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Strategic bioprospecting of *Ctenolepis garcinii* for metabolic and infectious disease intervention using HPLC and GC-MS analysis

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ABSTRACT

Diabetes mellitus, a common metabolic disorder, demands the development of safe and affordable therapeutic agents. Traditional medicinal plants provide valuable bioactive compounds with antidiabetic and antimicrobial potential. *Ctenolepis garcinii*, a climber from the Cucurbitaceae family used in South Indian and Sri Lankan medicine, remains scientifically underexplored. This study investigates its phytochemical composition and biological activities using phytochemical, chromatographic, antimicrobial, and molecular docking approaches, emphasizing its antidiabetic and antimicrobial efficacy. Ethanolic extracts of *C. garcinii* underwent qualitative and quantitative phytochemical screening, FTIR, UV–Vis, HPLC flavonoid profiling, GC-MS volatile compound identification, antimicrobial testing against *Streptococcus aureus* and *Candida albicans*, and molecular docking targeting SPA and Sky proteins. Phytochemical analysis revealed major constituents such as alkaloids (25.90%), flavonoids (21.50%), and phenols (18.40%). FTIR confirmed hydroxyl, aliphatic and aromatic hydrocarbons, ethers, halogens, and disulfides. UV–Vis analysis showed strong absorption at 353 nm and 407 nm, indicating phenolic and flavonoid presence. HPLC detected flavonoids including naringin (15.45 µg/mL), quercetin, kaempferol, and luteolin. GC–MS identified 20 compounds, notably tetracosanal (39.55%), 3-methylene-1-oxa-spiro[3,6]decane, and 3-methyl-2-(2-oxopropyl)furan. Antimicrobial assays showed moderate inhibition zones (8 mm for *S. aureus*, 7 mm for *C. albicans*). Docking studies revealed strong binding affinities of 3-methyl-2-(2-oxopropyl)furan with SPA (-7.4 kcal/mol) and Sky proteins (–8.3 kcal/mol). Overall, *C. garcinii* demonstrates a rich phytochemical profile with promising antidiabetic and antimicrobial potential. The identified compounds, supported by docking results, highlight its ethnomedicinal relevance and warrant further pharmacological and toxicological studies for drug development.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. With a global rise in diabetic prevalence, particularly in low- and middle-income countries, there is growing demand for cost-effective, safe, and naturally derived therapeutic agents. Traditional medicinal plants have emerged as valuable resources for identifying biologically active compounds with potential antidiabetic effects.

Ctenolepis garcinii (Tamil vernacular name Kollankovai) is a lesser-studied climbing plant found in South India and Sri Lanka, traditionally used for its medicinal benefits in treating fever, inflammation, and infections. However, its antidiabetic potential remains underexplored in scientific literature. Preliminary phytochemical screening of allied species in the Cucurbitaceae family has revealed the presence of bioactive constituents such as cucurbitacins, flavonoids, and triterpenoids, many of which are known for their therapeutic efficacy, including antidiabetic activity (Yadav *et al.,* 2010). These plants also show strong antioxidant activity, protecting pancreatic β-cells from oxidative stress. Additionally, hepatoprotective, antimicrobial, and anticancer properties have been reported due to various phytoconstituents (Sharma *et al.*, 2011). However, its phytochemical profile and antidiabetic potential remain scientifically underexplored.

Natural compounds such as flavonoids, alkaloids, phenolics, and saponins have been reported to possess antioxidant, antihyperglycemic, and antimicrobial activities (Patel *et al.*, 2012; Prior *et al.*, 2005). These bioactivities are often mediated via inhibition of carbohydrate-hydrolyzing enzymes, enhancement of insulin sensitivity, and protection of pancreatic β-cells from oxidative damage (Nijveldt *et al.*, 2001; Kumar and Pandey, 2013).

In this study, the ethanolic extract of *Ctenolepis garcinii* was subjected to qualitative and quantitative phytochemical analysis, Flavonoid profiling using HPLC, bioactive compound identification through GC-MS, *in vitro* antimicrobial activity and *in silico* molecular docking to evaluate interactions between selected compounds and diabetes-relevant target proteins.

