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Exploring *Ctenolepis garcinii* as a natural anti-diabetic agent: A phytochemical, biochemical and molecular docking approach

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ABSTRACT

Diabetes mellitus is a major global health challenge, and plant-derived bioactive compounds offer promising therapeutic alternatives. *Ctenolepis garcinii* is traditionally used in ethnomedicine, but its anti-diabetic potential has not been systematically validated. This study aimed to evaluate the physicochemical parameters, phytochemical constituents, fluorescence characteristics, flavonoid profiling, *in vitro* α-amylase inhibitory activity, and molecular docking interactions of *Ctenolepis garcinii*. Physicochemical analysis included determination of moisture content, ash values, and extractive values. Quantitative phytochemical screening measured flavonoids, alkaloids, tannins, saponins, phenols, crude fiber, ash, and pectic substances. Fluorescence analysis of powdered samples was performed using chemical reagents under visible and UV light. Thin Layer Chromatography (TLC) identified flavonoids using quercetin and rutin as standards. *In vitro* α-amylase inhibitory activity was assessed by the DNSA method at concentrations 100–500 µg/ml, with acarbose as standard. Molecular docking was conducted to evaluate the binding affinity of rutin with human pancreatic α-amylase (PDB: 1B2Y). Physicochemical analysis showed moisture content 1.97% and total ash 32%, reflecting mineral richness. Phytochemical screening revealed high amounts of tannins (32.4%), pectic substances (23.8%), saponins (16.5%), and flavonoids (10.2%). Fluorescence analysis provided distinct diagnostic features under different reagents. TLC confirmed the presence of flavonoids with Rf values comparable to quercetin (0.85) and rutin (0.65). *In vitro* α-amylase inhibition was dose-dependent, with 87.0 ± 2.10% inhibition at 500 µg/ml, nearly equivalent to acarbose (92.41 ± 0.81%). Molecular docking showed rutin exhibited strong binding affinity (-8.80 kcal/mol) with α-amylase, forming interactions with critical residues (Gly306, His305, Tyr151, Asp300, etc.). *Ctenolepis garcinii* exhibits significant anti-diabetic potential, attributable to its rich flavonoid and tannin content. The combination of *in vitro* and *in silico* findings supports its inhibitory effect on carbohydrate-hydrolyzing enzymes, validating its ethnomedicinal use. Further *in vivo* and clinical studies are recommended to establish its therapeutic efficacy and safety.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes is rising at an alarming rate, with projections estimating 783 million cases by 2045 (International Diabetes Federation, 2021). Persistent hyperglycemia can lead to severe complications, including cardiovascular disease, nephropathy, neuropathy, and retinopathy, underscoring the urgent need for effective and safer therapeutic interventions.

Currently available antidiabetic drugs, such as sulfonylureas, biguanides, and α-glucosidase inhibitors, are associated with limitations including gastrointestinal disturbances, hypoglycemia, and decreased efficacy with long-term use (Bailey, 2015). In this context, medicinal plants have gained increasing attention as complementary and alternative therapies for diabetes management due to their accessibility, low cost, and diverse bioactive constituents (Patel *et al*., 2012).

Ctenolepis garcinii (Cucurbitaceae), commonly used in traditional medicine, has been reported for its diverse pharmacological properties. Despite its ethnomedicinal importance, scientific validation of its anti-diabetic potential remains limited. Phytochemicals such as flavonoids, tannins, and saponins are known to modulate carbohydrate metabolism by inhibiting carbohydrate-hydrolyzing enzymes, particularly α-amylase and α-glucosidase (Tadera *et al*., 2006; Sales *et al*., 2012). Therefore, exploring the phytochemical composition and biological efficacy of *C. garcinii* could provide insights into its potential as a natural therapeutic agent.

This study aimed to evaluate thephysicochemical characteristics, phytochemical profile, fluorescence analysis, flavonoid identification, in vitro α-amylase inhibitory activity, and molecular docking interactionsof *Ctenolepis garcinii*. The integration of experimental and computational methods provides a comprehensive understanding of the plant's bioactive potential against diabetes mellitus.

MATERIALS AND METHODS

Collection and preparation of plant material

Ctenolepis garcinii, a species of plant, was discovered in and around S.T.E.T. College Herbal Garden, Sundarakkottai, Tamil Nadu, India. The Flora of Presidency of Madras was used to identify the plant, and Dr. S. John Britto, of the RAPINAT Herbarium and Center for Molecular Systematics at St. Joseph's College in Tiruchirappalli, validated the identification (Voucher number of the specimen, DS 011).

