

EXTRACTION, CRYSTALLIZATION, PRESERVATION, PELLETIZING AND QUANTIFICATION OF HYDROXY CITRIC ACID FROM *GARCINIA CAMBOGIA*

E. A. H. E. Edirisinghe, S. B. Nawarathna, R. A. U. J. Marapana, J. Jaysinghe
*Department of Food Science and Technology, Faculty of Applied Sciences,
University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.*

Abstract— Hydroxycitric acid (HCA), both free acid and lactone forms is present in the fruit rind, of *Garcinia* species and it is known to help to prevent obesity. HCA is isolated from dehydrated *Garcinia cambogia* fruit rind at 60°C, by the defined most efficient extraction method of refluxing for 5 hours in soxhelt apparatus using 99% ethanol at 60°C. acid base titration with 0.5M KOH_(eth) determined The total acid content of the extracted solutions was 0.50 – 0.51g g per 1 g. most stabilized precipitate of the salt of HCA was formed with 0.05M ethanolic KOH and it was able to crystallized into a dry form by drying in a rotary vacuum evaporator, below 40°C. The formed dry precipitate was further preserved by incorporating anti caking agent at the ratio of 1:3 (vacuum dried potassium salt of HCA: corn starch). The amount of HCA in the isolated precipitate and the extract was analyzed by HPLC and UV-Visible Spectroscopy and obtained graph was compared with standard potassium salt of HCA. The λ max was obtained at 208 nm when the solution of isolated HCA was scanned between 200 – 400 nm. Content of HCA present in the plant extract has been found to be in the range of 42 - 44% by using HPLC with 0.01M hydrochloric acid as mobile phase with a flow rate of 0.5ml/min using UV detection at 208 nm. Retention time of HCA was observed at 4.535 min in selected mobile phase. Pellets were obtained by mixing aqua edible gum solution with the preserved powder of HCA salt in 1: 4 ratio and drying at 60°C for 12 hours after molding in to pellet shape.

Index Terms— *Garcinia cambogia*, High Performance Liquid Chromatography (HPLC), Hydroxy citric acid (HCA) , Potassium hydroxide (KOH), Ultra Violet (UV)

I. INTRODUCTION

Acid hydroxycitric (HCA) is a major component in the fruit rind, of *Garcinia cambogia*, which grown quite popular in Southeast Asia, to use as spice for cooking. The plant contains various chemical constituents such as Xanthenes, Benzophenones and plant acids like Hydroxy Citric Acid, Maleic acid, Citric acid. [8] Hydroxycitric acid is used for weight reduction, particularly for overweight people. The acid is known to be soluble in water and alcohol and the free acid is unstable and is converted to its more stable lactone form. For consumer products, the free acid is often stabilized by forming salts of hydroxycitric acid [6]. Traditionally its dried fruits are used as anti obesity agent to reduce the cholesterol level, prevent the fat accumulation and improve the blood lipid level by boosting the excessive glucose metabolism in the body into lipid. The novel researches have discovered that the Hydroxycitric acid in the fruit is the active compound act on increases glycogenesis, gluconeogenesis, fat oxidation, [2] suppresses the fatty acid synthesis, food intake, and induced weight loss. (1)

Effectiveness of the HCA in weight lost has been studied as invitro and invivo on human. The dosages of *G. cambogia* extract in clinical trials ranged from 1,500 to 4,667 mg/day (25 to 78 mg/kg/day). The equivalent hydroxycitric acid (HCA) dose in the trials ranged from 900 to 2,800 mg/day (15 to 47 mg/kg/day). Significant effectiveness was observed among these studies when 2800mg dosage was administrated. Decrease in Body weight and BMI (5-6%), Food intake, total cholesterol, low-density

lipoproteins, triglycerides, serum leptin levels and increase in high-density lipoprotein levels and excretion of urinary fat metabolites were observed within 8 weeks [7].

Therefore objective of the present work is to identify the productive extraction method for HCA from *Garcinia cambogia*, crystallization, preservation and quantification of the HCA amount in the extract and a pelletizing method.

