VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 709-717 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

In Vitro Anti-Inflammatory and Anti-Diabetic Effects of Ethyl Acetate Extract of *Acalypha indica* Linn.

Jhansi Rani V.[1]*, Udayavani S.[2], Govindarajan R.[2], Vijetha P.[1], Ravichandra S.[1], Subbarao J.[1] and Yazdan S.K.[1]

[1]Department of Pharmaceutical Chemistry, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India
[2]Department of Pharmaceutical Chemistry, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India

**Corresponding Author*

Received: 21st February, 2024; **Accepted:** 21st June, 2024; **Published online:** 17th September, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.067

Abstract: *Acalypha indica* Linn. leaves (Euphorbiaceae) were extracted by successive solvent extraction with increasing solvent polarity, i.e. petroleum ether (0.9%), benzene (1.20%), chloroform (2.70%), ethyl acetate (4.4%), methanol (5.1%) and water (3.8%). Alkaloids were detected in ethyl acetate, chloroform, methanol and water fractions. Amino acids were found in ethyl acetate, methanol and water fractions, and flavones were found in ethyl acetate and methanol leaves extracts. The ethyl acetate extract was subjected to solvent-solvent separation using hexane (0.8%), benzene (1.43%), chloroform (1.68%), and methanol (2.12%). The ethyl acetate leaves extract was screened for various phytochemical constituents and estimated total flavonoid and alkaloid content has found to be 113.7 ± 2 mg/g and 76.4 mg/g, respectively. The ethyl acetate leaves extract was carried out for *in vitro* anti-inflammatory activity by Egg albumin denaturation method and anti-diabetic activity by alpha–amylase inhibition assay and the results showed significant anti-inflammatory activity at same concentration due to the presence of flavonoids and alkaloid contents and anti-diabetic activity was less significant when compared to standard drugs.

Keywords: *Acalypha indica*, Ethyl acetate, Alpha–amylase assay, Egg albumin denaturation method, Euphorbiaceae

Citation: Jhansi Rani V., Udayavani S., Govindarajan R., Vijetha P., Ravichandra S., Subbarao J. and Yazdan S.K.: *In vitro* anti-inflammatory and anti-diabetic effects of ethyl acetate extract of *Acalypha indica* Linn. Intern. J. Zool. Invest. 10(2): 709-717, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.067

Introduction

The fresh *Acalypha indica* (Euphorbiacae) plant has wide variety of nutrients such as carbohydrates, proteins, vitamins, and fat. This plant also contains mineral micronutrients. *Acalypha indica* contains high iron, and is also a good source of zinc, copper, nickel, and chromium for those who suffer from mineral shortages. This plant is good for hydrating the body because it has

a high moisture content of up to 80% and a total ash value of 16%. This low-cost, green vegetable can offer a decent nutritional balance. A significant phenolic content is also present in *Acalypha indica*, including geraniin and corilagin. Chebulagic acid and glucogallin were found to be effective antioxidants. Approximately 80% of people living in rural areas rely on herbal medicines for their primary medical needs. Dried leaves of *Acalypha indica* were prepared into a poultice to treat wounds and bedsores, according to traditional practices (Jagatheeswari *et al*., 2013) of the plant, which also claim to cure rheumatism, bronchitis, and asthma, among other illnesses. The root of *Acalypha indica* is used as a strong purgative, febrifuge, tonic, and astringent. With their laxative and anti-periodic qualities, leaves are used to treat eczema, ringworm, jaundice, ulcers, and skin outbreaks on the outside. The roots are used to treat blood dysentery, migraines, joint discomfort, and chest pain. An extract from the roots can reduce blood sugar levels by up to 30%. The herb's anti-inflammatory, anti-helminthic, antibacterial, antifungal, antioxidant, neuro-protective, anti-venom, and antiulcer activities are all additional qualities.

In the present study, *Acalypha indica* Linn. leaves were extracted by successive solvent extraction with increasing solvent polarity, i.e. petroleum ether (0.9%), benzene (1.20%), chloroform (2.70%), ethyl acetate (4.4%), methanol (5.1%) and water (3.8%). The ethyl acetate leaves extract was screened for various phytochemical constituents and assayed for *in vitro* anti-inflammatory activity by Egg albumin denaturation method and anti-diabetic activity by alpha–amylase inhibition.

Materials and Methods

Successive Solvent Extraction:

Successive solvent extraction uses a Soxhlet apparatus (Mohammed, 2018) for extraction. The siphon arm of Soxhlet apparatus was closed by glass wool or cotton to prevent the entry of particulate matter into the arm. The powdered material to be extracted was placed in the thimble. The boiling flask was attached to the thimble properly and was placed on the heating mantle. To the other side the condenser was attached and the whole assembly set properly. Sufficient quantity of the solvent (150 ml) was poured from the top on the plant material.

