

Estimation and Comparison of Amount of Organic Acids from Dried Leaves of *Garcinia cambogia*, *Garcinia indica*, *Garcinia xanthochymus*, and *Garcinia morella* by High-Performance Liquid Chromatography

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ABSTRACT

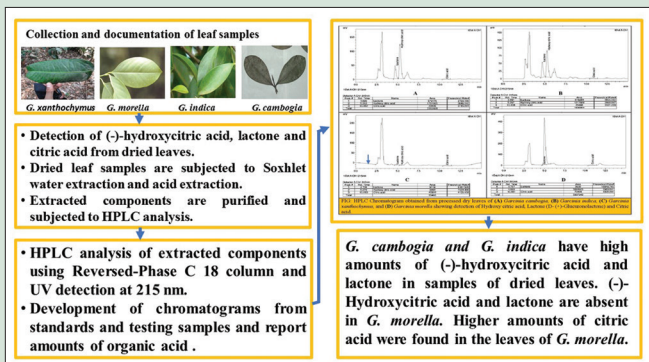
Background: Pharmacological properties of (-)-hydroxycitric acid (HCA) derived from plants are extensively investigated for preventing lipid storage and controlling appetite. The *Garcinia* species is most sought out species to recover (-)-HCA. **Objectives:** Organic acids from dried leaves of *Garcinia cambogia*, *Garcinia indica*, *Garcinia xanthochymus*, and *Garcinia morella* were extracted and analyzed by high-performance liquid chromatography. **Materials and Methods:** Oven-dried leaves of the four plant samples were subjected to Soxhlet water extraction and further acid extraction. After evaporation, the residue was mixed with 50 ml, 30% orthophosphoric acid. 20 µL of the sample was injected to a reversed-phase C 18 column under gradient elution solvent containing 0.2 M sodium sulfate with a pH of 2.5 adjusted with dilute H₂SO₄. At a flow rate of 0.5 mL/min, reading was taken using ultraviolet detection at 215 nm. Chromatograms of (-)-HCA, lactone, and citric acid were obtained for standards and the samples. **Results:** The amount of (-)-HCA, lactone, and citric acid in the dry leaves of *G. cambogia* was estimated at 7.95%w/w, 3.25%w/w, and 0.13%w/w, respectively. In *G. indica*, the components were estimated at 5.71%w/w, 3.21%w/w, and 0.07%w/w, respectively. In the dry leaves of *G. xanthochymus*, it was estimated at 0.02%w/w, 0.06%w/w, and 0.18%w/w. In *G. morella*, it was estimated at 0.0%w/w, 0.01%w/w, and 0.29%w/w. *G. cambogia* and *G. indica* have high amounts of (-)-HCA and lactone in samples of dried leaves. (-)-HCA and lactone are absent in *G. morella*. Higher amounts of citric acid were found in the leaves of *G. morella*.

Key words: Citric acid, *Garcinia*, high-performance liquid chromatography, hydroxycitric acid, lactone, organic acids

SUMMARY

- Organic acids from dried leaves of *Garcinia* species from the Western Ghats were analyzed by high-performance liquid chromatography. Extraction protocol and analysis of amounts of (-)-hydroxycitric acid, lactone, and citric acid

recovered from *Garcinia cambogia*, *Garcinia indica*, *Garcinia xanthochymus*, and *Garcinia morella* are reported. The protocols can be utilized in recovering HCA, which is a pharmacologically important drug. The research can boost cultivation of these species around these areas.



Abbreviations Used: HCA: Hydroxycitric acid; HPLC: High-performance liquid chromatography.

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INTRODUCTION

Obesity has a negative effect on human health. There is an ever-increasing need for research in anti-obese drugs as the effectiveness of the drugs for Humans available in the market today is not clearly justified. Sales of dietary supplements have increased over the years. Particularly, the use of dietary supplements extracted from plants has gained importance. Compounds such as galactomannan polysaccharide, (-)-hydroxycitric acid (HCA), xanthigen, and epigallocatechin are extensively used for their pharmacological properties of preventing lipid storage, controlling appetite, and increasing exercise endurance in humans. (-)-HCA is extracted from fruits of the plant *Garcinia cambogia* and is used worldwide as a dietary supplement drug for anti-obesity effect. *Garcinia* the genus belongs to the family *Guttiferae* and has many economically important species. The plant species selected for the present investigation are *G. cambogia*, *Garcinia indica*, *Garcinia xanthochymus*, and *Garcinia morella*. The plants that are selected are important forest trees and these are cultivated in the areas surrounding the Western Ghats regions in

