Plasticity for (-) - Hydroxycitric acid (HCA) content in ecotypes of Garcinia indica (Kokum) of Western Ghats

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Abstract

Western Ghats of India is the centre for biological diversity many plant species including Garcinia indica. Biologically important compound (-) - Hydroxycitric acid (HCA) is a popular anti-obesity compound extracted from the fruits of Garcinia indica. The present study was conducted to understand the extent of variation for the content of (-)-HCA in the fruits of Garcinia indica ecotypes prevailing in Western Ghats of Karnataka. The autoclave assisted extraction and the spectrophotometric analysis of 120 ecotypes of G. indica revealed significant variation in HCA content in the fruits. The average per cent HCA content on dry weight basis in fruits of G. indica was 17.90. The average mean HCA content of G. indica ecotypes in Uttara Kannada region (regarded as Uttara Kannada population) was highest (20.60%), compared to other ecotypes. The results indicated that there is a good genetic plasticity for HCA content among the ecotypes of G. indica prevailing in Western Ghats of Karnataka and there exists ample opportunity to exercise selection for the genotypes with higher (-)-HCA content.

Keywords: Hydroxycitric acid, Garcinia indica, Western Ghats

Introduction

The genus Garcinia comprises about 250 species in which 43 species are found in India, distributed across the tropical forests and adjoining main lands, and exhibit fair amount of morphological and phytochemical diversity Anu et al., 2016 [1], Parthasarathy et al., 2014 [11] and Sivu et al., 2017 [17]. The species Garcinia indica is endemic to Western Ghats of India is an important species of the genus Garcinia (Shameer et al., 2016) [14]. Garcinia species in general and Garcinia indica in particular are known to have rich diversity in their phytochemical traits of food and nutritional importance to human (Jena et al. 2002) [6] (Anu et al., 2016) [1]. Among the chemical compounds found in Garcinia species, the major compound is (-) - Hydroxycitric acid (HCA) which is used as an anti-obesity principle (Jayaparaksha 2002). It is a derivative of citric acid and a chiral compound (Jena et al. 2002) [6]. HCA has been found to have anti-obesity activity by inhibiting lipid synthesis in the body (Lewis and Neelakantan, 1965) [8]. The compound (-)- Hydroxycitric acid is chiral in nature and has an additional –OH group when compared to citric acid. Further, there are also two asymmetrical carbons rendering the compound to assume four isomer forms; all these confirmations possibly impart health benefit properties to the molecule (Gogoi et al., 2014) [13]. Primarily the (-)-HCA has made the fruits of Garcinia species and their products popular in international market for anti-obesity properties in human.

Presence of two hydroxyl and three carboxyl groups makes (-) -HCA an unstable compound and therefore it easily tends to form lactones with cations like Ca²⁺, K⁺ etc. (Antony et al., 1998). The Potential of weight loss property in human upon consuming (-) -HCA is aids by their ability of increasing lipid metabolism (Shrikantan et al., 2014) [16]. ATP citrate oxaloacetate lyase (citrate lyase) is involved in catalyzing the cleavage reaction of citrate into oxaloacetate and acetyl CoA (Jena et al., 2002) [6]. By inhibiting the activity of the citrate lyase enzyme and making Acetyl CoA unavailable for lipid synthesis, (-) -HCA acts as an anti-
obesity factor. However, the (-)-HCA content in the fruits of Garcinia indica vary from region to region and across the genotypes / ecotypes (Jayaprakasha 2002; Kureshi et al. 2019; Seethapathy et al. 2018) [4, 7, 13]. Assessment of genetic plasticity for (-)-HCA content among the natural populations of G. indica provides an insight into the natural sources of this valuable compound, its inheritance and distribution within and between the populations in its natural habitat. Estimation of (-)-HCA content is done from leaves and fruits, especially dried fruit rinds using various extraction procedures. The fruit rinds of Garcinia species contain higher amount of (-)-HCA compared to other parts of (-)-HCA (Jayaprakasha et al., 2003) [5]. We report the nature and extent of variation for the (-)-HCA content in the ecotypes of the Garcinia indica prevailing in the Western Ghats covering Karnataka state in India.