II. MATERIALS AND METHODS

Preparation of *Garcinia cambogia* for HCA extraction

Non salted, non-smoked ripen *Garcinia* fruits were taken and the fleshy part was removed. Then the rind was taken and cut into small pieces and dried in dehydrator at 60°C for 24 hours.

Identification of a productive extraction method for HCA from *Garcinia cambogia* and quantification of total acid content

Extraction was carried out in different methods to identify the most productive extraction method.

Subsequent extraction method: Hot water (at 60°C) and cold water (room temperature) was used as the medium for the extraction process. In the process, 8g of *Garcinia* pieces were added to 50ml of distilled water and kept for 1 hour. Then the extract was filtered out and the filter cake was undertaken to the same process 3 times. Extraction process was repeated separately for the two temperature conditions and they were maintained in the same temperature throughout the extraction time periods. The extracts were collected separately after filtering through filter paper.

Soxhlet extraction: Exactly 12.5g of dried *Garcinia* pieces were taken and was transferred to extraction thimble and plugged the end of the thimble with cotton wool. It was placed in soxhlet extraction apparatus which was fixed to round bottom flask, filled with 100ml of distilled water and allowed to reflux for 5 hours at 60°C.

Distillation in pressure cooker: Exactly 10g of dried *Garcinia* rind pieces were taken and was cooked with about three volumes of water in an autoclave (10 lb/in²) for 15 minutes. The resulting extract was filtered through a paper filter and the extract was collected.

After extracting the acid by the above mentioned procedures, the total acid content of the extracted solutions were determined by acid base titration with

0.5M KOH_(aq) solution. Same procedure was repeated to the extracts, which were pectin precipitated by alcohol precipitation. By the results obtained the most productive method was identified.

Ethanol extraction: According to the results of the above extraction methods, the most productive method was identified and acid extraction was carried out under same condition using the solvent as 99% absolute ethanol. The total acid content was determined by acid base titration with 0.05M ethanolic KOH solution and the pH was measured to determine the end point of the titration.

Crystallization of HCA

The soxhlet extracted solutions of *Garcinia c.* in ethanol medium and water medium were treated with NaOH, KOH and CaCl₂ solutions in aqua medium and ethanolic medium at 0.5M and 0.05M concentrations to obtain the precipitate. Then the characteristics and the yield of the precipitate were observed.

Preservation of the crystallized salt of HCA

The obtained most stabilized precipitate was treated in different ways to identify the best preservation method.

Under normal atmosphere: The precipitate was kept under normal atmosphere.

In desiccator: The precipitate was kept in desiccator with silica gel.

Oven dried at 60°C: The precipitate was dried in a dehydrator at 60°C for 12 hours

Dried under vacuum: The precipitate was dried in a rotary vacuum evaporator, below 40°C

Quantification of HCA content

By UV-Visible Spectroscopy

The solution of 800ppm concentration of standard potassium hydroxy citrate (Potassium salt of HCA) and solution of 1014ppm concentration extracted potassium hydroxy citrate (Potassium salt of HCA) from *Garcinia cambogia* fruit extract (vacuum dried for precipitate) in 0.1M hydrochloric acid was scanned between 190 – 400 nm against blank disk separately. The absorption and vibration bands were observed at various frequencies and reported graph (Fig.IV) was compared with standard graph (Fig V) [5]

Content analysis by High Performance Liquid Chromatography

The solution of 800ppm concentration of standard potassium hydroxy citrate (Potassium salt of HCA) and solution of 1014ppm concentration extracted

potassium hydroxy citrate (Potassium salt of HCA) from *Garcinia cambogia* fruit extract (vacuum dried for precipitate) in 0.01N hydrochloric acid were prepared. Exactly 10 µl solutions of standard and test sample were injected in HPLC instrument using C18 column as stationary phase and 0.1M hydrochloric acid as mobile phase at a flow rate of 0.5 ml/min with UV detection at 208 nm.[3][4] The resultant graph was shown at Fig VI. The percentage content of HCA in fruit extract was calculated.