South India. The fruits of these species are economically important. These species of trees are also considered as an important forest species. The ethnobotanical and pharmacological effects of *Garcinia* fruits are investigated by scientist extensively.^[1,2] The pharmacological effects of the fruits and leaves have been extensively used in ethnomedicinal investigation by many tribes that are inhabitants of this region. The presence of these fruits in regular food preparations in the states of

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Karnataka and Kerala in India has made researcher investigate these plants extensively. The leaves of these plant species have important organic acids and this fact is reported in the ethnomedicinal folklore. Organic acids have many roles in the plant metabolism.^[3] They are end products and intermediates of metabolic pathways in plants. The recent interest in the fruits of *G. cambogia* for extraction of anti-obese drug, which contains (-)-HCA, has increased the exploitation of fruits from the forest trees in the Western Ghats area of South India.^[4,5] Effectiveness of this dietary supplement in reducing body weight has been extensively investigated.^[6] Tapping the potential of these drugs from the leaves is a viable source in extractions. Derivatives of (-)-HCA are used in combination drugs that are prescribed for increasing weight loss and correcting the abnormalities of lipid metabolism.^[7,8] There are four isomers of HCA: (+)-HCA and (-)-HCA and (+)-allo-HCA and (-)-allo-HCA. The (-)-HCA isomer is the one found in *Garcinia* fruits and leaves.^[9] It is proven that the rind of the fruit from *Garcinia cambogia* contains high amounts of HCA. Lactones from plants have been used in drugs targeting cardiovascular disease and cancer.^[10] In plant leaves, lactones are mainly saturated and unsaturated γ - and δ -lactones. Various methods of high-performance liquid chromatography (HPLC) elucidation and estimation of organic acids from *Garcinia* plants are investigated and suggested by researchers.^[11] In the present investigation, a modified rapid method is developed and used to estimate the amount of HCA, lactone, and citric acid from leaves of four different plants.^[12] The investigation also gives a comparative account of HCA, lactone, and citric acid from leaves of the four species. This study suggests that leaves can be a potential plant part from which extraction of the organic acids can be achieved for commercial isolation of HCA. The work also indicates the quantity of HCA, lactone, and citric acid in %w/w present in the leaves of these species.

MATERIALS AND METHODS

Materials

Leaf samples from *G. cambogia*, *G. indica*, *G. xanthochymus*, and *G. morella* were collected from Virajpet Taluk, Kodagu District, Karnataka, India. The GPS location of the trees is recorded in Table 1. The collected leaf samples were identified in the Department of Botany, St. Joseph's College, and herbarium was documented at the Department of Biotechnology, St Joseph's College. The leaves were oven-dried at 45°C for 8 h under forced air ventilation. Extraction of organic acids was done with HPLC grade water, orthophosphoric acid, and sulfuric acid. Waters Cortecs, reversed-phase C 18, 2.7 μ m with spherical solid-core, 4.6 mm \times 150 mm column was used for separation.

Preparation of sample for analysis using high-performance liquid chromatography

Dried leaves of the four samples were subjected to Soxhlet water extraction by taking 10 g in 50 ml double distilled H₂O and at 100°C boiling for 45 min. The extract was subjected to evaporation and residue was mixed with 50 ml, 30% orthophosphoric acid.

Preparation of standards for comparative analysis using high-performance liquid chromatography

Working standards were prepared by ethylenediamine salt of HCA, lactone (D-(+)-Glucuronolactone), and citric acid obtained from Merck life Sciences Pvt. Ltd. Mumbai.

Five milligrams of ethylenediamine salt of HCA was dissolved by adding 5 mL of 50% H₂SO₄ which was made up to 10 mL volume by HPLC grade water. The stock solution was prepared with 500 μ g/mL of HCA. Lactone standards were prepared by dissolving the lactone (D-(+)-glucuronolactone)

Table 1: Location of plants on GPS coordinates

Plant species	GPS coordinates	
<i>Garcinia cambogia</i>	12°05'26.0"N	76°02'03.1"E
<i>Garcinia indica</i>	12°05'30.8"N	76°01'59.3"E
<i>Garcinia xanthochymus</i>	12°05'33.0"N	76°01'48.5"E
<i>Garcinia morella</i>	11°59'41.5"N	76°04'01.9"E