Material and Methods

Fruits of Garcinia indica ecotypes from random locations covering Western Ghats of the Karnataka state of India were collected in the peak fruiting season of 2016-17 and the fruits were stored at 4 °C until their actual use. The sampling locations are presented in Table 1. A set of 120 fruit samples each in replications were used for final analysis to estimate the (-)-HCA content. Chemicals used for the sample preparation were: 4N Sodium hydroxide, 50 per cent Calcium hydroxide, 10 per cent activated charcoal, 1N H₂SO₄ and 5 per cent Sodium metavanadate, Potassium hydroxyxycitrate bicarbonate (Standard for (-)-HCA).

Preparation of samples for extraction of (-)-HCA: The method described by Gogoi et al. (2014) [3] was followed for extraction of HCA with few minor modifications. About 5 g of chopped rinds of each fruit sample was dried to one third of their fresh weight (Figure 2 A and B). Powdered rinds were added to 15 ml of double distilled water and autoclaved twice (121 °C, 15 PSI.). The liquid extract was separated and treated with 10 per cent activated charcoal. The thick concentrated liquid was filtered. The residue was washed with small portion of distilled water and combined with the filtrate. The filtrate was neutralized with 4N NaOH solution which was maintained at pH 7.5. Fifty per cent solution of CaCl₂, was added and stirred well. Precipitated residue was filtered through 125 mm pore size filter paper and dried (Figure 1 C). Weight of the dried pellets of HCA lactones was recorded. Accurately weighed 0.29 g of HCA lactone was dissolved in 5 ml of 1 N H₂SO₄ and diluted to 25 ml with distilled water. The sample solution was decolorized using 10 per cent activated charcoal. The solution was filtered into a 50 ml standard flask, washed the residue with small portion of distilled water and made up to the volume. The standard Potassium hydroxyxycitrate bicarbonate salt equivalent to 0.0429 g of the free acid was weighed accurately and dissolved in 5 ml of 1 N H₂SO₄, and 25 ml of distilled water was added. It was filtered and transferred into a 50 ml volumetric flask and made up to the volume using distilled water. Linear working standards (0.5 ml, 1 ml, 1.5 ml and 2 ml) were prepared from the stock standard solution.

Quantification of HCA content by UV double-beamed spectrophotometer

One ml each of the prepared working standard solutions was added to 0.9 ml of 5 per cent Sodium metavanadate and 1ml double distilled water to estimate the HCA content present in the given sample (Figure 2 D). Absorbance value at 467 nm (Antony et al., 1998) was noted for all the working standards and a linear graph was plotted with the absorbance values to obtain a standard curve (Figure 2). Similarly, absorbance values for samples were recorded and the HCA content in samples was estimated using the following formula;

\[
\text{HCA content (per cent)} = \frac{\text{Absorbance value} \times \text{quantity of sample in the test solution} \times \text{factor}}{\text{Volume of the sample}} \times 100
\]

Factor = Pellet quantity obtained after precipitation with Ca (OH)₂ / 5 g of sample

Analysis of variance (ANOVA) and normal distribution of probability were worked out for the results obtained from HCA quantification.

Results

Relative levels of (-)-HCA content in the fruit samples on dry weight basis was estimated and analysed. The normal distribution of HCA content in ecotypes of G. indica species revealed a symmetrical bell-shaped curve (Figure 3). Per cent HCA content on dry weight basis in fruit samples of different ecotypes of Garcinia indica is presented in Table 2. The analysis of variance (ANOVA) revealed significant differences among the ecotypes of Garcinia indica for the (-)-HCA content in fruits (Table 3). The average mean per cent (-)-HCA content on dry weight basis was 17.90. The ecotype from Sirsi (Uttara Kannada) population recorded highest per cent (-)-HCA content of 25.46 and lowest per cent (-)-HCA content (10.53) was recorded in an ecotype from Mudigere (Chikmagalur) population.

The samples were divided into populations based on Districts as units. Descriptive statistics for HCA content among the samples of Garcinia indica representing different populations is presented in Table 4. The average mean for (-)-HCA content of populations of G. indica ecotypes based on geographical region was highest for the population from Uttara Kannada (20.60). The lowest average mean for (-)-HCA content was recorded in population from Belgaum (16.39). These results indicated that there is a fair amount of genetic plasticity for per cent (-)-HCA content among the ecotypes of G. indica in Western Ghats of Karnataka.

