Therapeutic Potential of *Garcinia cambogia* Ethanolic Extract in the Management of Diabetic Foot Ulcers: A Phytochemical and Pharmacological Evaluation

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Abstract

Diabetic foot ulcers (DFUs) are serious complications of diabetes mellitus, often resulting from peripheral arterial disease and neuropathy, with high risks of infection and amputation. Effective management is complex and costly, requiring multidisciplinary care. Current treatments focus on blood glucose control, wound care, and improving circulation. *Garcinia cambogia*, a traditional medicinal plant widely used in Kerala and other Asian regions, possesses various pharmacological properties including antioxidant, anti-inflammatory, and antidiabetic effects. This study investigates the therapeutic potential of *Garcinia cambogia* ethanolic extract in the treatment of DFUs, aiming to provide a natural, cost-effective alternative for managing diabetic complications and promoting wound healing. The present study explores the phytochemical profile and biological activities of ethanol extracts from *Garcinia cambogia* fruit. Preliminary phytochemical screening revealed the presence of several bioactive compounds, including tannins, flavonoids, sterols, terpenoids, phenols, glycosides, carbohydrates, cardiac glycosides, and phlobatannins. Quantitative analysis indicated a total phenolic content of 34.77±2.65 mg gallic acid equivalent and a flavonoid content of 26.65±0.98 mg quercetin equivalent. In vitro α-amylase inhibition assays demonstrated the antidiabetic potential of the extract, with an IC_{50} value of 46.60±0.98 µg/ml, closely approaching that of the standard acarbose (IC_{50} = 61.37±1.01 µg/ml). Cytotoxicity was evaluated using an MTT assay on 3T3-L1 cell lines. The extract did not exhibit cytotoxicity at lower concentrations and even enhanced cell viability, suggesting a protective or proliferative effect. Morphological changes were observed with increasing concentrations, yet cell death remained minimal. Furthermore, the wound scratch assay demonstrated enhanced cell migration capabilities upon treatment with 6.25 and 12.5 µg/ml of the extract. A 1.6-fold and threefold increase in wound closure was observed, respectively, indicating potential wound healing and regenerative properties. Collectively, these findings support the therapeutic potential of *Garcinia cambogia* extract, particularly for its antioxidant, antidiabetic, cytoprotective, and wound-healing effects. The presence of diverse phytochemicals provides a foundation for further investigation into its pharmacological applications.

Keywords: Diabetic Foot Ulcers (DFUs), *Garcinia cambogia*, Antidiabetic activity, Wound healing, Phytochemicals.

Introduction

Diabetes Mellitus is a chronic condition marked by persistent hyperglycemia resulting from impaired insulin secretion, action, or both [1]. A major complication is diabetic foot, defined by the International Working Group on the Diabetic Foot (IWGDF) as infection, ulceration, or tissue damage in the lower extremities of individuals with diabetes, often linked to peripheral artery disease or neuropathy. Around 15% of diabetics develop foot ulcers, with 14%–24% requiring amputation due to complications such as bone infection [2] Hyperglycemia worsens these conditions by causing hemoglobin glycation, vascular narrowing, and erythrocyte membrane changes, which hinder oxygen delivery and promote

tissue damage [3]. Managing diabetic foot ulcers (DFUs) is a growing challenge in clinical practice, requiring a multidisciplinary approach for optimal care. Standard treatments include glycemic control, infection management, wound debridement, drainage, dressings, and angioplasty for peripheral artery disease [4]. Treatment is both complex and costly, with global DFU management expenses exceeding $1 billion annually—surpassing those of many common cancers. Timely and coordinated care focusing on restoring blood flow, controlling infection, and reducing wound pressure is essential to prevent severe outcomes, including amputation [5]

Garcinia cambogia is also known as Garcinia gummi-gutta (L.) N. Robson, a botanical remedy utilized by Kerala traditional healers, was highlighted. Known by its Tamil name Kodumpuli and its English name Malabar Tamarind, it is a member of the Clusiaceae family. Along with the subtropical regions of Asia, such as China, Malaysia, and the Philippines, it is extensively dispersed throughout the Indian state of Kerala, especially in the Western Ghats, from the Konkan south to Travancore east [6] *Garcinia cambogia* is found to possess anticancer [7,8] antiobesity [9], antioxidant [10], anti –ulcer [11], anti-inflammatory [12] and cardioprotective properties [13] . In view of medicinal importance of *Garcinia cambogia*, the present study aims at investigating the effect its ethanolic extract against diabetic foot ulcer.

