useful in identifying suitable accessions for commercial extraction of bioactive compounds like HCA, anthocyanin, and phenolics for food and pharmaceutical industries.

2. Materials and methods

2.1. Plant material

Plants of *G. indica* collected from different locations in two states in India, namely Karnataka and Maharashtra, and grown in the experimental fields of Indian Council of Agricultural Research - Indian Institute of Horticultural Research, Bengaluru, Karnataka, were chosen for the study. Of the six accessions used, Red and Local had been raised from grafts and Vengurla, Amruth Kokum, IIHR-1, and IIHR-2, from seedlings. Thirteen-year-old plants were used in this study. Mature fully ripened fruits were harvested manually, with 15 fruits from each accession forming a replication. Three independent replicates were used to conduct biochemical analysis for each accession. From each accession, the rind, or the outer hard cover that covers the pulp of the fresh fruit (supplementary material 1), was removed and thoroughly homogenized in an electric blender until it turned into smooth pulp. The rind was used for the study because it is the edible part and is rich in anthocyanin. The homogenized samples were stored kept in a freezer at −70 °C and used within a week. All the analysis were done in triplicate.

2.2. Chemicals and reagents

All phenolic acid standards–caffeic acid, 2,4-dihydroxybenzoic acid, chlorogenic acid, ferulic acid, gallic acid, gentisic acid, *o*-coumaric acid, *p*-coumaric acid, 4-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, *trans*-cinnamic acid, vanillic acid, sinapic acid, 3-hydroxybenzoic acid, benzoic acid, ellagic acid, flavonoid standards–apigenin, catechin, hesperetin, kaempferol, luteolin, myricetin, naringenin, quercetin, rutin, umbelliferone, epicatechin, epigallocatechin, cyanidin standard, hydroxycitric acid (HCA) standard, DPPH (1,1-diphenyl-2-picrylhydrazyl), and TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine) were procured from Sigma-Aldrich, St. Louis, Missouri, USA. Chromatographic or MS-grade organic solvents were used for the analysis. Analytical grade sodium carbonate, Folin- Ciocalteau's phenol reagent, aluminium chloride, sodium nitrite, sodium hydroxide, sodium acetate, ferric chloride, sodium sulphate, hydrochloric acid (conc.), acetic acid (glacial), formic acid, and methanol were purchased from Merck KGaA, Darmstadt, Germany. Water purified in the Milli-Q (Millipore) system was used for mobile phase preparation.

2.3. Determination of hydroxycitric acid content

The extraction and estimation of hydroxycitric acid was carried out as per the method described by Jayaprakasha and Sakariah (2002). Separation and estimation of HCA was carried out using C-18 reverse phase column (250 × 4.6 mm) with 8 mM sulfuric acid as mobile phase using UV detection at 210 nm. The flow rate was 1.0 mL/min, and the sample injection volume for HPLC (Shimadzu Nexera X2 ultra high-performance liquid chromatograph) was 20 μl. Retention time of HCA and total run time was 4.28 min and 10 min, respectively (supplementary material 2). Quantitative estimation of HCA was done against HCA calibration standard.

2.4. Extraction and analysis of anthocyanin

Anthocyanin was extracted following Shivashankara, Jalikop, and Roy (2010) with slight modification. Fruit rind (5 g) was ground in a pestle and mortar with 1% formic acid in methanol till clear extract was obtained and the volume was made up to a known quantity. Total anthocyanin content estimation was done according to the pH differential method described by Giusti and Wrolstad (2001). The extract dilution was made with 0.2 mol/L of potassium chloride buffer (pH 1.0) and with 0.2 mol/L of sodium acetate buffer (pH 4.5), and further incubated for 30 min at room temperature in dark. The absorbance was read at 520 and 700 nm using a UV–vis spectrophotometer (PG Instruments Corporation, UK). Total anthocyanin content was expressed as mg of cyanidin per g fw of sample.

Further, 500 μl of the extract was dried under vacuum flash evaporator (Model Hei-VAP Advantage, M/s Heidolph Instruments GmbH & CO. Schwabach, German), to remove acidified methanol and diluted with Milli-Q water. The extraction was again carried out by washing with 3 mL of ethyl acetate in a separating funnel thrice. The upper layer was discarded, and the aqueous layer collected and dried under nitrogen flow using low volume concentrator (Caliper LifeSciences, USA). Further it was dissolved in mobile phase and injected to an Acquity UPLC-H class coupled with TQD-MS/MS from M/S Waters, USA after filtration through 0.2 μm nylon filter. All the operations were carried out under dark conditions to avoid degradation of pigments.

2.5. Extraction of phenolic compounds

As per modified method of Weidner, Amarowicz, Karamac, and Fraczek (2000), total phenolics and flavonoids were extracted from 5 g of ground rind with 25 mL of 80% methanol after keeping in dark for 48 h at room temperature. After repeatedly grinding in pestle and mortar, till the debris became colourless, the extract was centrifuged at 10000 g for 15 min at 4 °C, and the supernatant was collected and made up to 50 mL. The pooled extract was used for the estimation of total phenolics and flavonoids. The total phenolics content was estimated by the Folin-Ciocalteau method at 650 nm using a UV–vis spectrophotometer (PG Instruments Corporation, UK) and expressed as gallic acid equivalents (Singleton, Orthofer, & Lamuela-Raventos, 1999). The total flavonoids content was estimated using $AlCl_3$ and $NaNO_2$ method at 510 nm and expressed in units of catechin equivalents (Zhishen, Mengcheng, & Jianming, 1999).

The individual phenolic acids and flavonoids for LC-MS/MS analysis were isolated from the extract using the method previously described by Weidner et al. (2000) and Chen, Zuo, and Deng (2001) with slight

modifications. The methanol extract was dried under vacuum at 45 °C using flash evaporator (Model Hei-VAP Advantage, M/s Heidolph Instruments GmbH & CO. Schwabach, German), and the residue was dissolved in water and extracted thrice using into 25 mL ethyl acetate. Ethyl acetate extract was evaporated completely under vacuum, and then 10 mL of 2 mol/L NaOH was added and allowed to be hydrolysed at room temperature for 10 h under a nitrogen atmosphere. After acidification to pH 2 using 5 mL 6 mol/L HCl and hydrolysis for 2 h in a boiling water-bath, the cooled extract was further extracted with ethyl acetate. The ethyl acetate layer was further extracted twice with 25 mL of 0.1 mol/L $NaHCO_3$. The ethyl acetate layer which carried the phenolic acids and flavonoids was evaporated to complete dryness under vacuum at room temperature. The residue was dissolved in 2 mL MS grade methanol, filtered through 0.2 μm nylon filter, and injected in LC-MS/MS (Waters, USA) for phenolic acids and flavonoids estimation.

2.6. Profiling of phenolic acids, flavonoids and anthocyanins using UPLC-MS/MS

Profiling of phenolic acids, flavonoids and anthocyanins was done by using Acquity UPLC-H class coupled with TQD-MS/MS from M/S Waters, USA. The overall system was controlled by the Mass lynx software. The mass spectra was obtained using both negative ionization (ES-) and positive ionization (ES+) mode. The analytical column was 2.1 × 50 mm UPLC BEH- C18 column (Waters) with 1.7 μm particles size protected by a Vanguard BEH C-18 (2.1 × 5 mm with 1.7 μm particle size) for phenolic acids, flavonoids, and anthocyanins. The column temperature was maintained at 25 ± 1 °C. Estimation of individual phenolic acid and flavonoid was done using MRM methods (supplementary material 3 and 4). The metabolites eluted from UPLC column were pumped directly to the TQD-MS/MS (Waters, USA) system and monitored. This system was optimized with specific cone voltage, capillary voltage for the individual compounds of analysis with source temperature 135 °C, de-solvation gas flow and temperature 650 L/h and 350 °C, respectively. Quantitative estimation of individual compound was done against calibration standards.