Method:

100 g of coarsely powdered leaves of *Acalypha indica* Linn. was added into thimble. Soxhlet apparatus was properly assembled. Petroleum ether (BP 60-80 °C) was first used for extraction. This solvent was run in the Soxhlet, until the plant powder further had no more solutes soluble in petroleum ether. The same process was repeated with benzene, chloroform, ethyl acetate, methanol and water. The contents of the boiling flask were collected separately after each solvent and were concentrated on HeidolphRotavac at 45°C under vacuum. The obtained extracts are shown in Figure 1. They were subjected to preliminary phytochemical tests. The per cent yield of different successive extracts are shown in Table 1 and phytoconstituents present in the different successive solvent extracts are shown in Table 2. Thin layer chromatography was used to do a preliminary screening of the extracts. (TLC) and phytochemical evaluation to know which compounds are present in the extracts. All solvent extracts were subjected to TLC using various mobile phases of solvent systems with varying polarity. Following this, plates were exposed to Iodine vapor for plate visualization, sprayed with a 10% H_2SO_4 in methanol solution, and heated for up to 5 min at 110°C in an oven. With the help of this reagent, the compounds were taken on different hues.

Methanolic Extract Fraction by the Solvent-Solvent Separation Method:

In a separating funnel, added the ethyl acetate extract and then stirred in the hexane solvent for 12 h. The precipitated layers and hexane fraction layer was separated after being set aside for 30 min. They are collected one by one in beakers. This process was repeated until the color of the ethyl

Table 1: Percentage yield of successive solvent extracts

S. No.	Extract	Percentage yield
1	Petroleum ether	0.9 %
2	Benzene	1.20%
3	Chloroform	2.70%
4	Ethyl acetate	4.4%
5	Methanol	5.1%
6	Water	3.8%

Table 2: Phytochemical evaluation for extracts obtained after Soxhalation of *Acalypha indica leaves*

S. No.	**Constituents**	**Extracts**					
		Petroleum ether	**Benzene**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Water**
1	**Alkaloids**	-	-	+	+	+	+
2	**Amino acids**	-	-	-	+	+	+
3	**Carbohydrades**	-	-	-	+	+	-
4	**Flavones**	+	-	-	+	+	-
5	**Glycosides**	-	-	-	-	-	-
6	**Phenols**	-	-		-	+	+
7	**Proteins**	-	-	-	-	-	-
8	**Saponins**	-	-	-	-	-	+
9	**Resins**	-	-	-	-	-	-
10	**Fixed oils and fats**	+	+	+	+	+	+
11	**Steroids**	-	-	-	-	-	-
12	**Tannins**	-	-	-	-	-	+
13	**Triterpenoids**	-	-	-	-	-	-

+ Detected; - Not Detected

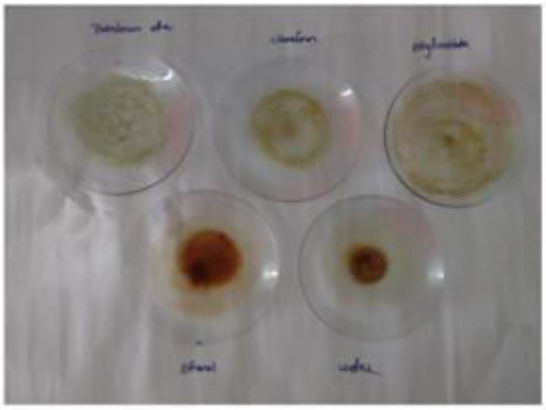

Fig. 1: Crude extracts of different solvents.

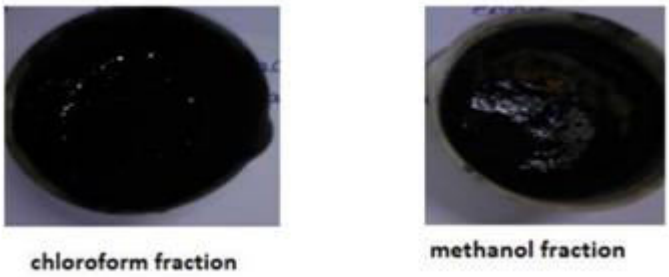

Fig. 2: Dried extracts of chloroform and methanol are fractionated from ethyl acetate extract.

Fig. 3: Methanolic fractions by TLC.

