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Article · January 2012

6 authors, including:

Utpala Parthasarathy
Indian Institute of Spices Research

Nandakishore O.P
FSSAI, Cochin, India

John Zachariah
Indian Institute of Spices Research

Parthasarathy Va
Bioversity International (CGIAR)

International Journal of Innovative Horticulture . 1(1):28-10, 2012

Chromatographic Fingerprinting and Estimation of Organic Acids in Selected *Garcinia* Species

Utpala Parthasarathy*, O.P.Nandakishore, R.Senthil Kumar, K.Nirmal babu, T.J Zachariah and V.A.Parthasarathy

Indian Institute of Spices Research,P.O.Merikunnu,kozhikod 673012, Kerala, India
Correspondingauthor: utpala@spices.res.in

Abstract

Key word

Introduction

Garcinia is a plant genus of the family Clusiaceae native to Asia, Australia, tropical and southern Africa, and Polynesia, includes about 300 species. About 35 species are found in India among which 17 are endemic. Many species are threatened due to habitat destruction (Cheek, 2004). It is an important medicinal crop because of the presence of many beneficial compounds like phenols and acids in the fruit rinds. Utpala *et al* (2010) quantified the presence HCA in the fruit rind of 8 species of Western Ghats & North East Himalayas. The present work focuses on the identification and quantification of organic acids present in the dried rinds of 8 different species from Western Ghats and NE. Himalayas.

Organic acids are the product of metabolism and stored in the vacuoles of cell, which are colourless, non-volatile and water soluble compounds and can be easily detected using acid indicators like bromocresol green. Common plant organic acids are malic, citric, tartaric, oxalic and ascorbic acids. Acidity plays an important part in the perception of fruit quality. It affects the sweetness of fruit by masking the taste of sugars (Lyon *et al*., 1993). A chemical fingerprint obtained by hyphenated chromatography, out of question, will become the primary tool for quality control of herbal medicines (Lazarowych and Pekos, 1998). Organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria. It lowers pH of cytoplasm and increases osmotic potential of cell making the cells stop functioning and get damaged. Hence acids like citric acid, tartaric acid and lactic acid is used as food preservatives and anti-microbial solutions (Brul and Coote, 1999). Organic acids are also used to cure Gastro Intestinal tract infections and control gut microflora. They are provided in cattle and poultry feeds to improve digestive health of animals without any adverse health-ethic impact and so can be used in foods for babies and ill-people (Dibner and Butin, 2002). In food and medicine industries, addition of organic acid increases shelf-life of foods and drugs and reduces chances of microbial contamination. They are preferred over benzoic acid derivatives as organic acids are non-toxic and highly water soluble (Patanen and Mroz 1999). Organic acids provide flavor to food. According to Silva *et al.* (2004), organic acids may have a protective effect against multiple diseases due to their antioxidant activity.

Considering the importance of organic acids and the *Garcinia* species in medicine and food, the present studies were conducted. This is the first report of such a study in *Garcinia.*

Materials and Methods

Dried fruit rinds of 8 species namely *G. gummi-gutta*, *G. tinctoria*, *G. indica*, *G. mangostana* and *G.subelliptica* of W.Ghats and *G. pedunculata* (Bor thekera), *G. lancifolia* (Rupohi thekera) and *G.kidya* (Kuji thekera) of N.E Himalayas were taken for the experiment.

5g of finely chopped rinds were refluxed with 20ml de-ionized water for 1 hour. Filtered and transferred extract in to 100ml standard flask. Repeated the extraction with fresh distilled water and pooled up the extracts. Final volume was then made up to 100ml with de-ionized water.Total acidity was estimated by acid-base titration method against 0.075M NaOH and phenolphthalein as the indicator.Detection of organic acids was performed by ascending paper chromatography using Whatman No.1 paper of about 20x5cm dimension. A proportionate mixture of n-butanol, formic acid and water was taken as the solvent (5:1:5). After completion, the paper was dried and sprayed with the indicator and re-dried. Indicator solution was 1% solution of Bromocresol green which was then made alkaline using Sodium hydroxide to obtain deep blue colouration. Rf values for each organic acids were calculated and compared with that of standard acids.Estimation of organic acids was performed using Reverse Phase HPLC. 50mM phosphate solution was taken as mobile phase for HPLC whose pH was adjusted to 2.1 using ortho-phosphoric acid. pH 2.1 was selected due to better resolution. Standard solutions for various organic acids were prepared separately with concentrations of malic acid (7000mg/l), citric acid (5000mg/l), oxalic acid (1000mg/l), ascorbic acid (1000mg/l), acetic acid (750mg/l), tartaric acid (5000mg/l) and succinic acid (5000mg/l) in de-ionized water and then filtered. Standards are stored at 4^{o}C. Succinic acid is used as internal standard and response factor were calculated for each acids.

