



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Article

Phytochemical Fingerprinting, Chromatographic Characterization, and Porcine Pancreatic Lipase Inhibitory Activity of a 70% Hydroethanolic Extract of *Garcinia cambogia* Formulation

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ARTICLE INFO

Published: 11 Sept 2026

Keywords:

Garcinia cambogia,
Phytochemical profiling,
Hydroethanolic extract,
FTIR, HPLC, Pancreatic
lipase inhibition, Anti-
obesity activity, Flavonoids,
Phenolic compounds.

DOI:

10.5281/zenodo.22703323

ABSTRACT

Garcinia cambogia has attracted considerable interest as a natural source of bioactive constituents with potential applications in the management of obesity. The present study was undertaken to investigate the phytochemical profile and analytical characteristics of a 70% hydroethanolic extract of *Garcinia cambogia*, along with its in vitro pancreatic lipase inhibitory activity. Preliminary phytochemical screening indicated the presence of various secondary metabolites, including flavonoids, tannins, phenolic compounds, alkaloids, glycosides, saponins, and terpenoids. Quantitative phytochemical analysis demonstrated the presence of appreciable amounts of phenolic and flavonoid constituents in the extract. FTIR spectral analysis further supported the presence of characteristic functional groups associated with hydroxyl, aromatic, and other bioactive phytoconstituents. HPLC analysis provided chromatographic evidence for the presence of selected phenolic and flavonoid markers, including catechol and quercetin, in the hydroethanolic extract. In addition, the extract exhibited concentration-dependent inhibition of porcine pancreatic lipase, indicating its potential to interfere with dietary lipid digestion. Overall, the findings demonstrate that *Garcinia cambogia* possesses a diverse phytochemical profile with measurable pancreatic lipase inhibitory activity, supporting its potential as a natural source for further investigation in obesity-related research.

INTRODUCTION

Natural products obtained from medicinal plants continue to attract considerable attention in drug

discovery and development because of their diverse chemical constituents and wide range of biological activities.^[1-3] Among these natural

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



resources, *Garcinia cambogia* has gained interest for its potential role in the management of obesity and related metabolic disorders.

Garcinia cambogia, a plant belonging to the family Clusiaceae, is traditionally valued for its medicinal and nutritional importance. The fruit rind is particularly known for its high content of hydroxycitric acid (HCA), along with other phytoconstituents such as flavonoids, phenolic compounds, tannins, and other secondary metabolites. These constituents have been associated with various biological activities, including antioxidant and potential anti-obesity effects.^[4-7]

The increasing prevalence of obesity has encouraged the search for safer and naturally derived agents that can interfere with key processes involved in lipid digestion and absorption. Pancreatic lipase is one of the major enzymes responsible for the hydrolysis of dietary triglycerides, making its inhibition an important approach in the investigation of potential anti-obesity agents^[8-10]

Phytochemicals characterization using modern analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and High-Performance Liquid Chromatography (HPLC) provides valuable information regarding the chemical composition and marker constituents present in plant extracts.

Despite extensive investigation of the anti-obesity potential of *Garcinia cambogia*, limited information is available integrating quantitative polyphenolic profiling, spectroscopic and chromatographic characterization with pancreatic lipase inhibitory activity of a hydroethanolic preparation under a unified experimental framework. Therefore, the present study was designed to investigate the phytochemical profile

and analytical characteristics of *Garcinia cambogia* using qualitative phytochemical screening, quantitative estimation, FTIR and HPLC analysis, along with evaluation of its in vitro pancreatic lipase inhibitory activity. The integrated approach provides a better understanding of the phytochemical's composition of the extract and its potential relevance in anti-obesity research.^[11-15]

2. METHODOLOGY:

2.1 SAMPLE PROCUREMENT AND PREPARATION OF EXTRACT:

2.1.1 PROCUREMENT AND IDENTIFICATION:

The traditional marketed formulation containing *Garcinia cambogia* was procured from the authenticated source for the present investigation. The sample was examined based on the information provided on the product label, including the botanical name, composition, and manufacturer details. The procured formulation was used as the test sample for subsequent extraction and phytochemical and pharmacological evaluation.

2.1.2 PREPARATION OF HYDROETHANOLIC-EXTRACT-MACERATION METHOD:

The powdered marketed *Garcinia cambogia* formulation was subjected to extraction using 70% hydroethanol as the extraction solvent by the maceration method. The required quantity of the sample was macerated with 70% hydroethanol for the 48 hrs with occasional stirring to facilitate the extraction of soluble phytoconstituents. The extract was then filtered to separate the insoluble residue, and the filtrate was concentrated under reduced pressure to obtain the *Garcinia cambogia*



hydroethanolic extract (GCHEC). The obtained extract was weighed and the percentage yield was calculated. The extract was subsequently stored in an airtight container under suitable conditions until further phytochemical, analytical, and in vitro pancreatic lipase inhibition-studies.



Fig 1: Weighing of Garcinia cambogia Powder for Maceration extraction



Fig 2: Hydroethanolic Extraction of Garcinia cambogia by Maceration method



Fig 3: Marc obtained after Maceration extraction



Fig.4: Final 70% hydroethanolic extract – Garcinia cambogia (GCHEE)



Fig 5: Primary Filtration by Muslin cloth and Secondary Filtration by Whattman filter paper

2.2 PRELIMINARY PHYTOCHEMICAL SCREENING:

The 70% hydroethanolic extract of *Garcinia cambogia* was subjected to preliminary phytochemical screening using standard qualitative chemical tests to identify the major classes of phytoconstituents present in the extract. The screening was carried out for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids, proteins, and anthraquinones. The observed colour changes, precipitate formation, or other characteristic reactions were recorded and interpreted according to standard phytochemical procedures.

