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Original Research Article

Pharmacognostic Characterization & Antiobesity Potential of Acalypha Indica: *In vitro* Evaluation & Network Pharmacology Analysis to Uncover Molecular Mechanism in Obesity Management

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Abstract: *Acalypha indica* has long been valued in traditional medicine, yet its potential in managing obesity and diabetes remains inadequately explored. This study aims to evaluate the pharmacognostic features and anti-obesity potential of *Acalypha indica* extracts obtained through ultrasound-assisted extraction (UAE). The extraction process was optimized using UAE to maximize the yield of bioactive constituents. Phytochemical profiling was conducted to assess total phenolic and flavonoid content. The anti-obesity activity was evaluated through in vitro pancreatic lipase and HMG-CoA reductase inhibition assays. Molecular docking and network pharmacology analyses revealed that key compounds such as Element, Anthraquinone, and Cholesterol interact with pivotal metabolic targets including NOS3, STAT3, and EGFR. The compound-target-pathway network illustrated a polypharmacological mode of action, where multiple bioactive synergistically regulate obesity-related pathways such as the Apelin signalling and progesterone- mediated oocyte maturation. *Acalypha indica* exhibits promising anti-obesity activity through dual enzyme inhibition and multi-target modulation of metabolic pathways. These findings support its therapeutic potential as a natural intervention in obesity management. Further in vivo and clinical validations are recommended to substantiate its efficacy and safety.

Keywords: Anti-Obesity, Anti-Oxidant Activity, *Acalypha Indica*.

INTRODUCTION

Obesity has emerged as a significant global health challenge, with its prevalence reaching alarming levels due to sedentary lifestyles, unhealthy dietary habits, and genetic predispositions. This multifaceted condition is characterized by an excessive accumulation of body fat, leading to severe comorbidities such as cardiovascular diseases, diabetes mellitus, and metabolic syndrome. Despite the availability of pharmacological treatments, these often pose challenges such as high costs, limited efficacy, and adverse side effects. Consequently, the search for safe, effective, and affordable therapeutic interventions has intensified, with medicinal plants offering a promising alternative.

Medicinal plants have been integral to traditional systems of medicine for centuries, owing to their diverse bioactive compounds and minimal side effects. Among these, *Acalypha indica*, a plant belonging to the Euphorbiaceae family, has garnered significant attention for its wide array of pharmacological properties Commonly known as the Indian nettle, *Acalypha indica* is widely distributed in tropical and subtropical regions and has been traditionally used in folk medicine to treat a variety of ailments, including respiratory disorders, gastrointestinal disturbances, and inflammatory conditions. The plant is rich in secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids, many of which exhibit potential therapeutic activities relevant to obesity management.

The anti-obesity potential of *Acalypha indica* can be attributed to its bioactive phytoconstituents, which may regulate lipid metabolism, inhibit adipogenesis, and enhance energy expenditure. In vitro studies are pivotal in understanding the biological activities of plant extracts and isolating specific compounds that contribute to their therapeutic effects. Moreover, advancements in computational biology and systems pharmacology have paved the way for network pharmacology.

In this context, the present study explores the Pharmacognostical characteristics and anti-obesity potential of *Acalypha indica* through a combination of in vitro assays and network pharmacology analysis. The Pharmacognostical evaluation ensures the quality, authenticity, and purity of the plant material, while the in vitro studies assess its bioactivity against obesity-related biomarkers. Simultaneously, network pharmacology elucidates the molecular interactions between the phytoconstituents of *Acalypha indica* and obesity- associated targets, unveiling its mechanistic pathways and therapeutic significance.

This dual approach not only highlights the relevance of *Acalypha indica* in managing obesity but also underscores the importance of integrating traditional medicine with modern scientific techniques. By bridging the gap between ethno pharmacology and molecular research, the study aims to contribute to the development of novel, plant-based interventions for combating obesity and its associated disorders.

MATERIALS AND METHODS

Source of data: The data will be collected through search engine, scientific journals and books/data base.

1. Collection and Authentication of Drug:

The plant will be collected from the appropriate area and authenticated.

2. Preparation of Extract:

The collected parts of the plant will be dried and processed into a coarse powder. The extraction of plant powder will be carried out using suitable solvents.

3. Phytochemical tests:

Various phytochemical tests will be performed by following the standard procedures for analyzing various phytochemicals such as alkaloids, Quinone, carbohydrates, cardiac glycosides, flavonoids, saponins, triterpenoids, sterols, phenols, fatty acids, and tannins.