For anthocyanin the condition of the MS was optimized for single ion reaction (SIR) and full scan mode by direct infusion of available standard, and preferably ESI + mode was found most effective to get the maximum abundant mass [M+H]+ at the cone voltage of 34 V, capillary voltage 4 V, R_f 0.1 V, with the source temperature 130 °C and de-solvation gas flow and temperature 600 L/h and 350 °C, respectively. Full scan was selected from the lower mass of 200–1100 amu. For anthocyanin identification from garcinia rind, sample was run in full

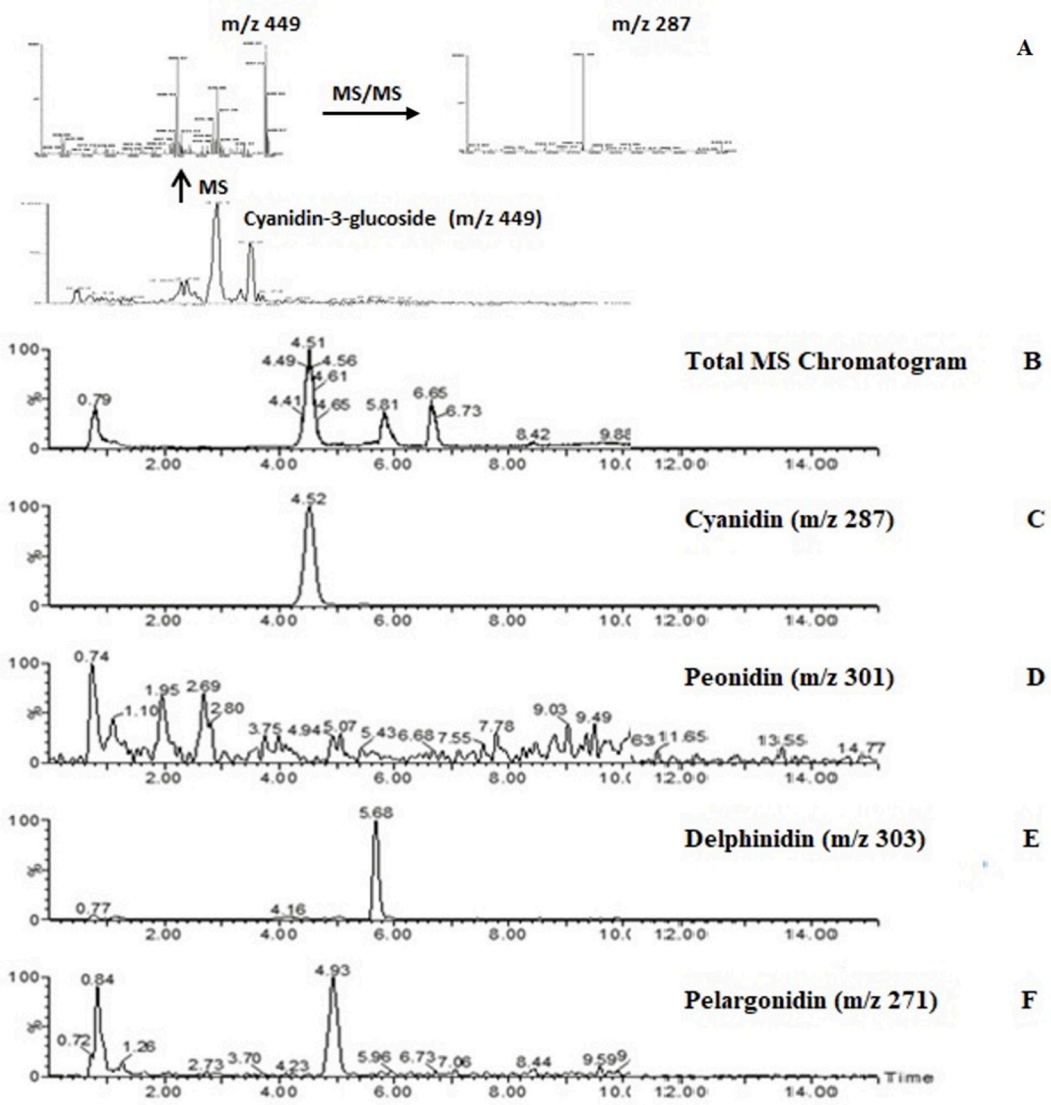

Fig. 1. (A) Fragmentation of cyanidin-3-glucoside (449 m/z) to daughter ion (287 m/z); (B) Total MS chromatogram of anthocyanin from *Garcinia indica* rind using LC-MS/MS; (C) Chromatogram of cyanidin; (D) Chromatogram of peonidin; (E) Chromatogram of delphinidin; (F) Chromatogram of pelargonidin.

mass scan ESI + mode and single ion reaction mode to find out the most abundant mass. Further, to find out their most abundant fragments the individual masses were broken down by adjusting collision energy. Fragmentation of cyanidin-3-glucoside is given in Fig. 1A. The compounds were identified by comparison of masses [M+H]+ and fragment ions with the reported data. Accordingly, some of the abundant anthocyanin compounds (belonging to cyanidin, peonidin, delphinidin and pelargonidin) were noted, and quantification (mg/g) was done comparing with standard cyanidin contents.

2.7. Determination of antioxidant potential

Total antioxidant potential was estimated both in terms of radical scavenging activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay as well as in terms of reducing power using FRAP (ferric-reducing antioxidant power) method. Free radical scavenging activity using DPPH assay was performed as described by Singh, Jyothi, Reddy, and Shivashankara (2018) and the absorbance was measured at 515 nm using UV–vis spectrophotometer (PG Instruments Corporation, UK). The percentage scavenge of DPPH by the sample extract was calculated by the following equation:

$$\%\text{Radical scavenging activity} = 100 \times (A_0 - A) / A_0$$

Where A_0 was the absorbance of the blank control (containing all reagents except the sample extract); A was the absorbance of the test sample.

The FRAP assay was performed according to the method described by Benzie and Strain (1996). The FRAP reagent consists of 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L $FeCl_3$ in the ratio 10:1:1 (v:v:v). 1.8 mL of FRAP reagent was mixed with 0.2 mL of plant extract, incubated at 37 °C for 30 min in a water bath. The intensity of colour developed was measured at 593 nm against reagent blank. In both the methods, ascorbic acid was used for standard curve preparation and antioxidant activity was expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

2.8. Data analysis

Data analysis was performed using a software package, namely Statistical Analysis System (SAS) ver. 9.3. Values are presented as mean ± SD of three replicates. Variability among the accessions for biochemical constituents were evaluated by ANOVA using PROC ANOVA. Tukey's test was used to compare the means of parameters analysed. The level of statistical significance was set at $P \leq 0.05$. Correlation analysis was done using PROC CORR in SAS to depict correlation between antioxidant potential and other parameters. Data was subjected to principal component analysis (PCA) by PROC PRINCOMP in SAS. For two-way hierarchical clustering and plotting heatmap, various library of R-programming language like, cluster, gplots, and RcolorBrewer have been used in R version 4.0.4. For clustering, a function hclust and the Ward method of clustering has been used. Scaling of data was done with log 2 data transformation and normalized data was used for plotting heat map using heatmap.2 function.

3. Results and discussion

3.1. Hydroxycitric acid

The six accessions differed significantly ($p < 0.05$) in their HCA content (Table 1), which ranged from 15.60 to 22.92 g/100 g; these values were similar to those reported by Jayaprakasha and Sakariah (2002), namely 10.3%–12.7% by HPLC and 12.48%–15.10% through titration. Vengurla was the richest source and IIHR-2, the poorest, the variation being probably due to genotypic differences. Hydroxycitric acid is also present in the rinds of other *Garcinia* species, namely *G. cambogia*, *G. atrovidis* (Jayaprakasha & Sakariah, 1998; Semwal, Semwal, Vermaak, & Viljoen, 2015), and *G. cowa* (Jena, Jayaprakasha, & Sakariah, 2002). The rind of *G. cambogia* is a prime natural source of HCA (10%–30%; Semwal et al., 2015)—*G. indica* 'Vengurla' was found to match that and is thus a promising source of HCA for commercial extraction.