Ctenolepis garcinii leaves were collected from Tamil Nadu in Thiruvannamalai District, India during January, 2023. Before being transported to the laboratory, the sample was placed in a plastic ziplock container and correctly labeled (Abreu *et al*., 2012). To get rid of the contaminants, the *Ctenolepis garcinii* leaves were washed several times using distilled water. After a thorough examination, the old parts of the leaves contaminated and damaged by fungi were removed. Using a combination of grinders, healthy crushed fruit were dried at room temperature.

Physicochemical analysis

Physicochemical parameters including moisture content, ash values (total ash, acid-insoluble ash, water-insoluble ash), and extractive values (alcohol-soluble and water-soluble) were determined as per the standard procedures outlined by the World Health Organization (WHO, 1998) and Kokate (2005).

Phytochemical analysis

Quantitative phytochemical estimations were performed to determine the concentration of flavonoids, alkaloids, saponins, phenols, tannins, crude fiber, ash content, and pectic substances following the standard protocols of Harborne (1998) and Trease and Evans (2002).

Fluorescence analysis

Fluorescence behavior of the powdered plant material was observed under visible and UV light at 0, 24, and 48 hours after treatment with various chemical reagents, as per the method described by Kokoshi *et al.* (1958). This served as a diagnostic tool for crude drug identification.

Thin layer chromatography (TLC) for flavonoids

Flavonoids were identified by TLC using suitable solvent systems. The Rf values of separated compounds were compared with standards quercetin and rutin. Spots were visualized under UV light and after spraying with detecting reagents (Wagner and Bladt, 1996).

***In vitro* anti-diabetic activity (α-Amylase inhibition assay)**

The α-amylase inhibitory activity of *C. garcinii* extract was evaluated using the DNSA (3,5-dinitrosalicylic acid) method described by Bernfeld (1955). Different concentrations of the extract (100–500 µg/ml) were tested, and percentage inhibition was calculated against the standard drug acarbose.

Molecular docking study

Molecular docking was performed to investigate the interaction of rutin (a major flavonoid identified) with human pancreatic α-amylase (PDB ID: 1B2Y). AutoDock Vina software was employed for docking studies (Trott and Olson, 2010). The binding affinity and amino acid interactions were analyzed using Discovery Studio Visualizer.

RESULTS AND DISCUSSION

The present study investigated the physicochemical, phytochemical, fluorescence, chromatographic, *in vitro* anti-diabetic activity, and molecular docking properties of Ctenolepis garcinii extracts, revealing significant therapeutic potential.

Physicochemical characteristics

The physicochemical analysis indicated that *C. garcinii* contains moderate moisture content (1.97%), which supports its stability against microbial contamination and deterioration during storage. The ash values, particularly the high total ash (32%) and acid-insoluble ash (22%), suggest the presence of inorganic matter, possibly reflecting the plant's mineral composition (Table 1).

Table 1. Physico-chemical characteristics of *Ctenolepis garcinii*

Parameters tested	Percentage yield (%)
Moisture content	1.97
Ash value	
Total ash	32
Acid insoluble ash	22
Water insoluble ash	21.66
Extractive value	
Alcohol soluble extractive	5.14
Water soluble extractive	2.92

Similar observations have been reported in traditional medicinal plants, where higher ash values correlate with enhanced therapeutic quality (Kokate, 2005; Harborne, 1998).

Phytochemical screening

A number of phytochemicals isolated from plant material are used inthe pharmaceutical drug industry today. The phytochemicals under investigation include secondary metabolites, many which are synthesized for plant defense and adaption to environmental stress. The phytochemicals can range from medicinally useful agents to treat varieties of diseases such as diabetes, malaria, anaemia (Fola, 1993).

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. In the present study was carried out on the *Ctenolepis garcinii* extract revealed the presence of medicinally active constituents. The phytochemical characters of *Ctenolepis garcinii* investigated and summarized in Table 2, Fig. 1. The presence of tannin, saponins, flavonoids, steroids, polyphenol, glycosides, terpenoids and alkaloids while anthroquinones and anthocyanins were absentin 70% ethanolic extract. The significant amount of flavonoids (60.00 mg/gm) and total phenol (210.95 mg/gm) were found in *Ctenolepis garcinii* represent.