Table 3: The % yield of various fractions obtained from solvent-solvent separation

S. No.	Fraction	% yield
1	Hexane	0.8%
2	Benzene	1.43%
3	Chloroform	1.68%
4	Methanol	2.12%

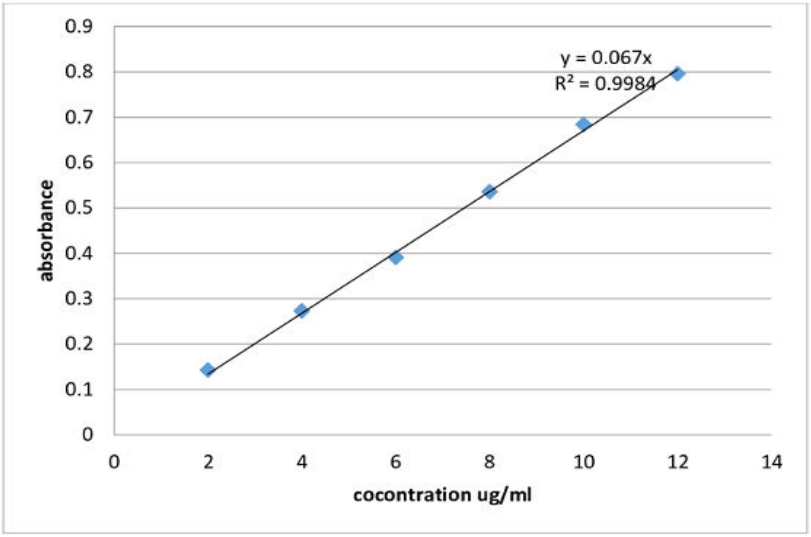

Fig. 4: Calibration curve of the estimated curve.

acetate layer lightens. Following the precipitate's addition to the separating funnel, benzene was stirred for 12 h before being set aside for 30 min. Precipitated layers and the benzene fraction layer split apart. These were gathered one at a time into beakers. Chloroform was stirred for 12 h and then left alone for 30 min. The precipitated layers and the chloroform fraction layer split apart. This process was continued until the precipitated methanol color lightens and was discarded. A rota vacuum evaporator was used to concentrate and dry the layers of methanol and chloroform and the extracts were shown in Figure 2 and percentage yields obtained were shown in Table 3.

Fractionated Compound by TLC:

Different fractions are collected (Sherma, 2000; Poole, 2003) from the solvent-solvent separation. Those fractions were subjected to thin layer chromatography. The mobile phase used benzene:ethyl acetate:methanol (6:3:1). The fraction showed 4 homogeneous spots. Those compounds were confirmed by the spectral data. The TLC plates are shown in Figure 3.

Determination of total Flavonoid Content:

The ethyl acetate extract was estimated for total flavonoid content (Jia *et al*., 1999) in it. Sodium nitrate 5%, Aluminium chloride 10%, 1M NaOH were used for colorimetric assay.

The principle of colorimetric assay used to determine total flavonoid content, lies in the fact that Aluminum chloride interact with the c-4 keto group and either c-3 or c-5 flavonoid forms acid stable complexes. Also, on complexation with the orthodihydroxyl groups in the A or B ring of Flavonoids, aluminum chloride forms acid liable complexes. The complexes so formed react with sodium nitrate in basic conditions to form a coloured product that can be measured spectrophotometrically at 510 nm. In addition to the addition of 150 L of sodium nitrite 5% solution, a total of 1 ml of plant extract was diluted with 200 L of distilled water. The mixture was incubated for 5 min, then 150 µl of aluminum chloride (10%) solution was added and kept in storage for 6 min. Then 2 ml of sodium hydroxide (1M) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and cooled to room temperature for 15 min. Absorbances were measured at 510 nm. The presence of flavonoids was detected by the appearance of pink colour. Using the standard curve (Fig. 4) and absorbance values (Table 3), the overall flavonoid content was calculated and expressed as rutin equivalent mg RE/g extract on dried weight basis.

Determination of total Alkaloid Content:

5 g of plant powder was taken in a separate 250 ml beaker, and 200 ml of methanol (Obadoni, and Ochuko, 2001) was added. The beaker was covered and let to stand for 4 h. This combination containing the solution was filtered, and a water bath was used to reduce the volume to one-quarter. Concentrated ammonium hydroxide was added to this sample drop by drop until the precipitation was fully formed. After letting the entire mixture settle, the precipitate was filtered out and weighed. This yielded the following proportion of total alkaloid content:

Total alkaloids as a percentage (%) = residue weight × 100 / sample weight

The computed outcomes are displayed in Table 4.