GPS: Global positioning system

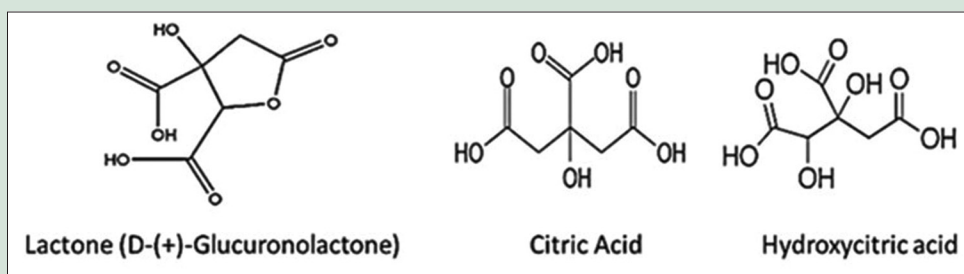
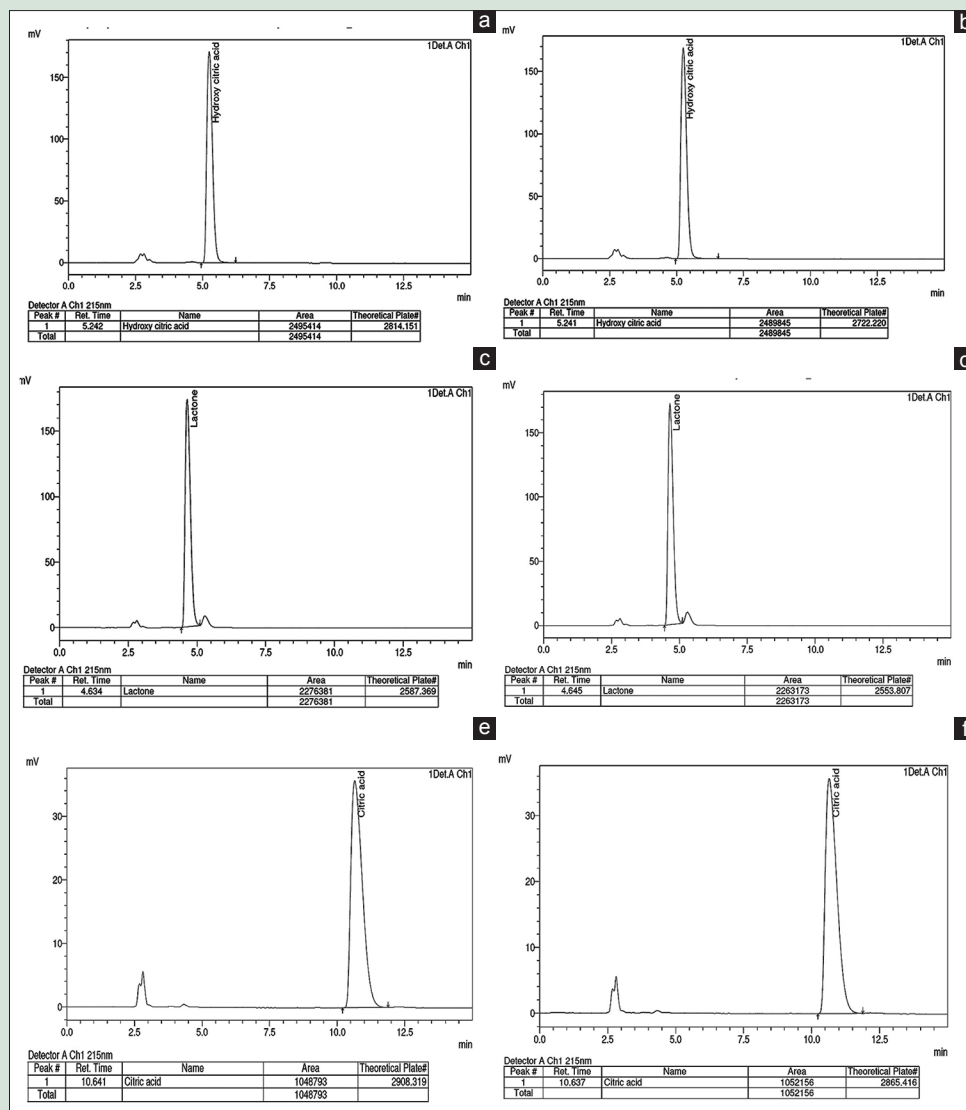
salts in HPLC grade water. Citric acid salts were also dissolved in HPLC grade water. A concentration of 500 μ g/mL was maintained for the standard solvents of lactone and citric acid.^[13]