3.2. Anthocyanins

The yield and composition of anthocyanins are important quality parameters as well as the key attributes for the industry. As pigments, anthocyanins are almost exclusively responsible for the red colour of the fruit; in the present study, their content (fresh weight) ranged from 4.47 mg/g in Red to 7.08 mg/g in IIHR-2 (Table 1); on dry weight basis, the corresponding concentrations were 17.88 mg/g and 28.32 mg/g. Accessions IIHR-2 and Red were significantly different having values which differ more than minimum significant difference (MSD) (Table 1). In general, all the accessions showed very high anthocyanin content, exceeding that reported in most of the fruits and vegetables (in mg/g, fresh weight: apple, 0.01–0.17; banana bracts, 0.32–2.50; black currants, 0.96–4.52; blueberries, 0.63–4.30; red radish, 0.11–0.60; grapes, 0.06–6.00; red raspberry, 3.92; and strawberry, 0.13–3.15. (Nayak et al., 2010; Yousuf et al., 2016). A few berries, however, are richer sources, namely blackberries (0.72–12.21 mg/g) and elderberries, 3.32–13.74 mg/g (Nayak et al., 2010). Our data clearly show *G. indica* to be superior

Table 1
Total phenolics, total flavonoids, anthocyanin, hydroxycitric acid (HCA), and antioxidant activity of *Garcinia indica* genotypes.

Genotypes	Code	Total phenols (mg GAE/g fw)	Total flavonoids (mg CE/g fw)	Anthocyanin (mg Cyanidin/g fw)	FRAP (mg AEAC/g fw)	DPPH (mg AEAC/g fw)	Hydroxycitric acid (HCA) (g/100 g fw)
G. indica "Red"	G1	9.50 ^{b} ± 0.28	36.42 ^{b} ± 1.04	4.47 ^{b} ± 0.50	12.16 ^{b} ± 0.42	4.39 ^{b} ± 0.86	16.70 ^{c} ±0.21
G. indica "Local"	G2	7.15^{d} ± 0.15	25.89 ^{cd} ± 0.67	5.28 ^{ab} ± 0.28	9.65 ^{c} ± 0.30	1.54 ^{c} ±0.06	21.02 ^{b} ± 0.05
G. indica "Amruth kokum"	G3	8.34^{c} ± 0.09	27.14 ^{c} ± 0.89	5.63 ^{ab} ± 1.71	12.74 ^{b} ± 0.20	3.44 ^{b} ± 0.13	15.71^{d} ± 0.03
G. indica "Vengrula"	G4	5.36^{e} ± 0.05	23.86 ^{d} ± 0.96	6.45 ^{ab} ± 0.59	7.37 ^{d} ± 0.17	1.32 ^{c} ± 0.03	22.92^{a} ±0.17
G. indica "IIHR-1"	G5	6.91^{d} ± 0.18	25.13 ^{cd} ± 1.13	5.10 ^{ab} ± 0.94	9.24 ^{c} ± 0.66	3.26 ^{b} ± 0.24	16.99^{c} ±0.06
G. indica "IIHR-2"	G6	12.60^{a} ± 0.37	41.48 ^{a} ± 0.93	7.08 ^{a} ± 0.84	18.69 ^{a} ± 0.64	5.65 ^{a} ± 0.63	15.60^{d} ± 0.07
Minimum Significant Difference (MSD)		0.599	2.6162	2.553	1.2235	1.248	0.3449

Values represent the mean ± standard deviation (n = 9) of three independent experiments.
Mean followed by the same superscripts are not significantly different referring to Tukey's HSD test ($p < 0.05$).
GAE: Gallic acid equivalents.
CE: Catechin equivalents.
FRAP: Ferric reducing antioxidant power.
DPPH: 1,1-Diphenyl-2-picrylhydrazyl radical scavenging ability.
AEAC: Ascorbic acid equivalents antioxidant capacity.

to many other natural sources as a colouring agent and therefore a promising source of anthocyanins in foods and dyes.

Individual anthocyanin compounds were identified using LC-MS/MS by comparing the mass-to-charge ratio (m/z) of each molecule and its fragmentation to published values (Cooke et al., 2006; Acevedo De la Cruz et al., 2012; De Rosso et al., 2008; Huang, Zhang, Qin, Le, & Wu, 2012; Lopes-da-Silva, de Pascual-Teresa, Rivas-Gonzalo, & Santos-Buelga, 2002; Mazzuca, Ferranti, Picariello, Chianese, & Addeo, 2005; Stein-Chisholm, Beaulieu, Grimm, & Lloyd, 2017; Tian, Giusti, Stoner, & Schwartz, 2006; Wu & Prior, 2005). A total of 38 anthocyanin compounds were identified (Table 2) through mass spectra. Overall, the identified anthocyanins represented four groups based on their m/z values, namely cyanidins (m/z 287), peonidins (m/z 301), delphinidins (m/z 303), and pelargonidins (m/z 271) (Fig. 1: C, D, E, F). The most prominent was cyanidin 3-sambubioside (MH^+581, major fragment, m/z 287), and the other characteristic pigments were as follows: cyanidin 3-glucoside (MH^+449, major fragment, m/z 287), cyanidin-3-pyranoside (MH^+449.107, major fragment, m/z 287), peonidin-3-arabinoside (MH^+433.112, major fragment, m/z 301), delphinidin-3-arabinoside (MH^+435.092, major fragment, m/z 303), and pelargonidin 3-glucoside (MH^+433, major fragment, m/z 271). We could locate no report of such detailed anthocyanin profiling for *G. indica*, although the presence of a few anthocyanin pigments has been reported. Our results are consistent with those obtained by Nayak, Srinivas, and Rastogi (2010), who reported that cyanidin 3-sambubioside and cyanidin 3-glucoside were the major anthocyanins in *G. indica*. In *G. mangostana* rind, cyanidin 3-sophoroside and cyanidin 3-glucoside were the major pigments (Azima, Noriham, & Manshoor, 2017). Anthocyanin has many beneficial properties; for example, anthocyanins are antioxidants, anti-tumour, and anti-ageing. Besides the use of anthocyanins as natural dyes, these coloured pigments are potential sources of pharmaceutical ingredients with various health benefits.

3.3. Total phenolics, flavonoids, and antioxidant activity

The amount of total phenolics and flavonoids varied significantly with the accession: total phenolics, as gallic acid equivalents (GAE), were maximum (12.60 mg/g) in IIHR-2 and minimum (5.36 mg/g) in Vengurla, as were total flavonoids, as catechin equivalent, the corresponding values being 41.48 mg/g in IIHR-2 and 23.86 mg/g in

Table 2
Anthocyanin compounds and mass spectrometry variables found in the rind of *Garcinia indica*.