MATERIAL AND METHODS

Collection of plant material

The plant material of Garcinia cambogia used in this study was collected from a local market, in Madhuranthakam.

Preparation of extract

The fruits were given time to dry. Following that, these 250g fruits were shade dried for three to five days without any contamination. After being ground into powder using an electronic grinder, the fruits were kept at 5° C in an airtight container until they were needed. Using a Soxhlet device, ethanol was used to extract the powdered fruit for a whole day. Following extraction, a Whatmann No. 1 filter paper was used to filter the liquid. The rotary vacuum evaporator was used to concentrate the filtrate to dryness at 40°C with decreasing pressure, and the powder was then stored at room temperature.

Figure 1: *Garcinia cambogia* dried fruit and Powdered Fruit

PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical screening of extract

The identification of active principles in medicinal plants is crucial for understanding their possible pharmacological effects. Hence the ethanolic *Garcinia cambogia* was screened for phytochemical [14].

Quantitative Estimation

Determination of total phenolic and flavonoid content

The Folin-Ciocalteau colorimetric technique was used to measure the total polyphenol content of the ethanol extract of *Garcinia cambogia* fruits. Using reference solutions of gallic acid, a standard curve was constructed [15-16]. in the same way, but without gallic acid. The absorbance was measured at 760 nm after one hour of incubation at room temperature, and the mean value was computed. *Garcinia cambogia* fruit's ethanolic extract's total flavonoid content was calculated using a method with a few minor adjustments. To create a standard curve, quercetin reference solutions were used [17]. After cooling, the turbidity was measured at 660 nm. The percentage inhibition of denaturation was calculated by using following formula,

% Inhibition = [(OD of test - OD of control)/OD of test] x 100.

ANTI DIABETIC ACTIVITY

Alpha-amylase inhibitory assay

The α-amylase inhibitory activity of the test samples **(KAqEE)** were carried out according to the standard method with minor modification. 100 µL of α- amylase solution (0.1

mg/mL) was mixed with different concentrations (10, 20, 40, 80, 160, and 320µg/mL) of test sample, reference standard (**Acarbose**) and control (without standard/test sample) and pre-incubated at 37 °C for 15 min. Then, 100 µL of starch solution was added to initiate reaction and incubation was done at 37 °C for 60 min., then 10 µL of 1 M HCl and 100 µL of iodine reagent were added to the test tubes. The absorbance of the mixture was measured at 565 nm. α-amylase inhibitory activity was measured using the formula,
% Inhibition = [(OD of test - OD of control)/OD of test] x 100

***IN VITRO* ASSAY FOR DFU**

MTT ASSAY

Viable cells are counted after labelling with a vital dye in order to determine the harmful effects of unknown substances in vitro. There are alternative techniques that rely on dyes and cellular activity, such as counting by automated counters and measuring radioisotope incorporation as a measure of DNA synthesis. To measure the activity of living cells, the MTT technique uses mitochondrial dehydrogenases [18].

PREPARATION OF TEST SOLUTION

For MTT assay, serial two fold dilutions (3.125 – 100 µg) were prepared from this assay.

CELL LINE CULTURE MEDIUM

3T3-L1 cell line was procured from NCCS, stock cell was cultured in DMEM medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37^{o}C until confluent.

PROCEDURE

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microliters plate, 100 µL of the diluted cell suspension (1 x 10^4 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37^{o}C for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37^{o}C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula, % viability = Sample abs/Control abs x 100.