4. Quantitative Determination of Secondary Metabolites:

a) Estimation of Total Flavonoid Contents: The total flavonoid content of the sample will be determined by Modified colorimetric Method.
b) Estimation of Total Phenolic Contents:
c) Determine the Total Phenolic Content by Folin Ciocaltu Method.

5. *In Silico* analysis

a) PPI interaction
b) Cluster analysis
c) Network pharmacology
d) Cytohubb analysis

6. Anti-obesity activity by utilizing *invitro* assay.

a) Pancreatic lipase inhibition assay.
b) HMG CoA reductase inhibition assay

7. To assess the *in-vitro* anti- oxidant studies

a) DPPH method
b) Nitric oxide method

List of Chemicals

Chemical Name	
Hydrochloric acid (HCl)	Merck Life Science Pvt. Ltd.
Methanol	Merck Life Science Pvt. Ltd.
Ethanol	Merck Life Science Pvt. Ltd.
Petroleum ether	Merck Life Science Pvt. Ltd.

Phloroglucinol HCl	Sigma-Aldrich
Sodium hydroxide	Merck Life Science Pvt. Ltd.
Quercetin	Sigma-Aldrich
Phosphate buffer	HiMedia Laboratories Pvt. Ltd.
Dinitro salicylic acid	Sigma-Aldrich
Gallic acid	Sigma-Aldrich
Fehling's A	HiMedia Laboratories Pvt. Ltd.
Fehling's B	Hi Media Laboratories Pvt. Ltd.
Aluminium chloride	Merck Life Science Pvt. Ltd.
Sodium nitrite	Merck Life Science Pvt. Ltd.
Sodium nitroprusside (SNP)	Sigma-Aldrich
Sulfanilamide	Sigma-Aldrich

Methods

1. Collection and Authentication of Plant Material:

Acalypha indica were procured from the local market in Kolhapur and authenticated by the Agharkar Research Institute, Pune, Maharashtra. The collected plant materials underwent a thorough preparation process, including washing to remove impurities, draining to eliminate excess moisture, and slicing for uniform drying. The sliced samples were then dried under controlled conditions to preserve their bioactive compounds. Once dried, the material was finely blended to obtain a uniform powder and subsequently sieved to ensure consistency. This prepared plant material was then utilized for further pharmacognostic and Anti-obesity studies, ensuring quality and reliability in experimental evaluations.

2. Processing of Plant Material:

Whole plant material was washed thoroughly with tap water. Further cleaned material was shade dried after complete drying, the plant material was made in to coarse powder and stored until further use.

3. Pharmacognostic Evaluation

Plant material was evaluated for various pharmacognostic parameters as mentioned below as per standard procedures.

3.1. Morphological Characteristics

The freshly collected plant material was subjected to organoleptic evaluation such as color, odour, taste and some extra Characteristics features are evaluated.

Preparation of Extract

The powdered plant material was first cleaned and chopped into smaller pieces before being subjected to shade drying. The extraction process was carried out by using a ultrasonic bath apparatus (LABMAN, Chennai, India). Hydroalcoholic solvent was used for the extraction process, and various experimental parameters were investigated. The independent variables included a solid-to-solvent ratio of 1:20, 1:25, and 1:30 g/mL, extraction temperatures of 40, 50, and 60 °C, and extraction durations of 15, 30, and 45 minutes. After extraction, the resultant mixture was cooled to room temperature and stored for subsequent analyses.

PRELIMINARY PHYTOCHEMICAL SCREENING

Initial phytochemical screening helps to identify the types of secondary metabolites that are present in plants. There is discussion of the numerous chemical tests that were performed [34, 35].

Test for Carbohydrates:

a) **Molisch's test:** Few drops of alcoholic alpha naphthol solution, few drops of concentrated sulphuric acid were added given sample was taken in a test tube. The test result gave a purple or violet-coloured.
b) **Benedict's test:** Added Benedict's reagent to the given sample in a test tube, heated the test tube in a boiling water bath. Red precipitation was formed.

Fehling's Test: Filtrates were heated with Fehling's A & B solutions; formation of red precipitate indicates the presence of reducing sugars.

Detection of Alkaloids Alkaloids Test

a) **Mayer's Test:** Mayer's reagent added to sample yellow ppt was formed.
b) **Dragendroff's Test:** Dragendroff's reagent added to sample orange ppt was formed.
c) **Wagner's Test:** Wagner's reagent added to sample reddish brown ppt was formed.
d) **Hager's Test:** Hager's reagent added to sample yellow ppt was formed.

***IN VITRO* ANTIOXIDANT ACTIVITY**

Assay Procedure

1. Reaction Mixture Preparation:

- A total of 1 mL of different concentrations of the test sample (or standard) was mixed with 1 mL of 10 mM SNP solution in PBS.
- The mixture was incubated at 25°C for 150 minutes under light to allow the production of nitric oxide.