Anthocyanin	MH^+ (m/z)	Fragments (m/z)	Anthocyanin content mg/g fw	Anthocyanin content mg/g dw	References
Cyanidin 3-sambubioside	581	287	3.482	13.930	Tian et al. (2006)
Cyanidin 3-glucoside	449	287	0.211	0.843	Wu and Prior (2005)
Cyanidin-3-pyranoside	449.107	287	0.206	0.826	Stein-Chisholm et al. (2017)
Cyanidin-3-(6″-acetoyl) glucoside	491	287	0.017	0.069	Wu and Prior (2005)
Cyanidin-3-(malonyl)-glucoside-5-glucoside)	697	535, 449, 287	0.011	0.045	Wu and Prior (2005)
Cyanidin-3-laminaribioside	611	287	0.024	0.094	Wu and Prior (2005)
Cyanidin-3-(6″-acetyl-pyranoside)	491.118	287	0.017	0.070	Stein-Chisholm et al. (2017)
Cyanidin-3-(6″malonoyl-laminiaribioside)	697	287	0.011	0.045	Wu and Prior (2005)
Cyanidin 3-rutinoside	595	449, 287	0.044	0.176	De Rosso et al. (2008)
Cyanidin-3-*O*-(6-*O*-acetyl)-5-*O*-diglucoside	653	287, 449, 611	0.012	0.046	Acevedo De la Cruz et al. (2012)
trans-Cyanidin-3-*O*-(6-*O*-*p*-coumaryl)-5-*O*-diglucoside	757	287, 449, 595	0.023	0.093	Acevedo De la Cruz et al. (2012)
trans-Cyanidin-3-*O*-(6-*O*-*p*-coumaryl)-glucoside	595	287, 449	0.034	0.136	Acevedo De la Cruz et al. (2012)
Cyanidin 3-diglucoside-5-glucoside	773	611, 449, 287	0.006	0.024	Wu and Prior (2005)
Cyanidin 3-(*p*-coumaroyl) diglucoside-5-glucoside	919	757, 449, 287	0.030	0.119	Wu and Prior (2005)
Cyanidin-3-malonylglucose-5-glucose	697	535, 449, 287	0.011	0.045	Lopes-da-Silva et al. (2002)
Cyanidin-3-*O*-(6-*O*-acetyl) pentoside	461	287	0.019	0.077	Mazzuca et al. (2005)
Peonidin-3-arabinoside	433.112	301	1.023	4.092	Stein-Chisholm et al. (2017)
Peonidin-3-(*p*-coumaroyl-glucoside)	609.160	301	0.111	0.443	Stein-Chisholm et al. (2017)
Peonidin 3-glucoside	463	301	0.022	0.089	Huang et al. (2012)
Peonidin-3-galactoside	463	301	0.022	0.089	Huang et al. (2012)
Peonidin-3-*O*-(6-*O*-acetyl)-glucoside	505	301, 463	0.017	0.070	Acevedo De la Cruz et al. (2012)
Peonidin-3,5-*O*-diglucoside	625	301, 463	0.010	0.040	Acevedo De la Cruz et al. (2012)
Delphinidin-3-arabinoside	435.092	303	0.307	1.227	Stein-Chisholm et al. (2017)
Dephinidin 3-rutinoside-5-galactoside	773	611, 465, 303	0.006	0.024	Wu and Prior (2005)
Dephinidin 3-glucoside	465	303	0.021	0.083	Wu and Prior (2005)
Dephinidin 3-rutinoside	611	465, 303	0.014	0.054	Wu and Prior (2005)
Dephinidin 3,5-diglucoside	627	465, 303	0.015	0.060	Wu and Prior (2005)
Dephinidin 3-galactoside	465	303	0.011	0.043	Wu and Prior (2005)
cis-Delphidin-3-*O*-(6-*O*-*p*-coumaryl)-5-*O*-diglucoside	773	303, 465, 611	0.006	0.024	Acevedo De la Cruz et al. (2012)
trans-Delphidin-3-*O*-(6-*O*-*p*-coumaryl)-5-*O*-diglucoside	773	303, 465, 611	0.004	0.014	Acevedo De la Cruz et al. (2012)
trans-Delphidin-3-*O*-(6-*O*-*p*-coumaryl)-glucoside	611	303, 465	0.014	0.054	Acevedo De la Cruz et al. (2012)
Methyl delphinidin glycoside	479	317	0.009	0.035	Cooke et al. (2006)
Pelargonidin 3-glucoside	433	271	1.029	4.116	Wu and Prior (2005)
Pelargonidin 3-diglucoside-5-glucoside	757	595, 433, 271	0.023	0.093	Wu and Prior (2005)
Pelargonidin 3-(acyl) rutinoside-5-glucoside	919	757, 579, 433, 271	0.021	0.083	Wu and Prior (2005)
Pelargonidin 3- rutinoside	579	433, 271	0.172	0.687	Wu and Prior (2005)
Pelargonidin 3-(caffeoyl) glucoside -5-glucoside	919	757, 433, 271	0.020	0.079	Wu and Prior (2005)
Pelargonidin 3-(feruloyl) diglucoside	771	271	0.016	0.065	Wu and Prior (2005)

Vengurla (Table 1). Two *in-vitro* assays (DPPH and FRAP) were used as complementary methods to evaluate the potential antioxidant activity. The results of DPPH assay of *G. indica* rind differed significantly with the accession and ranged from 1.32 mg/g to 5.65 mg/g (fresh weight, expressed in terms of ascorbic acid equivalent antioxidant capacity), being maximum (5.65 mg/g) in IIHR-2 and minimum in Vengurla and Local (Table 1). The FRAP assay also showed the same pattern, the values being 18.69 mg/g in IIHR-2 and 7.37 mg/g in Vengurla (Table 1).

3.4. Phenolic acids and flavonoids

A total of 30 phenolic compounds, comprising 18 phenolic acids and 12 flavonoids, were identified in the accessions of *G. indica* (Table 3).

3.4.1. Phenolic acids

A chromatogram of prime phenolic acids is given in Fig. 2. Each of the 18 phenolic acids belonged to one of the four groups, namely hydroxyl cinnamic acid derivatives, monohydroxy benzoic acid derivatives, dihydroxybenzoic acid derivatives, or trihydroxy benzoic acid derivatives. The major compound was *p*-coumaric acid, followed by *o*-coumaric acid, protocatechuic acid, salicylic acid, caffeic acid, ferulic acid, and *trans*-cinnamic acid.

Among the hydroxyl cinnamic acid derivatives, the amount of *p*-coumaric acid ranged from approximately 3.3 mg/g to 8.1 mg/g, the highest value being recorded in IIHR-2 (Table 3). The amount of *o*-coumaric acid ranged from approximately 1.6 mg/g (in Vengurla) to 4.0 mg/g (in IIHR-2). Other phenolic acids in this group were in meagre amounts: caffeic acid, ferulic acid, and *trans*-cinnamic acid. Sinapic acid and chlorogenic acid were present only in negligible amounts. In mono hydroxyl benzoic acid derivatives, salicylic acid was the predominant phenolic acid, followed by 4-hydroxy benzoic acid and 3-hydroxy benzoic acid. Protocatechuic acid was the major dihydroxy benzoic acid derivative, being maximum in Amruth Kokum; other members of this group were present in low amounts: 2,4-dihdroxy benzoic acid, vanillic acid, and gentisic acid. Gallic acid was predominant trihydroxy benzoic acid derivative.

Plant phenolics are natural antioxidants and exhibit a variety of functions, such as growth, development, and defence. They are also precursors of other crucial bioactive molecules frequently used for therapeutic, cosmetics, and food industries (Kumar & Goel, 2019). Plant phenolics are commercially exploited in food and pharmaceutical industries because of their various biological activities. For example, *p*-coumaric acid was characterized as the principal tyrosinase inhibitor; it inhibits the oxidation of L-tyrosine and L-3,4-dihydroxyphenylalanine (L-DOPA), which are the precursors of melanin biosynthesis (Lim, Ishiguro, & Kubo, 1999). Tyrosinase inhibitors can suppress the browning of fruits and vegetables and also melanogenesis in mammals (Zolghadri et al., 2019). Experimental evidence suggests *p*-coumaric acid is a potent inhibitor of tyrosinase, particularly toward human enzymes (An, Koh, & Boo, 2010), than other conventional tyrosinase inhibitors such as arbutin and kojic acid, so has good potential to be used as a skin-lightning element in cosmetics (Boo, 2019). Because of its various applications in food and pharmaceutical industries, researchers are keen to identify more potent sources of tyrosinase inhibitors.

