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Organic acid profile of *Garcinia gummi-gutta* (L) Rob. from central Western Ghats of India

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Abstract
Forests are a rich reserve of compounds that can be used as pharmaceuticals and nutraceuticals. The genus Garcinia, which belongs to the Clusiaceae family, has about 200 species that are found throughout the world's tropics, mostly in Asia, Africa, and Polynesia *Garcinia gummi-gutta* (L) Rob. is the most commercially exploited for organic acids with its vast economic importance worldwide. Hydroxycitric acid (HCA) is known to have excellent anti-obesity property. Field study was conducted to determine the effect of different locations and its site factors on organic acid content of *G. gummi-gutta* fruits in 9 different sites of Uttara Kannada, Karnataka. A statistically significant influence of sites conditions on the organic acid content. The Organic acids were found higher in site S_1(with altitude 646.3 m MSL, high precipitation and average temperature of 24 °C), the highest of 7.85±0.23% (-)-Hydroxycitric acid lactone (HCA-L) with the average 7.15±0.57% and 21.52±1.28% of HCA with the average of 18.47±1.50% was found respectively. The HCA and HCA-L had very strong positive correlation (r = 0.84 to 0.98) with site factors (except mean annual temperature).

Keywords: *Garcinia gummi-gutta*, organic acid profile, locations, HPLC, central Western Ghats, Karnataka

1. Introduction
Garcinia gummi-gutta (L) Rob. (syn. *Garcinia cambogia*). It belongs to family Clusiaceae, it is also known as Uppage (Kannada) locally. It is an evergreen tree of small to medium size, grows up to 12 m tall with leaves of shining dark green colour. The flowering season lasts from January to March, while fruit production begins in June and July. Fruits usually grow into ovoid shape with the diameter of 5 cm, ripe fruits are yellow, having 6 to 8 grooves and about 6 to 8 seeds covered by succulent aril (Singh, 1993) [20].
Distribution ranges between southwards of Konkan to eastwards of Travancore in Western Ghats. Apart from home range it's been introduced in subtropical Malaysia, China and Philippines (Chuah *et al.,* 2013) [3]. According to Lewis and Neelekantan (1965) [14], hydroxycitric acid (HCA) is a key organic acid that predominates in the fruit rinds of *G. gummi-gutta*. HCA helps to reduce obesity by suppressing appetite (Preuss *et al.,* 2004; Kovacs *et al.,* 2006) [19, 12] and enhancing energy outlay (Leonhardt & Langhans, 2002) [13]. The fleshy fruit rind of the tree is the richest natural source of potential anti-obesity plant metabolite (-)-Hydroxycitric acid (HCA) along with minute quantity of citric, tartaric, and malic acids (Lewis & Neelekantan, 1965; Jayaprakash & Sakaraiah, 2000) [14, 8].
Lab rats (Ohia *et al.,* 2001) [17] and other experimental animals with slower weight gain and less body fat accumulation have also been recorded after HCA usage (Kim *et al.,* 2008; Amin *et al.,* 2011; Kim *et al.,* 2008) [10, 1, 11]. HCA has the property to lower blood lipids such as cholesterol and triglycerides. Additionally, there have been no reported negative consequences from human usage (Maia-Landim *et al.,* 2021) [15]. There are wide variations in naturally distributed *G. gummi-gutta* populations. The present study was aimed to understand the organic acid chemical diversity existing among *G gummi-gutta* growing in Uttara Kannada district of Karnataka.

2. Material and Methods
2.1 Study area
The study was conducted in Uttara Kannada district of Karnataka, a part of Central Western Ghats. Nine sites were randomly selected, in each site five trees were chosen as replications. Trees of 50-80cm girth class were selected to maintain the uniformity among the samples. The rainfall in these sites ranged between 2300 to 4722 mm per year and the altitude ranges

between 418.7 to 646.3 m (Table 1).

Table 1: Geographical and climatic information of study area in Uttara Kannada district, Karnataka

Site	Altitude (m)	Mean annual rainfall (mm)	Annual rainy days	Mean annual temperature (℃)
S_1	646.3	3800 - 4722	120	24
S_2	610.7	3800 - 4722	120	23
S_3	623.3	3800 - 4722	120	24
S_4	516.2	2950 - 3667	100	25
S_5	508.9	2950 - 3667	100	25
S_6	522.7	2950 - 3667	100	25
S_7	460.8	2300 - 3263	95	27
S_8	437.8	2300 - 3263	95	27
S_9	418.7	2300 - 3263	95	27

2.2 Estimation of Organic acid profile (High Performance Liquid Chromatography)

Analysis was carried out using High Performance Liquid Chromatography (HPLC-Shimadzu chromatographic system - LC 8A) instrument.