Comparative analysis using high-performance liquid chromatography

200 μ L of the standard samples was filtered using a 0.45- μ filter and 20 μ L of each was injected to a Shimadzu chromatographic system LC 8A model dual pump, photodiode array detector (SPD-M10AVP), and Class LC-10 software. HPLC results were obtained through Waters Cortecs, reversed-phase C 18, 2.7 μ m with spherical solid-core, 4.6 mm \times 150 mm column under gradient elution solvent containing 0.2 M sodium sulfate with a pH of 2.5 adjusted with dilute H₂SO₄. The flow rate of 0.5 mL/min is maintained and reading taken using ultraviolet (UV) detection at 215 nm. Different trail sets were injected to obtain the accuracy in evaluation.

RESULTS

(-)-HCA, lactone, and citric acid in the dried leaves were extracted from the processed leaves. The collected leaf samples are from areas which have an average rainfall of 2000 mm. The GPS coordinates of the location of the plants are recorded [Table 1] from the regions of the Western Ghats belt in South India. Various extraction protocols have been investigated by researchers. The stability of (-)-HCA after these extractions is also investigated.^[14] The present protocol is a rapid method that is adopted by construction from the ethnomedical knowledge heard among the people of that region. The amounts of organic acids extracted are prerequisites for herbal formulations. The pharmacological activities of organic acids are reported even in minute quantities.^[15] The structural details of the organic acids are shown in Figure 1. The representation of data from two trails sets of HPLC chromatograms obtained for the standards are shown in Figure 2. The representation of HPLC chromatograms for the dried leaves of the four species of plants are shown in Figure 3. The values obtained for recovery data of organic acids [Table 2] is represented in w/w%. The detecting wavelength is 215 nm. Data of retention time in minutes are taken from two trails sets. Based on the area obtained, the amounts were calculated using the lab solution software Shimadzu Analytical (India) Pvt. Ltd.^[16,17] Quantitative analysis was obtained by measuring the peak responses data of standards [Table 3] to that of the testing extracts [Table 4]. The values from two trails were recorded, and the average mean was obtained as the final amount. As reported in Table 2, the amount of (-)-HCA, lactone, and citric acid in the dried leaves of *G. cambogia* was estimated at 7.95%w/w, 3.25%w/w, and 0.13%w/w, respectively. The amount of (-)-HCA in *G. indica* was estimated at 5.71%w/w, 3.21%w/w, and 0.07%w/w, respectively. The fruits from *G. cambogia* and *G. indica* yield up to 65%w/w and 23%w/w of (-)-HCA.^[18] The amount of (-)-HCA, lactone, and citric acid in *G. xanthochymus* was estimated at 0.02%w/w, 0.06%w/w, and 0.18%w/w, respectively. The results show that the amount of HCA and lactone recorded is nil. The presence of citric acid is seen in the extraction and this is not a derivative salt of HCA. The amount of (-)-HCA, lactone and citric acid in *G. morella* was estimated at 0.0%w/w, 0.01%w/w, and 0.29%w/w, respectively. *G. morella* does not contain HCA and lactone in dried leaf samples. *G. cambogia* and *G. indica* have high amounts of (-)-HCA and

**Figure 1:** Structure of organic acids**Figure 2:** High-performance liquid chromatogram representation: (a and b) standard ethylenediamine salt of hydroxy citric acid, (c and d) standard lactone (D-(+)-Glucuronolactone), and (e and f) standard citric acid

lactone in samples of dried leaves. (-)-HCA and lactone are absent in *G. morella*. High amounts of citric acid were found in the leaves of *G. morella*.

DISCUSSION

Rapid and sensitive methods in isolation of HCA and lactones from the fruits of *G. indica* have been developed.^[19] In the current investigation,

a different method has been reported in isolating HCA from the leaves. HCA preparation as tablets has been used to treat obesity and attempts to check its effects on rats have shown positive results.^[20] The HCA from leaves recovered here can be utilized for the same set of formulations. The formulations can be tested on rats for its activity on lipids. The application of HPLC to recover medicines from plants have been achieved in Chinese medication.^[21] In this investigation, HPLC has helped in recovery of the