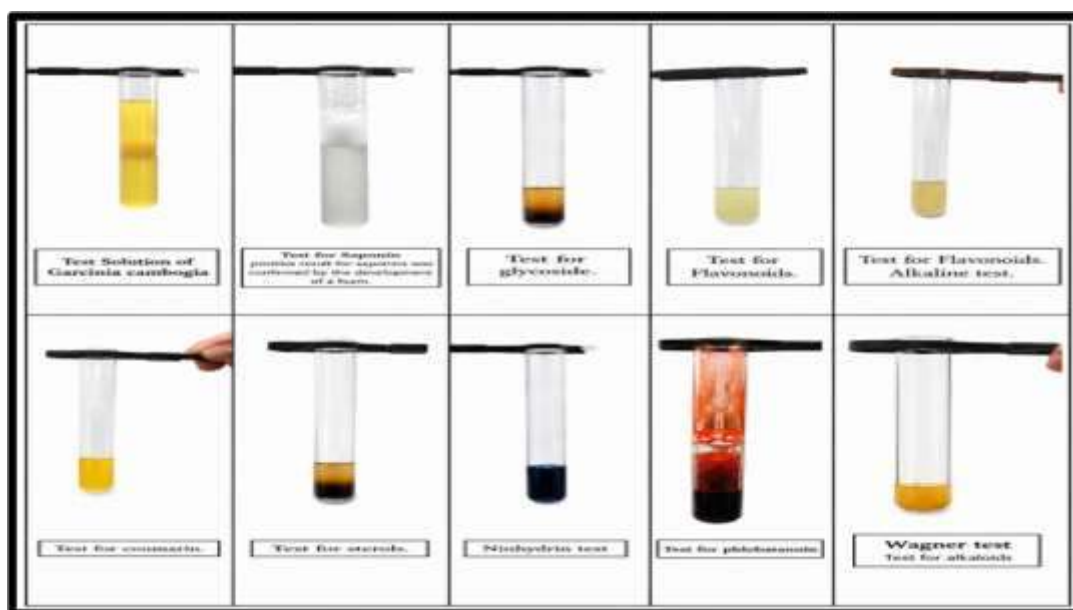


Fig 6: priliminary phytochemical screening

2.3 QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS:

2.3.1 DETERMINATION OF TOTAL FLAVONOID CONTENT:

The total flavonoid content of the 70% hydroethanolic extract of *Garcinia cambogia* was determined using the aluminium chloride colorimetric method. An aliquot of the extract was pipetted into a series of test tubes, and the volume was adjusted with distilled water. Sodium nitrite solution (5%; 0.3 mL) was added to each tube and allowed to react for 5 min at room temperature. Subsequently, aluminium chloride solution (10%; 0.06 mL) was added and the mixture was incubated for another 5 min. Sodium hydroxide (1 M; 0.25 mL) was then added, and the final volume was adjusted with distilled water. The absorbance was measured at 510 nm against a reagent blank using a UV-visible spectrophotometer. Quercetin was used as the reference standard, and the total flavonoid content of the extract was calculated from the calibration curve and expressed as mg quercetin equivalent (QE) per gram of extract.^[16]

2.3.2 DETERMINATION OF TOTAL PHENOLIC CONTENT:

The total phenolic content of the 70% hydroethanolic extract of *Garcinia cambogia* was determined using the Folin–Ciocalteu colorimetric method. An appropriate aliquot of the extract was transferred into a 10 mL glass tube, and the volume was made up to 3 mL with distilled water. Folin–Ciocalteu reagent (0.5 mL) was subsequently added, followed by 2 mL of 20% sodium carbonate solution. The mixture was allowed to develop a blue colour due to the reduction of the phosphomolybdic and phosphotungstic components of the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions. The reaction mixture was warmed for 1 min and then allowed to cool. The absorbance was measured at 650 nm using a UV-visible spectrophotometer. Catechol was used as the reference standard, and the total phenolic content was calculated from the calibration curve and expressed as mg catechol equivalent (CE) per gram of extract.^[17]

2.3.3 DETERMINATION OF TOTAL TANNIN CONTENT:

The total tannin content of the 70% hydroethanolic extract of *Garcinia cambogia* was estimated using the ferric chloride–potassium ferricyanide method. The presence of tannins was initially confirmed by standard ferric chloride and gelatin tests. For quantitative estimation, 0.1 g of the extract was transferred into a 100 mL volumetric flask, followed by the addition of 50 mL distilled water. The mixture was boiled for 30 min and subsequently filtered through a cotton filter. The filtrate was transferred into a 500 mL volumetric flask, and the volume was made up to the mark with distilled water. An aliquot of 0.5 mL was transferred into a vial, followed by the addition of 1 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and 1 mL of 1% ferric chloride ($FeCl_3$). The volume was then adjusted to 10 mL with distilled water. After 5 min, the absorbance was measured spectrophotometrically at 720 nm. Tannic acid was used as the reference standard, and the total tannin content was calculated from the standard calibration curve and expressed as mg tannic acid equivalent (TAE) per gram of extract.^[18]

2.4 FOURIER TRANSFORM INFRARED (FTIR) ANALYSIS:

FTIR analysis was carried out to characterize the major functional groups present in the 70% hydroethanolic extract of *Garcinia cambogia*. The extract was subjected to FTIR spectral analysis over the mid-infrared region, and the characteristic absorption bands were recorded and interpreted based on their corresponding functional groups. The spectrum showed prominent bands at approximately 3421, 2924, 1706, 1612 and 1280 cm^{-1} , corresponding broadly to O–H, C–H, C=O, C=C and C–O stretching vibrations, respectively. The observed spectral pattern supported the presence of hydroxyl-containing, carbonyl-containing and other oxygenated organic constituents in the extract.^[19]

2.5 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS:

HPLC analysis was performed for the chromatographic profiling and identification of selected phenolic and flavonoid marker compounds present in the 70% hydroethanolic extract of *Garcinia cambogia*. The analysis was carried out using a reversed-phase Luna C8 column (5 μm , 150 $\times$ 4.6 mm) with UV detection at 275 nm. The mobile phase consisted of 0.4% orthophosphoric acid and methanol in the ratio of 40:60. The flow rate was maintained at 0.8 mL/min, the column temperature was maintained at $50 \pm 5^\circ C$, and the injection volume was 50 μL . A dilution factor of 10 was applied to the sample before chromatographic analysis.^[20]

2.5.1 PREPARATION OF STANDARD SOLUTIONS AND CALIBRATION:

Standard stock solutions of tannic acid, catechol and quercetin were prepared at a concentration of 1000 $\mu g/mL$ using methanol as the solvent. The stock solutions were appropriately diluted to obtain the required working concentrations for chromatographic analysis. The retention times of the individual standards were recorded and used for comparison with the chromatographic profile of the *Garcinia cambogia* extract. Under the optimized chromatographic conditions, tannic acid, catechol and quercetin showed retention times of approximately 2.722, 4.249 and 6.779 min, respectively.^[21]

2.5.2 SAMPLE ANALYSIS:

The 70% hydroethanolic extract of *Garcinia cambogia* was diluted appropriately and injected into the HPLC system under the optimized chromatographic conditions. The resulting chromatogram showed a prominent peak at a retention time of approximately 2.628 min, with a



peak area of 44,717. The observed peak exhibited a tailing factor of 1.144 and a theoretical plate count of 7084, indicating satisfactory chromatographic performance. Comparison of the sample chromatogram with the reference standards provided evidence for the presence of chromatographically detectable phenolic constituents in the extract. Quantitative analysis of the identified marker compounds was performed using their respective calibration curves.