2.2.1 Preparation of sample for analysis

Under forced air ventilation, the fruit rinds were dried in oven at 45 °C for 8 hours. The samples' dried rind was extracted with Soxhlet water extraction mentod by boiling 10 g of it in 50 ml of double-distilled water for 45 minutes at 100 °C. After the extract was dried, the residue was combined with 50 ml of 30% orthophosphoric acid.

2.2.2 Standards for High performance liquid chromatography

Citric acid, D-(+)-Glucuronolactone, and the ethylene-diamine salt of HCA were purchased. The ethylene-diamine salt of HCA in 5 mg was dissolved in 5 mL of 50% H_2SO_4, which was then added to 10 mL of HPLC grade water to make the desired volume. A stock solution of 500 g/mL of HCA was created. By dissolving the lactone (D-(+)-Glucuronolactone) salts in HPLC grade water, lactone standards were created. Salts of citric acid were also dissolved in water of HPLC quality. The lactone and citric acid standard solvents were kept at a 500 g/mL concentration. (Jayaprakash and Sakariah, 2002) [9].

2.2.3 Analysis using High performance liquid chromatography

A dual pump, photodiode array detector (SPDM10AVP), and Class LC10 software-equipped HPLC-Shimadzu chromatographic system (LC 8A model) were used to filter 200 µL of the standard samples through a 0.45-inch filter. Twenty litres of each were then fed into the system. Results from HPLC were obtained using a Waters Cortecs reversed phase C-18, 2.7 µm with sperical solid core, 4.6 mm 150 mm column, and a gradient elution solvent with 0.2 M sodium sulphate and a pH of 2.5 that was adjusted using diluted H_2SO_4. The reading was obtained using ultraviolet (UV) detection at 215 nm while the flow rate of 0.5 mL/min was maintained. To achieve accuracy in evaluation, various trial sets were inserted.

Based on the area obtained, the amounts were calculated using the lab solution software Shimadzu Analytical (India) Pvt. Ltd. Quantitative analysis was obtained by measuring the peak responses data of standards to that of the testing extracts. The values from two trials were recorded, and the average mean was obtained as the final amount (Ong, 2004; Nour *et al.*, 2013) [18, 16].

2.3 Data analysis

The data were subjected to one factor ANOVA using both 'SPSS' and 'R-software', additionally 'Tukey's range test' was done, and 'Compact Letter Display' (CLD) was assigned to each variable. Further data visualizations were done using "ggplot2" package in R-software.

3. Results and discussion

3.1 Organic acid profile of fruit rind

The organic acids *viz*. (-)-Hydroxycitric acid lactone (HCA-L), Hydroxycitric acid (HCA), and Citric acid were estimated from dried rind samples (Figure 1) using HPLC. Percent share of these organic acids were presented in Table 2. The mean HCA of 18.47±1.90%, HCA-L of 7.15±0.57%and Citric acid of 0.13±0.04% were found respectively. The highest (-)-Hydroxycitric acid lactone (HCA-L) was found in site S_3 (7.94±0.43% w/w) and has no statistically significant difference between site S_1, maximum Hydroxycitric acid (HCA) in site S_1 (21.52±1.28% w/w) and Citric acid content in S_2 (0.18±0.03% w/w) was observed. In similar findings the range of HCA was at 10 to 30 percent (Anju & Rameshkumar, 2017; Lewis and Neelekantan, 1965; Devi *et al.*, 2012) [2, 14, 5], which in turn agrees with our results with an overall average HCA of 18.47±1.90%. However, There's been a report of up to 42 to 44 percent of HCA from *G. gummi-gutta* rind (Edirisinghe *et al.*, 2015) [7], which is way more than what we found from our study.

The observations indicated a higher percent presence of major organic acids (HCA and HCA-L) in site S_1 of altitude 646.3 with lower temperature, high rainfall, and total rainy days compared to other study sites. A very strong positive correlation was observed between HCA and HCA-L with site factors (*viz*. altitude, mean annual rainfall and annual rainy days) except mean annual temperature, where strong negative correlation was observed in case of HCA. With respect to fruit and rind parameters, only the rind thickness had strong positive correlation with HCA and HCA-L (Figure 2).

The environmental factors might have some kind of influence on chemical composition. The process of metabolism and secondary metabolite accumulation will be greatly influenced by important environmental factors like altitude, temperature, incident light, humidity, and precipitation. These environmental differences in different locations directly contribute to the difference and variation in active ingredient contents (Dong *et al.*, 2011; Wang *et al.*, 2014) [6, 21].