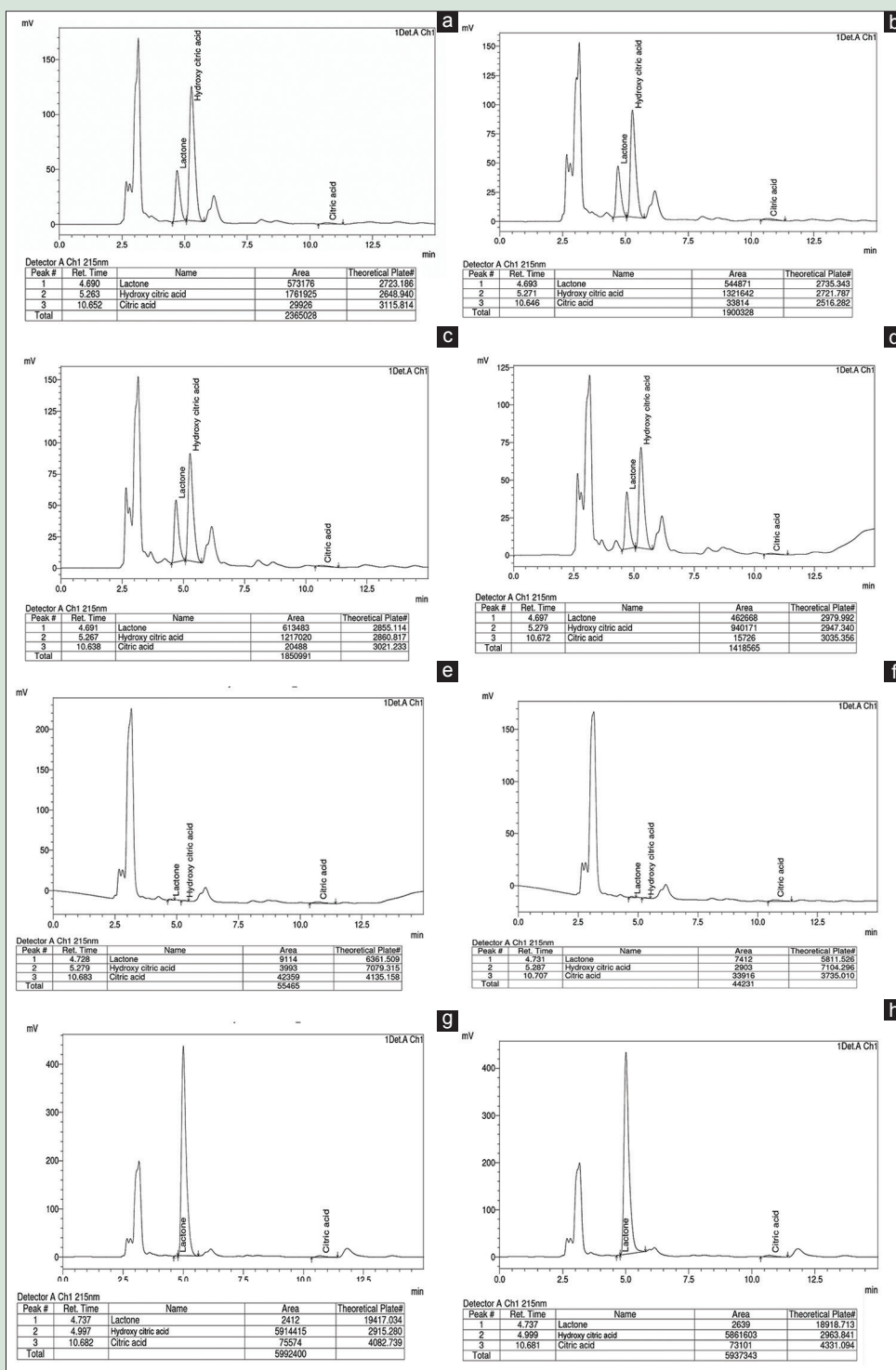


Figure 3: High-performance liquid chromatogram representation of processed dry leaves showing detection of hydroxy citric acid, lactone (D-(+)-Glucuronolactone), and citric acid in *Garcinia cambogia* (a and b); *Garcinia indica* (c and d); *Garcinia xanthochymus* (e and f); and *Garcinia morella* (g and h)

components from the leaves of the *Garcinia* species. HCA recovered from fruits of *G. cambogia* was formulated to about 0.5 g of salts from a 1 g containing solution.^[22] The highest recovery in the present investigation was 7.95%w/w; it will account to about 0.07% final formulation. *G. indica* fruit formulations containing lactones ranging from 0.5% to 2% have shown antidepressant and anxiolytic effects in rats.^[23] The highest recovery of lactones has been 3.25%w/w and can be utilized for comparative study

after formulations by testing it against rats. Considering the fact that leaves are not used in extraction, the current investigation suggests that it is a viable option. This is the first attempt in comparison and *G. cambogia* leaves have the highest amount. Harvesting these leaves can yield organic acids and exploitation of the fruits will reduce in forest areas. The attempt to extract the organic acids from dried leaves will become an alternative to the traditional methods involving fruits. The utility of leaves in