This integrative approach aims to validate the traditional claims associated with *Ctenolepis garcinii*, identify novel bioactive compounds and explore their therapeutic potential against diabetes and microbial pathogens.

MATERIALS AND METHODS

Collection of plant materials

The powdered form of *Ctenolepis garcinii* was procured in January 2023 from herbal S.T.E.T Women's College (Autonomous), Sundarakkottai, Mannargudi, Thiruvarur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli (Kamble et al., 2011).

Preliminary phytochemical screening

Standard procedures as outlined by Harborne (1998) were followed to detect the presence of alkaloids, flavonoids, phenols, tannins, steroids, glycosides, saponins, anthraquinones, terpenoids and reducing sugars.

Quantitative estimation of phytoconstituents

The extract was analyzed for Alkaloids (expressed as Atropine equivalents), Flavonoids (Quercetin equivalents), Phenols (Gallic acid equivalents), Tannins (Tannic acid equivalents), Saponins (Diosgenin equivalents), Colorimetric assays were employed, and absorbance values were measured using a UV–Vis spectrophotometer.

FT-IR analysis

The ethanolic extract was filtered and to determine the functional groups present in *C. garcinii*, Fourier Transform Infrared Spectroscopy (FT-IR) analysis was carried out. The FT-IR spectra were recorded in

the range of 4000–400 cm^{-1}. The characteristic peak values and their corresponding functional groups were identified and tabulated.

UV-VIS analysis

To investigate the presence of chromophoric compounds, UV-VIS spectrophotometric analysis was performed. The extract was dissolved in ethanol and scanned in the wavelength range of 200–800 nm using a UV-VIS spectrophotometer (Kumar and Roy, 2020; Sowndharajan and Kang, 2013). The absorbance at various wavelengths was recorded and the peaks corresponding to maximum absorbance (λmax) were noted.

HPLC analysis of flavonoids

The ethanolic extract was filtered and injected into an HPLC system with a C18 column. A gradient mobile phase of acetonitrile and water with 0.1% trifluoroacetic acid was used. Standard flavonoids (naringin, rutin, quercetin, kaempferol, etc.) were used to identify and quantify the compounds based on retention time and peak area.

GC-MS analysis

The extract was analyzed using GC-MS to identify volatile bioactive components. The instrument was operated with helium as the carrier gas. Compounds were identified by comparing the mass spectra with NIST database references.

Antimicrobial assay

The antimicrobial activity was evaluated using the well diffusion method against *Streptococcus aureus* (Gram-positive bacteria) and *Candida albicans* (fungus). The zone of inhibition were measured and compared to standard antibiotic controls.

RESULTS AND DISCUSSION

Qualitative analysis

The preliminary phytochemical analysis of *Ctenolepis garcinii* ethanol extract revealed the presence of several bioactive compounds, notably alkaloids, flavonoids, phenols, tannins, saponins, anthraquisnones, steroids, and glycosides, while terpenoids, quinones, and reducing sugars were absent. These findings support the traditional medicinal use of *C. garcinii* and suggest its potential for pharmacological activities, especially in the context of metabolic disorders and oxidative stress (Table 1).

Table 1. Preliminary phytochemical screening for the ethanolic extract of *C. garcinii*

Chemical constituent	Ethanol extract
Alkaloids	+
Flavonoids	+
Terpenoids	-
Phenol	+
Tannins	+
Quinones	-
Saponins	+
Reducing sugar	-
Anthraquinones	+
Steroids	+
Glycoside	+

(+) present; (-) absent

The presence of flavonoids is particularly significant due to their well-documented antioxidant, anti-inflammatory, and antidiabetic properties. Studies have shown that flavonoids can inhibit carbohydrate-hydrolyzing enzymes and improve insulin sensitivity, making them valuable in the management of diabetes mellitus (Patel *et al.*, 2012; Nijveldt *et al.*, 2001).

