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Isolation and Characterisation of Hydroxy Citric Acid from the Fruit Extract *Graciana Cambogia*

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KEYWORDS
Garcinia cambogia, Hydroxy Citric Acid

ABSTRACT:
The *Garcinia cambogia* fruit rind, often referred to as Garcinia gummi-gutta, has long been used as a flavoring and as an anti-inflammatory, particularly in the treatment of obesity. Many active chemical ingredients, including terpenoids and hydroxycitric acid, are found in the fruit of G. Cambogia. The goal of this study was to employ column chromatography to separate hydroxycitric acid from ripened dried fruit extract of Garcinia Cambogia. Hydroxyl citric acid was isolated using methanolic extraction amounted 1.8 gm . The solvent system of toluene, ethyl acetate, and acetic acid (5:4:1) was used for the isolation process. Spectrophotometric analysis was used to quantify the isolated hydroxycitric acid and thin layer chromatography was used to assess its purity. The outcomes showed that hydroxycitric acid may be obtained from *Garcinia Cambogia.*

Introduction

The production of beneficial bioactive secondary metabolites—which are crucial for both individual and societal health—can be largely attributed to medicinal plants. The chemical components in plants that have a specific physiological effect on humans are what give them their therapeutic qualities. The dried fruit of the Garcinia cambogia tree, which grows in the woods of south Asia and south India, is the source of the extract. Hydroxycitric acid is the primary active component (HCA). It inhibits lipogenesis, which stops carbs from being metabolized into fats. It increases the body's synthesis of glycogen in the liver, burns extra fat, and reduces appetite to prevent overeating[1].

Originating in Southeast Asia, Garcinia Cambogia was once known by the scientific name Garcinia cambogia (Gaertn.) Desr. (Clusiaceae). In many Asian nations, the fruit rind is used as a traditional treatment for a variety of conditions, including constipation, piles, rheumatism, oedema, irregular menstruation, and intestinal parasites[2]. It is also frequently used as a food preservative, flavoring agent, or food-bulking agent. Many organic acids, benzophenones, and xanthones were isolated from the plant based on previous phytochemical findings. Numerous scientific investigations have also suggested biological activity, including anti-obesity, hypolipidaemic, and anticancer action, among many other things[1].

Garcinia cambogia-containing commercial items sprung onto the scene and attracted a lot of attention from both favorable and negative media sources. It is clear that there is ambiguity surrounding the use of this plant, particularly as more data come to light[3]. An incident of potential serotonin toxicity is described in a prominent media piece titled "Garcinia cambogia: weight-loss supplement may be toxic to some." [4] The author may or may not ascribe this to the concurrent use of G. cambogia and an antidepressant. Hydroxycitric acid (HCA) ranges from 20 to 60% in garcinia supplements, and many of these products don't just contain Garcinia cambogia as their main active ingredient. When evaluating the effectiveness, safety, and quality of these products, this should also be taken into account. [5] There have also been reports that the advertised concentration of HCA in many products is less than the value given. The primary ingredient in the fruit rind that may be responsible for its

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weight-loss function is HCA, an α-, β-dihydroxy tricarboxylic acid. [6]

The fruit has a 10% to 30% HCA content that can be separated as a lactone, a mineral salt, or in its free form. On the market, HCA can be found in a variety of salt forms, including combinations of calcium, magnesium, and potassium. [7] Another possible source of natural HCA is the presence of HCA in a variety of bacterial species. Citric acid can also be used as a starting material to synthesize hydroxycitric acid. After being dehydrated, citric acid becomes aconitic acid, which is then oxidized to produce hydroxycitric acid [20]. HCA has been shown to prevent weight gain by blocking adenosine triphosphate (ATP)-citrate lyase, which is the enzyme in charge of converting citrate into oxaloacetate and acetyl-coenzyme A (acetyl-CoA), a building block of fatty acid synthesis . [8]