WOUND HEALING ASSAY IN *GARCINIA CAMBOGIA* FRUIT EXTRACT

The migration rates of 3T3-L1 cells were assessed by the scratch assay method. The cell density of 2×10^5 cells was seeded into each well of a 6 well plate and incubated with complete medium at 37 °C and 5% CO_2. After 24 h of incubation, the monolayer confluent cells were scrapped horizontally with a sterile P200 pipette tips. The debris was removed by washing with PBS. The cells were treated with test sample with various concentrations (6.25µg and 12.5µg) and standard CIPLADIN (25µg and 50µg) by diluting with serum-free DMEM. The cells without treatment were used as the control, respectively. The scratch induced that represented wound was photographed at 0 h using phase contrast microscopy at 10x magnification at 0h, before incubation with the test sample. After 24 h of incubation, the second set of images was photographed. To determine the migration rate, the images were analysed and percentage of the closed area was measured and compared with the value obtained at 0 h. An increase in the percentage of the closed area indicated the migration of cells. Experiments were performed in the triplicate manner and the data were recorded and analysed statistically [19].

Wound closure (%) = Measurement at 0 h - Measurement at 24 h/ Measurement at 0 h x 100

RESULTS & DISCUSSION

Phytochemical analysis of *Garcinia cambogia* fruit extract

The phytochemical analysis of the ethanolic extract of *Garcinia cambogia* fruit is shown in Table 1. While saponins, alkaloids, coumarin, proteins and amino acids, and anthraquinone are partially present in the extract, the results of phytochemical screening clearly demonstrate the presence of tannins, flavonoids, sterols, terpenoids, phenols, glycosides, carbohydrates, cardiac glycosides, and phlobatannin.

Table 1: Qualitative phytochemical analysis of Ethanol extracts of *Garcinia Cambogia* fruit

S.No	Phytochemical Constituents	Inference of Ethanol Extracts
1	Tannins	++
2	Saponins	+
3	Flavonoids	++
4	Sterols	++
5	Terpenoids	++
6	Phenols	++
7	Alkaloids	+
8	Glycosides	++
9	Coumarins	+
10	Carbohydrates	++
11	Proteins and amino acids	+

12	Phlobatannin	++
13	Cardiac glycosides	++
14	Anthraquinone	+

i) ++ Indicates – present ii) + Indicates – slightly present

Qualitative Analysis of Phytochemical Constituent of Ethanol Extracts of *Garcinia cambogia* fruit

Garcinia cambogia extract has been used in Indian medicine to treat a variety of ailments, such as ulcers, leukemia, hemorrhoids, diarrhea, and dysentery [20]. Plant species belonging to the genus Garcinia are good suppliers of bioactive compounds. Secondary metabolites, such as flavonoids and phenols, are responsible for much of their medicinal effects. The most significant secondary metabolites of fruits are flavonoids and phenols, which have therapeutic use [21]. Due to their high phenolic content, most fruits have substantial antioxidant properties. Due to their high phenolic content, most fruits have substantial antioxidant properties [22]

Quantitative Estimation of Ethanol Extract of *Garcinia Cambogia* Fruit

Ethanol extracts were subjected to a quantitative phytochemical investigation, and established techniques were employed to estimate their quantities.

Table 2: Total Phenol and Flavonoid Content of Ethanol Extract of *Garcinia Cambogia* Fruit

Sample	**Total Phenol (mg/g)**	**Total Flavonoid (mg/g)**
Ethanol extract(GCEE)	34.77±2.65	26.65±0.98

It was determined that the total phenolic and flavonoid content (Table 2) was 34.77±2.65 mg, which is comparable to the standard gallic acid equivalent and 26.65±0.98 mg quercetin equivalent. Polyphenols are useful parts of plants because of their hydroxyl groups, which allow them to scavenge free radicals. *Garcinia cambogia* extracts aid in the scavenging of free radicals of different ROS because they include secondary metabolites like flavonoids. The presence of bioactive metabolites in the fruit of our plant of interest, *Garcinia cambogia*, is therefore preliminary evidence for its possible use. The creation of naturally occurring antioxidant compounds from plants is currently of great interest to scientists.