Anti-Diabetic Activity by Alpha-Amylase Inhibitory Activity:

For 10 min, 30 µl of plant extracts, phosphate buffer (as the control), or an Acrabose positive control were combined with 10 µl of pig pancreatic amylase solution (0.1 mg/ml) (Ademiluyi and Oboh, 2013; Konappa *et al*., 2020) and incubated at 37 °C. Following the addition of 40 µl starch solution to start the reaction, the 96-well plate was incubated for 30 min at 37 °C. Next, 20 µl of 1 M HCl and 75 µl of iodine reagent were added. The mixture's absorbance was measured at 580 nm; the results are displayed in Table 5 and Figure 4.

Egg Albumin Denaturation Method:

Table 4: Phytochemical evaluation of methanol and fractionated extracts of *Acalypha indica* leaf

S. No.	Constituents	Extracts	
		chloroform fraction	methonalic fraction
1	Alkaloids	+	+
2	Amino acids	-	+
3	Carbohydrades	+	-
4	Flavonids	+	+
5	Glycosides	+	-
6	Phenols	-	+
7	Proteins	-	+
8	Saponins	-	-
9	Resins	+	-
10	Fixed oils and fats	-	+
11	Steroids	+	-
12	Tannins	-	+
13	Triterpenoids	-	-

+ Detected; - Not Detected

Table 5: absorbance obtained by total flanovoid content estimation

S. No.	Concentration in µg/ml	Absorbance
1	2	0.143
2	4	0.273
3	6	0.391
4	8	0.536
5	10	0.684
6	12	0.796

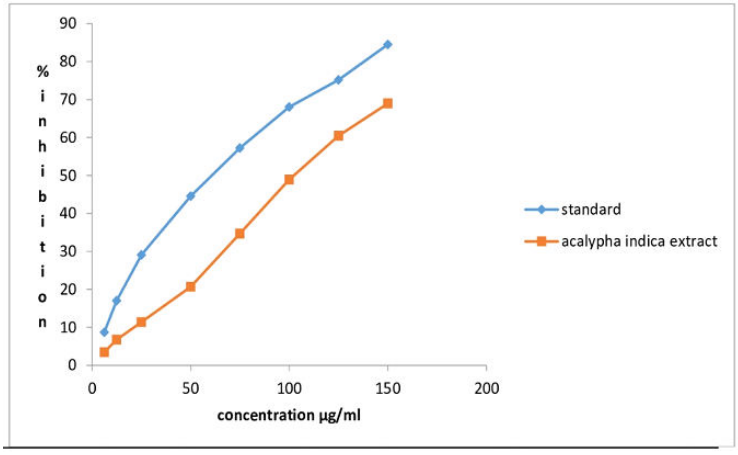

Fig. 5: Calibration curve for standard and *Acalypha indica* leaf anti diabetic activity.

Table 6: Evaluation of anti diabetic activity in ethyl acetate extract of *Acalypha indica* Linn.

S. No.	Concentration in µg/ml	Standard		*Acalypha indica* extract	
		Absorbance	% inhibition	Absorbance	% inhibition
1	6.25	0.868	8.72	0.918	3.47
2	12.5	0.789	16.99	0.887	6.73
3	25	0.675	29.04	0.843	11.36
4	50	0.527	44.56	0.754	20.72
5	75	0.407	57.22	0.621	34.70
6	100	0.304	68.04	0.486	48.90
7	125	0.236	75.19	0.376	60.46
8	150	0.147	84.51	0.295	68.98
IC_{50} Value		**71.82 µg/ml**		**106.38 µg/ml**	

Table 7: Evaluation of anti-inflammatory activity in ethyl acetate extract of *Acalypha indica* Linn.

S. No.	**Concentration in µg/ml**	**Standard**		***Acalypha indica* extract**	
		Absorbance	**% Protein denaturation inhibition**	**Absorbance**	**% Protein denaturation inhibition**
1	25	0.838	9.50	0.900	2.96
2	50	0.762	17.48	0.837	9.54
3	75	0.651	29.10	0.743	19.40
4	100	0.509	44.08	0.590	35.53
5	125	0.393	56.30	0.507	44.29
6	150	0.293	66.74	0.393	56.28
7	175	0.228	73.64	0.307	65.32
8	200	0.157	81.03	0.231	73.34
IC_{50} Value		**118.91µg/ml**		**140.00 µg/ml**	

5 ml of the test solution (Saranya *et al*., 2018) was added to 2.8 ml of PBS solution, 0.2 ml of fresh hen's egg albumin, and 2 ml of various extract strengths, resulting in final concentrations of 25, 50, 75, 100, 125, and 150 µg/ml. The control group utilized an identical volume of de-ionized distilled water. Subsequently, the solutions were heated to 70 °C for five minutes after being incubated for fifteen minutes at 37 ± 2 °C. Their absorbance was measured at 660 nm after cooling. Aspirin, or acetyl salicylic acid, was utilized as a standard and was handled in a similar way for absorbance measurements, with concentrations ranging from 25 to 200 µg/ml.