Pelletizing of salt of HCA

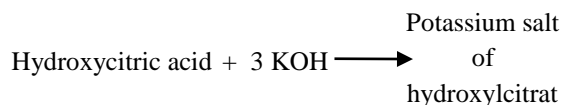
The preserved precipitates were further analyzed by incorporating anti caking agent (corn starch) in order to form a dry powder form salt, for pelletizing purposes and more stabilized salt, to store under normal atmospheric conditions. For the purpose corn starch was used with different ratios to determine the best combination for the preservation.

Pelletizing of the precipitate contains HCA salt with corn starch, was carried out using gelatin as an edible gum. The gum was mixed with water (in 1:1 ratio) and the prepared gum solution was mixed with the precipitate contains HCA salt with corn starch. (In 1: 4 ratio). Here the amount of precipitate added was calculated according to the content of HCA and the amount of HCA needed to be contained within the tablet. Then the mixture was molding into the shape or formed into a thin sheet and cut into different shapes using a mold. Then the formed pellets were dried in a dehydrator at 60°C for 12 hours.

III. RESULTS AND DISCUSSION

Identification of a productive extraction method for HCA from *Garcinia cambogia*

According to the functional groups in the HCA, it contains 3 acid groups and therefore it can combine with 3 hydroxyl groups.



In the water extraction method, color and the acid level in the extracted solutions were decreased among the

subsequence 3 extracted solutions. According to the results of the extraction methods used, highest acid amount per 1g of dried *Garcinia* rind was obtained through soxhelt extraction method.

Table 1: Total acid content in *Garcinia c.* extract, obtained by different extraction methods in water medium

Extract ion method	Water extracti on (hot)	Water extracti on (cold)	Soxhelt extracti on (water)	Pressu re cookin g
With pectin (g of acid extracte d per 1g of <i>Garcini a</i>)	0.0355	0.0323	0.2884	0.2006
Without pectin (g of acid extracte d per 1g of <i>Garcini a</i>)	0.0320	0.0289	0.2408	0.1698

According to the result of the above experiment, most productive extraction method was soxhelt extraction method and therefore it was used for the ethanol extraction process. During the titration process of the ethanol extract of the *Garcinia c.*, with 0.05M ethanolic KOH, precipitate was observed throughout the titration process and the pH changes determined that the amount of acid extracted is 0.513g g per 1 g of dried *Garcinia c.* rind.

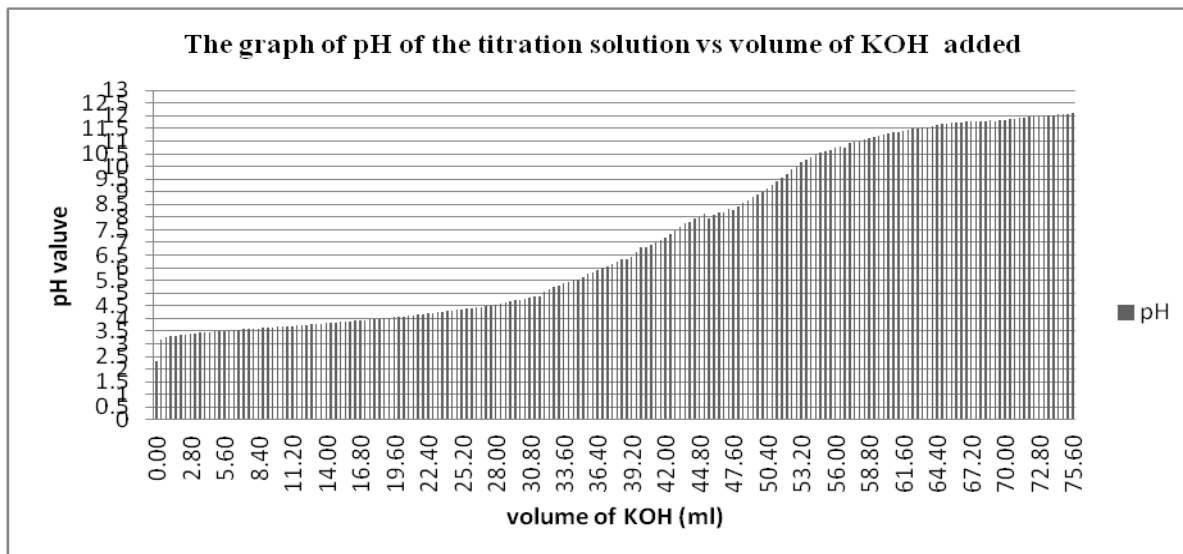


Figure 1: pH change of the ethanolic *Garcinia c.* Solution during the titration with 0.05M, ethanolic KOH