2.5.3. PREPARATION OF STANDARDS:

Stock : 1000 µg/mL (tannic acid, catechol, quercetin).

Dilutions : 5–100 µg/mL.

Table 1: HPLC Analysis Instrumentation and Conditions.

Parameter	Condition
Column	Luna C8 (5 µm, 150 × 4.6 mm)
Mobile phase	0.4% Orthophosphoric acid : Methanol (40:60)
Flow rate	0.8 mL/min
Column temperature	50 ± 5°C
Detection wavelength	275 nm
Injection volume	50 µL

2.6 Antiobesity Activity:

The following *in-vitro* model were carried out to evaluate antiobesity activity.

a) Porcine pancreas lipase enzyme inhibition assay:^[22]

Garcinia cambogia extracts were prepared at different concentrations in 0.01 M Tris-HCL buffer. Porcine pancreatic lipase was dissolved in 0.01 M Tris-HCL buffer (25 units/mL). The substrate was prepared using the modified method of Fox and Stepaniak. Briefly, olive oil (10% v/v) was mixed with Arabic gum mixture (10% w/v in 0.1 M Tris-HCL buffer, pH 8, 0.5 M NaCl, and 20

mM CaCl₂) using a homogenizer. Inhibition of PL by plant extracts was determined using the method reported by Fukumoto et al. with some modifications. Lipase solution (0.2 mL) was allowed to react with 0.5 mL of plant extract for 30 minutes at 4°C. Substrate emulsion (2 mL) was then added and incubated for 30 minutes at 37°C. One-mL acetone and ethanol (1:1) mixture was used to stop the reaction and titrated with 0.02 M NaOH until reached pH 9.4. Auto titrator was used to perform the titrations (Metrohm, 785 DMP Titrino). The experiment was repeated thrice for each sample extract. The amount of free fatty acid (FFA) liberated was reflected by the amount of base required by the incubation mixture which is equivalent to PL activity. Control Sample was equivalent to 100% enzyme activity. Percent inhibition was calculated based on the following equation:

$$\% \text{ inhibition} = 100\% - [(V_{\text{sample}}/V_{\text{control}})] \times 100]$$

Where,

V_{sample} is the amount of base added to sample,

V_{control} is the amount of base added to control



Fig.7: Weighing of chemical reagents



Fig.8: Preparation of stock solutions

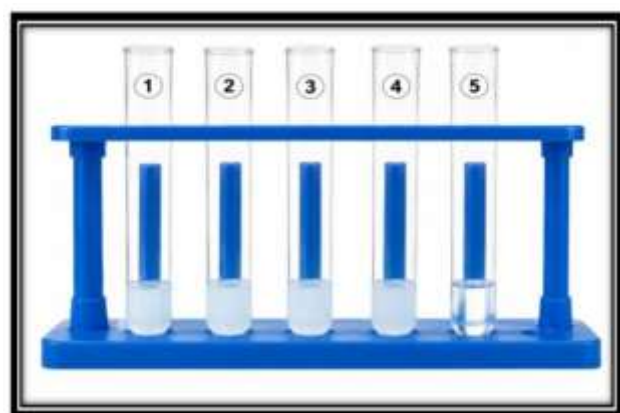


Fig.9: Test samples prepared before incubation



Fig.10: Sample test tube Reaction mixture after 2nd incubation

3. RESULTS:

3.1 PRELIMINARY PHYTOCHEMICAL SCREENING:

Table 2: Preliminary phytochemical screening of 70% GCHEE

Sr. No	Types of Phytochemical constituents	70% ethanolic extract
1	Flavonoids	++
2	Terpenoids	+
3	Phenolic compounds	++
4	Cardiac Glycosides	+
5	Tannins	+
6	Carbohydrates	+
7	Saponins	+
8	Phlobatannins	-
9	Sterols	+
10	Amino acids	+
11	Coumarins	-

+++ Better response, ++ More clarity, + Present, – Absent

The preliminary phytochemical screening of the 70% hydroethanolic extract of *Garcinia cambogia* revealed the presence of a diverse range of phytoconstituents. Flavonoids and phenolic compounds showed a comparatively stronger response (++), while terpenoids, cardiac glycosides, tannins, carbohydrates, saponins, sterols, and amino acids were detected with a positive response (+). Phlobatannins and coumarins were not detected in the tested extract. The presence of flavonoids and phenolic compounds, together with other secondary metabolites, indicates the phytochemical richness of the extract and supports its suitability for further quantitative, spectroscopic, chromatographic, and *in vitro* pancreatic lipase inhibition studies

3.2 QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS:

Table 3: Quantitative estimation of phytoconstituents in 70% GCHEE.

Parameter	Wavelength	70% HE – GCHCA
Total Phenolics	650 nm	10.2 mg/g

Total Flavonoids	510 nm	82.14 mg/g
Total Tannins	700 nm	16.09 mg/g (TAE)

The quantitative analysis of the 70% hydroethanolic extract of *Garcinia cambogia* demonstrated the presence of appreciable levels of phenolic, flavonoid, and tannin constituents. Among the estimated phytoconstituents, flavonoids showed the highest concentration at 82.14 mg/g, followed by tannins at 16.09 mg /g and total phenolics at 10.2 mg /g. These findings indicate that the extract contains a considerable proportion of flavonoid and other polyphenolic constituents, which may contribute to its observed

biological activity and support further investigation of its potential anti-obesity effects.

Spectroscopic Determination of total phenolic, flavonoids and tannin content:

The total phenolic content of 70% hydroalcoholic extract of *Garcinia cambogia* was 10.2 mg/g expressed as equivalent to catechol. Similarly flavonoid content was found to be 82.14 mg/g expressed as equivalent to quercetin and the Tannin content was found to be 16.09 mg/g expressed as equivalent to tannic acid as table.8 and shown in Fig.no 16 , 17 and 18 respectively.

Table 4: Total phenolic, flavonoid and tannin content of 70% GCHEE.