According to a number of studies, HCA helps people lose weight without activating the central nervous system. It's clear that opinions on Garcinia cambogia's safety and effectiveness are divided. [9] This article provides a brief summary of the scientific data that is currently available regarding the toxicity and biological activity of Garcinia cambogia and HCA, as well as significant phytochemical, botanical, and other characteristics. [10]

Materials and Methods

Collection and processing of plant samples

The fruit of *Garcinia cambogia* and were collected from the local area during the month of November. The taxonomic identification of plant material was done and identification no. was 2023-074 and date of authentication 20th Jan 2023. The fruit rinds of *Garcinia cambogia* and were washed with water, shade dried at room temperature and powdered coarsely. Pericarp (fruits) of G. cambogia was dried and it was powdered coarsely in a mixer grinder. The powder was stored in an airtight container and used for further extraction. About 100 g of powder of the pericarp was extraction was carried out using organic solvents; Petroleum ether and 80% methanol for 4-5 hours and 40-60oC temperature of the heat¬ing mantle were adjusted. After the extraction process, the extract of sample were filtered and concentrated to dryness. Extracts were collected in air tight container. [11]

Qualitative Phytochemical Estimation of Extracts

Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids, Proteins and Anthraquinone were performed . Mayers test, Wagner s test for Alkaloids, Shinodas test for flavonoids, Benedicts test, Molisch s test for carbohydrates, Keller-Killani test for cardiac glycosides, Froth test for saponins, Lead acetate test for tannins, Salkowski test for terpenoids, Ninhydrin test and Biuret test for protein and Ammonia test
for anthraquinone were performed[12].(Table 1)

Quantitative Phytochemical estimation

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. The *G. cambogia* fruitsextracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically. The flavonoid content was determined using Aluminium chloride metho. 0.5 ml of *G. cambogia* fruits extracts solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. [13,14] After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. Then the mixture was diluted up to 5 mL with distilled water mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight**.(Table-2)(Graph1 &2)**

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Isolation

Thin Layer Chromatography

Thin Layer Chromatography of *Garcinia Cambogia* extract was carried out on TLC plates of silica gel 60 F_{254} pre coated with layer thickness of 0.2 mm using different solvent systems. Spots were applied manually using capillary tube, plates were air dried using air blower and TLC chamber were developed at room temperature with respective solvent systems. Spots on TLC plates were visualised with spraying reagent: sulphuric acid solution, then in UV light. R_f values were calculated .[8] **(Fig 1)**

$$R_f \text{ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Solvent system developed in preliminary TLC for *GC* methanolic extract in which the maximum spots were visible in Toluene: Ethyl acetate: Acetic acid (5:4:1) mobile phase with std. terpenoid. So that Toluene: Ethyl acetate: Acetic acid (5:4:1) solvent was taken as mobile phase for column chromatography[17]

Column chromatography

Methanolic extract was subjected to silicagel column chromatography for isolation of Terpenoid. from*Garcinia Cambogia* extract. A vertical glass column made of borosilicate material was used for chromatography. The column was rinsed with the acetone and was completely dried before packing. Column was packed using wet packing technique using silica gel (60-120) as the adsorbent. Slurry was prepared using toluene and was poured in to the column. 1gm of extract was added over the top of the column. Gradient elusion technique was followed for column chromatography. [15] The column was eluted with Toluene: Ethyl Acetate: Acetic acid (5:4:1) number of elutes were collected. The fractions/elutes collected were concentrated and TLC was performed to identify the presence of single compound**.(Table-3)**

Characterization

UV-visible Spectroscopy

The isolated fraction of sample was diluted to 1:10 with the same solvent. The extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded. The isolated fraction (C) of *GC*Extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded. [16]**(Graph-2)**

FT-IR

To establish the presence of the functional groups in the isolated fraction (C) of GC Extract, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The sample was dried and ground with KBr pellets and analyzed on Thermo Nicolet model 6700 spectrum instrument. A disk of 200 mg of KBr was prepared with a mixture of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 400 - 4,000 cm-1[10]. **(Graph3,Table4)**

NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction to identify the structure of the compound present in the isolated fraction. ^{1}H NMR spectra of synthetic compounds were recorded on NMR Spectrometer (Bruker AV 500, at 500.130 MHz).NMR spectroscopy was performed for the isolated fraction (C) of GC Extract to identify the structure of the compound present in the isolated fraction. JEOL RESONANCE NMR spectroscopy for this purpose was Fourier Transform Nuclear Magnetic Resonance spectroscopy [11].**(Graph-4)**

Mass Spectroscopy

Mass spectrometry converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of the compound was Bruker Daltonik, Benchtop easy-to-use, high performance Electrospray Ionization Quadrupole time-of-flight LC MS spectrometer. Mass spectrometry converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of isolated fraction (C) of *GC* Extract was recorded on mass spectrometer instrument OTOF-MS[12].**(Graph-5)**

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RESULT

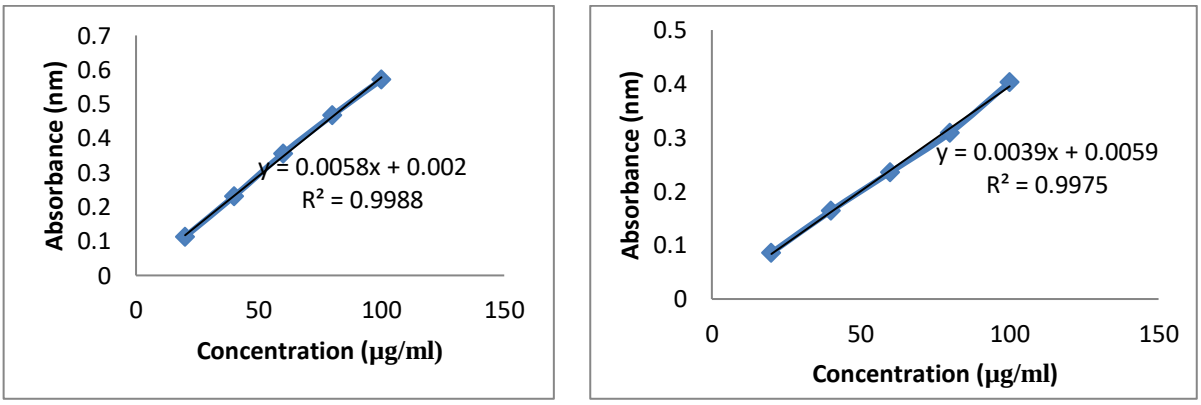
Table 1 :Qualitative analysis of phytochemicals present in the extract of *G. cambogia*

Compounds	Methanolic Extract
Flavonoids	++
Terpenoids	+
Phenols	++
Tannins	-
Cardiac glycosides	+
Carbohydrates	+
Saponin	+
Amino acids	+
Phlobatannin	-
Sterols	+
Coumarin	-

+++ presentin higher amt; ++ moderately present ; + present ; - means absent

Table 2 : TPC and TFC of the *Garcinia Cambogia fruit* extract

Concentration (µg/ml)	Absorbance (760 nm) (gallic acid)	Absorbance (510nm) (rutin)	Total Phenolic content (mg/gm equivalent to Gallic acid)	Total Flavonoid content (mg/gm equivalent to Rutin)
20	0.113	0.086	Absorbance	Absorbance
40	0.231	0.164	0.2275±0.002	0.1190±0.003
60	0.356	0.236	TPC	TFC
80	0.467	0.309	45.10	38.00
100	0.571	0.403		



(a) (b)

Graph 1: represent standard curve a and b of Gallic acid and Rutin

Preliminary TLC preparation for the estimation of active constitutes –

TLC of *Garcinia Cambogia* Methanolic extract
Mobile Phase- Toluene: Ethyl acetate: Acetic acid (5: 4: 1)

