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QUALITATIVE PHYTOCHEMICAL SCREENING AND IN–VITRO EVALUATION OF ANTI-HELMINTHIC ACTIVITY USING HYDROALCOHOLIC EXTRACT AND AQUEOUS EXTRACT OF ACALYPHA INDICA LEAVES

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ABSTRACT

The hydroalcholic extract and aqueous extract of Acalypha indica is used as antihelminths. An antihelmintic (also called antihelmintic) is a drug or substance used to expel or destroy parasitic worms (helminths) from the body, typically by either stunning or killing them without causing significant harm to the host. These drugs are commonly used in both human and veterinary medicine to treat infections caused by roundworms, tapeworms, and flukes.The present study screening of bioactive constituents from Acalypha indica utilizing an array of hydroalcholic extract and aqueous extract alongside an evaluation of their anthelmintic efficacy. Qualitative and the presence of different bioactive components such as alkaloids, flavonoids, tannins, saponins, and glycosides, indicative of the plant's diverse metabolic collections. The anthelmintic potential of the extracts was rigorously assessed employing Pheretima posthuma as a biological model, with results demonstrating both extracts at different concentrations of extract, along with compare standard antihelmintic drug praziquantel as evidenced by significant paralysis and mortality rates. This investigation provides a foundation for the isolation and structural elucidation of the bioactive molecules, potentially advancing novel therapeutic agents.

KEYWORDS: Acalypha indica, Phytochemical investigation, Antihelmintic activity, hydroalcholic extract, Pheritima posthuma.

INTRODUCTION

Anthelmintic activity refers to the ability of substances to eliminate or inhibit parasitic worms (helminths), which cause significant health problems, particularly in tropical and subtropical regions. Helminth infections contribute to malnutrition, stunted growth, and economic losses in humans and animals, making anthelmintic drugs essential for public health and veterinary medicine.

Classification of Helminths

- **Nematodes (Roundworms)** – e.g., Ascaris lumbricoides, Trichuris trichiura.
- **Cestodes (Tapeworms**) – e.g., Taenia solium, Echinococcus.
- **Trematodes (Flukes**) – e.g., Schistosoma, Fasciola hepatica.

Types & Mechanism of Anthelmintic Drugs

- Broad-spectrum anthelmintics – Effective against various helminths (e.g., albendazole, ivermectin).
- Narrow-spectrum anthelmintics – Target specific helminths (e.g., praziquantel for trematodes/cestodes).

Mechanisms of Action

1. Neuromuscular Disruption – Causes worm paralysis (e.g., levamisole, pyrantel).
2. Energy Metabolism Inhibition – Disrupts glucose uptake (e.g., albendazole, mebendazole).
3. Membrane Integrity Disruption – Alters calcium permeability, leading to parasite death (e.g., praziquantel).

Plant profile (Acalypha indica)

Acalypha indica (Indian Nettle) – A Natural Anthelmintic

Fig 1.

Taxonomy and Botanical Description

Taxonomy.

1. Kingdom: Plantae
2. Phylum: Tracheophyta
3. Class: Magnoliopsida
4. Order: Malpighiales
5. Family: Euphorbiaceae
6. Genus: Acalypha
7. Species: Acalypha indica

A medicinal plant from the Euphorbiaceae family, used traditionally for various ailments, including deworming.

Phytochemicals & Activities

Drug profile: Acalypha indica leaves extract

Flindersine

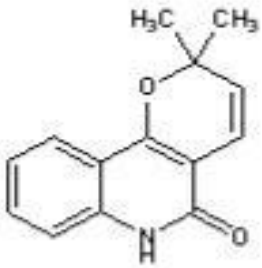

Fig .2.

Active constituent: Flindersine

Additional Names: 2,2 dimethyl-a-pyrano(5¢,6¢,3,4)-2(1H)-quinolone

Molecular Formula: C14H13NO2

Molecular Weight: 227.26

Percent Composition: C 73.99%, H 5.77%, N 6.16%, O 14.08%

Other phytochemicals: Alkaloids, flavonoids, tannins, saponins, terpenoids, sterols.