HPLC Instrument and Conditions

HPLC system comprising of Shimadzu LC-10AT pump, SPD-10A VP UV-VIS detector, SCL-10A VP controller and C-18 reversed phase column was used for analysis. Analyses were made at 214nm wavelength at a flow rate of 1.00ml per minute.

Results and Discussion

The paper chromatograms are given in Fig.1. The HPLC chromatograms are given in Fig.2. In HPLC, the conversion factors for each organic acids were calculated using the peak area information and the concentration of the concerned organic acids as well as the internal standard (succinic acid). The species showed a great variation in the number of peaks and their relative area. Among the Western Ghats species (*G.gummi-gutta* , *G.tinctoria,G.indica and G.Subelliptica)* along with the Malayan species *G.mangostana*, the peak corresponding to HCA (Peak No.4) had the largest area while in case of the species from NE Himalayas, the Peak3 corresponding to malic acid was the largest. In case of *G.tinctoria*, the largest peak was for citric acid with small peak for HCA. In all the species, the peaks corresponding to tartaric acid, oxalic acid and ascorbic acid were small.

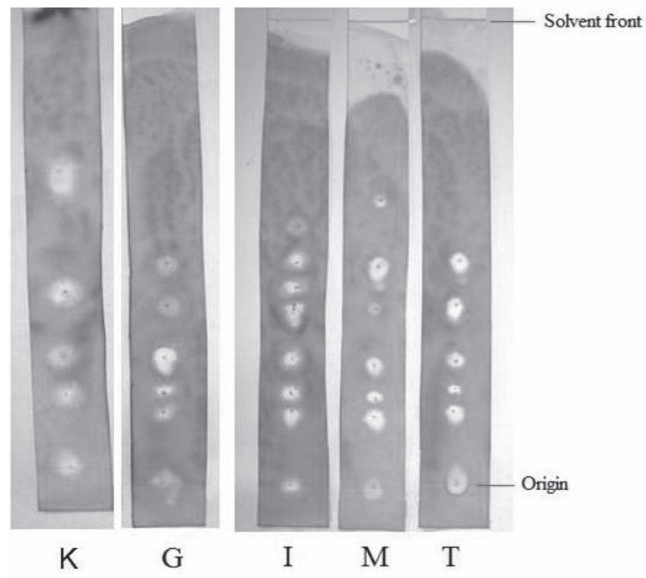


Fig.1: Paper Chromatograms for organic acid detection

Table 1. Retention factor of organic acids in Garcinia samples

Sample	No.of spots	Rf values
G. gummigutta	5	0.15, 0.20, 0.26, 0.37, 0.45
G. indica	7	0.16, 0.22, 0.26, 0.36, 0.40, 0.45, 0.67
G. tinctoria	5	0.15, 0.20, 0.25, 0.37, 0.45
G. mangostana	6	0.14, 0.22, 0.26, 0.37, 0.46, 0.59
G. kidya	5	0.13, 0.20, 0.24, 0.38, 0.45
G. pedunculata	6	0.15, 0.21, 0.25, 0.36, 0.45, 0.59
G. lancifolia	6	0.16, 0.21, 0.24, 0.38, 0.44, 0.54
G. subelliptica	6	0.14, 0.20, 0.24, 0.36, 0.45, 0.59

The Rf values of standard acids were found to be oxalic acid (0.14), tartaric acid (0.21), malic acid (0.45), citric acid (0.38), hydroxycitric acid (0.24) and ascorbic acid (0.60). These values were found to be in agreement with that reported by Harborne (2005) with deviation of +- 0.01 to 0.07.