Alkaloids, another major group identified, are known for their broad-spectrum biological activities, including hypoglycemic, analgesic, and antimicrobial properties. They can enhance insulin secretion and reduce blood glucose levels by modulating glucose metabolism (Abdel-Sattar *et al.*, 2020). Phenolic compounds and tannins have also been widely reported for their ability to act as natural antioxidants by donating hydrogen atoms to free radicals, thereby reducing oxidative damage in tissues (Prior *et al.*, 2005). This antioxidant capability is crucial in mitigating complications associated with chronic diseases such as diabetes, cardiovascular disease and cancer.

The detection of saponins and steroids indicates possible membrane-modifying effects and hormonal activity, which might contribute to the therapeutic

properties of the plant. Saponins have been reported to exert cholesterol-lowering effects and possess immune-stimulating properties (Man *et al.*, 2010). Meanwhile, anthraquinones, which were also present, are known to possess laxative, antimicrobial, and cytotoxic properties (Evans, 2002). Importantly, the absence of terpenoids and quinones does not diminish the therapeutic potential, but suggests that ethanol as a solvent may preferentially extract polar compounds, possibly leaving behind some of the non-polar constituents.

Quantitative analysis

The quantitative assay (Table 2) further supports these observations by determining the concentrations of selected bioactive constituents such as alkaloids (25.90 ± 0.73%) were the most abundant phytochemical, which aligns with previous findings that alkaloid-rich plant extracts exhibit strong antidiabetic, analgesic and antimicrobial activities. These compounds can modulate glucose metabolism and stimulate insulin secretion (Abdel-Sattar *et al.*, 2020). Flavonoids (21.50 ± 0.25%) were also present in high quantities. These compounds are known for their antioxidant, anti-inflammatory, and anti-diabetic effects.

Flavonoids act by scavenging free radicals and inhibiting α-glucosidase and α-amylase enzymes, which can help regulate postprandial blood glucose levels (Patel *et al.*, 2012; Nijveldt *et al.*, 2001). Phenolic content (18.40 ± 0.64%) is also substantial, reflecting the antioxidant capacity of the extract. Phenolic compounds protect cellular components from oxidative damage and contribute to reducing the risk of chronic diseases including diabetes, cancer, and cardiovascular disorders (Prior *et al.*, 2005). Tannins (4.15 ± 1.46%) contribute to the extract's astringent and antimicrobial properties. They also exhibit antidiabetic effects by improving glucose uptake and regulating metabolic enzymes (Chung *et al.*, 1998). Saponins (2.25 ± 2.26%), although present in lower concentrations, are noteworthy due to their cholesterol-lowering, immunostimulatory, and antidiabetic properties. They also promote pancreatic β-cell regeneration and insulin secretion (Man *et al.*, 2010). These results support the potential of *Ctenolepis garcinii* as a source of natural therapeutic agents.

Table 2. Quantitative analysis of phytoconstituents of ethanolic extract of *C. garcinii*

Extracts	Quantitative determination of chemical constituents (%)				
	Alkaloids	Flavonoids	Saponins	Tannins	Phenols
Ethanol extract	25.90±0.73	21.50±0.25	2.25±2.26	4.15±1.46	18.40±0.64

Table 3. FT-IR spectral peak values and functional groups obtained for the extract of *C. garcinii*

Extracts prepared in	Peak values	Functional groups	Interpretation
Ethanol	3425.43	-OH group	Alcohols
	2927.07	C - H stretching	Alkanes
	2103.04	C = C stretching	Terminal alkyne
	1631.68	C = C group	Alkenes
	1490.61	C – F stretching	Halogen
	1401.60	C - H stretching	Ethers
	1127.27	C – O group	Ethers
	1054.73	C – O group	Ethers
	933.94	C - H stretching	Aromatic compounds
	899.81	C – O group	Ethers
	828.69	C – Cl stretching	Halogen
	719.01	C – Cl stretching	Aliphatic chloro compounds
	438.14	S – S stretching	Aryl disulfides