Results and Discussion

Successive solvent extraction was performed in a Soxhlet apparatus by using petroleum ether, benzene, chloroform, ethyl acetate, methanol and water. The obtained extracts were weighed and the percentage yield was calculated. The extracts were then subjected to preliminary phytochemical analysis and the phyto-constituents present in the different successive solvent extracts were determined. Preliminary phytochemical analysis of successive solvent extracts are given in Table 2. After soxhalation extracts from different polarities of solvents were subjected to phytochemical evaluation in order to find out the chemical constituents.

Solvent – Solvent Separations:

The ethyl acetate extract of *Acalypha indica* Linn. leaves were subjected to solvent-solvent

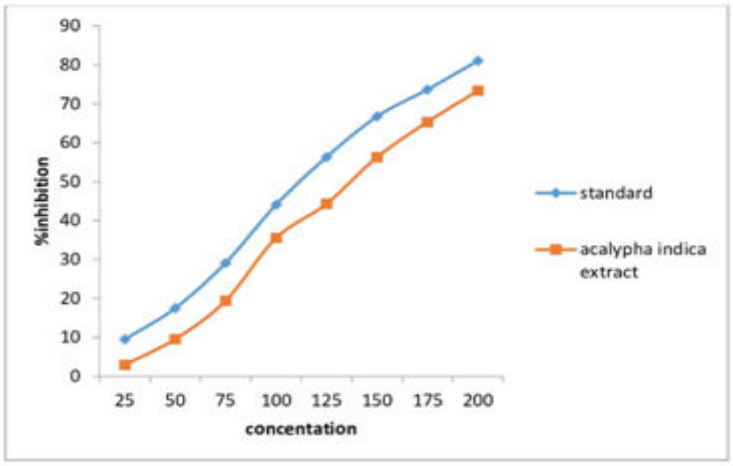

Fig. 6: Calibration curve for standard and *Acalypha indica* anti-inflammatory activity.

separation using hexane, benzene, chloroform, and methanol. The per cent yield were calculated and shown in Table 3. The phytochemical evaluation of the fraction was performed and results are shown in Table 4.

Estimation of total Flavonoid Content:

The ethyl acetate extract was estimated for its flavonoid content. The calibration curve was plotted by 2, 4, 6, 8, 10, and 12 µg/ml concentration of standard (rutin) flavonoid solutions. The calibration curve absorbance is shown in Table 5 and test solution was used for estimating the total flavonoid content in the ethyl acetate extraction. From the calibration curve, ethyl acetate extract was estimated to contain 113.7 ± 2 flavonoids.

Estimation of Total Alkaloid Content:

Total alkaloids as a percentage (%) = residue weight × 100 / sample weight

Weight of residue is 3.82 mg and amount estimated in mg/g is 76.4

Evaluation of anti-diabetic activity:

Anti diabetic activity was observed in the following concentrations 6.25, 12.5, 25, 50, 75, 100, 125, 150 µg/ml. In that extension the following test solution and standard showed less significant when compared with control. The anti diabetic activity exerted by ethyl acetate extract of *Acalypha indica* Linn. was also found to be dose dependent and the results obtained are given in the Table 6.

Evaluation of Anti Inflammatory Activity:

Anti-inflammatory activity was observed at concentrations 25, 50, 75, 100, 125, 150, 175, 200 µg/ml. In that extension phase the test solution and standard showed equal significant activity when compared with control as shown in Figure 6. The anti-inflammatory activity exerted by ethyl acetate extract of *Acalypha indica* Linn. is shown in Table 7.

Conclusion

Acalypha indica Linn. leaves were extracted and separated by appropriate methods. The ethyl acetate leaves extract has screened for various phytochemical constituents and estimated total flanovoid and alkaloid content was found to be 113.7 ± 2 mg/g and 76.4 mg/g, respectively. The ethyl acetate leaf extract was assayed for *in vitro* anti-inflammatory and anti diabetic activities and the results showed less significant activity when compared to standard drugs due to the presence of flavonoids and alkaloid contents.

Acknowledgements

The authors wish to thank the authorities at Hindu College of Pharmacy, Guntur for constant support

to complete this work.

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