Anti-Diabetic Activity

The alpha amylase inhibitory assay was carried out to assess the anti-diabetic activity of crude extracts of *Garcinia cambogia* fruits.

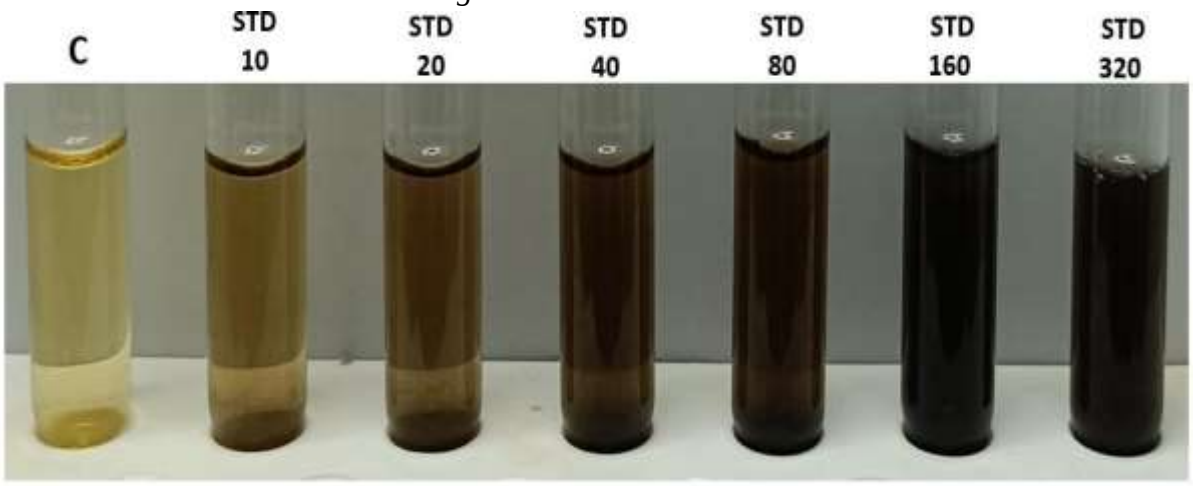

Figure 2: Alpha Amylase Inhibitory Assay of Standard –Acarbose

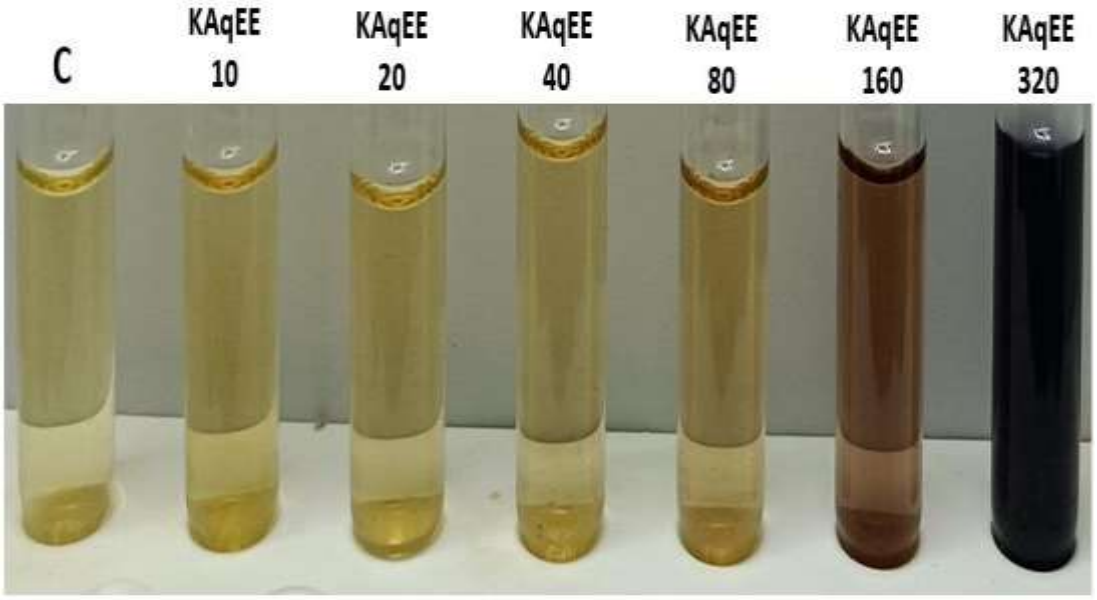

Figure 3: Alpha Amylase Inhibitory Assay of Ethanol Extract of *Garcinia cambogia* Fruit

Table 3: Alpha Amylase Inhibitory Assay for Crude Ethanol Extract of *Garcinia Cambogia* Fruit

Conc.(µg/ml)	KAqEE	Acarbose
10	15.64±3.18	61.37±1.01
20	46.60±0.98	81.82±0.18
40	77.16±0.49	86.21±0.15

80	82.99±0.16	90.98±0.07
160	95.50±0.01	95.01±0.019
320	96.29±0.01	96.57±0.00

Data are presented as mean ± SD values of triplicate determinations.

Table 4: IC 50 Value of Alpha Amylase Inhibitory Assay for Different Crude Extracts of *GCF*

Sample	**IC50 Value**
KE	**24.64**
Standard	**1.15**

The obtained data are represented in the table based on the concentration of the sample, increases the turbidity which in turn increases the optical density are showed in the image. Acarbose are used as standard at a concentration 100 µg/ml showed 96.57% inhibitory effects on the α- amylase activity with an IC50 value 61.37±1.01 at 1.15µg/ml. The ethanol extracts of *Garcinia cambogia* exhibited 96.29±0.01 at 320µg/ml concentration with an IC50 values 46.60±0.98 at 24.64µg/ml.

MTT ASSAY OF *GARCINIA CAMBOGIA* FRUIT EXTRACTS

MTT Assay over 3T3-L1 cell line

The crude ethanol extract of *Garcinia cambogia* fruit was treated over the 3T3-L1 cell lines & observed under microscope (magnification 20X) to identify the percentage of cell death after 24 hrs incubation period. The cells were observed with slight modification even at least concentration, the increase in dosage from 6.25µg to 100µg shows slightly alters the morphology of cells & percentage of cell viability. The results of the MTT assay (Table 8) indicated that the viability of 3T3-L1 cell lines showed a significant difference after treatment with various concentrations of the extract, which demonstrated a decrease in cell viability compared to the control group. Therefore, it seems that the extract not only lacks a toxic effect on the cells, but may also enhance survival at lower concentration.

Table 5: Represents the Mean±SD of *Garcinia Cambogia* fruit extract

SAMPLE	**CONCENTRATON**	**MEAN± SD**	**% Viability**
Control	100	100**±1.00**	100

Extract	6.25	93.61±0.18	92.69
	12.5	84.64±0.37	84.59
	25	73.18±0.46	73.59
	100	59.77±0.46	59.68

