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Original Research Article

ISOLATION AND CHARACTERIZATION OF CHEMICAL CONSTITUENTS OF *Garcinia cambogia* AS MARKERS

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Abstract: Background and Objectives: *Garcinia cambogia* (Guttiferae), generally known as Malabar tamarind is a dried fruit rind commonly found in Western ghats of south India. It extends from Konkan southward to Travancore and in the forests of Nilgiris. It is highly regarded for their medicinal properties in the indigenous systems of medicine.
The present study deals with the isolation and characterization of the chemical constituents of *Garcinia cambogia.*
Methodology: Authenticated aqueous extract of *Garcinia cambogia* is collected from Pharmed Medicare, Bangalore. Phytochemical screening of extract has been done. Isolation of phytoconstituents by column chromatography and/or alternate method of isolation was carried out with aqueous extract. The isolated compound was identified by comparing the HPLC fingerprint of the sample with the standard chromatogram of (-)-HCA.
Results: One compound has been isolated and estimated as (-)-Hydroxy Citric Acid by comparing the HPLC data of sample and standard.
Conclusion: The isolation procedure used is suitable to isolate the marker constituent from aq extract of *Garcinia cambogia.*
Keywords: *Garcinia cambogia*, (-)-Hydroxy Citric Acid, HPLC.

Introduction: Plants have been used as a source of medicine by man from ancient times. Initially, these formed the bulk of folk or ethnomedicine, practiced in India and some other parts of world like China, the middle east Africa and south America. Later a considerable part of this indigenous knowledge was formulated, documented and eventually passed in to the organized system of medicine such as Ayurveda, Unani, Siddha or some other systems outside India. Plants have granted mankind a large divergence of potent drugs to assuage suffering from diseases.
As the herbal drugs contain so many chemical compounds, it is essential to separate out those

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compounds, which are responsible for therapeutic effect and are called active constituents.

Garcinia cambogia (Guttiferae) is a small or medium sized tree with rounded crown and horizontal or drooping branches; leaves dark green and shining, elliptic obvate, 2-5 inches long and 1-3 inches broad. Fruits ovoid, 2 inches in diameter, yellow or red when ripe, with 6-8 grooves surrounded by a succulent aril. The fruits are edible, but too acidic to be eaten raw. They are valued for their dried rind. It is rich in acids which contains tartaric acid 10.6%, reducing sugars (as glucose) 15%, and phosphoric acid (as calcium triphosphate) 1.52% of the total acids present in the material, nearly 90% is non- volatile.

Chemical constituents of the herb[6]: (-)- Hydroxy Citric Acid. (-)- Hydroxy Citric Acid Lactone. Garcinol. Isogarcinol. Cyanidin 3 sambubioside.

Marker constituents:

(-)- Hydroxy Citric Acid.

Methodology

Collection of plant material: Authenticated aqueous extract of *Garcinia cambogia* was collected from Pharmed Medicare, Bangalore.

Collection of solvents: The following solvents were collected from Pharmed Medicare, Bangalore.

1. Methanol HPLC grade 2.Acetonitrile HPLC grade 3.Millipore water 4.Disodium Sulphate 5.Standard

Collection of materials: Different kinds of glass wares and membrane filters were collected from Pharmed Medicare, Bangalore.

Collection of equipments: Different kinds of equipments viz., Analytical-weighing balance, HPLC system, Column, Sonicator, Water purification system, Vacuum filter pump, Glass vacuum mobile phase system, Water bath, Sample filtration assembly were used throughout the experiment. All were collected from Pharmed Medicare, Bangalore.

Phytochemical screening.