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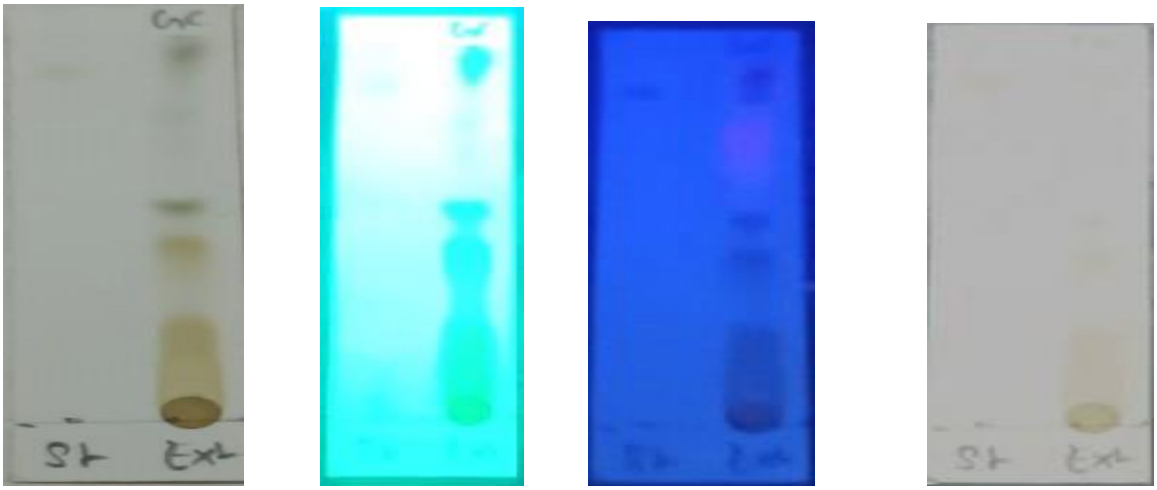

Short-UV (254nm) **Long-UV (365 nm)** **Visible**

Figure1: TLC estimation by UV lamp for GCwith Std. Terpenoid.

(Std.= Standard,GC = Garcinia Cambogia)

Table 3: Fraction collected from Column Chromatography of *GC* Methanolic extract

Eluent composition	Fraction collected	Remarks
Toluene: Ethyl Acetate: Acetic acid (5:4:1)	01 (A)	White creamy coloured mixture of compound
	02 (B)	Greenish coloured mixture of compound
	03 (C)	Light Yellowish coloured mixture of compound
	04 (D)	Greenish coloured mixture of compound
	05 (E)	Light Greenish coloured mixture of compound
	06 (F)	Very Light Greenish coloured mixture of compound
	07 (G)	Yellowish coloured mixture of compound
	08 (H)	White creamy coloured mixture of compound
	09 -10 (I) (I1, I2)	Yellowish coloured mixture of compound
	11 (J)	Light Yellowish coloured mixture of compound
	12 (K)	White creamy coloured mixture of compound