FT-IR analysis

The FT-IR analysis of the ethanolic extract of *C. garcinii* revealed several distinct absorption bands corresponding to various functional groups. Prominent peaks included 3425.43 cm^{-1} indicating the presence of hydroxyl (-OH) groups (Coates, 2000), suggesting alcohols or phenolic compounds, 2927.07 cm^{-1} corresponding to C–H stretching,

characteristic of alkanes, 2103.04 cm^{-1} due to C≡C stretching, attributed to terminal alkynes, 1631.68 cm^{-1} representing C=C stretching, associated with alkenes, Peaks at 1490.61 cm^{-1} and 828.69 cm^{-1} suggested the presence of halogen-containing groups like C–F and C–Cl stretching, indicating halogenated compounds, multiple peaks such as 1401.60 cm^{-1}, 1127.27 cm^{-1}, 1054.73 cm^{-1}, and 899.81 cm^{-1} were indicative of C–O bonds, pointing to ether linkages, The peak at 933.94 cm^{-1} was assigned to C–H bending in aromatic compounds, The low-intensity peak at 438.14 cm^{-1} suggested the presence of aryl disulfides (S–S stretching) (Movaliya and Rathod, 2019; Tiwari *et al.*, 2017) (Table 3, Fig. 1).

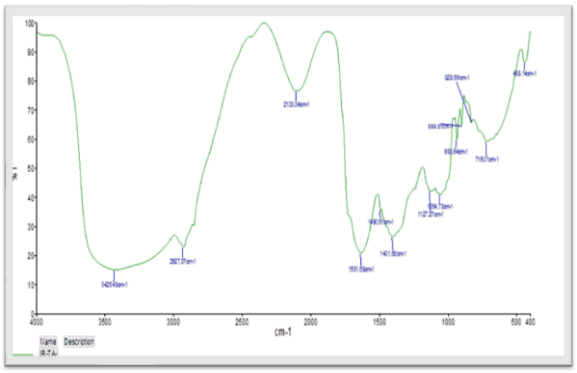

Fig. 1. FT-IR spectral peak values and functional groups obtained for the extract of *C. garcinii*

The presence of diverse functional groups like alcohols, alkanes, alkynes, alkenes, ethers, halogenated compounds, and sulfur-containing groups supports the chemical complexity of the plant extract (Manikandan *et al.*, 2015; Devi *et al.*, 2018). These functional groups are known to contribute to various biological activities, thereby underlining the pharmacological potential of *C. garcinii*.

UV-VIS spectral analysis

The UV-VIS spectral analysis of the pure ethanolic extract of *C. garcinii* showed several distinct absorption peaks. The strong absorption peaks at 353 nm and 407 nm suggest the presence of conjugated systems, likely flavonoids or phenolic compounds, known for characteristic absorbance in the UV-visible region (Table 4, Fig. 2). The peak at 665 nm (with moderate absorbance) might indicate the presence of chlorophyll-related compounds or other conjugated pigments (Abdel-Aziz *et al.*, 2018; Raj *et al.*, 2016).

The UV-VIS spectrum thus confirms that the ethanolic extract of *C. garcinii* contains a range of chromophoric compounds, which may contribute to its biological activities, including antioxidant potential (Ghasemzadeh *et al.*, 2011).

Table 4. UV-VIS peak values of extract of *C. garcinii*

Wavelength (nm)	Absorbance	Functional groups	Interpretation
353	1.309	Aromatic rings with –OH, C=O conjugation	Strong UV band I typical of flavonols / phenolic acids
407	1.469	Carotenoids (conjugated C=C)	Carotenoids or porphyrin-type pigments
504	0.1	Anthocyanins or carotenoid shoulders (conjugated C=C	Carotenoids
535	0.062	Positively charged flavylium cation	Likely trace amounts of anthocyanins
609	0.108	Extended conjugated macrocycles, possible pheophytins or pigment aggregates	Minor porphyrin-type derivatives
665	0.630	Porphyrin macrocycle (strong Q-band	Moderate-to-strong band characteristic of chlorophyll a

HPLC analysis

HPLC analysis of flavonoid profile

The HPLC analysis revealed the presence of nine flavonoid compoundsincluding Naringin (15.45 µg/mL), Rutin (8.60 µg/mL), Quercetin (5.49 µg/mL), Kaempferol (4.66 µg/mL), Luteolin (7.48 µg/mL), Apigenin (5.49 µg/mL), Catechin (6.78 µg/mL), Daidzein (2.33 µg/mL), Hesperetin (5.30 µg/mL). These flavonoids are well-documented for their pharmacological properties. Quercetin, kaempferolandluteolin, in particular, have shown remarkable efficacy in reducing hyperglycemia, modulating inflammatory cytokines, and improving lipid profiles in diabetic models (Kumar and Pandey, 2013; Li *et al.*, 2016). Naringin, the most abundant flavonoid identified, is known for its antioxidant and hepatoprotective activities (Chanet *et al.*, 2012; Fan *et al.*, 2020; Singh *et al.*, 2010).