Total acids obtained were highest in *G. kidya/Cowa* 28% followed by *G. gummi-gutta* and *G. pedunculata.* (15.8%) while it is less in *G. mangostana* (4.5%) (Table 2). Common plant organic acids are malic, citric, tartaric and ascorbic acids. Acidity plays an important part in the perception of fruit quality. It imparts not only the sour taste to the fruit, but also masks the sugars in the ripened fruit (Lyon et al., 1993).

Hydroxycitricacid (HCA) 1,2-dihydroxypropane-1,2,3-tricarboxylic acid is well known acid of Garcinia is present in all the species more than 1%, except in *G. tinctoria* and *G. mangostana*. *G. gummi-gutta* is having Highest 10.48%. *G. kidya/Cowa* and *G. indica* is also having mordarately high 8.97 and 6.13 respectively. Hydroxycitric acid is a potent anti-obesity compound and reduces lipid and sugar

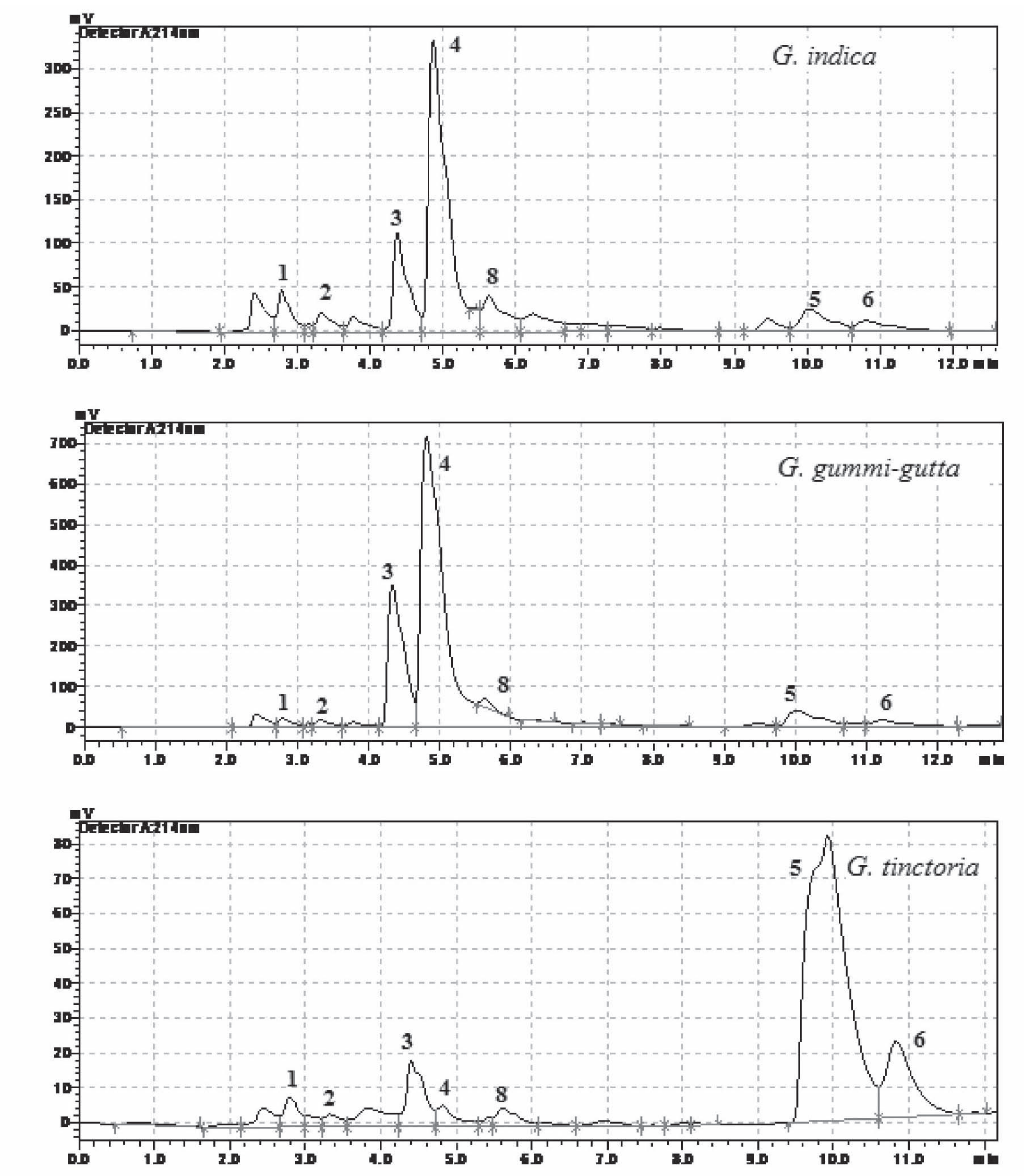
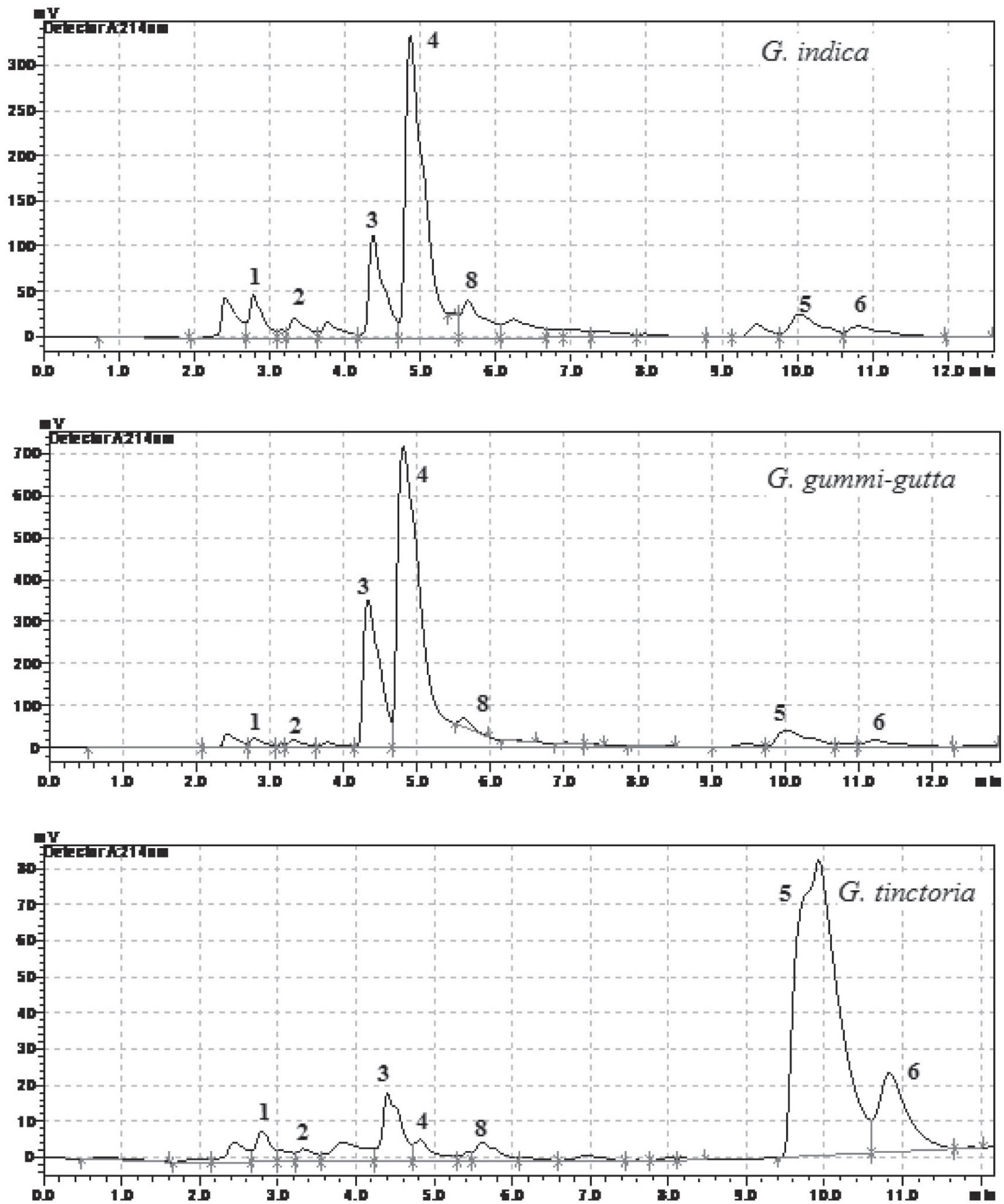