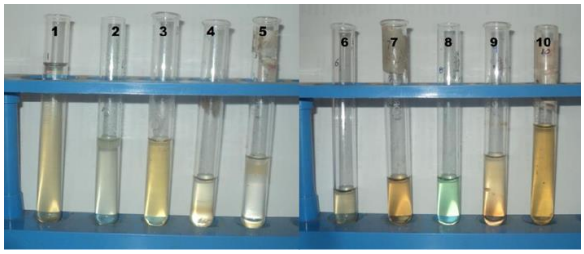
Quantitative phytochemical screening revealed substantial levels of tannins (32.4%), pectic substances (23.8%), and saponins (16.5%), along with flavonoids (10.2%) and alkaloids (4.8%) (Table 3). These bioactive compounds are known for their antioxidant, anti-inflammatory, and anti-diabetic

properties (Ghasemzadeh and Ghasemzadeh, 2011; Kumar *et al.*, 2012).

Table 2. Qualitative phytochemical analysis of *Ctenolepis garcinii* extract

Phytochemicals	Ethanolic extract
Tannin	+
Saponin	++
Flavonoids	++
Steroids	+
Terpenoids	++
Alkaloids	+
Antroquinone	-
Polyphenol	++
Glycoside	+
Anthocyanins	-

(+) Present (++) High concentration (–) Absent



1. Tannin, 2. Saponin, 3. Flavonoids, 4. Steroids, 5. Terpenoids, 6. Alkaloids, 7. Anthroquinone, 8. Polyphenol, 9. Glycoside, and 10. Anthocyanins

Fig. 1. Phytochemicals

Table 3. Quantitative phytochemical analysis of *Ctenolepis garcinii*

Phytochemical	Concentration in percentage (%)
Flavonoids	10.2
Alkaloids	4.8
Saponins	16.5
Phenols	6.6
Tannins	32.4
Crude fiber	5.5
Ash content	5.8
Pectic substances	23.8

Particularly, tannins and flavonoids are widely reported to modulate carbohydrate metabolism and inhibit digestive enzymes, making them promising anti-diabetic agents.

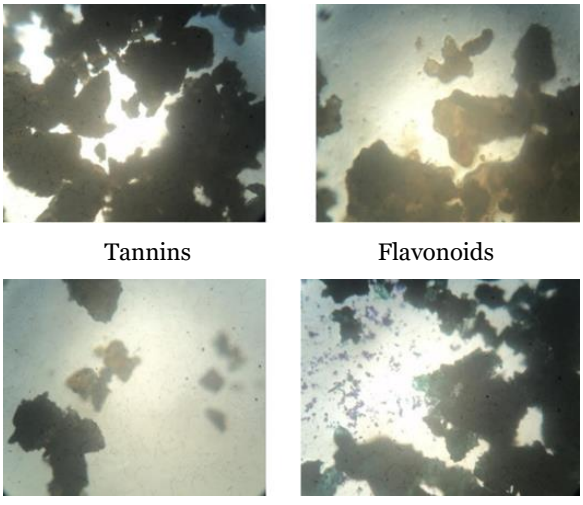
Histochemical analysis of powder in *Ctenolepis garcinii*

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytocompounds (Krishnan *et al.,* 2001). In the present study, *Ctenolepis garcinii* were treated with specific chemicals and reagents. The *Ctenolepis garcinii* powder treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids, treated with few drops toluidine blue reagent gave Blue green / red colour indicates polyphenol, treated with few drops H_2SO_4 reagent gave yellow colour indicates saponin (Table 4 and Fig. 2). This results further confirmed the presence of phytochemicals in *Ctenolepis garcinii.*

Table 4. Histochemical analysis of powder in *Ctenolepis garcinii* powder

Phytochemicals	Results
Tannin	++
Flavonoids	+
Terpenoids	+
Polyphenol	++

(+) Presence; (++) present with high intensity of the colour



Tannins Flavonoids

Terpenoids Polyphenol

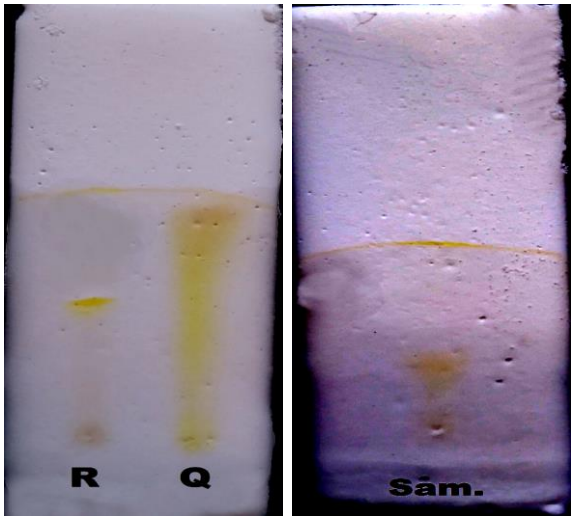
Fig. 2. Histochemical analysis of powder in *Ctenolepis garcinii* powder

John Peter Paul (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner). Histochemical analyses of the plant

were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Results of histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light.