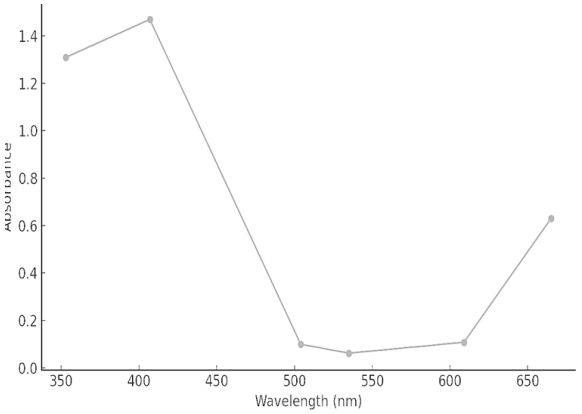

Fig. 2. UV-VIS spectra of *C. garcinii* plant extract

Table 5. Flavonoid compounds identified in the extract from *C. garcinii* by HPLC

RT (min)	Area (mAU)	Compound	Concentration (µg/mL)
4.6	215.68	Naringin	15.45
5.2	330.85	Rutin	8.60
7.0	448.52	Quercetin	5.49
8.1	204.44	Kaempferol	4.66
9.0	380.78	Luteolin	7.48
10.0	189.58	Apigenin	5.49
12.01	320.09	Catechin	6.78
14.0	59.74	Daidzein	2.33
15.6	380.15	Hesperetin	5.30

The identification of rutin and catechin, both strong antioxidants, further emphasizes the extract's ability to counteract oxidative stress, which is a key pathological feature in chronic diseases like diabetes and cardiovascular disorders. Moreover, apigeninandhesperetin contribute to the anti-inflammatory and neuroprotective effects associated with dietary flavonoids (Salehi *et al.,* 2019; Khamble*et al.*, 2017; Zhang *et al.*, 2012).

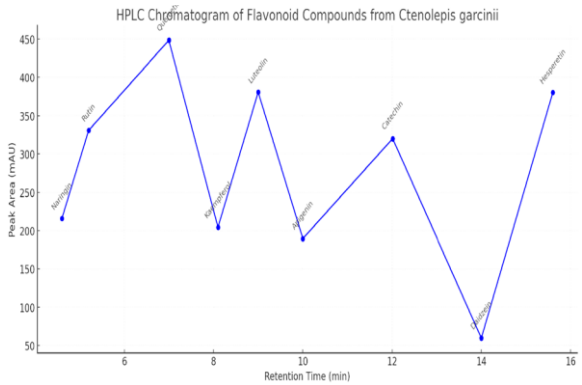

Fig. 3. HPLC chromatagram for *Ctenolepis garcinii* extract

The flavonoid-rich profile obtained through HPLC also supports the traditional claim of this plant's use in managing ailments associated with inflammation and oxidative stress. These compounds may act via multiple mechanisms including enzyme inhibition (α-amylase, α-glucosidase), free radical scavengingand modulation of glucose transporters (Tiwari *et al.*, 2013; Chen *et al.*, 2019).

GC-MS analysis

The GC-MS profiling revealed the presence of 20 bioactive compounds, with Tetracosanal (39.55%) and 3-Methylene-1-oxa-spiro[3,6]decane (11.00% and 20.01%) being the most abundant (Table 6, Fig. 4).