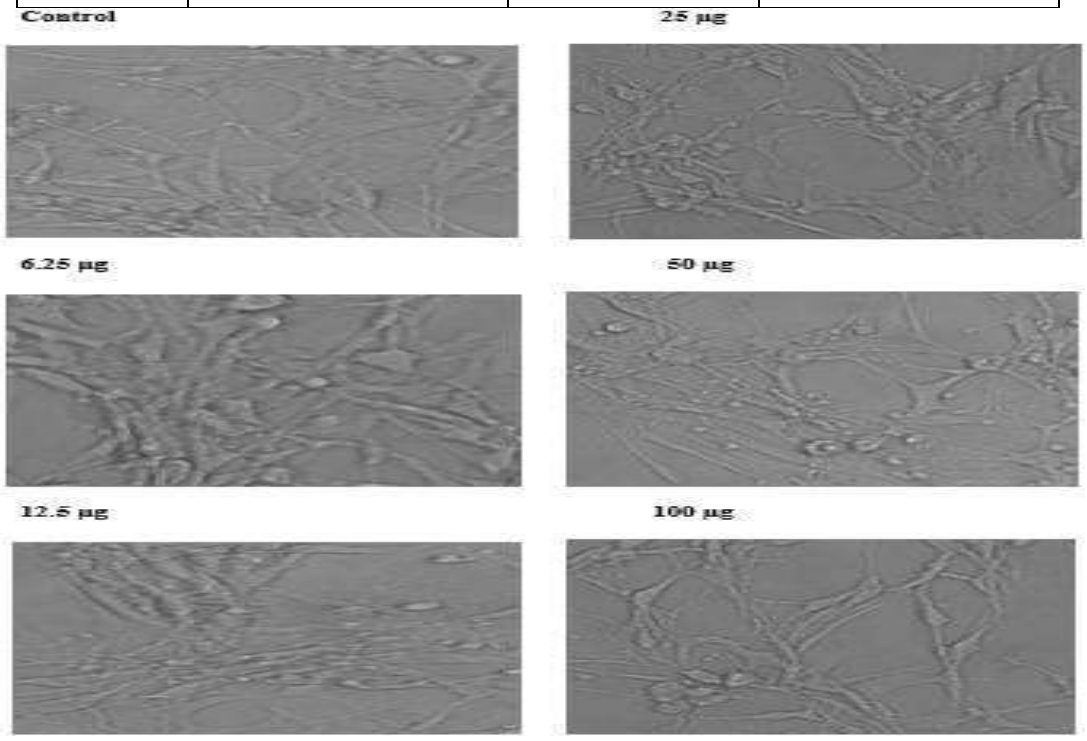

Figure 4: MTT Assay of *Garcinia Cambogia* fruit Extract

The **3T3-L1 cell line** were treated with various concentration of crude ethanol extract of G***arcinia cambogia*** fruit observed under microscope (magnification 20X) after 24 hrs incubation periods to calculate the number of cell death.