Pharmacological Properties:

- Anthelmintic,
- Antimicrobial,

- Anti-inflammatory,
- Antioxidant,
- Anticancer,
- Respiratory benefits.

Drug Profile: Praziquantel (Standard Anthelmintic Drug)

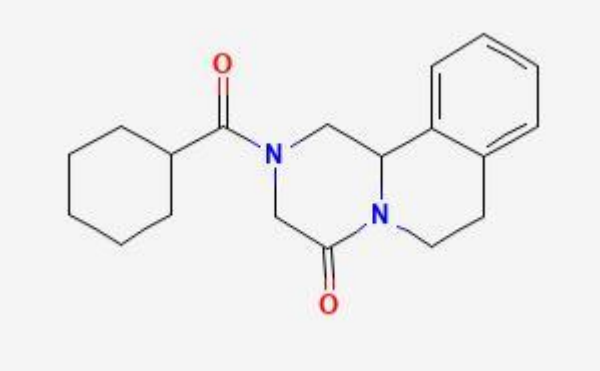

Fig 3: Praziquantel.

General Information

Generic Name: Praziquantel

Brand Names: Biltricide, Distocide, Prazitel, Praziquantel Tablets

Drug Class: Anthelmintic (Anti-parasitic)

Molecular Information

IUPAC Name: 2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one.

Molecular Formula: $C_{19}H_{24}N_2O_2$

Molecular Weight: 312.41 g/mol

Chemical Classification: Isoquinoline derivative

Structural Formula

The chemical structure of praziquantel consists of.

- Pyrazinoisoquinoline core
- Cyclohexanecarbonyl substituent
- Lactam (cyclic amide) functional group

Mechanism of Action of Praziquantel: Praziquantel is an anthelmintic drug primarily used to treat infections caused by schistosomes (blood flukes) and a variety of cestodes

(tapeworms). It acts by disrupting the parasite's membrane integrity, inducing muscle paralysis, and leading to worm death.

Increases Calcium Permeability – Causes calcium influx, leading to muscle contraction and paralysis.

Damages the Tegument – Weakens the parasite's protective covering, exposing it to immune attacks.

Paralysis and Dislodgement – Prevents the parasite from attaching to host tissues, leading to expulsion.

Disrupts Energy Metabolism – Interferes with glucose metabolism and ATP production, causing energy depletion.

Enhances Immune Response – Exposes hidden antigens, triggering immune system attacks for parasite clearance.

Uses: Treats schistosomiasis, liver flukes, and tapeworm infections.

Pharmacokinetics: Rapidly absorbed, metabolized by the liver, and excreted in urine.

Side Effects: Nausea, dizziness, abdominal pain.

Drug Interactions: Affected by CYP3A4 inducers (Rifampin, carbamazepine) and inhibitors (cimetidine, ketoconazole).

Both synthetic (praziquantel) and natural (Acalypha indica) anthelmintics play crucial roles in treating helminth infections. Because of both having similar mechanism of action.WHO emphasizes integrated control strategies, including mass drug administration and improved sanitation, to reduce the global burden of helminthiasis.

MATERIALS AND METHODS

Collection of plant

The entire plant leaves of Acalypha indica were collected from road sides of our campus and surroundings of (Sri Krishnadevaraya university).

Plant authentication

After collection of plant leaves of Acalypha indica,typically involves a systematic approach to confirm its identity and ensure it matches the intended species.So that we approach Department of botany in (SK university) to confirm the taxonomical identities to the plant.

Chemicals and reagents

All the chemicals and reagent used for our entire experiment work are procured from our college lab (SK university college of pharmaceutical sciences).

Selection of earthworms

The adult earth worms of Pheretima posthuma was used to carry out anthelmintic evaluation. The worms are collected from moist soil near surroundings of Akuthotapalli village.Then the worms are washed with saline water to remove the feacal matter. The worms about 8cm length and 0.5 to 0.7 cm width was selected for the experiment. Finally the worms used for evaluation of anti helmentic activity.