Discussion

The extraction of (-)-Hydroxycitric acid from the dried rinds of Garcinia indica by autoclaving the samples worked efficiently for better estimation of HCA content. Autoclaving the samples help in effective release of the target contents and enabling their detection (Gogoi et al., 2014) [3]. The spectrophotometric estimation has some limitations such as inaccurate quantification due to reaction of other similar compounds and time dependence of the developed colour by the reaction. Nonetheless, a proper extraction of the target compound from the tissue into the solution, such as the process of autoclaving, evolved better capture and accounting in the final estimation. Similar results for (-)-HCA content estimation in fruit rinds of Garcinia indica (12 per cent) was reported by Pandey et al. (2015) [10]. The variation in (-)-HCA content among the ecotypes of Garcinia indica was significant across the samples of different natural populations of Western Ghats. The high HCA content of Garcinia indica in the sub-populations of Northern regions of Western Ghats of Karnataka such as Uttara Kannada and Udupi Districts pointed at the genetic diversity of Garcinia indica. The variation in (-)-HCA content across geographic populations may be due genotypic variation and adoption to different
regions where the environmental conditions have favoured maximum expression of the trait (Priyadevi et al., 2012). The survey conducted by Parthasarathy et al. (2013) revealed that *Garcinia indica* and *Garcinia cambogia* were present throughout Western Ghats, extended from Konkan valley in North of Maharashtra to Malabar Coast in South of Kerala and ecotypes of *Garcinia indica* was predominant in Konkan region. The normal distribution of ecotypes with more number of ecotypes having the (--)HCA content near to mean (--)HCA content in ecotypes of *G. indica* also reveals that the content of HCA in fruits is symmetrically distributed in natural populations of the species in Western Ghats of Karnataka.

Table 1: Details of locations covered to collect *Garcinia indica* samples in Western Ghats of Karnataka

| Sl. No. | District name       | No. of locations | Location names                                                                 |
|---------|---------------------|------------------|---------------------------------------------------------------------------------|
| 1       | Uttara Kannada      | 10               | Karasulli, Yana, Kumta, Dandeli, Sirsi, Siddapura, Yellapur, Honnawara, Ankola, Karwar and Ramnagar |
| 2       | Udupi               | 3                | Mandarthi, and Kokkarne                                                        |
| 3       | Kodagu              | 6                | Kodlipet, Chettalli, CHES-Chettallli, Madikeri, Valnoor and Coorg                 |
| 4       | Hassan              | 1                | Sakleshpur                                                                     |
| 5       | Chikmagalur         | 5                | Koppa, Mudigere, Kadur, Sringeri, and Chattanahalli                            |
| 6       | Dakshina Kannada    | 5                | Ullala, Kouduiar, Puttur, Kaniyoor, Madibidre and Machina Belthangadi            |
| 7       | Belgaum             | 2                | Belgaum and Anmod                                                               |
| 8       | Goa                 | 1                | Ela                                                                             |
| 9       | Shivamogga          | 1                | Aagumbe                                                                        |
| 10      | Dharwad             | 1                | Dharwad                                                                        |
| 11      | Kasargod (Kerala)   | 1                | Kasargod                                                                        |

Table 2: Relative content of (--)HCA (per cent on dry weight basis) in fruit rinds of different ecotypes of *Garcinia indica* estimated by spectrophotometer method