Table 2: Recovery data of the organic acids, (-)-hydroxycitric acid, lactone, and citric acid in *Garcinia cambogia*, *Garcinia indica*, *Garcinia xanthochymus*, and *Garcinia morella*

Parameter	<i>Garcinia cambogia</i> Assay (%w/w)	<i>Garcinia indica</i> Assay (%w/w)	<i>Garcinia xanthochymus</i> Assay (%w/w)	<i>Garcinia morella</i> Assay (%w/w)
HCA	7.95	5.71	0.02	Not detected
Lactone	3.27	3.21	0.06	0.01
Citric acid	0.13	0.07	0.18	0.29

HCA: Hydroxycitric acid

Table 3: Recorded values in trails for retention time and area for standard ethylene diamine salt of hydroxycitric acid, standard lactone (D-(+)-glucuronolactone), and standard citric acid

Contents	Trail number	Detecting (nm)	Retention time (min)	Area
Standard hydroxycitric acid	1	215	5.242	2495414
	2	215	5.241	2489845
Standard lactone	1	215	4.645	2263173
	2	215	4.634	2276381
Standard citric acid	1	215	10.641	1048793
	2	215	10.637	1052156

Table 4: Recorded values in trails for retention time and area for the organic acids, (-)-hydroxycitric acid, lactone, and citric acid in *Garcinia cambogi*, *Garcinia indica*, *Garcinia xanthochymus*, and *Gracinia morella*

Sample	Trail number	Type organic acid	Detecting (nm)	Retention time (min)	Area
<i>Garcinia cambogia</i>	1	HCA	215	5.2634	1,761,925
		Lactone	215	4.690	573,176
		Citric acid	215	10.652	29,926
	2	HCA	215	5.271	1,321,642
		Lactone	215	4.693	544,871
		Citric acid	215	10.645	33,814
<i>Garcinia indica</i>	1	HCA	215	5.267	1217,020
		Lactone	215	4.691	613483
		Citric acid	215	10.638	20488
	2	HCA	215	5.297	940171
		Lactone	215	4.697	462668
		Citric acid	215	10.672	15726
<i>Garcinia xanthochymus</i>	1	HCA	215	5.297	9114
		Lactone	215	4.728	3993
		Citric acid	215	10.683	42359
	2	HCA	215	4.731	2903
		Lactone	215	5.287	7412
		Citric acid	215	10.707	33916
<i>Garcinia morella</i>	1	HCA	215	4.997	5914415
		Lactone	215	4.737	2412
		Citric acid	215	10.682	75574
	2	HCA	215	5861603	4.999
		Lactone	215	2639	4.737
		Citric acid	215	73101	10.681

HCA: Hydroxycitric acid

recovery of these pharmacological important compounds will also boost cultivating the plants at horticultural level.

CONCLUSION

The amount of organic acids in leaves is less compared to the amounts in the fruits. The leaves may not serve as a commercial sample that can be used for extractions of these organic acids. This is because the organic acid recovery is less from leaves.^[24] The current investigation suggests a rapid method to extract organic acids from leaves and can be further explored to recover more amounts. A comparative quantitative account from the same procedure in extraction and analysis used for leaves across species is recorded for the first time in this investigation. However, most of the commercially available HCA is from fruit samples. Leaves would be the next source. The amount of (-)-HCA in *G. cambogia* and

G. indica is 7.95% w/w and 5.71% w/w which was the highest among the species investigated. The intake of HCA at levels up to 2800 mg/day is safe for human consumption.^[25] Hence, oven-dried leaves can be used for extractions of (-)-HCA by method suggested in the procedure and utilized for the same. The leaves of *G. morella* contain 0.29%w/w citric acid, this amount reported is less, but it can still be used for extractions. The investigation can be attempted in different months of the year, and the data can be correlated to see if the organic acids would increase or decrease in different seasons. Further stability analysis of HCA, lactone and citric acid in comparison to fruits can be investigated.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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