Detection of Carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test b) Benedict's test c) Fehling's test

Detection of alkaloids: The small portion of aqueous extract was stirred with a few drops of dil.HCl and filtered and then subjected to test for alkaloids.

a) Dragendorff's Test b) Mayer's Test c) Hager's Test d) Wagner's Test

Detection of glycosides: Extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test b) Legal's test

Detection of saponins: Foam Test

Detection of phytosterols: a) Salkowski's Test b) Libermann Burchard's test

Detection of fixed oils and fats: Stain Test

Detection of resins: Acetone-water Test

Detection of phenols: Ferric Chloride Test

Detection of tannins: Gelatin Test

Detection of flavonoids: a) Alkaline Reagent Test b) Lead acetate Test c) Shinoda Test

Detection of proteins and amino acids

a) Xanthoproteic Test b) Ninhydrin test c) Biuret Test

Detection of Citric Acid:

a) To 2 ml test solution, add one drop dil. NH_4OH and excess cadminum chloride solution. Boil for 15 min. in boiling water bath. White gelatinous ppt observed.
b) To 2-3 ml test solution add one drop of dil. NH_4OH and excess $AgNo_3$ solution. Boil for 15 min. Blackish silver mirror formed.
c) To 2 ml. test solution, add few drops Denige's reagent and few drops dil. $KMnO_4$. Heat Permanganate color disappears.

Results of Phytochemical screening are tabulated in table No. 1.

Isolation of phytoconstituents

1. By Column chromatography

The aqueous extract collected from Pharmed Medicare was subjected to column chromatography using different solvent

systems. The fractions collected were further chromatographed using silica gel G as a stationary phase to isolate non-polar constituents.

Column chromatography was done by using a glass column. The dimensions of the column was 27X40mm. The column was packed with silica gel by wet packing method wherein a padding of cotton was placed at the bottom of the column and then it was filled with eluting solvent of the lowest polarity (pet ether). Then the required amount of stationary phase (silica gel) was poured into the column to form a bed of silica. The solvent was eluted to the top of the bed. The extract was then poured on to the bed of silica, a layer of cotton covered it again and more amount of solvents was poured over it, the column was then eluted gradiently. The column chromatography consists of following steps:

1. **Pre-column preparation:** The pre-column preparation included adsorption of the selected extract/ fraction, charging and saturation of the column.
   a. Adsorption of the extract: The extract selected for fractionation was adsorbed on stationary phase in ratio 1:1 and dried at 60°C in oven.
   b. Charging of column: A glass column was selected and rinsed with the solvent. A cotton layer was placed at the bottom and the column was charged with the solvent and stationary phase. The silica gel was used in the ratio (1:10) of the extract to make the gel bed for complete separation. The solvent was eluted up to the level of column bed and the dried extract was charged in the column. Another layer of cotton was placed over the charged matter to prevent the disturbance of the extract bed while pouring the eluting solvent from the top.
   c. Saturation of the column: The charged column was left for 6 hrs. for complete saturation and removal of air bubbles to make the bed static.
2. **Elution:** The charged column was then eluted with different mobile phases with gradual increase in polarity/non-polarity. The fractions collected were dried in concentrator/rotavapor (Buchi). The dried fractions were then weighed and kept for further used. All the fractions were subjected to TLC for the identification of the desired bands.

Column requirements: -

- Stationary phase – silica gel G (60-120 mesh)
- Mobile phase – pet ether, benzene, chloroform and methanol
- Charged material – aq extract
- Volume of fraction – 150ml
- Visualization - Iodine, anisaldehyde sulfuric acid

Procedure: The column was first eluted with 100% pet ether. The polarity of mobile phase was gradually increased with Benzene, Chloroform, MeOH, EtOH. The fraction collected were concentrate. The desired concentrated fractions were screened for phytoconstituents. The desired concentrated and dried fractions were kept in container with suitable label and kept for further use.

Manufacturing Process of Calcium Salt of (-)-Hydroxy Citric Acid

- The dried rind of Garcinia cambogia is extracted with demineralised water (4 times) in a pressure extractor.
- The material and extraction medium is kept at a pressure of 10lb/in^2 for 30 to 45 min. under forced circulation. Live steam can also be used for building up the required pressure.
- The extract is drained through candle filter and marc is further extracted under identical conditions till rind tastes sour.
- The total filtered liquid is concentrated in an evaporator to 20-25 % solids. It is then adjusted to pH 4.0 by 10% aqueous sodium hydroxide solutions.
- It is left over night for separation of resinous material.