Fig. 2: HPLC chromatograms for Garcinia organic acid profiling



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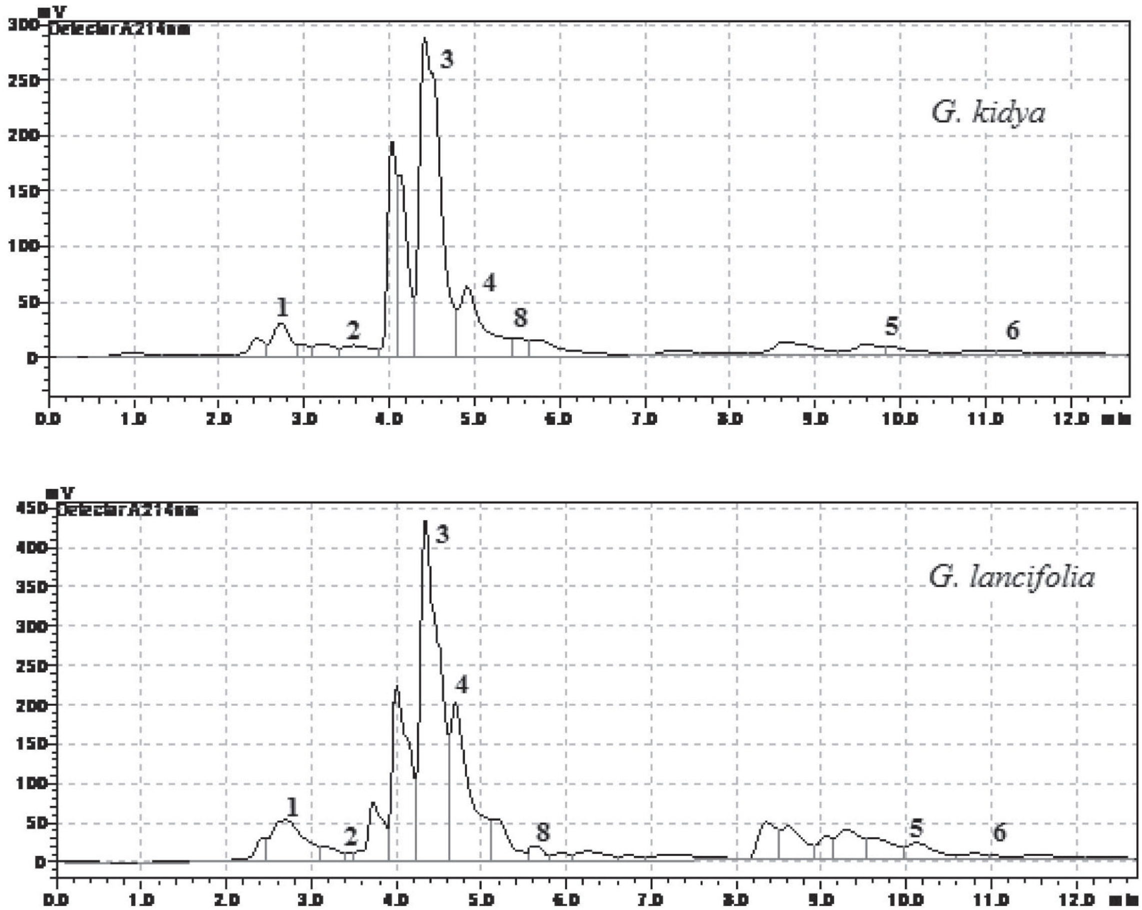

Fig. 2: HPLC chromatograms for Garcinia organic acid profiling

levels in blood. Sullivan & Triscari (1977) reported that (-) HCA lowered body fat level with no loss of body protein or lean mass in test animals that had been experimentally made obese. Thus a natural product of *Garcinia* the Hydroxycitric acid is working wonders in reducing weight and obesity. (HCA) which is a potent metabolic regulator of obesity and it also lowers blood lipids such as cholesterol and triglycerides and hence is increasingly becoming important industrially, commercially and medicinally