Sr. No	Particulars	Standard curve	Absorbance at	70% ethanolic extract	R ² Value
1	Phenol	Catechol	650 nm	10.2mg/g	0.989
2	Flavonoid	Quercetin	510 nm	82.14mg/g	0.917
3	Tannin	Tannin acid	700 nm	16.09mg/g	0.992

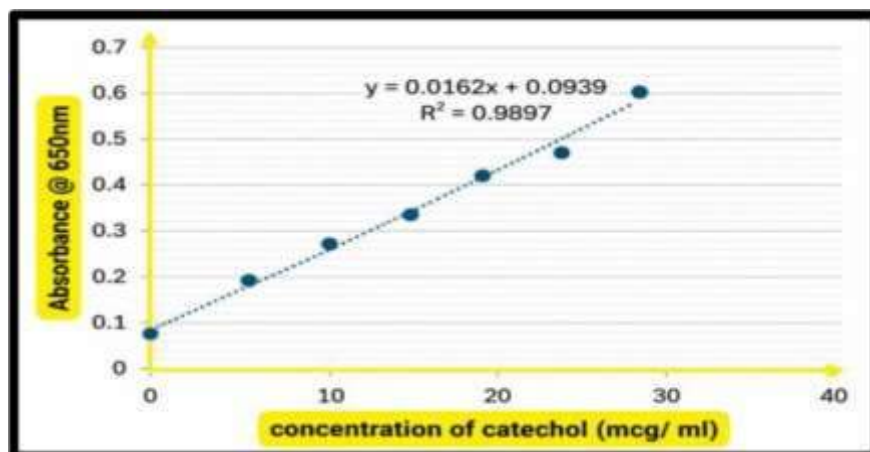


Fig.11: Standard graph for catechol

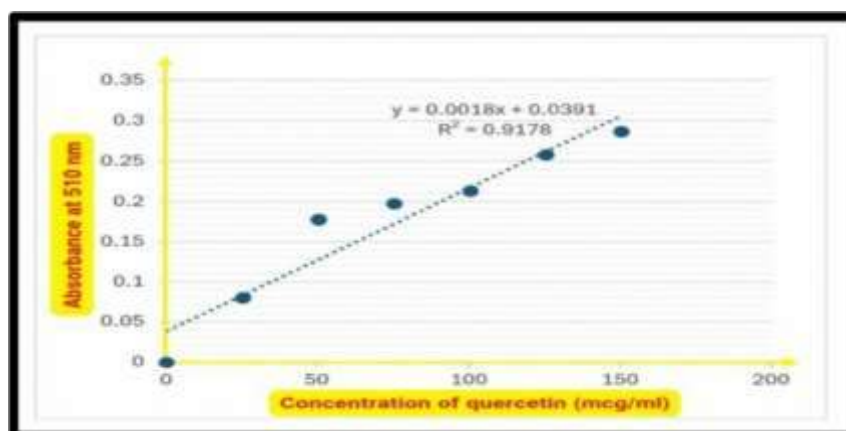


Fig.12: Standard graph for quercetin

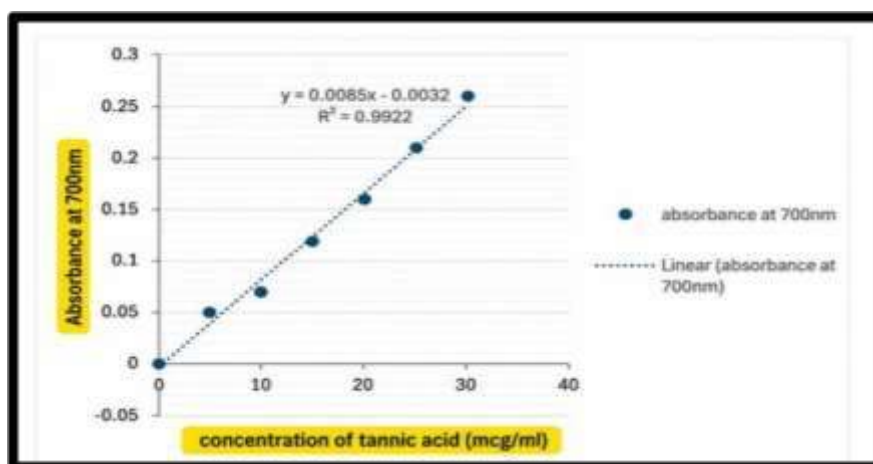


Fig.13: Standard graph for tannic acid.

3.3 FTIR SPECTROSCOPIC ANALYSIS:

FTIR analysis of the 70% hydroethanolic extract of *Garcinia cambogia* revealed several characteristic absorption bands corresponding to different functional groups present in the extract. A broad absorption band at 3297 cm^{-1} can be attributed to O–H stretching vibrations, suggesting the presence of hydroxyl-containing compounds such as phenolics and flavonoids. The band observed at 2902 cm^{-1} corresponds to C–H stretching vibrations of aliphatic groups. An absorption band around 1633 cm^{-1} may be associated with C=C stretching vibrations of aromatic or conjugated structures, supporting the presence of phenolic and flavonoid constituents.

The band near 1413 cm^{-1} can be assigned to C–H bending vibrations. Several prominent bands observed in the region of $1227\text{--}1020\text{ cm}^{-1}$ are characteristic of C–O stretching vibrations, which may indicate the presence of alcohols, ethers, glycosides, and other oxygen-containing phytoconstituents. The multiple absorption bands observed in the lower wavenumber region ($978\text{--}519\text{ cm}^{-1}$) represent the fingerprint region and reflect complex molecular vibrations associated with various constituents of the plant extract. Overall, the FTIR spectrum supports the presence of hydroxyl, aliphatic, aromatic, and C–O-containing functional groups in the 70% hydroethanolic extract of *Garcinia cambogia*, consistent with its observed phytochemical profile.

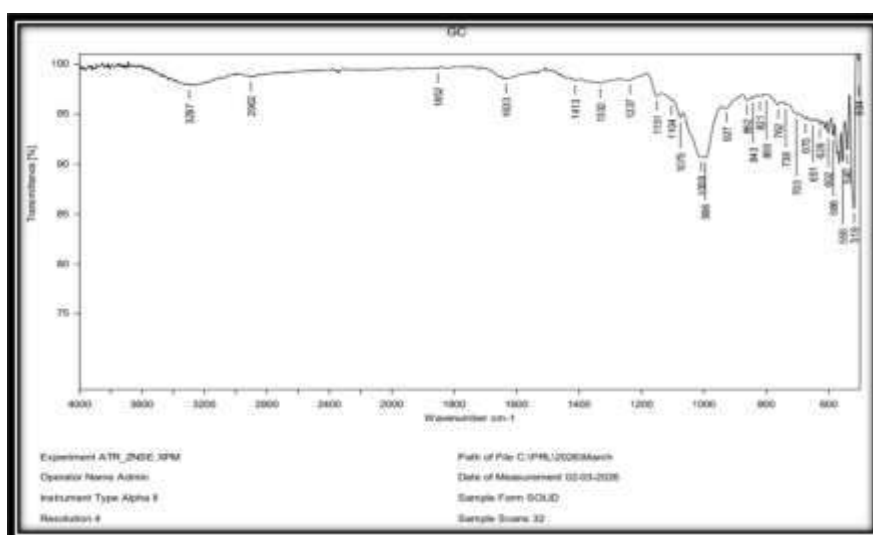


Fig.14: FTIR Spectrum of 70% GCHEE.