2. Nitric Oxide Detection:

- After incubation, 0.5 mL of the reaction mixture was mixed with 0.5 mL of freshly prepared Griess reagent.
- The mixture was incubated in the dark for 10 minutes at room temperature.

3. Absorbance Measurement:

- The absorbance of the pink-coloured complex was measured at 546 nm using a UV-Visible spectrophotometer.
- A control (without test sample) and blank (without SNP) were also prepared.

Calculation of % Inhibition

The percentage inhibition of nitric oxide production was calculated using the following equation:

% Inhibition= [(Control-Test)/Control] x 100.

Where:

- Ac = Absorbance of the control (without extract)
- As = Absorbance of the sample or standard

***IN VITRO* ANTI-OBESITY ACTIVITY Assay Procedure:**

1. Sample Preparation:

- Add 20 µL of test sample to a 96-well plate.
- Add 20 µL of Orlistat as the positive control.
- Add 20 µL of DMSO as the negative control.

2. Enzyme Reaction: o Add 20 µL of pancreatic lipase solution to each well. o Pre-incubate the plate at 37°C for 15 minutes.

3. Substrate Addition: o Add 100 µL of p-NPP substrate solution to initiate the reaction. o Incubate at 37°C for 30–60 minutes.

4. Measurement: o Measure absorbance at 405 nm or 410 nm using a microplate reader.

RESULTS AND DISCUSSION

Morphological Evaluation of *Acalypha indica*

Acalypha indica, commonly known as Indian Copperleaf or Indian Acalypha, is a medicinal plant belonging to the Euphorbiaceae family. Morphological evaluation involves examining its macroscopic and organoleptic characteristics, which help in identification and authentication.

Figure 1: *Acalypha indica*

1. Morphological Features

Table 1: Morphological Features of *Acalypha indica*

Characteristic	Description
Root	Taproot system, thin and fibrous roots.
Stem	Erect, slender, green or purplish, branching, slightly hairy.
Leaves	Simple, alternate, ovate-lanceolate, 2– 6 cm long, serrated margins, green with a reddish tinge.
Flowers	Small, greenish, in axillary or terminal spikes, unisexual, with male and female flowers in the same inflorescence.
Inflorescence	Catkin-like, with male flowers at the top and female flowers at the base.
Fruit	A small, three-lobed capsule containing tiny seeds.
Seeds	Tiny, dark brown or black, oval in shape.

Table 2: Total Flavonoid content

Concentration (µg/mL)	Absorbance
0	0
2	0.085
4	0.154
6	0.249
8	0.330
10	0.398
Sample	**Absorbance**
Sample	0.83

Table 3: Phytochemical Screening of *Acalypha indica* Extract

Phytochemical Constituents	Test Performed	Result (+/−)
Alkaloids	Dragendorff's test	+
Flavonoids	Shinoda test	+
Tannins	Ferric chloride test	+
Saponins	Froth formation test	+
Steroids	Liebermann- Burchard test	+
Terpenoids	Salkowski test	+
Phenols	Ferric chloride test	+
Glycosides	Keller- Killiani test	+
Carbohydrates	Molisch's test	+
Proteins & Amino acids	Biuret test	–

Table 4: Physicochemical Properties of *Acalypha indica*

Parameter	Result (%)
Moisture Content	6.8 ± 0.5
Total Ash Value	8.2 ± 0.3
Acid Insoluble Ash	1.5 ± 0.2
Water Soluble Ash	3.9 ± 0.4
Alcohol-Soluble Extractive	12.5 ± 0.6
Water-Soluble Extractive	18.3 ± 0.5
Ether-Soluble Extractive	7.1 ± 0.4