Malic Acid occurs naturally almost in all fruits. It is generated during fruit metabolism. Malic acid is used as an ingredient in skin preparations (combined with benzoic acid and salicylic acid) for the removal of dead skin from ulcers, burns, and wounds. Malic acid is also an ingredient in artificial saliva preparations. Malic acid is also present in good concentration in *G. kidya/Cowa, (*14.32%), G. lancifolia (10.02%) and in *G. pedunculata* (8.95%). It is interesting to note that all Himalayan Samples are having more percentage of malic acid than Western Ghats samples. G. lancifolia is known as *Rupohi thekara* in Assam and the extract is used for skin beautification. Malic acid is added to many foods and candies to give them a more tart taste. Fruity-tasting candies and confections rely on malic acid as a flavoring agent, as do some sodas. Malic acid can be used to preserve certain flavors, or enhance flavors in processed foods that lose some of their natural flavoring during the processing, like canned fruits. A study conducted in 2009 published by a group of doctors in the "Journal of Clinical Biochemistry and Nutrition" suggested that malic acid is beneficial in lowering blood pressure. The Food and Drug Administration identifies malic acid as being an effective pain reliever for certain conditions, like the pain sustained from an ischemic reperfusion injury. It can also act as an anti-inflammatory agent (www.livingstrong.com).

Citric acid is nominal in all the species except in *tinctoria* where HCA is less and Citric acid is high. *G. mangostana* is having considerably high 1.42%. All Himalayan species are having more than 1%. Citric acid is a weak organic

Table 2: Organic Acid profile of Garcinia species

Sample	Total acidity (g %)	HCA (g %)	Malic acid (g %)	Oxalic acid (g %)	Citric acid (g %)	Tartaric acid (g %)	Acetic acid (g %)	Ascorbic acid (g %)
G. gummi-gutta	15.81	10.48	4.62	0.18	0.62	0.11	0.07	trace
G. indica	8.11	6.13	1.67	0.63	0.79	0.51	0.31	trace
G. mangostana	4.49	0.26	0.44	0.73	1.42	1.66	0.26	0.41
G. tinctoria	8.95	0.05	0.31	0.37	6.00	0.10	0.04	trace
G. subelliptica	9.16	1.16	3.77	0.92	0.81	0.88	1.22	4.61
G. kidya/Cowa	28.00	8.97	14.32	0.60	1.35	1.80	0.23	trace
G. lancifolia	12.17	1.93	10.02	1.70	1.45	0.07	0.14	trace
G. pedunculata	15.92	1.02	8.95	0.51	1.30	0.12	trace	1.54

acid. Citric acid sold in a dry powdered form is commonly sold in markets and groceries as "sour salt", due to its physical resemblance to table salt. It has use in culinary applications where an acid is needed for either its chemical properties or for its sour flavor, but a dry ingredient is needed and additional flavors are unwanted (e.g., instead of vinegar or lemon juice). A solution with a 6% concentration of citric acid will remove hard water stains from glass without scrubbing. In industry, it is used to dissolve rust from steel. It can be used in shampoo to wash out wax and coloring from the hair. (Frank, 2005).

Oxalic acid and oxalates are present in many plants, including tea and cocoa. Within the sub-group of 'weak acids', oxalic Acid is relatively strong .Oxalic acid is a chemical that can be a dangerous in high doses but not in moderate levels. In *G.indica* ,oxalic acid percentage is comparatively high (0.63%). The Himalayan sample *G. lancifolia* is having good amount of *oxalic* acid (1.7%). It is also used for juice preparation. Oxalic acid has many uses, but it is widely used as a wood bleach because it removes stains without removing the natural color of the wood. It was identified as the acid which kills cancer cells without harming normal cells (www.youtube.com/watch).

Ascorbic acid is present only in traces in Garcinia species except *G.Subelliptica* and *G.pedunculata*. Ascorbic acid which is also known as vitamin C is a good antioxidant and anti-ageing chemical. It is an important co-factor for several enzymes and essential for collagen synthesis. It is used to prevent colds and ulcers. It is known to enhance immunity. It is used in cosmetic industries for reducing ageing symptoms and enhancing wound healing (Garret and Grisham, 2005).Sometimes ascorbic acid is put on their skin to protect it against the sun, pollutants, and other environmental hazards.