Table 5. Fluroscence analysis of *Ctenolepis garcinii*

Particulars of the treatment	Under ordinary light		
	0 Hours	24 Hours	48 Hours
Powder as such	Green	Green	Green
Powder + water	Green	Dark green	Black
Powder + conc.H_2So_4	Black	Brown	Dark brown
Powder + Acetone	Sandal	Pale green	Green
Powder + Acetic acid	Green	Pale green	Dark green
Powder + $Fecl_3$	Dark green	Pale green	No change
Powder + NaOH	Green	Pale green	No change
Powder + $CHCl_3$	Sandal	Pale sandal	Sandal
Powder + Sodium nitrite	Pale green	Brown	No change
Powder + NaCl	Pale grey	Green	Pale green
Powder + NH_3OH	Brown	Dark brown	Pale brown
Powder + Picric acid	Yellow	Green	No change
Powder + Iodide	Grey	Pale green	No change
Powder + Conc.HCl	Pale brown	Dark brown	Black
Powder + 1N HCl	Green	Pale green	Dark green
Powder + 1N KOH	Dark grey	Green	Dark green



Standard Sample

Fig. 3. Identification of flavonoids using thin layer chromatography (TLC) from *Ctenolepis garcinii* extract

Table 6. Identification of flavonoids using thin layer chromatography (TLC) from *Ctenolepis garcinii* extract

Fraction color	Qualitative analysis flavonoids	Rf value
Light yellowish brown	++	0.67
Standard (Q: Quercetin)		0.85
Standard (R: Rutin)		0.65

Fluorescence and TLC analysis

The fluorescence analysis demonstrated distinct color changes under various reagents and light conditions, providing a reliable tool for crude drug identification. Fluorescence analysis has been established as a simple and cost-effective method for authentication of medicinal plants (Sharma *et al.*, 2011). TLC profiling further confirmed the presence of flavonoids, with Rf values comparable to standard quercetin (0.85) and rutin (0.65). The extract fraction displayed a strong spot (Rf 0.67) (Table 6, Fig. 3), indicating a high concentration of flavonoid compounds, consistent with the quantitative phytochemical results (Table 5).

CONCLUSION

The present study comprehensively evaluated the physicochemical, phytochemical, fluorescence, TLC, in vitro anti-diabetic, and molecular docking properties of *Ctenolepis garcinii*. Physicochemical parameters revealed acceptable quality indices, with moderate moisture content (1.97%) and high total ash value (32%), reflecting the presence of mineral constituents. Phytochemical analysis highlighted a

rich composition of bioactive compounds, particularly tannins (32.4%), pectic substances (23.8%), saponins (16.5%), and flavonoids (10.2%), which are well-documented for their therapeutic potential.

Fluorescence analysis under different reagents and light conditions provided diagnostic characteristics for the crude drug, while TLC confirmed the presence of flavonoids with Rf values corresponding to quercetin and rutin standards.

The *in vitro* anti-diabetic study demonstrated significant alpha-amylase inhibitory activity of *C. garcinii* extract in a concentration-dependent manner, with 87% inhibition at 500 µg/ml, comparable to the standard acarbose (92.41%). Molecular docking studies further validated these results, showing that rutin exhibited strong binding affinity (-8.80 kcal/mol) with pancreatic alpha-amylase, interacting with key catalytic residues, thus providing a mechanistic basis for its anti-diabetic action.

Collectively, the findings establish *C. garcinii* as a potential natural source of bioactive compounds with anti-diabetic activity, supported by both experimental and computational approaches. The high flavonoid and tannin content appears to play a crucial role in enzyme inhibition and glucose metabolism modulation.

However, while the *in vitro* and *in silico* results are promising, further *in vivo* studies and clinical evaluations are essential to validate safety, efficacy, and dosage standardization. These insights reinforce the ethnomedicinal relevance of *C. garcinii* and pave the way for its development as a phytotherapeutic candidate in diabetes management.

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