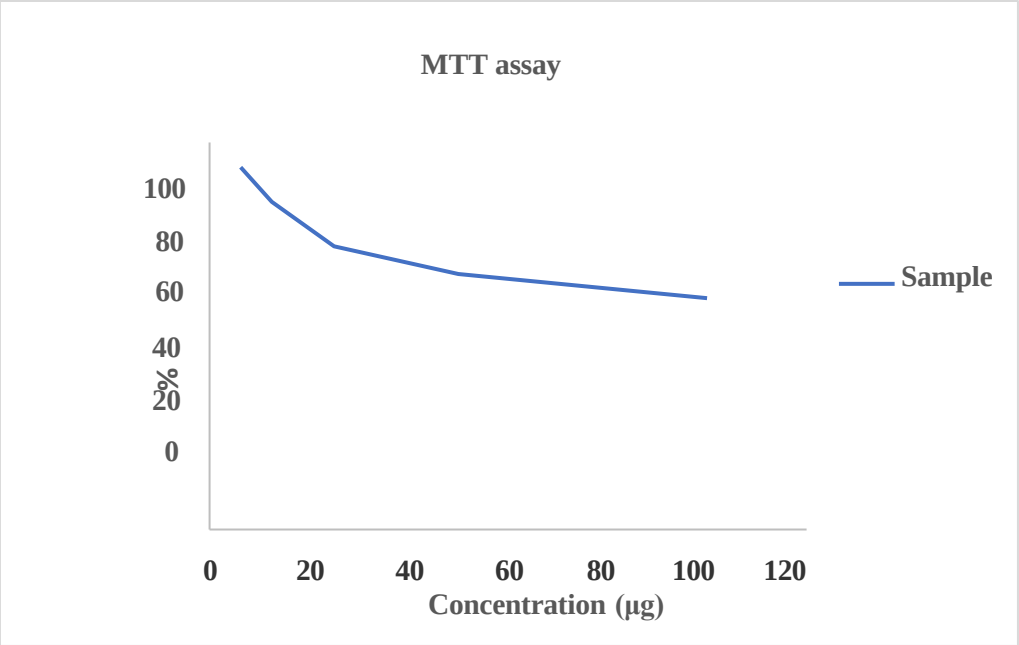

Figure 5: MTT Assay of *Garcinia Cambogia* fruit extract

Garcinia cambogia induced cytotoxicity in **3T3-L1 cell lines fibroblast cell lines were represented in Figure 5.** The cell was treated with various concentrations of extract. The graph values are expressed as % of inhibition of cell death to identify the IC50 of the extracts.

The IC50 of the extract was found to be at 100 µg of concentration. it has been indicated that *Garcinia Cambogia* extracts have less toxicity in non-cancerous cells compared to cancer cells due to a high IC50 in normal cells.

WOUND SCRATCH ASSAY OF *GARCINIA CAMBOGIA* FRUIT EXTRACT:

Garcinia cambogia extract increased migration ability of 3T3-L1 was assessed by wound scratch assay .The cell migration assay demonstrated a significant enhancement in the migratory capacity of 3T3-L1 following treatment with an extract concentration of 6.25 &12.5 µg/ml. Specifically, the closure percentage in treated concentration 12.5 µg/ml exhibited a threefold increase ($P < 0.05$) compared to the control group, while treated with 6.25 µg/ml showed a 1.6-fold increase ($P < 0.05$) compared to their respective controls.