Tartaric acid is high in *G.kydia* and at moderate level in *G.pedunculata*. It occurs in traces in other species. Tartaric acid is among the fruit acids used as a flavoring ingredient in soft drinks. It also adds tartness in foods. Tartaric acid is used in the tanning of leather. As a di-acid and a di-alcohol, it can be used in polymeric products, including lacquers. Additionally, it is used in textile printing and blueprinting applications (http://www.ehow.com/about_5349916_use-tartaric-acid.html)

Conclusion

Acidity plays an important part in the perception of fruit quality. It affects not only the sour taste of the fruit but also sweetness, by masking the taste of sugars. In the recent years Garcinia is more valued as medicinal crop because of the presence of HCA. Studies on the presence of other acids like Malic acid, Citric acid, oxalic acid and tartaric acid will improve the importance of the crop. HPLC chromatographic studies indicated that the *Garcinia* species showed a great variation in the number of peaks and their relative area. Among the Western Ghats species (*G.gummi-gutta* **,** *G.tinctoria,G.indica and G.Subelliptica)* along with the Malayan species *G.mangostana*, the peak corresponding to HCA (Peak No.4) had the largest area while in case of the species from NE Himalayas, the Peak 3 corresponding to malic acid was the largest. In case of *G.tinctoria*, the largest peak was for citric acid with small peak for HCA. In all the species, the peaks corresponding to tartaric acid, oxalic acid and ascorbic acid were small. Total acids obtained were highest in *G. kidya/Cowa* 28% followed by *G. gummi-gutta* and *G. pedunculata* (15.8%) while it is less in *G. mangostana* (4.5%).

References

American Heritage Dictionary: malic acid, www.answers.com

Brul, S.; Coote, P. 1999. Preservative agents in foods, mode of action and microbial resistance mechanisms. *Intl. J. Food Microbiology,* 50**:**1–17.

Cheek, M. (2004). Garcinia kola. In: IUCN 2004. IUCN Red List of Threatened Species. Downloaded on 23 December 2008.

Dibner, J. J. and Butin, P. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J. Appl. Poultry Res*., **11**: 453–463.

Frank H. V. (2005), Citric Acid, *Ullmann's Encyclopedia of Industrial Chemistry,* Weinheim: Wiley-VCH. http://onlinelibrary.wiley.com/

book/10.1002/14356007

Garret. R.H. and Grisham.C.M. 2005. *Nutrition Biochemistry*, pp 597-600.

Harborne, J.B. 2005. *Phytochemical methods: A guide to modern techniques of plant analysis*, Springer, Germany. p. 155-156.

http://www.livestrong.com/article/28688-uses-malic-acid/

Lazarowych, N.J. and P. Pekos, 1998. Use of fingerprinting and marker compounds for identification and standardization of botanical drugs: strategies for applying pharmaceutical HPLC analysis to herbal products. *Drug Inf. J.*, **32:**497-512

Lyon ,B.G., Robertson, J.A. and Meredith, F.I. 1993. Sensory descriptive analysis of cv. Cresthaven peaches: maturity, ripening, and storage effects. *J. Fd. Sci*., **58:**177-181.

Patanen, K. H. and Mroz, Z. (1999). Organic acids for preservation. In Block, S. S.. *Disinfection, sterilization & preservation (5th ed.).* Philadelphia: Lea Febiger. ISBN 0683307401.

Silva, B. M., Andrade, P.B., Valentão, P., Ferreres, F., Seabra, R.M., and Ferreira, M.A. 2004. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: Antioxidant activity. *J. Agricultural and Food Chemistry,* **52**:4705-4712.

Sullivan, A.C. and Triscari, J. 1977. Metabolic regulation as a control for lipid disorders. influence of (-) hydroxy citrate on experimentally induced obesity in the rodent. *Am. J. Clin. Nutr.,* **30:**767–776.

Utpala, P., Asish, G.R., Jayarajan, K., Aravind, R. Krishnamoorthy, B. and Mathew, P.A. 2010. Isozyme diversity of *Garcinia gummigutta* (L.) N. Robson in Western Ghat region, South India. *J. Spices and Aromatic Crops.*, **19:**29-33.