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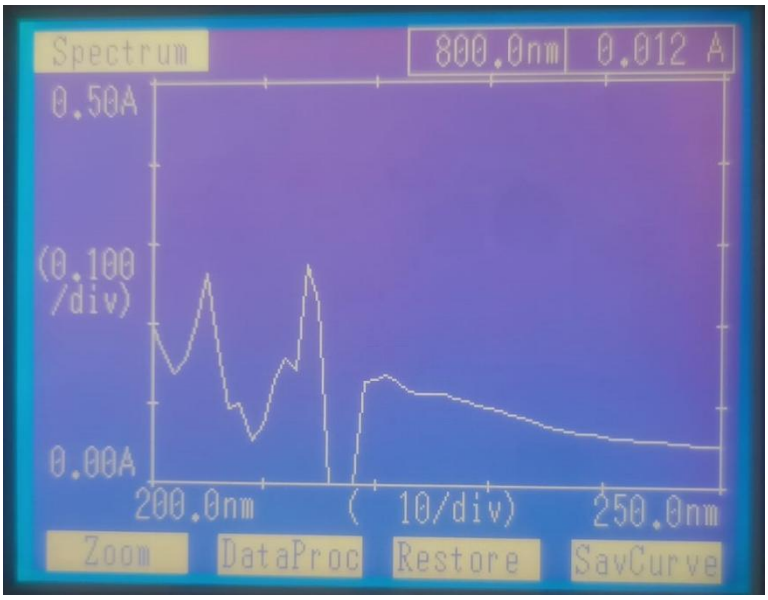
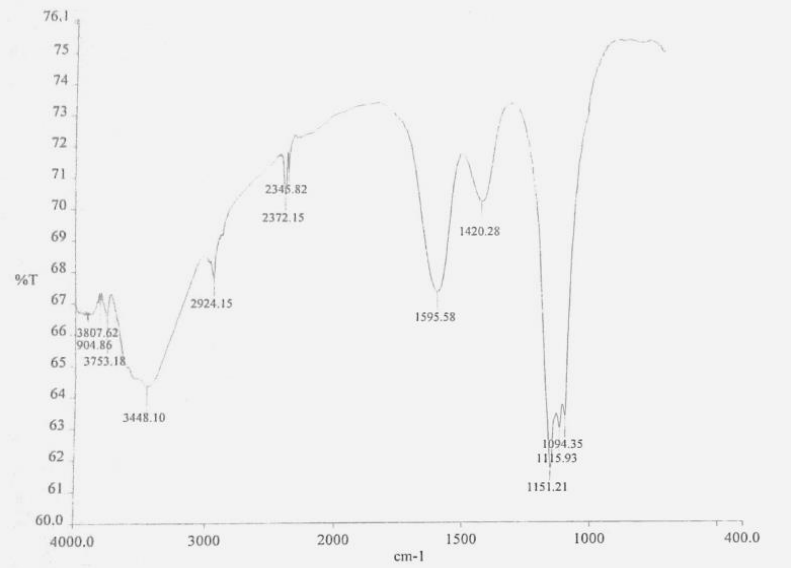


Graph 2: Active constitutes estimation By UV- Spectra of (A) fraction of GC Methanolic extract after column chromatography



Graph 3: IR spectra of the isolated **fraction (C) of G cambogia Methanolic extract**

Table 4: FTIR- Spectrum Frequency Range of the isolated **fraction (C) of GC Methanolic extract**

Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
C	3550-3200 (cm^{-1})	3448.10	Strong, Broad	O-H stretching	Hydroxyl Group