Table 5: FTIR Functional Group Analysis and Peak interpretation of 70% GCHEE.

Major Peaks and Their Functional Group Interpretation				
Observed Peak (cm ⁻¹)	Functional Group	Vibrational Mode	Possible Phytochemical Source	Contribution / Significance
3297	O-H stretching	Stretching	Phenols, Alcohols, Carboxylic acids (HCA)	Indicates presence of hydroxyl groups; responsible for antioxidant activity and hydrogen bonding
2902	C-H stretching	Stretching	Alkanes, Terpenoids, Sterols	Confirms presence of aliphatic compounds and lipidic constituents
1852	C=O stretching	Stretching	Carboxylic acids, Acid anhydrides	Indicates carbonyl compounds such as hydroxycitric acid derivatives
1633	C=C stretching / Amide I	Stretching	Flavonoids, Aromatic compounds, Proteins	Associated with conjugated double bonds; indicates aromatic and proteinaceous compounds
1413	O-H bending	Bending	Phenols, Organic acids	Associated with phenolic compounds and carboxyl groups
1332	C-O stretching	Stretching	Phenols, Alcohols	Indicates C-O bond in phenolic and alcoholic compounds
1237	C-O stretching	Stretching	Esters, Ethers, Alcohols	Associated with ester linkages and aliphatic alcohols
1151, 1104	C-O stretching	Stretching	Carbohydrates, Glycosides	Indicates glycosidic linkages and sugar moieties
1075, 1009, 996	C-O / C-C stretching	Stretching	Carbohydrates, Glycosides, Polysaccharides	Confirms presence of carbohydrate structures and glycosides
927 – 800	Aromatic C-H bending	Bending	Polyphenols, Flavonoids	Indicates substitution in aromatic rings of phenolic compounds
703 – 628	C-H bending	Bending	Aromatic compounds	Associated with out-of-plane bending of aromatic C-H bonds
586 – 519	Skeletal vibrations	Bending	Complex organic molecules	Represents fingerprint region of various bioactive compounds

3.4 HPLC ANALYSIS

HPLC analysis of the 70% hydroethanolic extract of *Garcinia cambogia* was performed using a reversed-phase Luna C8 column (150 × 4.6 mm, 5 µm) with UV detection at 275 nm. The chromatographic profile of the extract showed several peaks, indicating the presence of multiple UV-absorbing phytoconstituents. A prominent peak was observed at a retention time of 2.628 min, with a peak area of 44,717. The chromatographic system showed a tailing factor of 1.144 and 7084 theoretical plates, indicating satisfactory peak shape and column efficiency under the applied conditions.

The extract chromatogram was compared with the chromatographic profiles of the selected reference standards, namely tannic acid, catechol, and quercetin. Their retention times were approximately 2.722, 4.249, and 6.779 min, respectively. The prominent peak at 2.628 min occurred close to the retention time of tannic acid; however, based on retention time alone, it is more appropriate to describe this as a tannic-acid-

corresponding peak rather than confirm its identity without additional spectral or reference-standard confirmation. The HPLC findings, together with the FTIR results and quantitative phytochemical estimation, provide complementary evidence for the presence of phenolic and flavonoid-related constituents in the *Garcinia cambogia* extract.

These phytoconstituents may contribute to the biological activity observed in the subsequent *in vitro* pancreatic lipase inhibition assay

Table 6: Standard Calibration Data for Tannic Acid, Catechol and Quercetin -Peak Area

Concentration (µg/mL)	Tannic Acid	Catechol	Quercetin
5	12,499	71,376	75,471
10	27,627	141,387	154,371
25	75,165	345,724	398,483
50	155,777	692,429	828,098
100	339,224	1,447,790	1,779,731

3.4.1 REGRESSION EQUATIONS :

- **Catechol:** $y = 0.0162x + 0.0939$; $R^2 = 0.9897$
- **Quercetin:** $y = 0.0018x + 0.0391$; $R^2 = 0.9178$



- **Tannic acid:** $y = 0.0085x - 0.0032$; $R^2 = 0.9922$

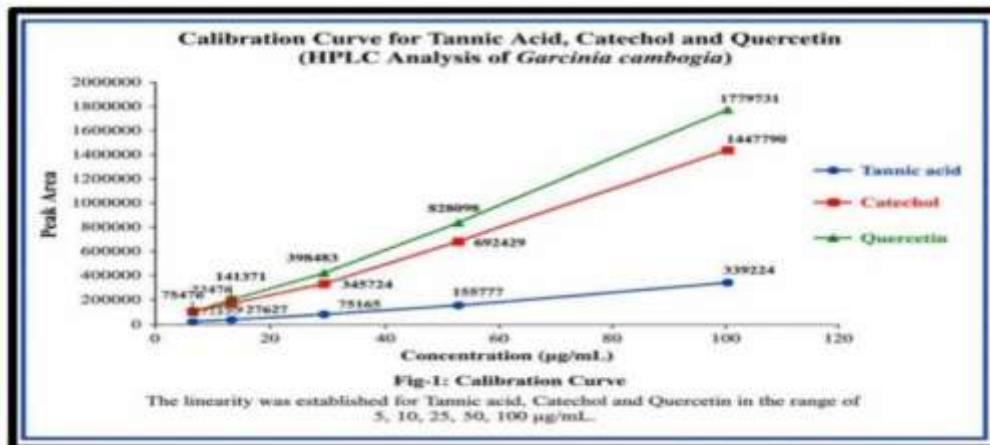


Fig.15: Standard Calibration Curve (Tannic acid, Catechol, Quercetin).

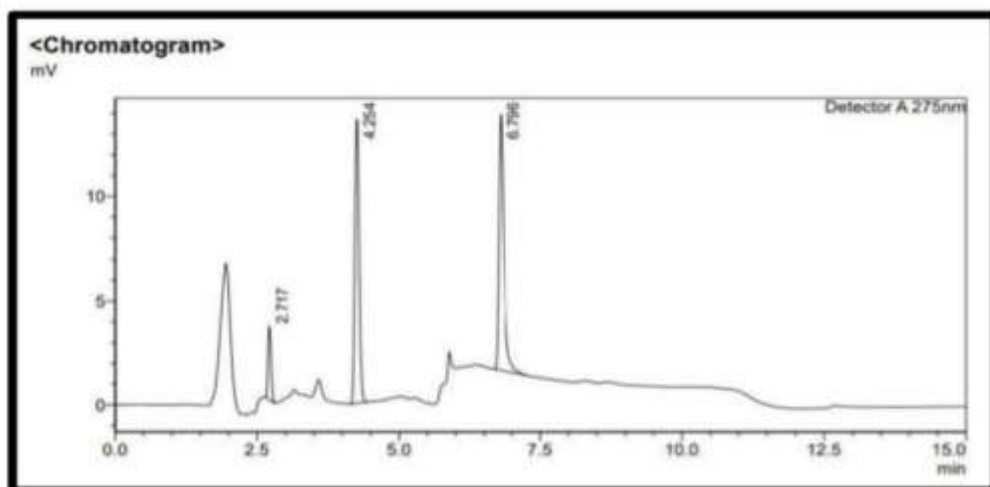


Fig. 16: Standard Chromatogram of Tannic acid, Catechol, Quercetin at 5 mcg.