| Sl. No. | Sample ID | Mean (%) | SD  |
|---------|-----------|----------|-----|
| 1       | GI_KAR1   | 22.37    | 0.18|
| 2       | GI_KAR2   | 21.66    | 0.21|
| 3       | GI_KAR3   | 11.50    | 0.50|
| 4       | GI_KAR4   | 13.69    | 0.11|
| 5       | GI_KAR5   | 12.28    | 0.50|
| 6       | GI_KAR6   | 13.47    | 0.14|
| 7       | GI_KAR7   | 17.59    | 0.15|
| 8       | GI_KAR8   | 15.18    | 0.30|
| 9       | GI_KAR9   | 17.29    | 0.10|
| 10      | GI_KAR10  | 11.41    | 0.14|
| 11      | GI_KAR11  | 10.57    | 0.09|
| 12      | GI_KAR12  | 17.11    | 0.20|
| 13      | GI_KAR13  | 11.42    | 0.11|
| 14      | GI_KAR14  | 12.67    | 0.15|
| 15      | GI_KAR15  | 14.02    | 0.09|
| 16      | GI_MAN2   | 17.54    | 0.53|
| 17      | GI_MAN3   | 14.82    | 0.31|
| 18      | GI_MAN8   | 14.54    | 0.76|
| 19      | GI_KAD1   | 14.34    | 0.56|
| 20      | GI_KAD2   | 12.48    | 0.14|
| 21      | GI_KAD3   | 14.64    | 0.127|
| 22      | GI_KAD4   | 14.41    | 0.16|
| 23      | GI_KAD5   | 17.11    | 0.92|
| 24      | GI_KOD1   | 13.55    | 0.10|
| 25      | GI_KOD2   | 17.57    | 0.75|
| 26      | GI_MAC    | 17.48    | 0.16|
| 27      | GI_CHE1   | 12.02    | 0.28|
| 28      | GI_KOP1   | 17.50    | 0.51|
| 29      | GI_MDB    | 17.43    | 0.61|
| 30      | GI_SKP    | 20.13    | 0.07|
| 31      | GI_MDG1   | 20.04    | 0.14|
| 32      | GI_YAN1   | 20.02    | 0.94|
| 33      | GI_KOK2   | 21.33    | 0.85|
| 34      | GI_KOD2   | 20.58    | 0.35|
| 35      | GI_GOA4   | 13.21    | 0.25|
| 36      | GI_DAN1   | 11.65    | 0.31|
| 37      | GI_KAN1   | 13.29    | 0.56|
| 38      | GI_KAN2   | 14.98    | 0.63|
| 39      | GI_KAN3   | 15.44    | 0.13|
| 40      | GI_KAN4   | 16.03    | 0.42|
| 41      | GI_KAN5   | 12.72    | 0.38|
| 42      | GI_KAN6   | 14.35    | 0.16|
| 43      | GI_KAN7   | 12.33    | 0.37|
| 44      | GI_KAN8   | 13.28    | 0.53|
|   |   |
|---|---|
| 45 | GI_KAN9 | 13.09 | 0.52 |
| 46 | GI_KAN10 | 21.54 | 0.21 |
| 47 | GI_KAN11 | 23.04 | 0.15 |
| 48 | GI_KAN12 | 13.40 | 0.45 |
| 49 | GI_KAN13 | 17.50 | 0.16 |
| 50 | GI_KAN14 | 20.78 | 0.86 |
| 51 | GI_KAN15 | 23.06 | 0.57 |
| 52 | GI_KAN16 | 16.07 | 0.48 |
| 53 | GI_KAN17 | 19.62 | 0.15 |
| 54 | GI_KAN18 | 19.39 | 0.10 |
| 55 | GI_KAN19 | 18.66 | 0.67 |
| 56 | GI_KAN20 | 19.76 | 0.23 |
| 57 | GI_YAN2 | 18.39 | 0.10 |
| 58 | GI_KAN21 | 19.41 | 0.87 |
| 59 | GI_KAN22 | 19.74 | 0.25 |
| 60 | GI_KAN23 | 14.50 | 0.10 |
| 61 | GI_KAN24 | 18.67 | 0.64 |
| 62 | GI_KAN25 | 15.67 | 0.26 |
| 63 | GI_KAN26 | 17.67 | 0.32 |
| 64 | GI_KAN27 | 16.68 | 0.20 |
| 65 | GI_KAN28 | 14.67 | 0.27 |
| 66 | GI_KAN29 | 13.79 | 0.42 |
| 67 | GI_KAN30 | 15.42 | 0.15 |
| 68 | GI_KAN31 | 12.61 | 0.23 |
| 69 | GI_KAN32 | 14.06 | 0.21 |
| 70 | GI_KAN33 | 17.06 | 0.14 |
| 71 | GI_KAN34 | 16.79 | 0.17 |
| 72 | GI_KAN35 | 15.65 | 0.61 |
| 73 | GI_KAN36 | 12.59 | 0.26 |
| 74 | GI_KAN37 | 13.61 | 0.62 |
| 75 | GI_KAN38 | 14.05 | 0.24 |
| 76 | GI_KAN39 | 12.90 | 0.15 |
| 77 | GI_KAN40 | 11.80 | 0.43 |
| 78 | GI_KAN41 | 10.53 | 0.71 |
| 79 | GI_KAN42 | 15.41 | 0.98 |
| 80 | GI_KAN43 | 15.25 | 0.22 |
| 81 | GI_KAN44 | 12.23 | 0.61 |
| 82 | GI_KAN45 | 15.91 | 0.46 |
| 83 | GI_KAN46 | 17.41 | 0.26 |
| 84 | GI_KAN47 | 17.05 | 0.32 |
| 85 | GI_KAN48 | 13.76 | 0.31 |
| 86 | GI_KAN49 | 13.52 | 0.22 |
| 87 | GI_KAN50 | 12.98 | 0.18 |
| 88 | GI_KAN51 | 23.68 | 0.31 |
| 89 | GI_KAN52 | 22.63 | 0.36 |
| 90 | GI_KAN53 | 24.06 | 0.09 |
| 91 | GI_KAN54 | 22.43 | 0.25 |
| 92 | GI_KAN55 | 23.78 | 0.87 |
| 93 | GI_KAN56 | 24.45 | 0.42 |
| 94 | GI_KAN57 | 24.72 | 0.30 |
| 95 | GI_KAN58 | 22.74 | 0.16 |
| 96 | GI_KAN59 | 23.40 | 0.32 |
| 97 | GI_KAN60 | 24.03 | 0.42 |
| 98 | GI_KAN61 | 24.57 | 0.46 |
| 99 | GI_KAN62 | 24.70 | 0.63 |
| 100 | GI_KAN63 | 23.91 | 0.34 |
| 101 | GI_KAN64 | 23.96 | 0.42 |
| 102 | GI_KAN65 | 23.50 | 0.93 |
| 103 | GI_KAN66 | 24.09 | 0.32 |
| 104 | GI_KAN67 | 24.41 | 0.31 |
| 105 | GI_KAN68 | 24.86 | 0.76 |
| 106 | GI_KAN69 | 20.66 | 0.38 |
| 107 | GI_KAN70 | 22.61 | 0.25 |
| 108 | GI_KAN71 | 21.41 | 0.82 |
| 109 | GI_KAN72 | 21.76 | 0.25 |
| 110 | GI_KAN73 | 23.60 | 0.32 |
| 111 | GI_KAN74 | 25.46 | 0.24 |
| 112 | GI_KAN75 | 24.54 | 0.37 |
| 113 | GI_KAN76 | 24.93 | 0.25 |
| 114 | GI_KAN77 | 22.66 | 0.27 |
| 115 | GI_KAN78 | 24.57 | 0.67 |
| 116 | GI_KAN79 | 25.27 | 0.45 |
| 117 | GI_KAN80 | 21.68 | 0.71 |
Table 3: Analysis of variance (ANOVA) of (-)–HCA content among ecotypes of *Garcinia indica*