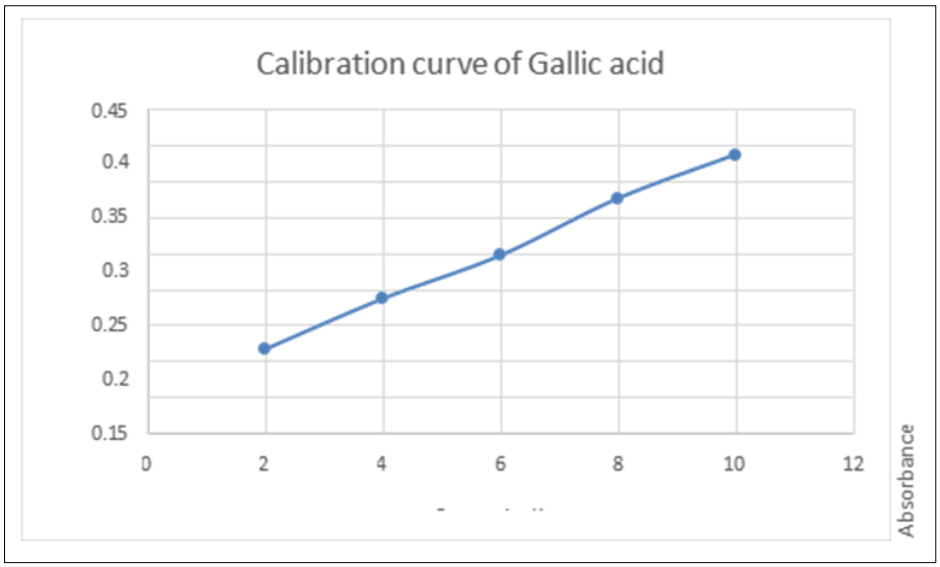

Fig. 1: Calibration curve of Gallic acid

The linear regression equation was found to be Y=0.034x+0.0481while the correlation coefficient was found to be 0.9985. The amount of phenol content present in the extract in terms mg GAE/g of extract was found to be 42.58±0.81 by using the above linear regression equation.

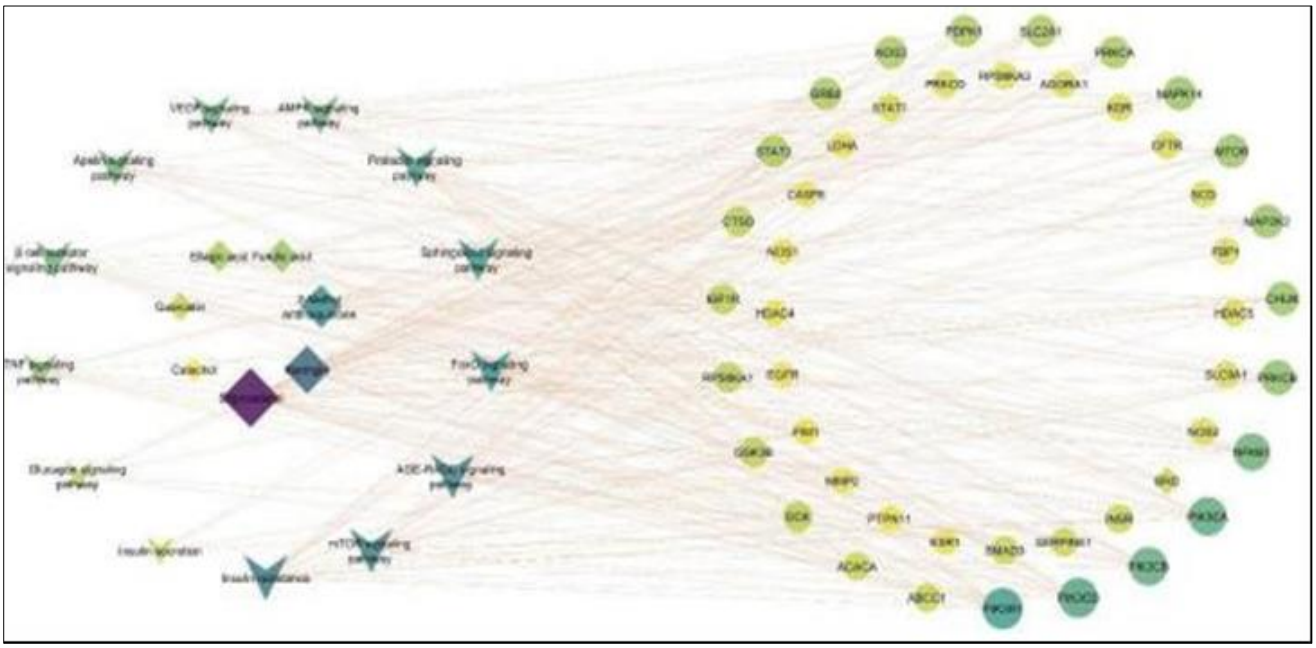

Fig. 2: Network construction between Phytoconstituents, Targets and Pathways in Anti-obesity.