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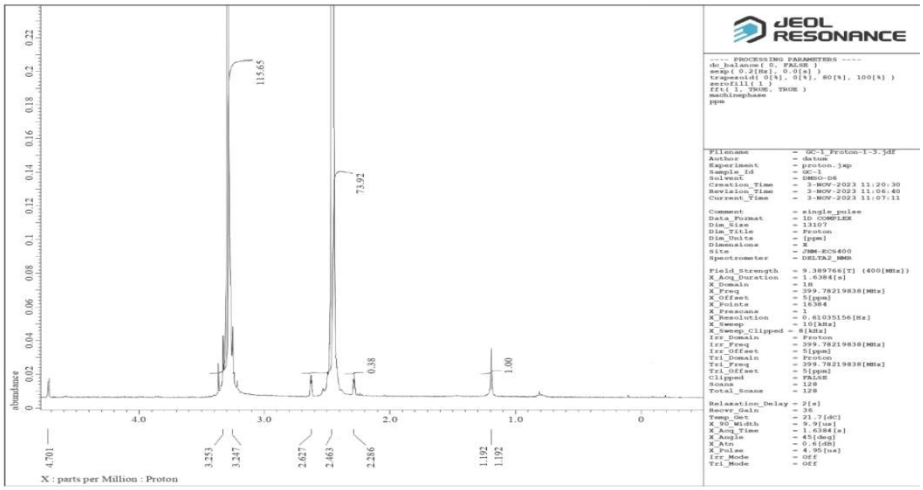
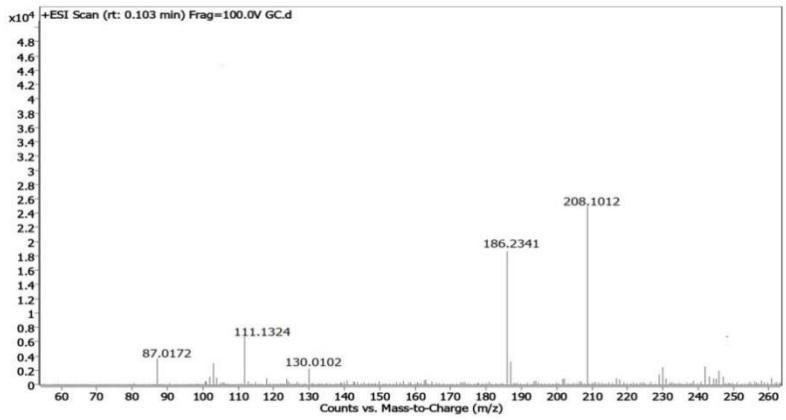
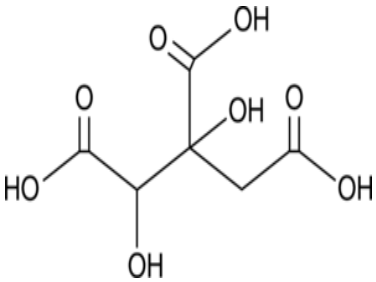
	3000-2840 (cm^{-1})	2924.15	Medium	C-H stretching	Alkane
	2400- 2000 (cm^{-1})	2372.15	Strong	C-H stretching	Alkane
	1600–1578 (cm^{-1})	1595.58	Medium	C-O stretching	Carbonyl group
	1440-1395 (cm^{-1})	1420.28	Medium	O-H bending	carboxylic acid
	1205-1124 (cm^{-1})	1151.21	Strong	C-O stretching	Alcohol
	1400- 1100 (cm^{-1})	1115.93	Weak	C-C stretching	Alkane
	1124-1087 (cm^{-1})	1094.35	Strong	C-O stretching	Alcohol



Graph 4 :^{1}H-NMR spectra of the **isolated Fraction (C) of GC Methanolic extract**





Graph 5: Mass spectra of the isolated Fraction (C) of GC Methanolic extractand structure of 1,2-dihydroxypropane-1,2,3-tricarboxylic acid

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Discussion

In the Preliminary TLC of *Garcinia Cambogia* Methanolic extractwas performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in Tolurene: Ethyl acetate: Acetic acid (5:4:1) with Std. Terpenoid. that were clearly visible bands in *GC* Methanolic extract. TheRf value were found to be 0.79 and 0.79 of *GC* and Std. Terpenoid. So that Tolurene: Ethyl acetate: Acetic acid (5:4:1) was taken as mobile phase for column chromatography. Active constitutes are isolated from column chromatography with the mobile phase of Tolurene: Ethyl acetate: Acetic acid (5:4:1) for *GC* to obtained Fractions 01 (A), 02 (B), 03 (C), 04 (D), 05 (E), 06 (F), 07 (G), 08 (H), 09-10 (I1 & I2), 11 (J) and 12 (K). Rf value Resulted after performing the TLC estimation is also done for the confirmation of active constituents in fractions (C) of *GC* with mobile phase Tolurene: Ethyl acetate: Acetic acid (5:4:1) by comparing with Std. Terpenoid. The collected Fractions were taken properly and do the UV spectrum. UV spectra of the isolated fractions (C) of *GC* was recorded over a scanning range of 200-800 nm and λmax of fractions (C) of *GC* was determined and the wavelength of *GC*, (C) fraction was found to be 205 and 215 nm. The Rf values of GCExtract with Std. Terpenoid. were found to be 0.79 and 0.79. Rf value Resulted after performing the TLC estimation was also done for the confirmation of active constituent in fraction (C) of GC Methanolic extract with mobile phase Toluene: Ethyl Acetate: Acetic acid (5:4:1)by comparing with Std. Terpenoid.