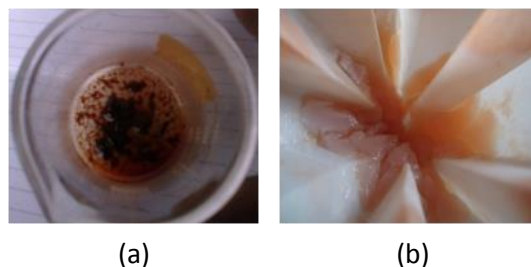
Crystallization of HCA

Water extract of *Garcinia c.* : At 0.05M concentration of aqua KOH, NaOH and CaCl₂ precipitate were not observed. At higher concentrations of aqua KOH and NaOH precipitate was obtained. But due to the hygroscopic nature of the formed salt, it dissolved rapidly by absorbing moisture from the medium. No precipitate was obtained in the addition of aqua CaCl₂, at the higher concentrations. In the addition of ethanolic KOH, NaOH and CaCl₂ same results were observed but the precipitate dissolved rapidly in the presence of water in the medium.

Ethanol extract of *Garcinia c.*: At low concentrations of ethanolic KOH and NaOH, precipitate was observed with small sized crystals with light pink color. At higher concentrations, precipitate was form as cluster. Crystals were red-brown crystals due to the present of color pigments in *Garcinia C.* extract. (Fig: 3) When the precipitate washed with ethanol the color removes gradually. It was also hygroscopic and dissolves in the presence of moisture (Dissolve by absorbing moisture in air). The precipitate formed with ethanolic KOH was more stable than the precipitate formed with ethanolic NaOH under the normal atmospheric condition. But the stability of the precipitate formed in ethanol medium was higher than the precipitate formed in water medium. No precipitate was obtained in the

addition of ethanolic CaCl₂, neither at the higher or lower concentrations.

Figure II: Formation of crystals at different concentrations of KOH (a)- at higher concentrations / (b)- at lower concentrations Preservation of the crystallized salt of HCA



Due to the highly hygroscopic nature of the formed crystals, it rapidly dissolved by absorbing moisture from the atmosphere. The same action was observed with the precipitate kept in desiccator at lower rate. The precipitate dried in a dehydrator at 60C⁰ was a light brown color solid with a hard sticky texture. Light pink / white colour powder form solid precipitate was obtained by drying the precipitate under vacuum. The best form of precipitate was formed by rotary vacuumed evaporation and it was stabilized under vacuum conditions. All the formed preprecipitates were dissolved within few seconds under normal atmospheric condition by absorbing moisture.

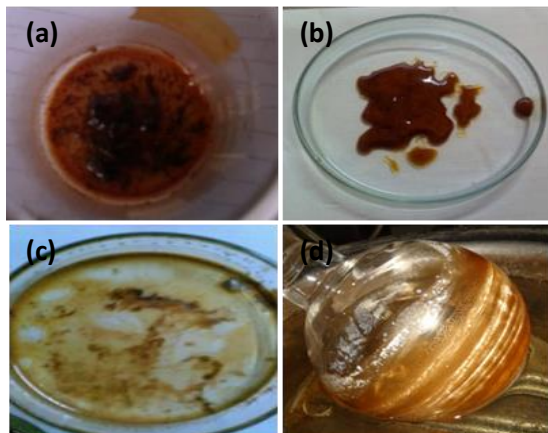


Figure III : Preservation of precipitate under different conditions a- Under normal atmosphere / b- dried in a desiccator / c- dried in a dehydrator at 60C⁰/ d- dried using rotary vacuum evaporation

Quantification of HCA content

Qualitative Analysis By UV-Visible Spectroscopy

The absorbance maximum was observed at 208nm; for the UV-Visible Spectrum for standard potassium salt of Hydroxy citric acid and it was 0.333A. For the test sample the absorbance was 2.523A at the same wavelength.