Hydroxycitric acid and citric acid is formed from carbohydrate in Garcinia fruit tissue and suggest that their initial accumulation and subsequent disappearance are related to the changes in the activity of the condensing enzyme and other enzymes of the citric acid cycle. However young fruits have more citric acid and mature fruits have more HCA. The decrease in citric acid in the ripe fruit suggests the possibility that this stage may perhaps be characterized by the presence of an enzyme system that affects the partial breakdown of citric acid (Deshpande & Ramakrishnan, 1960) [4].

The different forms of organic acids such as HCA-L, HCA, and Citric acid may be due to the process of concentration along with evaporation in Garcinia species leading to lactonization of regular - HCA to HCA-L. HCA-L (lactone) is way less biologically active (Jayaprakash & Sakariah, 2002) [9].

Table 2: Percent (w/w) Organic acid content of *Garcinia gummi-gutta*fruit rind

Site	Organic acids (in percentage)					
	Hydroxycitric Acid (@ p<0.05)	Retention time (min)	Hydroxycitric acid lactone (@ p<0.05)	Retention time (min)	Citric Acid (@ p<0.05)	Retention time (min)
S_1	21.52±1.28 ^{a}	5.263	7.85±0.23 ^{a}	4.690	0.13±0.05 ^{ab}	10.652
S_2	20.61±1.04^{a}	5.268	7.23±0.29 ^{ab}	4.694	0.18±0.03 ^{a}	10.650
S_3	20.45±1.20^{a}	5.258	7.94±0.43 ^{a}	4.689	0.11±0.03 ^{b}	10.641
S_4	17.61±0.59^{b}	5.264	6.94±0.60 ^{b}	4.691	0.15±0.02 ^{ab}	10.653
S_5	17.38±0.43^{b}	5.266	7.24±0.41 ^{ab}	4.697	0.12±0.03 ^{b}	10.651
S_6	17.94±0.44^{b}	5.260	6.76±0.36 ^{b}	4.695	0.13±0.02 ^{ab}	10.657
S_7	16.58±0.35^{b}	5.257	6.72±0.41 ^{b}	4.689	0.1±0.02 ^{b}	10.649
S_8	16.89±0.41^{b}	5.259	6.94±0.38 ^{b}	4.686	0.09±0.02 ^{b}	10.654
S_9	17.23±0.31^{b}	5.267	6.75±0.42 ^{b}	4.689	0.14±0.02 ^{ab}	10.656
Mean	18.47±1.90		7.15±0.57		0.13±0.04	

Fig 1: Overview of typical Garcinia fruit rind (Fresh and dried)

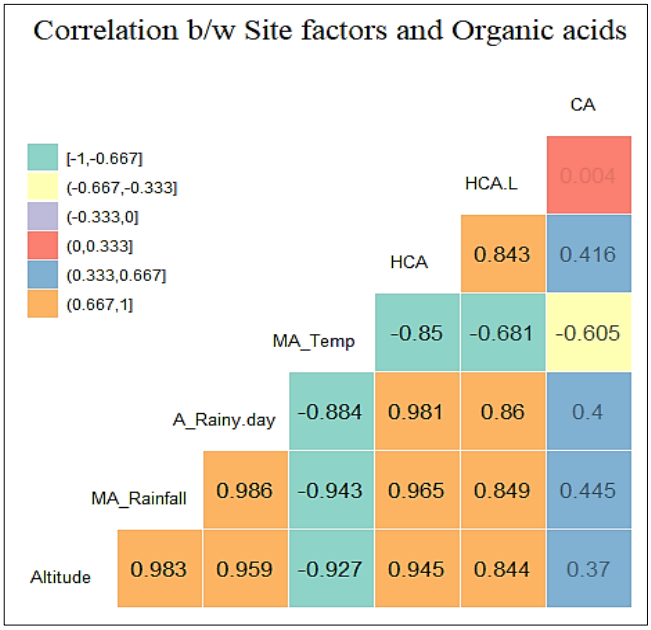

Fig 2: Correlation matrix of Organic acids with Site factors

Conclusion

The environmental factors might have influence on organic acid profile of *Garcinia gummi-gutta*. HCA is the predominant commercially important organic acid observed through HPLC. The site (S_1) with high altitude and precipitation with lower temperature found to have higher percent availability of major organic acid content within the fruit rind. The HCA and HCA-L had very strong positive correlation with site factors (except mean annual temperature). The different environmental conditions might have a direct impact on organic acid composition within the plant in case of our study. Further study is needed to determine particular reason causing this phenomenon.

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