| Source of Variation | DF | MS   | F-cal | F-critical |
|---------------------|----|------|-------|------------|
| Treatments          | 119| 59.25| 848.01| 1.43**     |
| Replications        | 240| 0.07 |       |            |
| Total               | 359|      |       |            |
| Standard error      |    | 0.22 |       |            |
| t tab 0.01 (240)    |    | 2.34 |       |            |
| Critical Difference |    | 0.51 |       |            |
| Co-efficient of Variance (CV) | | 0.25 |       |            |

**- significant at 1%**

Table 4: Mean, range and standard deviation for (-) - HCA content in different sub populations (regions) of *Garcinia indica*

| Sub populations | Regions     | Mean (%) | Minimum (%) | Maximum (%) | Standard Deviation |
|-----------------|-------------|----------|-------------|-------------|--------------------|
| POP 1           | Uttara Kannada | 19.11    | 12.05       | 23.22       | 4.25               |
| POP 2           | Udupi       | 15.97    | 9.07        | 23.96       | 4.83               |
| POP 3           | Kodagu      | 15.32    | 10.53       | 19.08       | 3.04               |
| POP 4           | Dakshina Kannada | 15.38    | 12.11       | 18.64       | 4.62               |
| POP 5           | Chikmagalur | 16.91    | 11.48       | 22.41       | 5.67               |
| POP 6           | Hassan      | 16.45    | 10.83       | 23.78       | 4.07               |
| POP 7           | Belgaum     | 16.38    | 9.03        | 23.36       | 4.73               |