In plants, *p*-coumaric acid is found in relatively low levels, from 0.5 mg/kg in blackberries to 830.3–1172.1 μg/g in *Juglans regia*, but in high levels in some species of mushrooms including *Ganoderma lucidum* (1386.4 mg/kg), *Termitomyces heimii* (3700 mg/kg), and *Cantharellus cibarius* (2420 mg/kg)—all the above quantities are dry weights (Pei, Ou, Huang, & Ou, 2016). In *G. indica, p*-coumaric acid content varied from 3266.67 mg/kg to 8093 mg/kg on fresh weight basis and from 13,066 mg/kg to 32,373 mg/kg on dry weight basis; the species is thus richer in *p*-coumaric acid than all the above sources and is a potential commercial source.

3.4.2. Flavonoids

All the 12 identified flavonoid compounds (Table 3) categorized into six groups, namely flavanones, flavones, flavonols, flavan-3-ols, flavonol glycosides, and coumarin derivatives. The main flavonoid found in *G. indica* was naringenin, followed by apigenin, quercetin, catechin, luteolin, hesperetin, and myricetin; the chromatograms of major flavonoids are given in Fig. 2.

Among the flavanones, naringenin was predominant, followed by hesperetin (Table 3): naringenin ranged from 1.5 mg/g in Vengurla to 6.8 mg/g in IIHR-2 and hesperetin, from 0.01 mg/g in IIHR-2 to 0.03 mg/g in Local. Among the two flavones identified, apigenin was predominant, ranging from 0.27 mg/g to 0.57 mg/g, IIHR-2 being the richest source. Among the three flavonols identified, quercetin was predominant, being significantly higher than myrecetin and kaempferol. In flavan-3-ols, catechin was predominant, followed by epicatechin and epigallocatechin. Umbelliferone was the single coumarin derivative identified in this study.

Flavonoids are known antioxidants and have antimutagenic, anti-inflammatory, anti-diabetic, antimicrobial, and antiviral effects. Flavonoids are commercially important for their health benefits, and their effects on mammals have been well studied (Panche, Diwan, & Chandra, 2016). Naringenin, a flavanone, has a diverse pharmacological activities and is known to inhibit microbial activity and that of G-6-P synthase, an important enzyme for the synthesis of cell walls in bacteria and fungi. Naringenin is a potential safe and natural preservative for food, pharmaceuticals, and cosmetics (Lather, Sharma, & Khatkar, 2020). The most important sources of naringenin (as given in the database of the United States Department of Agriculture and for every 100 g of fresh weight) are grapefruit (53 mg), pomelo (24 mg), pomelo juice (1.94–132.86 mg), and orange (3.65–45.42 mg) and Mexican oregano (335–418 mg/100 g dry weight) (Bhagwat, Haytowitz, & Holden, 2014). With naringenin content of 152–682 mg/100 g fresh weight, *G. indica* can also serve be a good source.

Additionally, rinds of *G. indica*, being rich in apigenin, can serve as a commercial source of this compound also. Apigenin has the potential to be anti-inflammatory, anti-toxicant, and anticarcinogenic, a combination that makes apigenin a potent therapeutic agent in treating such diseases as rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, and autoimmune disorders and various type of cancers (Ali, Rahul, Naz, Jyoti, & Siddique, 2017). Currently, rich sources of apigenin for commercial extraction are dried leaves of parsley (1174–13,506 mg/100 g; Bhagwat, Haytowitz, & Holden, 2014), *Adinandra nitida* (2500 mg/100 g; Liu, Ning, Gao, & Xu, 2008), and leaves of *Cajanus cajan* (13.2 mg/100 g; Fu et al., 2008)—with 111.8–231.8 mg/100 g (dry weight), *G. indica* can also be a new and potential source of apigenin.

3.5. Correlation analysis

Total phenolics and total flavonoids were significantly and positively correlated to their antioxidant capacity as determined by FRAP ($r = 0.96992$ for phenols; $r = 0.87487$ for flavonoids) and DPPH assays ($r = 0.89017$ for phenols; $r = 0.84743$ for flavonoids) (Table 4). Significant linear correlations between total phenolics content and antioxidant capacities were demonstrated in many plants (Othman, Muktar, Ismail, & Chang, 2014). On the other hand, we found no significant correlation between total anthocyanins and antioxidant activity ($r = 0.34276$ for FRAP; $r = 0.04215$ for DPPH). This finding corroborates the findings of Fawole, Opara, and Theron (2011) but not those of Zhang, Deng, Xu, Lu, and Wang (2016), who found total anthocyanins to be closely correlated to antioxidant capacity. Besides, the two assays, FRAP and DPPH, were also significantly correlated ($r = 0.85548$). Hydroxycitric acid content was significantly and negatively correlated to total phenols ($r = -0.73544$) and to antioxidant capacity as assayed by both FRAP ($r = -0.72991$) and DPPH ($r = -0.85048$) but was correlated neither to flavonoids content ($r = -0.60567$) nor to anthocyanins ($r = 0.07925$)

Table 3
Phenolic acids and flavonoids in different accessions of *Garcinia indica*.