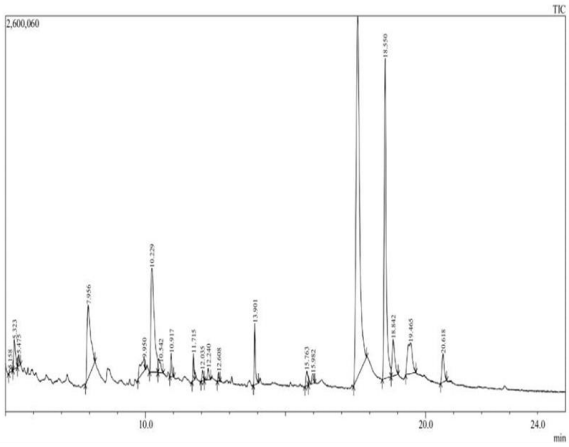

Fig. 4. Spectral graph of GC-MS analysis of *C. garcinii* extract

Other significant constituents include L-(+)-Ascorbic acid (8.16%), Isopropyl myristate (4.54%), Oleic acid (2.12%) and Palmitic acid (0.84% and 0.76%), Tetracosanal, a long-chain aliphatic aldehyde, exhibits antimicrobial and insecticidal activities (Jeyasankar, 2014; Jeyaprakash and Chinnaswamy *et al.*, 2017), 3-Methylene-1-oxa-spiro[3,6]decane is a spiro compound with known antioxidant and cytotoxic potential and ascorbic acid (Vitamin C) acts as a powerful antioxidant and co-factor in enzymatic reactions.Fatty acids like oleic acid and palmitic acid play roles in lipid metabolism regulation and cell membrane stabilization, with implications in anti-inflammatory and hypoglycemic actions (Bays *et al.*, 2008) and Isopropyl myristate and isopropyl

decanoate are esters often used in pharmaceutical formulations due to their penetration-enhancing properties and low toxicity profiles (Ramalakshmi and Muthuchelian, 2011; Pavithra and Vadivukkarasi, 2015; Rajeswari and Murugan, 2012).

Table 6. Phytocomponents identified in *Ctenolepis garcinii* [GC-MS]

Peak	R.Time	Area	Area%	Height	Height%	A/H	Name
1	5.158	128988	0.24	21567	0.28	5.98	trans-Z-alpha-Bisabolene epoxide
2	5.323	886564	1.67	194323	2.49	4.56	4-Hexen-3-one, 4,5-dimethyl- (CAS) 4,5-DIM
3	5.475	242786	0.46	76678	0.98	3.17	1,2,3-Propanetriol, 1-acetate (CAS) 1-ACETO
4	7.956	4321047	8.16	482547	6.19	8.95	L- (+)-Ascorbic acid
5	9.950	952230	1.80	85526	1.10	11.13	3-Methyl-2-(2-oxopropyl) furan
6	10.229	5824897	11.00	681834	8.75	8.54	3-Methylene-1-oxa-spiro [3,6]decane
7	10.542	523875	0.99	63992	0.82	8.19	Hexadecane
8	10.917	433089	0.82	152055	1.95	2.85	6-Octadecenoic acid
9	11.715	443822	0.84	176784	2.27	2.51	Hexadecanoic acid (CAS) Palmitic acid
10	12.035	255242	0.48	68739	0.88	3.71	Didodecyl phthalate
11	12.240	281744	0.53	73882	0.95	3.81	Limonene dioxide 4
12	12.608	130898	0.25	58267	0.75	2.25	9-Eicosyne (CAS)
13	13.901	1120829	2.12	402520	5.16	2.78	9-Octadecenoic acid (Z)- (CAS) Oleic acid
14	15.763	470211	0.89	96571	1.24	4.87	1,10-Decanediol (CAS) Decane-1,10-diol
15	15.982	399673	0.76	64305	0.82	6.22	Hexadecanoic acid (CAS) Palmitic acid
16	17.578	20933137	39.55	2368544	30.39	8.84	Tetracosanal
17	18.550	10592421	20.01	2099150	26.93	5.05	3-Methylene-1-oxa-spiro [3,6] decane
18	18.842	1544024	2.92	239545	3.07	6.45	Trans-5-Hexyl-1,4-dioxane-2-carboxylic acid
19	19.465	2403926	4.54	201773	2.59	11.91	Isopropyl myristate
20	20.618	1044379	1.97	186190	2.39	5.61	Isopropyl decanoate

Antimicrobial activity

The ethanolic extract showed moderate antimicrobial activityagainst *Streptococcus aureus*, a zone of inhibition of 8 mm was observed 10 mmagainst *Candida albicans*, it exhibited a 7 mm zone of inhibition compared to the positive control 11 mm.