Preparation of plant extract

Whole plant extraction process is done by "Maceration process ".

Materials Required

1. Dried and powdered plant material (e.g., Acalypha indica leaves)
2. Solvent (e.g.Distilled water and hydroalcoholic mixture)
3. Glass rod
4. Petridish & Petriplate
5. Filter paper or muslin cloth
6. Funnel
7. Beakers or volumetric flasks
8. Earthworms
9. Standard drug (Praziquantel)

Procedure

Maceration is a simple and widely used method for extracting bioactive compounds from plant material by soaking it in a solvent.

Here's the step-by-step procedure.

- The fresh whole plants were rinsed two times in tap water and finally with distilled water in order to remove debris.
- Then leaf, stem, flower and root parts were separated with fine knife. All these parts were air-dried in the laboratory for 5 days at 37ºC.

- Air dried samples were than pulverized by an electric blender into a fine powder.Whole plant extraction process is done by "Maceration process "
- Take 250 ml Erlenmeyer flasks are taken and add Acalypha indica fine powder into the flasks.
- Then individual solvents are dissolve in each Erlenmeyer flasks.Here powder and solvent ratio is taken as 1:10 w/v %.
- Prepare extract by using hydroalcholic solvent mixture ratio(70 : 30)70ml propanol and 30ml water. Instead of hydroalcholic solvent mixture.Prepare aqueous extract by using 100ml (distilled water).
- Then dissolve it properly by using glass rod. Cover the aluminium foil to the flasks containing sample to avoid evaporation of solvent.
- Then keep it for 3 days with frequently stirring for every 24 hours.
- Then sample is filtered by using buchner funnel along with whatmanns filter paper.
- Collect the extract and store in beaker.
- Prepare the dilutions by using stock sample extract.
- Dilutions are prepared 1mg/ml, 2mg/ml ,3mg/ml, 4mg/ml, 5mg/ml.

Phytochemical investigation

Qualitative determination of alkaloids, flavonoids, glycosides, saponins, tannins, steroids, phenols, and proteins from leaves of Acalypha indica were screened using standard.

Phytochemical screening procedures

Identification tests for alkaloids

1.Dragendorff's test

- Add 1 ml of an extract to a test tube
- Carefully add a few drops of Dragendorff's reagent to the test tube .
- Observe for the formation of a reddish-brown precipitate, which indicates the presence of alkaloids.

2 Hagers test

- Take few drops of Hager's reagent (saturated picric acid solution) added to 2 ml of the respective plant extract.
- Bright yellow precipitate formation indicated the existence of alkaloids.

Identification tests for flavonoids

1. Shinoda test

- 1ml of each extract was added to a little of magnesium and a few drops of concentration chlorohydride acid were carefully along the walls of the tube.
- Appearance of red colour indicates the presence of flavonoids.

2. Lead acetate test

- Add a few drops of 10% lead acetate solution to 1 mL of plant extract.
- It is appeared as yellow precipitate indicates presence of flavonoids.

Identification tests for tannins

1. Fecl3 test:

- Take 0.5 ml of each extract was mixed with 1 ml of distilled water then treated with few drops of FeCl3.
- Formation of green precipitate indicates the presence of tannins.

Identification tests for glycosides

1.Liberman burchard test.

- Mix a crude extract with a few drops of acetic anhydride.
- Boil the mixture and then cool it .Add concentrated sulphuric acid along the sides of the test tube.
- Observe for formation of a brown ring at the junction of the two layers.

2. Coumarin test

- Add 1 mL of distilled water and NaOH to 0.5 mL of crude extract.
- A yellowish colour indicates the presence of glycosides.

Identification test for cardiac glycosides

1. Legal test:

- A chemical test that involves mixing 2 mL of extract with a solution of 0.5 ml of glacial acetic acid and 2-3 drops of ferric chloride.
- Then, 1 mL of concentrated H2SO4 is added along the walls of the test tube.
- The presence of cardiac glycosides is indicated by a deep blue colour at the junction of the two liquids.