A. B. C. D

Fig 1: Steps in preparation of samples for HCA content estimation; A) Dried rinds of *Garcinia indica*, B) Powdered dried rinds, C) HCA lactone pellets and D) Colour development after Sodium meta vanadate reaction with HCA in the sample

![Standard linear curve](image)

Fig 2: Standard linear curve plotted for the linear working standard solutions (0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml) of Potassium hydroxycitrate bicarbonate at 467 nm absorbance in UV double-beamed spectrophotometer. The $R^2$ value above 0.8 (0.9941) confirms the linearity of the standard curve plotted for the estimation of HCA content.
Fig 3: Normal distribution of HCA content among *G. indica* ecotypes

Supplementary materials

Table 1: Details of *Garcinia indica* samples collected from different regions of Karnataka

| Sl. No. | Sample | Sample ID | Location | District/State | GPS Coordinates |
|---------|--------|-----------|----------|----------------|-----------------|
| 1       | *G. indica* | GI_KAR1   | Karasulli | Uttara Kannada | 14° 34' 30.8316'' N 74° 48' 33.1992'' E |
| 2       | *G. indica* | GI_KAR2   | Karasulli | Uttara Kannada | 14° 34' 30.8388'' N 74° 48' 33.1632'' E |
| 3       | *G. indica* | GI_KAR3   | Karasulli | Uttara Kannada | 14° 34' 30.828'' N 74° 48' 33.1524'' E |
| 4       | *G. indica* | GI_KAR4   | Karasulli | Uttara Kannada | 14° 34' 30.8172'' N 74° 48' 33.174'' E |
| 5       | *G. indica* | GI_KAR5   | Karasulli | Uttara Kannada | 14° 34' 30.8244'' N 74° 48' 33.1488'' E |
| 6       | *G. indica* | GI_KAR6   | Karasulli | Uttara Kannada | 14° 34' 30.8136'' N 74° 48' 33.1236'' E |
| 7       | *G. indica* | GI_KAR7   | Karasulli | Uttara Kannada | 14° 34' 30.8028'' N 74° 48' 33.1668'' E |
| 8       | *G. indica* | GI_KAR8   | Karasulli | Uttara Kannada | 14° 34' 30.7956'' N 74° 48' 33.1344'' E |
| 9       | *G. indica* | GI_KAR9   | Karasulli | Uttara Kannada | 14° 34' 30.8532'' N 74° 48' 33.1056'' E |
| 10      | *G. indica* | GI_KAR10  | Karasulli | Uttara Kannada | 14° 34' 30.8464'' N 74° 48' 33.138'' E |
| 11      | *G. indica* | GI_KAR11  | Karasulli | Uttara Kannada | 14° 34' 30.846'' N 74° 48' 33.1524'' E |
| 12      | *G. indica* | GI_KAR12  | Karasulli | Uttara Kannada | 14° 34' 30.8464'' N 74° 48' 33.138'' E |
| 13      | *G. indica* | GI_KAR13  | Karasulli | Uttara Kannada | 14° 34' 30.8244'' N 74° 48' 33.1668'' E |
| 14      | *G. indica* | GI_KAR14  | Karasulli | Uttara Kannada | 14° 34' 30.8064'' N 74° 48' 33.1344'' E |
| 15      | *G. indica* | GI_KAR15  | Karasulli | Uttara Kannada | 14° 34' 30.8028'' N 74° 48' 33.1812'' E |
| 16      | *G. indica* | GI_MAN2   | Mandarthi | Udupi           | 13° 29' 48.4476'' N 74° 48' 35.172'' E |
| 17      | *G. indica* | GI_MAN3   | Mandarthi | Udupi           | 13° 29' 47.7852'' N 74° 48' 36.5256'' E |
| 18      | *G. indica* | GI_MAN8   | Mandarthi | Udupi           | 13° 29' 46.9572'' N 74° 48' 35.136'' E |
| 19      | *G. indica* | GI_KAD1   | Kadur     | Chikmagalur     | 13° 33' 12.0528'' N 76° 0' 40.0248'' E |