UV-Spectra of isolated fraction (C) of *GC* Methanolic extract was recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. UV spectra of the isolated fraction was recorded in solvent as Toluene: Ethyl acetate: Acetic acid (5:4:1) over a scanning range of 200-800 nm and λmax of isolated compound were determined. The Blank was Toluene: Ethyl acetate: Acetic acid (5:4:1). The wavelength of isolated fraction (C) of *GC* Methanolic extract was found to be 205 and 215 nm.

The IR Spectra of isolated fraction (C) of GC Methanolic extract showed that -OH group Strong, Broad peak appeared at 3448.10 cm-1, C-H stretching peaks of Alkane at 2924.15 & 2372.15 cm-1. The Carbonyl group C-O stretching peak at 1595.58 cm-1, O-H bending peak of carboxylic acid at 1420.28cm-1, C-O stretching peak of Alcohol at 1151.21 and 1094.35 cm-1 and C-C stretching peak of Alkane at 1115.93 cm-1

In ^{1}H NMR spectra of isolated fraction (C) of *GC* Methanolic extract showed that ^{l}H-2 protons appeared at 1.15-1.25 (2H, 1.19 (s), 1.19 (s)) ppm, ^{l}H-1 proton appeared at 2.28 (d) ppm, ^{l}H-1 proton appeared at 2.46 (dd) ppm, ^{l}H-1 proton appeared at 2.62 (d) ppm, ^{l}H-2 protons appeared at 3.20-3.30 (3.24 (s) ppm, 3.25 (s) ppm), and ^{l}H-1 proton appeared at 4.70 (dd) ppm)

A mass spectrum of isolated Fraction (C) of *GC* Methanolic extract was recorded on Mass Spectroscopy. Mass spectra of isolated Fraction (C) of *GC* Methanolic extract showed molecular ion [M^{+}] peaks at mlz 208.1012 which obtained 1,2-dihydroxypropane-1,2,3-tricarboxylic acid compound in which presence of carbons (C_8), Hydrogens (H_8) and Oxygen (O_8). Finally the molecular formula of isolated Fraction (C) of *GC* Methanolic extract was found to be $C_6H_8O_8$ according to their frangments

From this physical, chemical and spectral investigation were confirmed the presence of 1,2-dihydroxypropane-1,2,3-tricarboxylic acidin Fraction (C) of GC Methanolic extract.

The Various examination of the GC Methanolic extract plant of Garcinia Cambogia belonging to the family Clusiaceae was effectively carried out. From this physical, chemical and spectral investigation were confirmed the presence of 1,2-dihydroxypropane-1,2,3-tricarboxylic acidin Fraction (C) of GC Methanolic extract.

Conclusion

The fruit of the Garcinia cambogia plant generally has significant amounts of the organic acid known as HCA, which is also stated to be the active ingredient on most product labels. Although a number of other chemicals, including carbogiol from the roots, have been isolated from different plant sections, the fruit is the one that is primarily exploited for commercial purposes. This widely used commercial medicinal plant's phytochemistry has been thoroughly investigated. With its many biological qualities, including its ability to

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decrease hunger and its ability to prevent obesity and hyperlipidemia, Garcinia cambogia extracts have been shown to be helpful to obese people in a variety of situations. Garcinia cambogia is safe to use, according to research on toxicity and findings from clinical trials. The majority of the unfavorable reports have to do with situations in which people ingested multiingredient mixtures and the outcome was not directly connected to any one component.In the current study, hydroxycitric acid was extracted in a significant amount from the extract and will be used to construct various dosage forms that will be beneficial in the treatment of obesity and as-yet-unreported liver illnesses.

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