Identification test for resins

1. Fecl3 test

- Dissolve a small amount of the resin sample in a suitable solvent (like Ethanol or water).
- Add a few drops of a dilute ferric chloride solution.A colour change usually to blue, green, purple, or red.

Identification test or proteins

1. Biuret test:

- Add 1–2 mL of the test solution to a clean, dry test tube.
- Add 1–2 mL of Biuret reagent to the test tube.
- Shake the solution.observe colour change.

Identification of carbohydrates

1.Benedict's test

- Add 1 millilitres of your sample solution to a clean test tube.
- Add 2 milliliters of Benedict's reagent to the sample.Heat the test tube in a boiling water bath or directly over a flame for 3–5 minutes.
- observe colour change.

Evaluation of Antihelmintic activity

Anthelmintic activity was carried on adult earthworm (Pheretima posthuma) of equal size. The appropriate dilutions of standard and sample solutions are prepared.The dilutions are done by using Acalypha indica leaves extract and antihelmintic activity was tested.11 petridish along with lid was taken and kept in hot air oven to sterilize them. Then place adult earthworms in each 11petridishes. Take 10ml of hydroalcholic solvent extract in different dilutions such as (1mg/ml,2 mg/ml,3mg/ml,4mg/ml,5mg/ml)pour them in each petridishes Simultaneously take another sets of petridishes for evaluate activity by using aqueous extract(1mg/ml,2mg/ml,3mg/ml,4mg/ml,5mg/ml)and standard drug solution. Allow the earthworm.And observe paralysis and death. Note paralysis and death time in (min) of each dilution.

RESULT

The phytochemical screening of Acalypha indica leaf extract was show the presence of various active constituents, it is showed in table. The phytoconstituents present in the leaf extract include saponins, terpenoids, flavonoids, cardiac glycosides, tannins and steroids.

Table 1: Qualitative phytochemical screening of Acalypha indica leaf extract.

S.NO	TESTS	RESULT
1.	Alkaloids	Positive
2.	Tannis	Positive
3.	Flavonoids	Positive
4.	Glycosides	Positive
5.	Resins	Positive
6.	Cardiac glycosides	Positive
7.	Carbohydrates	Positive
8.	Proteins	Positive

Table 2: Antihelmintic activity of Acalypha indica leaves extract.

S.No	Type of plant extract	Concentration of extract (mg/ml)	Time of paralysis (min)	Time of death (min)
1.	Hydro alcoholic	01	75.2±1	77 .0±1
2.	Hydro alcoholic	02	57.2±1	59.5±1
3.	Hydro alcoholic	03	46.1±1	47.0±1
4.	Hydro alcoholic	04	26.3±1	28.6±1
5.	Hydro alcoholic	05	17.6±1	19.2±1
1.	Aqueous	01	82.0±1	84.0±1
2.	Aqueous	02	56.0±1	58.0±1
3.	Aqueous	03	44.3±1	46.2±1
4.	Aqueous	04	36.6±1	38.0±1
5.	Aqueous	05	31.2±1	33.6±1
1.	Praziquantel (standard drug)	05	14.3±1	15.6±1

The worms paralysis time was noted included when it was lost their motility and fading away of their body colours, finally the death time was noted.

Whole process of Acalypha indica leaves extraction








Whole process of In – vitro evaluation of Antihelmintic activity

CONCLUSION

The anthelmintic activity of Acalypha indica using hydroalcoholic extract and aqueous extract against Pheretima posthuma showed that both extracts caused paralysis and death of the worms within a specific time period. The time required for paralysis and death varied for

each extract, indicating differences in their potency and mode of action. The plant contains bioactive compounds with anthelmintic properties, which interfere with the neuromuscular activity of Pheretima posthuma, leading to paralysis and eventual mortality. The observed activity may be due to the presence of phytochemicals such as alkaloids, flavonoids, tannins, and saponins, which are known to have anthelmintic effects. The standard drug praziquantel was used as a reference. It indicate that hydro alcoholic extract and aqueous extracts exhibited anthelmintic activity, leading to immobilization and mortality of the worms.

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