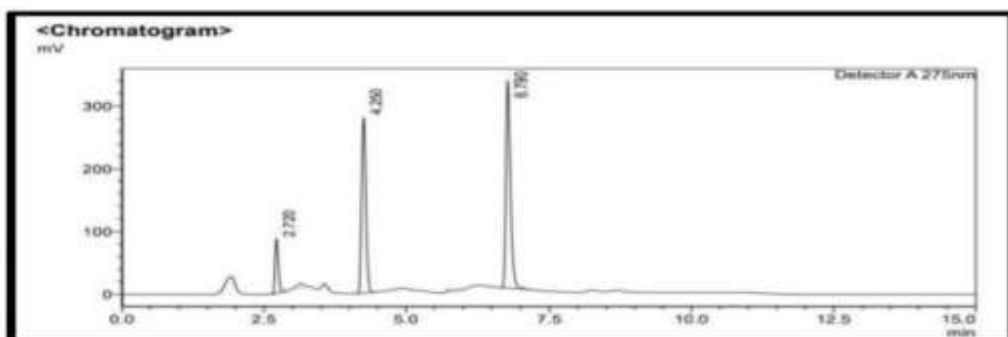


Fig.17: Standard Chromatogram of Tannic acid, Catechol, Quercetin at 100 mcg.

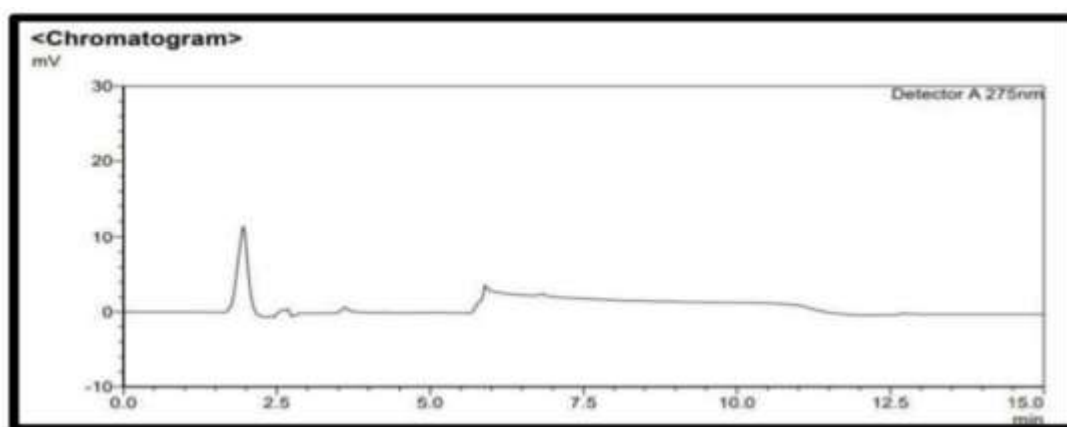


Fig.18: Blank Chromotogram (Methanol).

3.4.2 SAMPLE ANALYSIS: *Garcinia cambogia* Hydroethanolic Extract

- Retention time : 2.628 min
- Peak area : 44,717
- Tailing factor : 1.144
- Theoretical plates : 7084

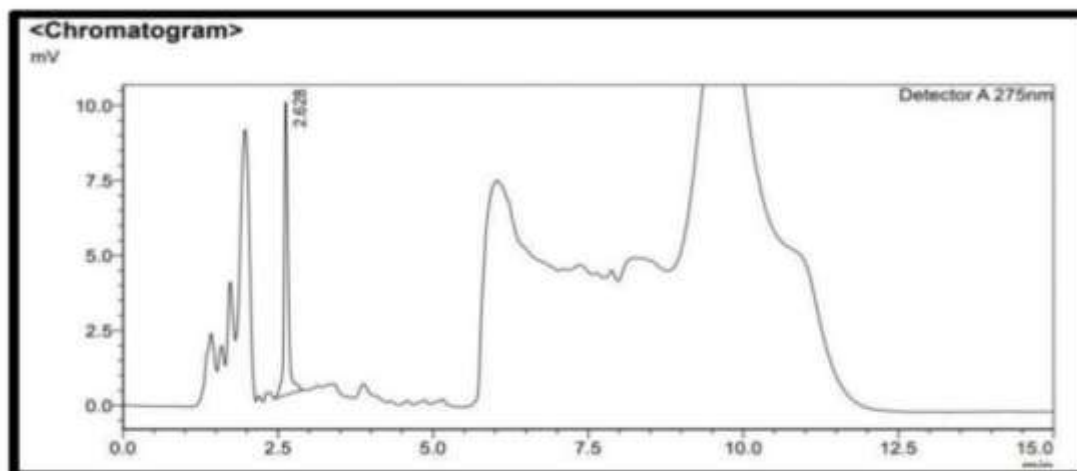


Fig.19: HPLC Chromatogram of 70% Hydroethanolic extract of *Garcinia cambogia*.