| 20      | *G. indica* | GI_KAD2   | Kadur     | Chikmagalur     | 13° 33' 9.6588'' N 76° 0' 39.9852'' E |
| 21      | *G. indica* | GI_KAD3   | Kadur     | Chikmagalur     | 13° 33' 12.4383'' N 76° 0' 38.1312'' E |
| 22      | *G. indica* | GI_KAD4   | Kadur     | Chikmagalur     | 13° 33' 12.2868'' N 76° 0' 36.432'' E |
| 23      | *G. indica* | GI_KAD5   | Kadur     | Chikmagalur     | 13° 33' 10.5624'' N 76° 0' 35.9676'' E |
| 24      | *G. indica* | GI_KOK1   | Kokkarne  | Udupi           | 13° 26' 37.1976'' N 74° 48' 35.6848'' E |
| 25      | *G. indica* | GI_KOD1   | Kodlipet  | Kodagu          | 12° 47' 48.588'' N 75° 53' 22.9308'' E |
| 26      | *G. indica* | GI_MAC    | MachinBelthangad | Dakshin Kannada | 12° 57' 35.946'' N 75° 12' 26.046'' E |
| 27      | *G. indica* | GI_CHE1   | Chettalli  | Kodagu          | 12° 22' 12.0144'' N 75° 49' 50.4336'' E |
| 28      | *G. indica* | GI_KOP1   | Koppar    | Chikmagalur     | 13° 31' 57.5688'' N 75° 21' 20.8548'' E |
| 29      | *G. indica* | GI_MDB    | Mudibide   | Dakshin Kannada | 13° 4' 7.6764'' N 75° 59' 36.96'' E |
| 30      | *G. indica* | GISKP     | Sakleshpur | Hassan          | 12° 56' 33.1188'' N 75° 47' 32.7228'' E |
| 31      | *G. indica* | GI_MDG1   | Mudigere   | Chikmagalur     | 13° 8' 9.3336'' N 75° 38' 23.1216'' E |
| 32      | *G. indica* | GI_YAN1   | Yana      | Uttara Kannada  | 14° 34' 10.6788'' N 74° 33' 28.962'' E |
| 33      | *G. indica* | GI_KOK2   | Kokkarne  | Udupi           | 13° 26' 38.7456'' N 74° 48' 56.804'' E |
| 34      | *G. indica* | GI_KOD2   | Kodlipet  | Kodagu          | 12° 47' 45.51'' N 75° 53' 27.4848'' E |
| 35      | *G. indica* | GI_GOA4   | Ela       | Goa            | 15° 17' 57.5376'' N 74° 7' 26.3856'' E |
| 36      | *G. indica* | GI_DAN1   | Dandeli   | Uttara Kannada  | 15° 14' 58.8408'' N 74° 37' 25.5646'' E |
| 37      | *G. indica* | GI_KAN1   | Kaniyoor, Puttur | Dakshin Kannada | 12° 43' 0.8976'' N 75° 22' 5.448'' E |
| 38      | *G. indica* | GI_KAN2   | Kaniyoor, Puttur | Dakshin Kannada | 12° 43' 0.0048'' N 75° 22' 1.704'' E |
| 39      | *G. indica* | GI_KAN3   | Kaniyoor, Puttur | Dakshin Kannada | 12° 42' 59.9292'' N 75° 22' 5.4084'' E |
| 40      | *G. indica* | GI_KAN4   | Kaniyoor, Puttur | Dakshin Kannada | 12° 42' 59.9292'' N 75° 22' 8.886'' E |
| 41      | *G. indica* | GI_KAN5   | Kaniyoor, Puttur | Dakshin Kannada | 12° 42' 55.7856'' N 75° 22' 3.4788'' E |
| 42      | *G. indica* | GI_KAN6   | Kaniyoor, Puttur | Dakshin Kannada | 12° 42' 51.5664'' N 75° 22' 3.5544'' E |
Conclusion
The plasticity for HCA content in fruit rinds of ecotypes of *Garcinia indica* in the present study suggested that there is a significant amount of diversity with respect to (-)-HCA content in ecotypes of *G. indica* in Western Ghats of Karnataka. The diversity for (-)-HCA content in fruits of ecotypes of *G. indica* is normally distributed across the geographical region of Western Ghats of Karnataka. These results can serve as basis for selection of ecotypes for a detailed estimation of (-)-HCA content using more robust methods and select the superior ecotypes for further evaluation.