- Next day it is filtered and adjusted to pH 9.0-9.2 by NaOH solution.
- It is heated in a reactor for 30 minutes and drained in a tank fitted with stirrer.
- 10% $CaCl_2$ solution is added with stirring to pH 6.0. Stirring is continued for 30 minutes.
- The precipitated calcium salt is filtered in a centrifuge or vacuum filtration equipment and washed with water till washings show no trace of $CaCl_2$ or $Ca(OH)_2$.
- The precipitate is dried in vacuum tray dryer at temperature not more than 60^0C.
- The dried material is pulverized, sifted to required mesh (80-100) and packed-yield 30-32.0%.

Characterization of the isolated compound: The isolated compound was identified by comparing the HPLC fingerprint of the sample with the standard chromatogram of (-)-HCA.

Fingerprint of isolated compound using HPLC: The fingerprints of isolated compound and of standard compound are given in annexure 1 and 2.

Results and Discussion

Phytochemical screening.

Table no. 1

Chemical Constituent	Tests	Extract (aq)
Alkaloids	1. Mayer's test	-
	2. Dragendorff's test	-
	3. Wagner's test	-
	4. Hager's test	-
Carbohydrates	1. Molisch's test	-
	2. Benedict's test	-
	3. Fehling's test	-
Glycosides	1.Modified Borntrager's	-
	2. Legal test	-
Saponins	1. Foam test	-
Phytosterols	1. Salkowski test	-
	2.Libermann Burchard	-
Phenols	1. Ferric Chloride test	-
Tannins	1. Alkaline Reagent	-
Flavanoids	1. Gelatin test	-
	2. Lead acetate test	-
	3. Shinoda test	-
Proteins	1. Xanthoproteic test	-
	2. Ninhydrin test	-
	3. Biuret test	-
Citric acid	1. Cadminum chloride test	+
	2. $AgNo_3$ test	+
	3. Denige's test	+

Isolation of Phytoconstituents: The isolation of phytoconstituents was experimented by column chromatography and alternate method of isolation of markers.

Table- 2: Details of column

Column size	Stationary Phase	Mobile phase	Charged material	TLC solvent	Visualization
600X27mm	Silica gel (60-120mesh)	PE ,Bz, $CHCl_3$ MeOH	Benzene extract Wt. 7gms	PE : Bz (3: 2)	ANS and I_2

Report

Although the isolation for compounds from *Garcinia cambogia* through column chromatography has been experimented but didn't got any compound by this method.

Hence the alternate method of isolation of marker compound from *Garcinia cambogia* had been carried out.

Paper Chromatography of isolatedcompound

Table No 3

Solvent system	Butanol: Acetic acid: Water (4:1:5)
Paper	Whatmann filter paper No. 1
Spray Reagen	Sodium Metavandate

Sample Preparation:

100 mg of sample is dissolved in 1 ml of water and dilute to 10 ml with methanol.

Paper chromatogram for isolated compound

Characterization of the isolated compounds

The isolated compound was identified by comparing the HPLC fingerprint of the sample with the standard chromatogram of (-)-HCA.

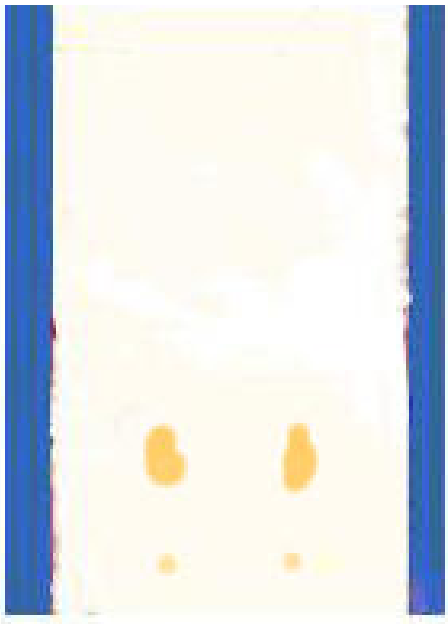
Data is given in annexure 1 and 2.