The 70% hydroethanolic extract of *Garcinia cambogia* was analyzed under the optimized chromatographic conditions. The chromatogram exhibited a prominent peak at a retention time of 2.628 min, with a peak area of 44,717, indicating the presence of a major chromatographically detectable constituent. The observed peak was close to the retention time of the tannic acid reference standard (2.722 min), suggesting a possible correspondence with tannic acid or a related phenolic constituent. However, definitive

identification would require confirmation using additional analytical evidence

Table 7: HPLC Chromatographic Parameters of 70% Hydroethanolic Extract of *Garcinia cambogia*

PARAMETER	VALUE
Retention time	2.628 min
Peak area	44,717
Tailing factor	1.144
Theoretical plates	7084

Table 8: Retention Time Comparison of 70% Hydroethanolic *Garcinia cambogia* Extract with Reference Standards

COMPOUND	RETENTION TIME
Tannic acid	2.722 min
Catechol	4.249 min
Quercetin	6.779 min

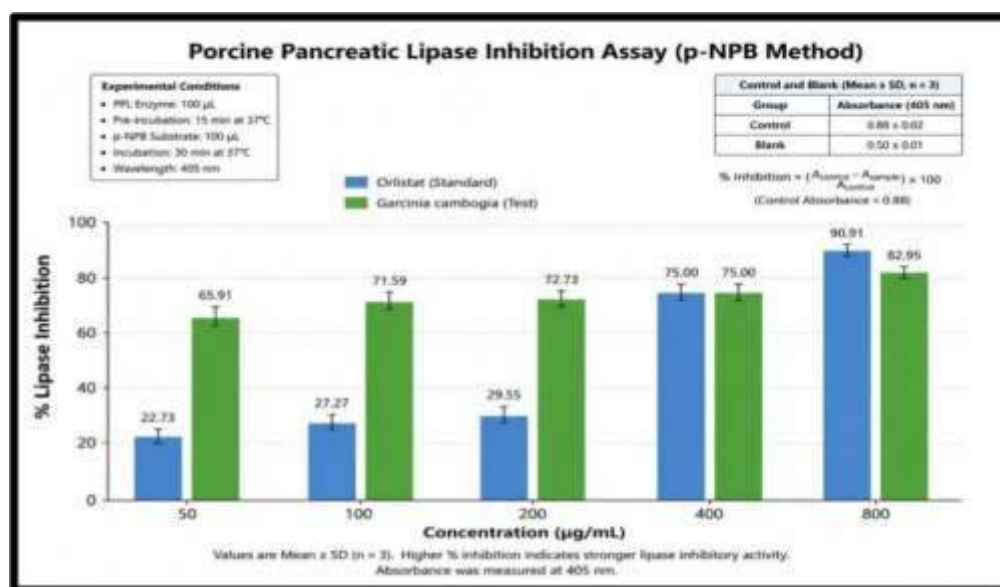
The prominent peak observed in the *Garcinia cambogia* extract at 2.628 min was close to the retention time of the tannic acid standard (2.722 min), suggesting a possible correspondence with tannic acid or a closely related phenolic constituent. Since the difference in retention time was approximately 0.094 min, the result supports the presence of a tannic-acid-related chromatographic component in the extract.^[23-24]

***In-Vitro* antiobesity activity : Porcine Pancreatic lipase enzyme inhibition assay :**

It is observed that 70% hydroalcoholic extract of *Garcinia cambogia* have demonstrated concentration dependent increase in the lipase inhibition property. The standard Orlistat showing increasing lipase inhibition from 22.73% to 90.91% at concentration of 50 – 800 µg/ml. The test extract exhibited 65.91%, 71.59%, 72.73%, 72.73%, 82.95% inhibition at 50, 100, 200, 400, and 800 µg/ml, which was comparatively lower than the standard Orlistat (90.91%). The results are summarised in the table 8 and graphically represented in fig.19

Table.9 Effect of 70% HE – GCHCA on Procine pancreatic lipase enzyme inhibition assay

Sr. No.	Concentration (1 mg/ml)	Orlistat % Inhibition	70% HE – GCHCA % Inhibition
1	50	22.73%	65.91%
2	100	27.27%	71.59%
3	200	29.55%	72.73%
4	400	75.00%	75.00%
5	800	90.91%	82.95%

**Fig.20: Porcine Pancreatic Lipase inhibition activity of 70% HE – GCHCA.**

4. DISCUSSION:

The present study was carried out to evaluate the phytochemical profile, analytical characteristics,

and *in vitro* anti-obesity potential of the 70% hydroethanolic extract of *Garcinia cambogia*. Preliminary phytochemical screening revealed the presence of several classes of phytoconstituents,



including flavonoids, phenolic compounds, terpenoids, cardiac glycosides, tannins, carbohydrates, saponins, sterols and amino acids, while phlobatannins and coumarins were not detected. Flavonoids and phenolic compounds showed comparatively stronger responses (++), indicating their appreciable presence in the extract. The broad phytochemical profile may be attributed to the ability of hydroethanol to extract constituents with different polarities and supports the potential biological relevance of the extract.^[25-26]

Quantitative estimation further supported the preliminary screening results. The 70% hydroethanolic extract contained 10.2 mg CE/g total phenolics, 82.14 mg QE/g total flavonoids and 16.09 mg TAE/g total tannins, with flavonoids representing the highest quantified fraction. The calibration curves showed good linearity for catechol ($R^2 = 0.9897$), quercetin ($R^2 = 0.9178$) and tannic acid ($R^2 = 0.9922$), demonstrating a satisfactory concentration–response relationship under the respective assay conditions. These polyphenolic constituents are of particular interest because phenolics and flavonoids have been associated with antioxidant activity and modulation of several biological targets.^[27-30]

FTIR spectroscopy provided additional structural information about the extract. The characteristic bands around 3297 cm^{-1} , 2902 cm^{-1} and 1633 cm^{-1} were associated with O–H, aliphatic C–H and C=C stretching vibrations, respectively, while bands in the lower wave number region suggested the presence of C–O-containing functional groups. The fingerprint region showed several additional absorptions, reflecting the chemically complex nature of the extract. These observations were consistent with the presence of hydroxyl-containing, aromatic and oxygenated

phytoconstituent indicated by the phytochemicals and quantitative analyses.^[31]

HPLC profiling further characterized the extract and showed a prominent peak at 2.628 min, with a peak area of 44,717. This peak was close to the retention time of the tannic acid standard (2.722 min), suggesting a possible tannic-acid-related or structurally similar phenolic constituent; however, retention-time similarity alone does not provide definitive compound identification. The chromatographic system showed a tailing factor of 1.144 and 7084 theoretical plates, indicating satisfactory peak shape and column efficiency. HPLC analysis also detected catechol ($1.076211\text{ }\mu\text{g/mL}$) and quercetin ($19.99322\text{ }\mu\text{g/mL}$) in the extract. The presence of quercetin is particularly relevant in view of the high total flavonoid content obtained by spectrophotometric estimation, although the two measurements represent different analytical parameters and should not be directly equated.

The *in vitro* porcine pancreatic lipase inhibition assay demonstrated appreciable activity of the extract. The 70% hydroethanolic extract produced 65.91%, 71.59%, 72.73%, 75.00% and 82.95% inhibition at concentrations of 50, 100, 200, 400 and $800\text{ }\mu\text{g/mL}$, respectively, indicating an overall concentration-related increase in inhibitory activity. Orlistat produced 22.73%, 27.27%, 29.55%, 75.00% and 90.91% inhibition at the corresponding concentrations. The extract therefore showed greater inhibition than orlistat at 50–200 $\mu\text{g/mL}$, equivalent inhibition at 400 $\mu\text{g/mL}$, and lower inhibition at 800 $\mu\text{g/mL}$. The maximum inhibition of 82.95% demonstrates substantial *in vitro* pancreatic lipase inhibitory activity, although the extract did not reach the activity of orlistat at the highest concentration tested.