Table 6: Percentage Migration of *GARCINIA CAMBOGIA*

CONCENTRAION (µg)	PERCENTAGE MIGRATION
Control	8.860759
12.5	12.98701
6.25	20

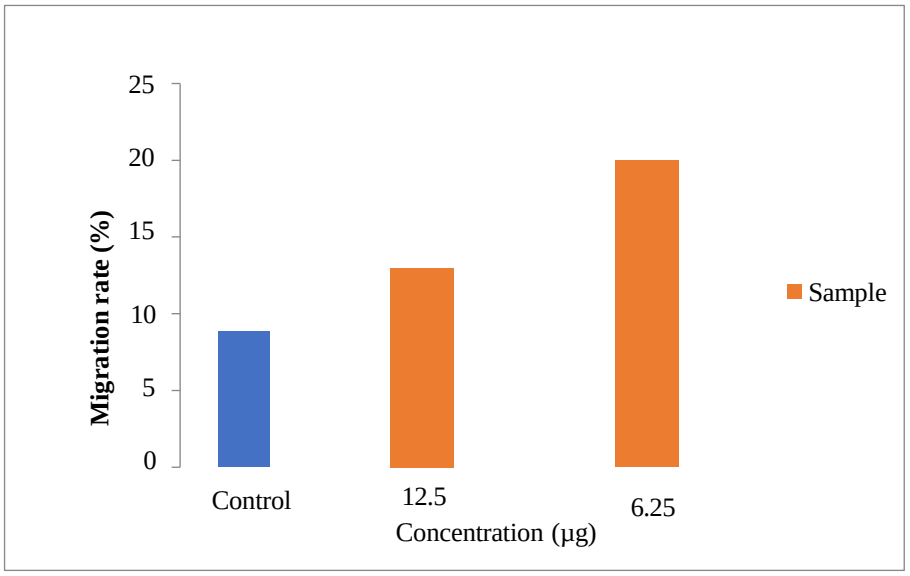

Figure 6: Wound scratch assay in *Garcinia Cambogia* fruit extract

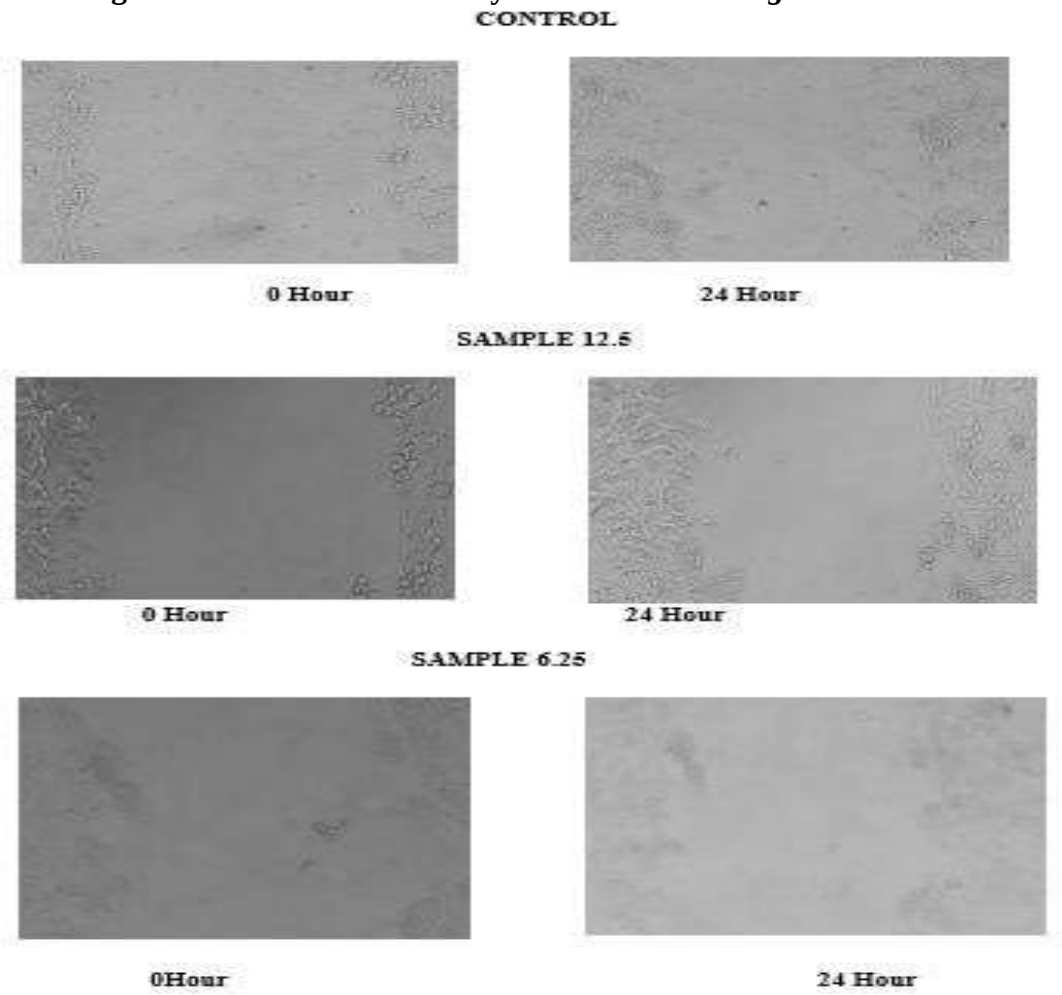

Figure 7: Images of the *in vitro* scratch migration assay in Fibroblast cell line immediately and 24 h after wound induction. Based on our result investigation, the extract of *Garcinia Cambogia* significantly promoted migration and proliferation of 3T3-L1 compared to control

cells (untreated cells) which proves the wound healing is enhanced by the extract.

Conclusion

Garcinia cambogia fruit extract has demonstrated antidiabetic properties and beneficial effects on the healing of diabetic foot ulcers, likely due to its anti-inflammatory, antioxidant and antibacterial activities. The study's findings suggest that the extract promotes wound healing by enhancing cell proliferation and migration, key processes in tissue repair. Additionally, the extract may stimulate angiogenesis in endothelial cells, further supporting tissue regeneration. These results highlight the extract's significant potential as a natural therapeutic agent for diabetic wound care. The observed pharmacological effects are likely attributed to the diverse phytochemicals present in the *Garcinia cambogia* fruit extract.

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