Table 7. Zone of inhibition of ethanol extract of *C. garcinii*

Name of the microorganism	Zone of inhibition in mm		
	Standard (mm)	Control	Sample (mm)
Streptococcus aureus	10	Nil	8

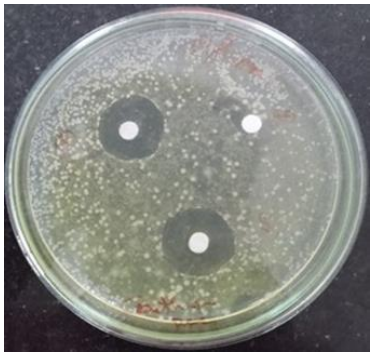

Fig. 5. Antibacterial activity of ethanolic extract of *C. garcinii* against *Streptococcus aureus*

Table 8. Zone of inhibition of ethanolic extract of *C. garcinii*

Organism	Positive	Sample	Negative
Candida albicans	11	7	-

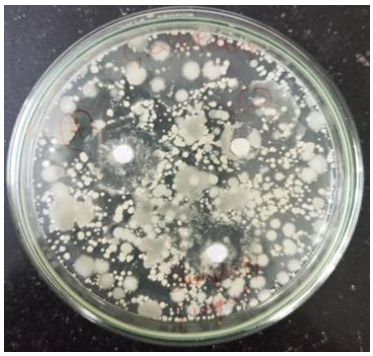

Fig. 5. Zone of inhibition of ethanolic extract of *Ctenolepis garcinii*

These results, although not as strong as standard antibiotics, indicate that *Ctenolepis garcinii* possesses inherent antimicrobial agents, likely contributed by flavonoids (e.g., quercetin, kaempferol) and fatty acids (oleic acid, palmitic acid), which are known to disrupt microbial membranes and interfere with essential microbial enzymes (Cushnie and Lamb, 2011).

CONCLUSION

The present study highlights the significant pharmacological potential of *Ctenolepis garcinii*, a traditionally used but scientifically underexplored medicinal plant. Through an integrative approach combining phytochemical screening, chromatographic profiling, antimicrobial assays and molecular docking, the ethanolic extract was shown to contain a rich composition of bioactive compounds, particularly flavonoids, alkaloids, and phenolics.

The FT-IR spectral analysis of the ethanolic extract of *Ctenolepis garcinii* confirmed the presence of multiple functional groups including hydroxyl, aliphatic and aromatic hydrocarbons, ethers, halogens, and disulfides. These findings suggest that the plant is rich in chemically diverse phytoconstituents, which may correlate with its reported therapeutic properties. Further phytochemical and biological investigations are warranted to isolate, identify, and validate the bioactive compounds responsible for the observed activities.

The UV-VIS spectrophotometric analysis of the ethanolic extract of *Ctenolepis garcinii* revealed significant absorption in both UV and visible regions, particularly at 353 nm and 407 nm, indicating the presence of phenolic and flavonoid compounds. The observed peaks suggest that the extract is rich in chromophoric phytoconstituents, supporting its potential pharmacological value. Further phytochemical profiling and bioactivity studies are recommended to isolate and characterize the active compounds.

HPLC and GC-MS analyses confirmed the presence of therapeutically important constituents such as naringin, quercetin, kaempferol, and 3-methyl-2-(2-oxopropyl) furan. These compounds are associated with antioxidant, antidiabetic and antimicrobial effects. The extract demonstrated moderate inhibitory activity against *Streptococcus aureus* and *Candida albicans*, supporting its antimicrobial efficacy.

In conclusion, *Ctenolepis garcinii* holds considerable promise as a source of natural therapeutic agents for managing metabolic and infectious diseases. Further isolation, structural elucidation, pharmacological validation, and toxicity studies are essential to advance its development into clinically viable phytopharmaceuticals.

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