Phenolic acids	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
	Fresh weight basis fw (μg/g)						Dry weight basis dw (μg/g)					
Caffeic acid	34.29^{def} ±3.78	16.11^{ef} ±0.98	30.91^{def} ±6.68	21.23^{ef} ±2.40	14.65^{ef} ±1.67	10.74^{f} ±2.01	137.15^{a} ±15.12	64.44^{bc} ±3.90	123.6^{a} ±26.73	84.92^{b} ± 9.62	58.60^{bcd} ± 6.69	42.96^{cde} ± 8.04
2,4-Dihydroxybenzoic acid	1.20^{cd} ± 0.26	0.59^{de} ± 0.10	0.12^{e} ±0.02	0.32^{de} ± 0.04	0.44^{de} ± 0.03	0.54^{de} ± 0.13	4.81^{a} ±1.02	2.36^{b} ± 0.38	0.48^{de} ± 0.07	1.28^{cd} ± 0.16	1.76^{bc} ±0.14	2.15^{bc} ±0.52
Chlorogenic acid	0.00^{a} ±0.00	0.00^{a} ±0.0	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ±0.00	0.00^{a} ± 0.00	0.00^{a} ±0.00	0.01^{a} ±0.01	0.00^{a} ±0.00
Ferulic acid	27.63^{c} ±6.03	31.56^{c} ±3.23	34.22^{c} ±5.05	23.20^{c} ±2.89	19.66^{c} ±1.51	19.51^{c} ±2.18	110.5^{ab} ± 24.13	126.2^{a} ±12.93	136.8^{a} ±20.20	92.82^{b} ± 11.56	78.62^{b} ± 6.05	78.02^{b} ± 8.73
Gallic acid	2.92^{e} ±1.05	2.11^{e} ±0.17	4.60^{de} ± 0.72	3.60^{de} ± 0.50	3.13^{e} ±0.32	3.04^{e} ±0.34	11.66^{bc} ±4.19	8.44^{cd} ± 0.69	18.40^{a} ±2.88	14.40^{ab} ± 2	12.52^{bc} ±1.30	12.16^{bc} ±1.37
Gentisic acid	0.13^{d} ± 0.01	0.97^{c} ±0.11	0.10^{d} ± 0.03	0.97^{c} ±0.06	0.52^{cd} ± 0.05	0.13^{d} ± 0.01	0.53^{cd} ± 0.05	3.88^{a} ±0.43	0.40^{d} ± 0.10	3.88^{a} ±0.25	2.08^{b} ± 0.21	0.53^{cd} ± 0.04
o-Coumaric acid	2963^{f} ±250	2201^{f} ±180	2522^{f} ±429	1633^{f} ±152	2105^{f} ±178	4046^{ef} ±481	11854^{b} ± 1002	8804^{cd} ± 720	10091^{bc} ±1716	6533de ± 611	8422^{cd} ± 712	16186^{a} ±1924
p-Coumaric acid	6027^{fg} ± 72	4502^{gh} ± 175	5101^{gh} ± 400	3266^{h} ± 230	4239^{gh} ± 211	8093^{f} ±290	24108^{b} ± 290	18009^{d} ± 701	20407^{c} ±1600	13066^{e} ±923	16958^{d} ± 845	32373^{a} ±1160
4-Hydroxy benzoic acid	3.76^{c} ±0.73	2.41^{c} ±0.67	2.53^{c} ±0.11	2.56^{c} ±0.51	2.12^{c} ±0.34	3.96^{c} ±0.32	15.03^{a} ±2.93	9.64^{b} ± 2.68	10.14^{b} ± 0.43	10.24^{b} ± 2.03	8.48^{b} ± 1.34	15.83^{a} ±1.28
Protocatechuic acid	110.0^{e} ±6.45	121.0^{de} ± 5.34	225.5^{d} ± 22.21	113.5^{e} ±12.59	161.4^{de} ± 6.14	115.4^{e} ±13.74	440.0^{c} ±25.81	484.0^{c} ±21.36	902.3^{a} ±88.83	454.0^{c} ±50.36	645.6^{b} ± 24.54	461.7^{c} ±54.96
Salicylic acid	91.48^{ef} ±4.75	75.28^{ef} ±8.40	132.3^{e} ±5.05	81.52^{ef} ±7.05	87.22^{ef} ±9.26	66.96^{f} ±3.55	365.9^{b} ± 19.01	301.1^{cd} ± 33.61	529.3^{a} ±20.20	326.1^{bc} ±28.20	348.9^{bc} ±37.04	267.8^{d} ± 14.21
Syringic acid	0.03^{f} ±0.02	0.22^{bcd} ± 0.03	0.07^{ef} ±0.01	0.07^{def} ±0.02	0.08^{def} ±0.02	0.05^{ef} ±0.01	0.12^{cdef} ±0.06	0.88^{a} ±0.11	0.26^{bc} ±0.06	0.28^{b} ± 0.07	0.33^{b} ± 0.06	0.19^{bcde} ± 0.04
trans-Cinnamic acid	15.21^{f} ±1.09	21.31^{f} ±1.82	81.61^{d} ± 7.70	37.31^{ef} ±4.12	61.23^{de} ± 2.80	30.46^{ef} ±2.95	60.84^{de} ± 4.36	85.24^{d} ± 7.28	326.4^{a} ±30.78	149.2^{c} ±16.50	244.9^{b} ± 11.21	121.8^{c} ±11.80
Vanillic acid	0.80^{b} ± 0.29	0.59^{b} ± 0.14	0.81^{b} ± 0.05	0.56^{b} ± 0.05	0.65^{b} ± 0.07	0.60^{b} ± 0.18	3.19^{a} ±1.17	2.36^{a} ±0.54	3.23^{a} ±0.18	2.22^{a} ±0.19	2.60^{a} ±0.29	2.39^{a} ±0.70
Sinapic acid	0.11^{d} ± 0.06	0.09^{d} ± 0.01	0.03^{d} ± 0.02	0.04^{d} ± 0.01	0.49^{b} ± 0.02	0.03^{d} ± 0.00	0.44^{b} ± 0.23	0.36^{bc} ±0.04	0.11^{d} ± 0.06	0.15^{cd} ± 0.05	1.96^{a} ±0.08	0.12^{d} ± 0.02
3-Hydroxy benzoic acid	0.02^{d} ± 0.02	0.11^{cd} ± 0.04	0.11^{cd} ± 0.03	0.02^{d} ± 0.01	0.29^{bcd} ± 0.02	0.16^{cd} ± 0.10	0.09^{cd} ± 0.07	0.44^{bc} ±0.17	0.43^{bc} ±0.12	0.08^{cd} ± 0.03	1.16^{a} ±0.07	0.64^{b} ± 0.39
Benzoic acid	1.71^{f} ±0.36	1.22^{f} ±0.19	4.72^{ef} ±0.46	2.51^{f} ±0.46	3.14^{ef} ±0.11	3.73^{ef} ±0.83	6.83^{de} ± 1.45	4.88^{ef} ±0.76	18.90^{a} ±1.85	10.04^{cd} ± 1.83	12.56^{bc} ±0.44	14.9^{2ab} ±3.33
Ellagic acid	1.55^{f} ±0.17	1.21^{f} ±0.26	3.51^{def} ±0.50	1.31^{f} ±0.26	1.21^{f} ±0.1	2.58^{ef} ±0.24	6.20^{c} ±0.67	4.84^{cde} ± 1.05	14.05^{a} ±2.00	5.24^{cd} ± 1.05	4.84^{cde} ± 0.40	10.32^{b} ± 0.97
Flavonoids												
Apigenin	279.7^{e} ±41.55	419.5^{e} ±103	468.7^{e} ±56.73	297.9^{e} ±11	509.3^{e} ±22.80	579.6^{de} ± 100	1118^{cd} ± 166	1677^{bc} ±412	1874^{ab} ± 226	1191^{c} ±44.01	2037^{ab} ± 91.19	2318^{a} ±403
Catechin	29.53^{b} ± 7.43	25.89^{b} ± 5.23	27.11^{b} ± 3.01	24.81^{b} ± 6.02	25.89^{b} ± 4.73	31.11^{b} ± 6.38	118.1^{a} ±29.73	103.5^{a} ±20.93	108.4^{a} ±12.02	99.24^{a} ±24.08	103.5^{a} ±18.91	124.4^{a} ±25.5
Hesperetin	17.35^{e} ±2.70	31.47^{cde} ± 5.86	26.12^{de} ± 2.52	20.39^{de} ± 6.20	24.33^{de} ± 2.09	15.26^{e} ±10.72	69.40^{bcd} ± 10.80	125.9^{a} ±23.45	104.5^{ab} ± 10.07	81.5^{abc} ±24.8	97.30^{ab} ± 8.36	61.03^{bcde} ± 42.88
Kaempferol	0.05^{bcde} ± 0.02	0.03^{e} ±0.01	0.03^{de} ± 0.01	0.04^{cde} ± 0.01	0.03^{de} ± 0.01	0.04^{cde} ± 0.01	0.19^{a} ±0.08	0.11^{abcde} ±0.05	0.14^{abcde} ±0.02	0.14^{abc} ±0.06	0.14^{abcd} ±0.05	0.15^{ab} ± 0.02
Luteolin	37.64^{bc} ±16.14	27.31^{c} ±6.41	24.78^{c} ±3.03	21.55^{c} ±4.33	29.87^{c} ±4.38	35.54^{bc} ±12.32	150.5^{a} ±64.56	109.2^{ab} ± 25.62	99.11^{abc} ±12.11	86.21^{abc} ±17.34	119.5^{a} ±17.5	142.1^{a} ±49.27
Myricetin	10.38^{de} ± 1.68	5.38^{e} ±0.66	7.23^{de} ± 0.85	5.38^{e} ±0.96	9.11d^{e} ± 1.64	14.42^{cd} ± 3.16	41.51^{b} ± 6.71	21.53^{cd} ± 2.64	28.93^{bc} ±3.40	21.53^{cd} ± 3.84	36.45^{b} ± 6.57	57.69^{a} ±12.64
Naringenin	5820^{e} ±231	2463^{f} ±212	3543^{f} ±387	1526^{f} ±90	2774^{f} ±155	6820^{e} ±277	23280^{b} ± 925	9852^{d} ± 851	14172^{c} ±1551	6107^{e} ±360	11096^{d} ± 622	27280^{a} ±1111
Quercetin	139.1^{b} ± 7.39	139.1^{b} ± 14.3	127.6^{b} ± 5.73	27.38^{c} ±6.25	135.1^{b} ± 8.94	137.2^{b} ± 8.78	556.5^{a} ±29.56	556.6^{a} ±57.41	510.5^{a} ±22.91	109.5^{b} ± 24.98	540.7^{a} ±35.76	548.9^{a} ±35.11
Rutin	4.30^{bcd} ± 0.95	2.65^{cd} ± 0.31	2.73^{cd} ± 0.25	3.02^{cd} ± 0.32	3.01^{cd} ± 1.99	2.19^{d} ± 0.74	17.21^{a} ±3.80	10.60^{abc} ±1.22	10.92^{abc} ±1.01	12.08^{ab} ± 1.26	12.03^{ab} ± 7.96	8.75^{bcd} ± 2.95
Umbelliferone	0.03^{bc} ±0.02	0.02^{b} ± 0.01	0.03^{bc} ±0.01	0.03^{bc} ±0.02	0.03^{bc} ±0.01	0.05^{bc} ±0.01	0.14^{abc} ±0.08	0.10^{abc} ±0.02	0.10^{abc} ±0.02	0.14^{ab} ± 0.08	0.13^{abc} ±0.02	0.19^{a} ±0.05
Epicatechin	3.72^{e} ±1.80	6.20^{de} ± 1.05	4.18^{e} ±0.68	2.89^{e} ±1.09	8.83^{cde} ± 0.24	3.52^{e} ±0.43	14.88^{cd} ± 7.21	24.79^{b} ± 4.23	16.73^{bc} ±2.72	11.57^{cde} ± 4.37	35.31^{a} ±0.95	14.10^{cd} ± 1.71
Epigallocatechin	3.03^{d} ± 1.53	20.23^{cd} ± 4.67	10.55^{d} ± 1.50	2.18^{d} ± 0.52	15.32^{d} ± 4.05	3.02^{d} ± 0.45	12.11^{d} ± 6.11	80.92^{a} ±18.70	42.19^{bc} ±6.01	8.73^{d} ± 2.09	61.30^{ab} ± 16.20	12.09^{d} ± 1.81