Table 6: Percentage inhibition of Nitric Oxide (NO) Scavenging Assay

Conc	% Inhibition ascorbic acid	% Inhibition *Acalypha indica*
10	37.415	29.184
20	51.543	40.703
30	62.484	50.966
40	77.064	61.656
50	86.274	68.959
50	19.486	29.708

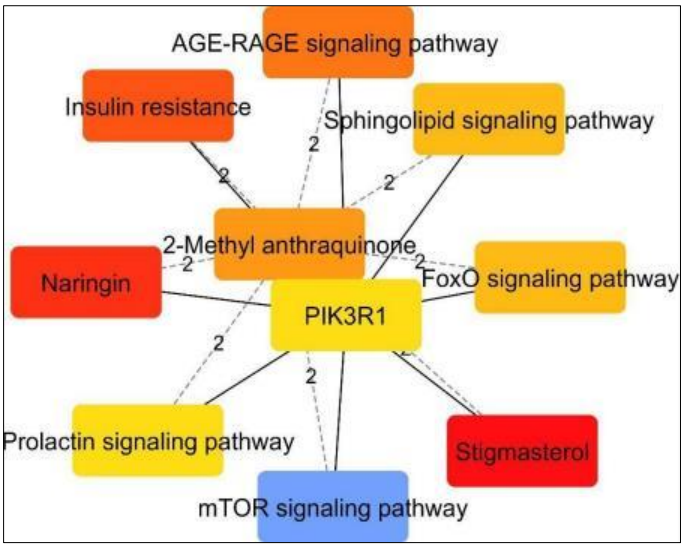

Figure 3: Gene Ontology (GO) analysis (cellular component)

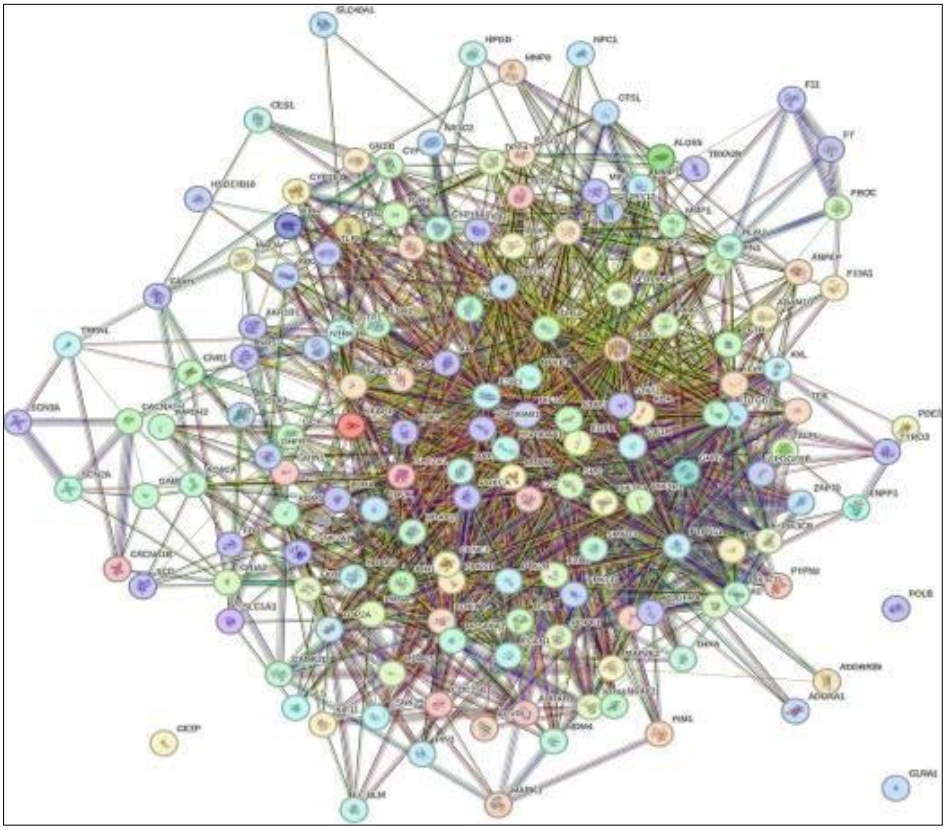

Figure 4: The Protein protein interaction of common targets between phytoconstituents and Anti-obesity. (A) *Acalypha indica* - Anti-obesity PPI network

CONCLUSION

This study investigated the pharmacognosy and anti-obesity potential of *Acalypha indica* using an optimized ultrasound-assisted extraction (UAE) method. Extraction parameters were meticulously optimized to enhance the yield of bioactive compounds, particularly total phenolic and flavonoid content, known for their therapeutic effects. In-silico analysis elucidated the anti-obesity mechanisms by revealing interactions between *Acalypha indica* phytoconstituents and key molecular targets involved in cholesterol metabolism. These findings suggest *Acalypha indica* as a promising natural candidate for obesity management, warranting further in vivo studies to confirm its efficacy and safety for pharmacological applications.

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