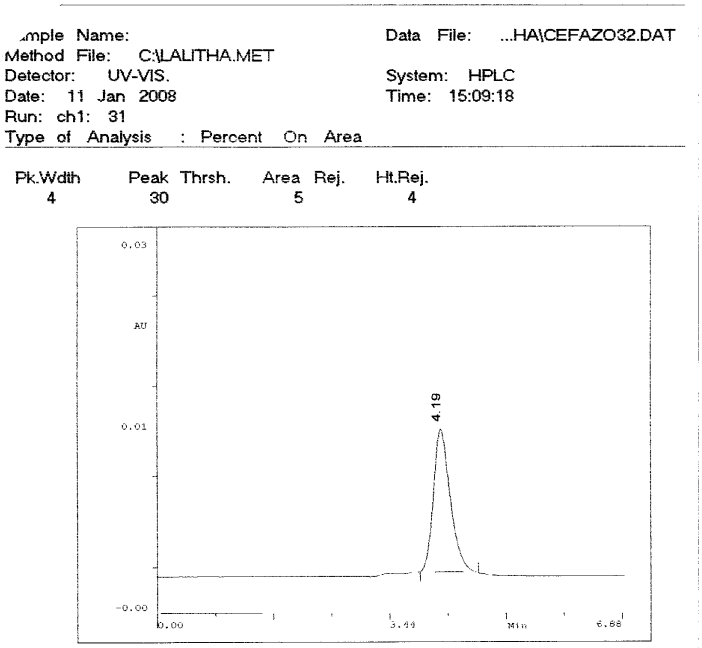
Fingerprint of isolated compound using HPLC:

The isolated compound was determined by HPLC. The spectrum is given in annexure 1.

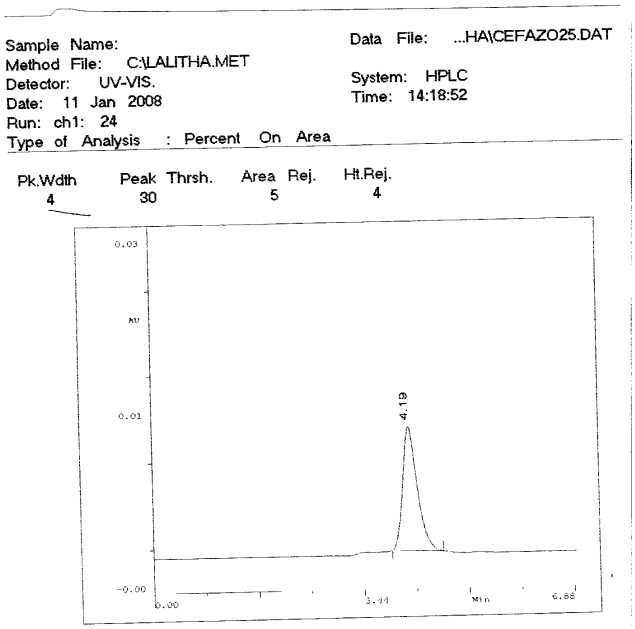
Sample Compound: - A nice peak has been observed at the retention time 4.19 and it covers the area 1191863.

Standard Compound: - Peak has been observed at the retention time of 4.19 and it covers the area 104429





Annexure- 1



Annexure- 2

Conclusion

- The present study is an attempt to study phytochemical investigation, which includes phytochemical screening, isolation and identification of constituents from aqueous extract of *Garcinia cambogia*.
- Aqueous extract was subjected to isolate compounds by column chromatography and/or alternate method of isolation. Marker was isolated by alternate method of isolation.
- The isolated compound was identified by comparing the HPLC fingerprint of sample with standard chromatogram of (-)- HCA.
- The method has been developed to validate the isolated compound by HPLC using Wakosil II C18 250 x 4.6mm, 5µm column and 4:1 of 0.01 M Disodium sulphate and methanol (pH 2.5 adjusted with H_2SO_4) as mobile phase at the wavelength of 212 nm.

The present study gives the precise method of isolation of marker from the *Garcinia cambogia* and its identification. And the developed HPLC method provides estimation of (-) – HCA. The present HPLC method is suitable for the quality control of the Marker in *Garcinia cambogia*.

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