Values represent the mean (n = 9) of three independent experiments.
Mean followed by the same superscripts are not significantly different referring to Tukey's HSD test ($p < 0.05$).
G1: *G. indica* "Red"; G2: *G. indica* "Local"; G3: *G. indica* "Amruth kokum"; G4: *G. indica* "Vengrula"; G5: *G. indica* "IIHR-1"; G6: *G. indica* "IIHR-2".

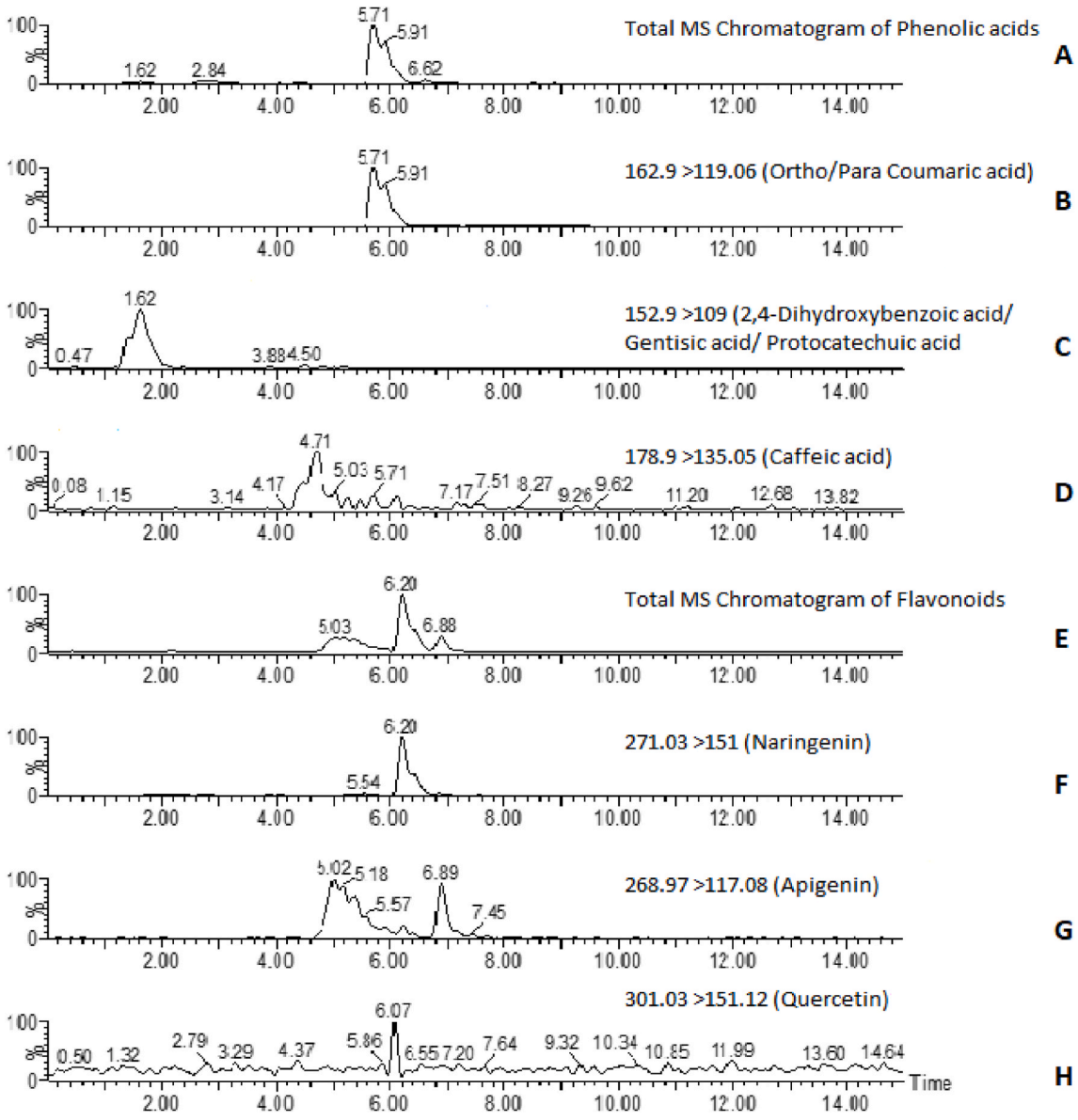

Fig. 2. Chromatogram of major phenolic acids and flavonoids from *Garcinia indica* rind using LC-MS/MS. (A) Total MS chromatogram of phenolic acids; (B) MS/MS chromatogram of ortho/para coumaric acid; (C) MS/MS chromatogram of protocatechuic acid; (D) MS/MS chromatogram of caffeic acid; (E) Total MS chromatogram of flavonoids; (F) MS/MS chromatogram of naringenin; (G) MS/MS chromatogram of apigenin; (H) MS/MS chromatogram of quercetin.

Table 4
Correlation between the total phenolics, total flavonoids, anthocyanin, hydroxycitric acid (HCA) content and antioxidant potential with 4 digitals after decimal point.

	Phenol	Flavanoid	Anthocyanin	FRAP	DPPH	HCA
Phenol	1.00000					
Flavanoid	0.94275	1.00000				
	<.0001					
Anthocyanin	0.22080	0.18757	1.00000			
	0.3786	0.4561				
FRAP	0.96992	0.87487	0.34276	1.00000		
	<.0001	<.0001	0.1638			
DPPH	0.89017	0.84743	0.04215	0.85548	1.00000	–
	<.0001	<.0001	0.8681	<.0001		
HCA	−0.73544	−0.60567	0.07925	−0.72991	−0.85048	1.00000
	0.0005	0.0077	0.7546	0.0006	<.0001	

FRAP: Ferric reducing antioxidant power.
DPPH: 2,2-Diphenyl-1-picrylhydrazyl radical scavenging ability.
HCA: Hydroxycitric acid.