The observed lipase inhibition may be associated, at least partly, with the flavonoid, phenolic and tannin constituents identified in the extract, as plant polyphenols can interact with enzymes and influence their activity. However, the present findings do not establish a direct causal relationship between any individual constituent and pancreatic lipase inhibition. Overall, the agreement between preliminary phytochemical screening, quantitative estimation, FTIR characterization, HPLC profiling and pancreatic lipase inhibition provides complementary evidence for the bioactive potential of *Garcinia cambogia*. These findings support its further investigation as a natural source of constituents relevant to anti-obesity research, while isolation studies, enzyme-kinetic investigations, *in vivo* evaluation and safety studies are required to establish the responsible constituents, mechanisms and therapeutic relevance.

5. CONCLUSION:

The present investigation provides an integrated evaluation of the phytochemical composition, analytical characteristics, and *in vitro* anti-obesity potential of the 70% hydroethanolic extract of *Garcinia cambogia*. Preliminary phytochemical screening demonstrated the presence of several classes of phytoconstituents, including flavonoids, phenolic compounds, terpenoids, cardiac glycosides, tannins, carbohydrates, saponins, sterols and amino acids, whereas phlobatannins and coumarins were not detected. The comparatively stronger responses observed for flavonoids and phenolic compounds indicate their appreciable presence in the extract.

Quantitative estimation confirmed the presence of total phenolics (10.2 mg CE/g), total flavonoids (82.14 mg QE/g), and total tannins (16.09 mg TAE/g), with flavonoids representing the highest quantified phytochemical group. The

corresponding calibration curves demonstrated good linearity for catechol ($R^2 = 0.9897$), quercetin ($R^2 = 0.9178$) and tannic acid ($R^2 = 0.9922$), supporting the reliability of the quantitative estimations under the experimental conditions. These findings provide a measurable chemical basis for the phytochemical richness of the extract.^[32-33]

FTIR analysis further supported the presence of diverse phytoconstituents through characteristic absorption bands associated with hydroxyl, aliphatic C–H, aromatic/conjugated and C–O-containing functional groups. HPLC profiling demonstrated a prominent peak at approximately 2.628 min, which was close to the retention time of the tannic acid standard (2.722 min), suggesting the possible presence of a related phenolic constituent. Furthermore, catechol (1.076211 µg/mL) and quercetin (19.99322 µg/mL) were detected in the analyzed extract. The observed tailing factor (1.144) and theoretical plate count (7084) indicated satisfactory chromatographic performance under the selected conditions.

Importantly, the 70% hydroethanolic extract demonstrated appreciable porcine pancreatic lipase inhibitory activity. The extract produced 65.91%, 71.59%, 72.73%, 75.00% and 82.95% inhibition at concentrations of 50, 100, 200, 400 and 800 µg/mL, respectively, demonstrating an overall concentration-related increase in enzyme inhibition. The standard drug orlistat produced 22.73%, 27.27%, 29.55%, 75.00% and 90.91% inhibition, respectively. Thus, the extract exhibited considerable inhibitory activity across the tested concentration range, although its maximum inhibition at 800 µg/mL was lower than that of orlistat. These findings provide preliminary evidence that the extract can interfere with pancreatic lipase, an important enzyme involved in dietary lipid digestion.



Collectively, the integration of phytochemical screening, quantitative estimation, FTIR characterization, HPLC profiling, and pancreatic lipase inhibition provides complementary evidence for the bioactive potential of *Garcinia cambogia*. The appreciable flavonoid content, measurable phenolic and tannin levels, detection of catechol and quercetin, and substantial pancreatic lipase inhibition suggest that the 70% hydroethanolic extract may serve as a promising natural source of phytoconstituents relevant to anti-obesity research. However, the present findings are based on an *in vitro* model and do not establish clinical efficacy. Further studies involving isolation and identification of active constituents, enzyme-kinetic and mechanistic investigations, *in vivo* anti-obesity evaluation, toxicity studies, and clinical investigations are necessary to establish the safety, efficacy, and therapeutic relevance of *Garcinia cambogia*.

6. LIMITATIONS AND FUTURE PERSPECTIVES:

The present study has certain limitations. The investigation was performed using a marketed formulation containing *Garcinia cambogia*, and therefore the findings may vary with differences in formulation, source, and composition. The study was also limited to a 70% hydroethanolic extract and preliminary phytochemical and analytical evaluation. Although FTIR and HPLC provided useful evidence regarding the phytochemical composition, definitive structural identification of individual constituents requires further confirmation. In addition, the pancreatic lipase inhibition assay represents an *in vitro* model and cannot directly establish

in vivo anti-obesity efficacy or clinical effectiveness. The specific phytoconstituent responsible for the observed enzyme inhibition

and their mechanism of action were not established.

Future studies should focus on isolation and structural characterization of the active constituents using advanced techniques such as LC-MS/MS and NMR spectroscopy. Enzyme-kinetic studies should be performed to determine the mechanism and potency of pancreatic lipase inhibition. Further *in vivo* anti-obesity, pharmacokinetic and toxicity studies are required to evaluate efficacy and safety. Well-designed clinical investigations would ultimately be necessary to establish the therapeutic potential and clinical relevance of *Garcinia cambogia* in obesity management.

ACKNOWLEDGEMENT:

The authors express their sincere gratitude to His Holiness Sha || Bhra || Shri Varasadyojatha Shivacharya Swamiji, President, TMAE Society, Harapanahalli, and Dr. T.M. Chandrashekharaiyah, Secretary, TMAE Society, Harapanahalli, for their valuable support and encouragement. The authors also extend their sincere thanks to Mr. Pratheek T M, Administrative Officer, Gnanagangothri Campus, Harapanahalli, and the Principal, S.C.S. College of Pharmacy, Harapanahalli, for their constant encouragement and support throughout the study.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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HOW TO CITE: Ittagi Shanmukha, C. M. Manu, E. Vinay Kumar Setty, Deepa Madagoud, Deepa M, Shoukat Ali, *Phytochemical Fingerprinting, Chromatographic Characterization, and Porcine Pancreatic Lipase Inhibitory Activity of a 70% Hydroethanolic Extract of *Garcinia cambogia* Formulation*, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 9, 1176-1193. <https://doi.org/10.5281/zenodo.22703323>