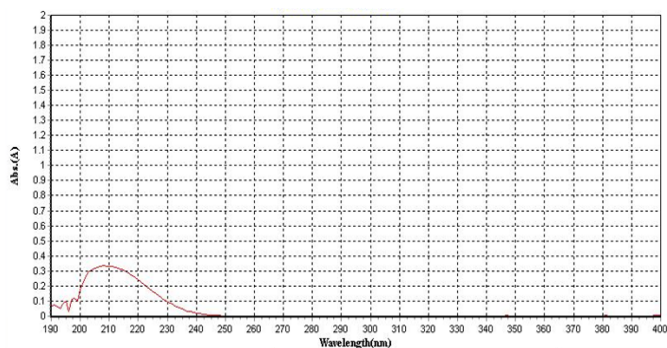


Figure IV: UV-Visible Spectrum for standard potassium salt of Hydroxy citric acid

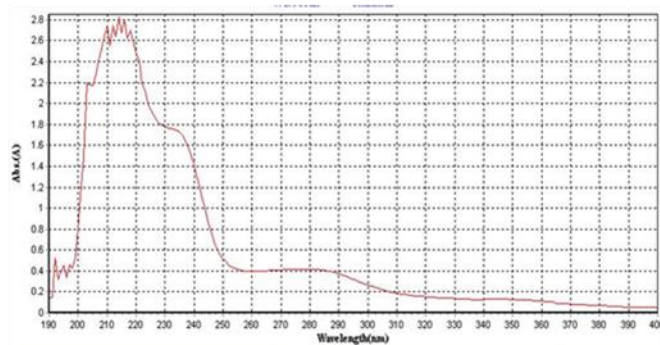


Figure V; UV-Visible Spectrum for extracted potassium salt of Hydroxy citric acid from *Garcinia cambogia* fruit extract

Content analysis by High Performance Liquid Chromatography

The absorbance maximum was observed at 208nm; hence 208 nm was used for HPLC detection. Retention time of HCA was observed at 4.535 min in selected mobile phase. Some other minor peaks were also observed which may be due to other acids present in plant. HCA was resolved as single peak and confirmed by spiking with standard HCA. The content of potassium salt of HCA in the precipitate was around 1300 – 1400 mg/g and the amount of HCA in the precipitate was Content of HCA in fruit extract was found to be 48 - 49%.