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References
1. Anu AP, Menon LN, Rameshkumar KB. Structural diversity of secondary metabolites in *Garcinia* species. In Diversity of *Garcinia* species in the Western Ghats: Phytochemical perspective. Rameshkumar, K. B. (Ed). JNTBGRI, Kerala, 2015, 196-201.
2. Ashish GR, Parthasarathy U, Zachariah J, Kokkat GC. A comparative estimation of (-) – Hydroxycitricacid in different species of *Garcinia*. The Hort. J. 2008; 21:26-29.
3. Gogoi A, Gogoi N, Neog B. Estimation of (-) - Hydroxyctic acid (HCA) in *Garcinia lanceaefolia* using novel HPLC methodology. Int. J. Pharm. Sci. Res. 2014; 5:4995-4999.
4. Jayaparaksha GK. Determination of organic acids in leaves and rinds of *Garcinia indica* (Desr.) by LC 2002; 28:379-384.
5. Jayaparaksha GK, Jena BS, Sakariah KK. Improved liquid chromatographic method for determination of organic acids in leaves, pulp, fruits, and rinds of *Garcinia*. Int. AOAC J. 2003; 5:1063-1068.
6. Jena BS, Jayaparaksha GK, Singh RP, Sakariah KK. Chemistry and biochemistry of (-)-Hydroxyctic acid from *Garcinia*. J. Agri. Food Chem. 2002; 50:10-22.
7. Kureshi AA, Dholakiya C, Hussain T, Mirgal A, Salvi SP, Barua PC et al. Simultaneous Indian *Garcinia* species using a validated UHPLC-PDA method. J AOAC Int. 2019; 102:1423-1434.
8. Lewis YS, Neelakantan S. (-)-Hydroxyctic acid-the principal acid in the fruits of *Garcinia cambogia*. Phytochem. 1965; 4:619-625.
9. Mishra. Antioxidant activity of *Garcinia indica* (kokum) and its syrup. Curr. Sci., 2006; 1:90-93.
10. Pandey R, Kumar B, Rameshkumar KB. Rapid estimation of bioactive constituents of *Garcinia* species in the Western Ghats using UHPLC-MS/MS Method. In Diversity of *Garcinia* species in the Western Ghats: Phytochemical perspective. Rameshkumar, K. B. (Ed). JNTBGRI, Kerala, 2015, 196-201.
11. Parthasarathy U. Morphological characterization of some important Indian *Garcinia* Species, Dataset Paper. 2014; 2-6-10.
12. Sathish M, Misra SM. Estimation of (-)-Hydroxycitric acid HCA in *Garcinia indica* chotty by HPLC method. Anal. Chem., 2008; 12(7):854-856.
13. Seethapathy G5, Tadesse M, Urumarudappa SKJ, SVG, Vasudeva R, Maltered KE et al. Authentication of *Garcinia* fruits and food supplements using DNA barcoding and NMR spectroscopy. Sci. Rep. 2018; 8:10561.
14. Shameer PS, Rameshkumar KB, Sivu AR, Sabu T, Pradeep NS, Mohanan N. Morphological, chemical and molecular taxonomy of a new *Garcinia* species- *Garcinia pushpangadaniana*, In Diversity of *Garcinia* species in the Western Ghats: Phytochemical Perspective. (Ed) Rameshkumar, K. B., JNTBGRI, Kerala, 2016, 196-201.
15. Shivkumar S, Sriraman S, Subhasree N, Dubey GP. *In vitro* assessment of antibacterial and antioxidant activities of fruit rind extracts of *Garcinia cambogia* L. Int. J. Pharm. Pharm. Sci. 2013; 5:254-257.
16. Shrikant BS. *Kokum* (*Garcinia indica*) and its many functional components as related to the human health: A review. J. Food Res. Technol. 2014; 2:130-142.
17. Sivu AR, Pradeep NS, Rameshkumar KB. Molecular characterization of *Garcinia* species in the Western Ghats, In Diversity of *Garcinia* species in the Western Ghats: Phytochemical perspective. Rameshkumar, K. B. (Ed). JNTBGRI, Kerala, 2017, 196-201.