In-Vitro Anti-Diabetic Activity of *Acalypha indica* by Using A - Amylase Inhibition Assay

G. Supriya Reddy^{1*}, Akula Sai Nandini¹, Bommidi Haripriya¹, Annela Shirisha¹, Keesari Sampath¹, V. V. Rajesham¹, T. Rama Rao¹ CMR College of Pharmacy, Kandlakoya (V), Medchal (M & D), Hyderabad, Telangana -501401.

Corresponding Author

G. Supriya Reddy, M. Pharm., (Ph. D).
Associate Professor,
Department of Pharmacology
CMR College of Pharmacy,
Kandlakoya (V), Medchal (M & D),
Hyderabad–501401, Telangana, India

Abstract:- Medicinal plants have been used as remedies for many illnesses for a very long time all throughout the world. Acalypha indica is a kind of plant with an inflorescence resembling catkins. Despite being a weed, Acalypha indica is recognized by the locals as a valuable source of medication for a number of therapeutic therapies. Acalypha indica Linn is a plant that may have the ability to lower blood glucose levels. Acalypha indica *Linn* is one of the alternative herbal treatments for type-2 diabetes mellitus. Acalypha indica extract can be used to treat type-2 diabetes in addition to metformin therapy as well as to avoid insulin resistance in healthy individuals. According to this research, the findings of the alpha amylase enzyme test using the ethanol and pet ether extracts of the Acalypha indica plant suggested possible anti-diabetic efficacy based on its inhibitory activity (%). When acarbose and the potential for its anti-diabetic effects were evaluated, pet ether extract shown a better potential for suppressing alpha amylase's enzymatic activity. This was in contrast to ethanol extract.

Keywords:- Acalypha indica, Diabetes, Phytochemical Screening, α - amylase inhibition assay.

I. INTRODUCTION

One of the most prevalent non-communicable diseases in the world is "diabetes mellitus". India faces a number of difficulties in managing diabetes, including a rising incidence of the condition in both urban and rural regions, a lack of public awareness of the disease, a lack of adequate medical facilities, a high cost of treatment, subpar glycemic control, and an increase in the frequency of diabetic complications. [1] Uncontrolled diabetes commonly produces hyper glycemia, or increased blood sugar, which over time seriously harms several bodily systems, including the neurons and blood vessels. [2]

Type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes resulting from endocrinopathies, steroid use, etc. are some of the different kinds of DM. Type 1

diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are the two primary subtypes of DM, and both are typically brought on by defects in insulin secretion (T1DM) and/or action (T2DM). T2DM is expected to affect middle-aged and older individuals who have chronic hyperglycemia as a result of poor lifestyle and nutritional choices, whereas T1DM is thought to manifest in children or teenagers. Since the pathophysiology of T1DM and T2DM is very diverse from one another, each type has a separate etiology, presentation, and course of treatment. [3]

The dried herb known as *Acalypha indica Linn* is a member of the *Euphorbiaceae* family. Acalypha indica is one of the largest and most diverse genera in the *Euphorbiaceae* family, having around 450 species. [4] Acalypha indica is one of the weed plants with significant medicinal benefits for use in human health applications. India, Sri Lanka, Thailand, and Pakistan are among frequent locations for it. The extracts from several plant components, including the leaves, roots, and stems, are used as medicines to treat a wide range of illnesses, including eye infections, respiratory issues, rheumatism, skin issues, and blood sugar levels. [5] The medicinal plant Acalypha indica thrives in moist, temperate, and tropical climates, mostly near the equator. [6]

II. MATERIALS AND METHODS

A. Collection of plant material:

Acalypha indica plant material was collected from local areas, washed with water, shade dried and ground to coarse powder and stored in an airtight container.

B. Extraction process:

20 gm of Acalypha indica powder was extracted with ethanol and petroleum ether separately and soaked for 7 days at room temperature and stirred occasionally and then filtered by using filter paper. The filtrate was collected in separate petri-dishes and was air dried at room temperature for 3 days. Phytochemical screening is performed using standard reagents.

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C. Phytochemical screening:

Standard protocols were used to conduct preliminary phytochemical screening of the plant extracts to determine the presence or absence of secondary metabolites like alkaloids, resins, fats and oils, phenolic compounds, flavonoids, saponins, tannins, and cardiac glycosides.^[7]

D. In vitro Anti-diabetic activity of Acalypha indica

➤ Anti-diabetic activity

Stock solution of A. indica was extracted with ethanol and petroleum ether using maceration, that solution is further treated with α -amylase solution for conducting *In-vitro* anti-diabetic activity.