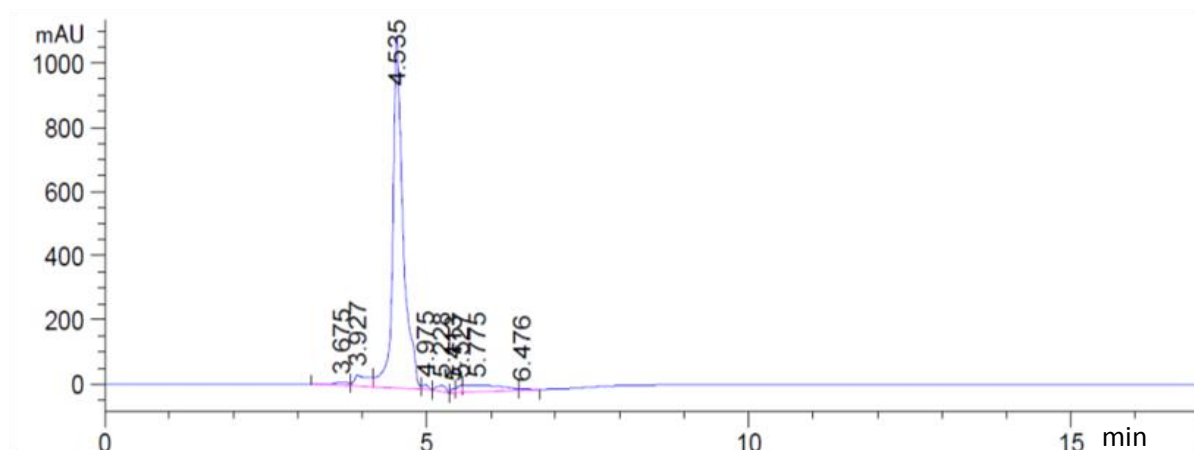


Figure VI: HPLC graph for extracted potassium salt of Hydroxy citric acid from *Garcinia cambogia* fruit extract

Pelletizing of salt of HCA

After treating the dried precipitate with the anti-caking agent (corn starch) the stability was increased and the precipitate was able to preserve without dissolving by moisture.

The oven dried precipitate was not mixed well with corn starch and the formed a cluster natured precipitate among the corn starch powder.

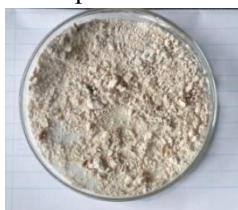


Figure VII: Precipitate formed by incorporating of oven dried precipitate and anti-caking agent (corn starch)

The precipitate kept under normal atmosphere and desiccators formed a sticky nature precipitate at lower ratios of corn starch and powder form precipitate at higher ratios of corn starch. But the ratio of corn starch needed to be incorporated to form the powder form was higher than 1: 6 (oven dried precipitate: corn starch).

Figure VIII: Preservation of desiccators dried



(a) - 1:3 ratio (b) – 1:6 ratio

precipitate using different ratios of anti-caking agent (ratio between corn starch: desiccators dried precipitate)

The best preserved precipitate was observed in Vacuum dried precipitate with corn starch. At lower ratios of corn starch, precipitate was sticky in nature and tends to absorb moisture with time. At higher ratios of corn starch, the precipitates formed in dry powder nature and it was stable under normal atmospheric conditions. The most stabilized dry powder precipitate with lowest corn starch amount gave at the ratio of 1:3 (vacuum dried precipitate: corn starch).



(a) 1:2 ratio (b) 1:3 ratio (c) 1:4 ratio

Figure IX: Preservation of vacuum dried precipitate using different ratios of anti-caking agent (ratio between corn starch: vacuum dried precipitate)

After mixing the edible gum solution with the precipitate preserved by vacuum drying and incorporated with corn starch it showed a non-sticky and non hygroscopic in nature. After molding and drying the pellets were in exact shape with rigid nature.



Figure X: Pellets formed by preserved precipitate and edible gum.

IV CONCLUSION

In summary, hydroxycitric acid was extracted from the dehydrated *Garcinia c.* rinds by using soxhelt extraction method. Extraction using 99% ethanol as solvent was defined as the most productive method by analyzing the total acid content and the amount of acid extracted is 0.513g per 1 g of dried *Garcinia c.* rind. Most stabilized precipitate or crystals of potassium salt of HCA was obtained by treating the extract with 0.05M KOH_(eth). The obtained crystals were highly hygroscopic in nature and therefore it was preserved by drying under vacuum, below 40°C and incorporation of anti caking agent (corn starch) in 1:3 ratio. Pellets were obtained by mixing aqua edible gum solution with the preserved powder of HCA salt in 1:4 ratio and drying at 60°C for 12 hours after molding in to pellet shape. The amount of HCA in the isolated precipitate and the extract was analyzed by HPLC and UV-Visible Spectroscopy and obtained graph was compared with standard potassium salt of HCA. The λ max was obtained at 208 nm when the solution of isolated HCA was scanned between 200 – 400 nm. Absorbance values were 0.333A and 2.523A for potassium salt of Hydroxy citric acid and test sample respectively. Content of HCA present in the plant extract has been found to be in the range of 42 - 44% by using HPLC with 0.01M hydrochloric acid as mobile phase with a flow rate of 0.5ml/min using UV

detection at 208 nm. Retention time of HCA was observed at 4.535 min in selected mobile phase.

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