(Table 4).

3.6. Principal component analysis and clustering

To increase the interpretability of the data set, 36 quantitative traits (phenolic and flavonoid compounds, total phenols, total flavonoids, anthocyanins, antioxidant activity by FRAP and DPPH, and HCA) were subjected to principal component analysis (PCA). Eigenvectors for the first four principal components (PC1 to PC4) are given in supplementary material 5. The first principal component (PC1) accounted for most of the variation: it explained 93.05% of the total variation and was positively correlated to HCA, apigenin, catechin, and hesperetin but negatively correlated to naringenin, *p*-coumaric acid, *o*-coumaric acid, phenol, flavonoids, FRAP, and DPPH (Fig. 3A). The second principal component (PC2) explained only 6.401% of total variation and was positively correlated to naringenin, *p*-coumaric acid, phenol, flavonoids, antioxidant activity by FRAP and DPPH, and HCA but negatively to apigenin (Fig. 3A). The biplot analysis of components showed that the parameters which lied in same quarter of the plot have a strong correlation. For example, total phenols, total flavonoids and antioxidant potential analysed by two methods had a significant positive correlation (Table 4), and they occupied the same quarter in the biplot (Fig. 3A).

Two-way hierarchal cluster analysis was performed using hclust function and the Ward method using R (Fig. 3B). The six accessions fell into two clusters: one comprised Red and IIHR-2, both rich in total phenols and with high antioxidant potential, whereas the other comprised accessions were low in total phenols and with poor antioxidant potential, namely Local, Amruth Kokum, Vengurla, and IIHR-1, although Local and Vengurla were rich in HCA.

Heat map enabled easy depiction of the variability of analysed parameters among the accessions. For example, IIHR-2 found to be richest source of anthocyanin, coumaric acid, naringenin, and apigenin which was clearly visible in heat map (Fig. 3B). Two-way hierarchal clustering and a heat map helped in choosing the best accessions with optimal quality parameters.

4. Conclusions

In the present study, principal bioactive compounds present in the fruit of *G. indica*, an edible fruit of medicinal value and native to Asia, were investigated using liquid chromatography-mass spectrometry (LC-MS). To our knowledge, this is the first report on *G. indica*, an underexplored species, as a rich source of hydroxycitric acid, colour pigments, and phenolic compounds, all of which make the species particularly promising in food and pharmaceutical industries. Anthocyanin and phenolic profiling of six accessions of *G. indica* made it possible to determine the composition of anthocyanin, phenolic acid, and flavonoids. Thirty phenolic compounds were successfully separated,

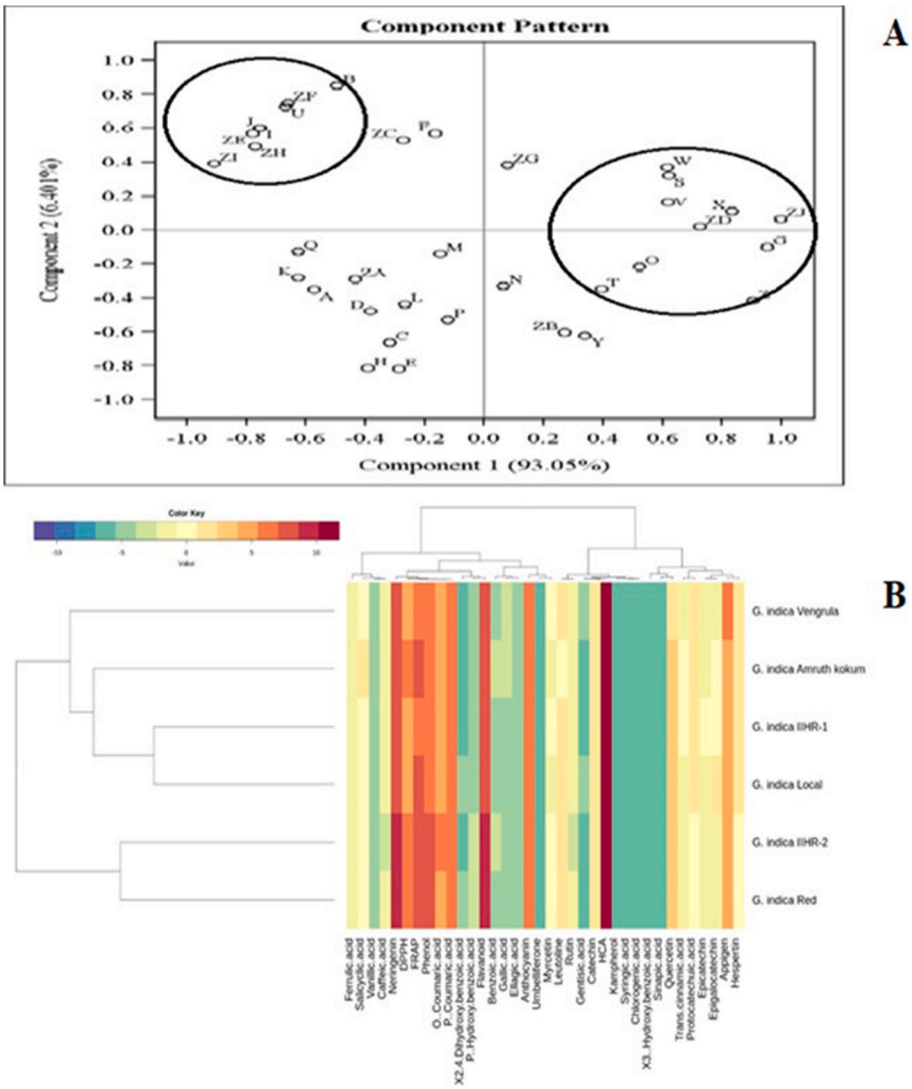

Fig. 3. Principal component analysis (PCA) and clustering of *Garcinia indica* accessions. (A) Biplot of PC1 and PC2 of *Garcinia indica* accessions based on biochemical constituents. ZJ– HCA; X–Catechin; T–Apigenin; I– *o*-Coumaric acid; J– *p*-Coumaric acid; U–Naringenin; ZE–Total phenol; ZF–Total flavonoids; ZH–FRAP; ZI–DPPH. Details of code for biochemical componentis given in supplementary material 5. (B) Two-way hierarchal clustering of *Garcinia indica* accessions using Ward's aggregation method in R programming.

identified, and quantified; these included *para* and *ortho* coumaric acid, naringenin, and apigenin. These compounds are known to be natural preservatives and anti-browning and therapeutic agents. Two-way hierarchal cluster analysis and heat map enabled easy selection of accessions with optimal quality parameters. Promising accessions were identified for hydroxycitric acid (*G. indica* 'Vengurla') and for anthocyanin and phenolic compounds (*G. indica* 'IIHR-2'). Such data on the biochemical composition of *G. indica* will help in promoting this fruit as a promising source of hydroxycitric acid, anthocyanin, coumaric acid, naringenin, and apigenin.

CRediT authorship contribution statement

Pritee Singh: Conceptualization, Investigation, Formal analysis, Methodology, Visualization, Data curation, Statistical analysis, Supervision, Validation, Writing – original draft, Writing – review & editing, Reviewing and Editing. **T.K. Roy:** Methodology, Validation. **C. Kanupriya:** Germplasm collection and maintenance, Formal analysis, Writing – review & editing. **P.C. Tripathi:** Germplasm collection and maintenance, Supervision. **Prakash Kumar:** Formal analysis, Statistical analysis. **K.S. Shivashankara:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful to ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India for providing financial support for this work. We are also thankful to Dr. Vasantha Kumar T., Division of Medicinal Crops, ICAR-IIHR, Bengaluru for some of the accessions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112999.

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