$\triangleright \alpha$ - amylase preparation

1mg of α – amylase powder in 2mL of water.

> DNSA color reagent preparation

Prepare by dissolving 1gm of 3, 5-dinitro salicylic acid in 50ml reagent grade water, Add 30gms of sodium potassium tartarate tetrahydrate. Add 20ml of 2N NaOH.

$\triangleright \alpha$ - Amylase inhibition assay

The extract was mixed with α – amylase solution along with sodium phosphate buffer & NaCl. Mixture was incubated at room temperature at 10 mins and few mL of starch solution (1%) in 0.02M sodium buffer was added. Result mixture was incubated at room temperature for 10 mins and the rection was terminated using DNSA color reagent. At this time, test tubes were placed in water bath and cooled until room temperature was attained. Mixture was then diluted with deionized water and absorbance was determined at 540 nm.

% inhibition of
$$\alpha$$
 – amylase = $\frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$

III. RESULTS AND DISCUSSION

Phytochemical screening

The results of the screening of ethanol and petroleum ether extracts of *Acalypha indica* are shown in Table 1 & 2 respectively.

Table 1: Phytochemical screening results of ethanolic extract of Acalypha indica

S. No.	Active constituents	Identification test	Inference
1.	Tannins	Braymer's test	Presence
2.	Cardiac	Keller – killani's	Presence
	glycosides	test	
3.	Flavonoids	FeCl ₃ test	Presence

The results of phytochemical screening of ethanol extract of Acalypha indica by maceration method contains various phytochemicals like tannins, cardiac glycosides, flavonoids show their potential by lowering the blood sugar level.

Table 2: Phytochemical screening results of petroleum ether extract of Acalypha indica

S. No.	Active constituents	Identification test	Inference
1.	Tannins	Braymer's test	Presence
2.	Cardiac glycosides	Keller – killani's test	Presence
3.	Flavonoids	FeCl ₃ test	Presence
4.	Saponins	Foam test	Presence

The results of phytochemical screening of petroleum ether extract of Acalypha indica by maceration method contains various phytochemicals like tannins, cardiac glycosides, flavonoids, saponins show their potential by lowering the blood sugar level.

\triangleright Inhibition of α -amylase activity

Table 3: Inhibition activity of α-amylase activity

S. No	Sample	Concentration (mL)	Inhibition (%)
1.	Standard Acarbose	0.5	55.8
2.	Ethanol extract	0.5	34.37
3.	Petroleum ether	0.5	53.12
	extract		

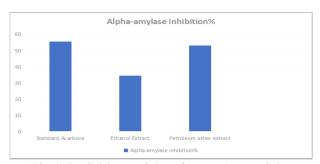


Fig 1: Inhibition activity of α -amylase activity

The results of the ethanol extract and pet ether extract of Acalypha indica plant showed potential activity as an anti-diabetic indicated by its inhibitory activity (%) based on the alpha amylase enzyme test. The potential for the anti-diabetic activity of acalypha indica plant and acarbose as a comparison showed different potentials where pet ether extract showed a stronger potential in inhibiting the enzymatic activity of alpha amylase compared to ethanol extract.

IV. CONCLUSION

The findings of this study showed that Acalypha indica aerial part extracts are highly concentrated in flavonoids, tannins, cardiac glycosides, and other phytochemicals. Through the use of various model systems and by evaluating the effectiveness of *Acalypha indica* against that of common medications, it's *In vitro* anti-diabetic potential has been validated.

The ethanol extract and petroleum ether extract of Acalypha indica plant showed potential activity as an anti-diabetic indicated by its inhibitory activity (%) based on the alpha amylase enzyme test. The potential for the anti-diabetic activity of Acalypha indica plant and acarbose as a comparison showed different potentials where petroleum ether extract (53.12%) showed a stronger potential in inhibiting the enzymatic activity of alpha amylase compared to ethanol extract (34.37%).

Overall, the present study results suggested that Acalypha indica ethanolic and petroleum ether leaf extract has potential Anti-diabetic activity. However, further preclinical studies have to be done along with in vivo studies for confirming the results.

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