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EVALUATION OF IN-VITRO ANTIHELMINTIC AND ANTIFUNGAL ACTIVITIES OF THE BARK EXTRACT OF THEVETIA PERUVIANA

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ABSTRACT

Introduction: The rising incidence of resistance to traditional antihelmintic and antifungal medications highlights the critical necessity to investigate natural plant-derived alternatives. *Thevetia Peruviana* commonly called as yellow oleander, it has lots of bioactive compounds i.e. triterpenoids, cardiac glycosides that have therapeutic effects.

Objectives: The study focus in the evaluation of anthelmintic and antifungal effects of *Thevetia Peruviana* by using acetonic bark extract and evaluate its potential by comparing it with standard agents.

Methods: *Bark of Thevetia Peruviana* was collected and extracted via soxhlet extractor using acetone. The anthelmintic activity was evaluate using *Eisenia Fetida* earthworm, by monitoring death and paralysis time at different conc. (20-80mg/ml) compared with the std. Albendazole (20mg/ml). Antifungal activity was assessed by agar well diffusion method using yeast strains. Zone of inhibition was recorded across concentrations of 100-500µg/ml.

Conclusions: The acetonic extract of thevetia peruviana shows dose dependent anthelmintic effect. The extract shows death and paralysis at 52 and 24 minutes, at 80mg/ml as compare to 46 and 34 minutes of albendazole respectively. The antifungal activity rises with concentration, at 500µg/ml maximum inhibition zone observed (26mm). The MIC shows effective antifungal activity at 125µg/ml.

KEYWORDS: Thevetia peruviana, antifungal, anthelmintics, Soxhlet, extraction, earth worms.

INTRODUCTION

Approximately 75–80% of the world's population still uses plant-based medications for basic medical treatment, primarily in developing nations. Due to the belief that medications made from plants have no negative side effects, they are less expensive, and they may be conveniently produced locally. According to the World Health Organization, the use of herbal medications is rising by two to three times worldwide.

Herbal preparations, herbal materials, final herbal products, and herbs containing primary active ingredients originating from various plant parts, including flowers, leaves, roots, stems, and seeds, are all considered herbal medicines by the World Health Organization [1].

The fruit is extensive black/red, like the fortunate nut of China, and the bloom is yellow with a structure resembling a trumphet. The plant's green stem is gradually becoming grey as it ages. The plant mostly grows in shadowy locations and areas with a well-maintained water supply [2]. Despite having a variety of pharmacological actions and being used as a medicinal plant, Thevetia peruviana is still not receiving enough attention. Thevetia Peruviana, popularly known as yellow oleander, is a member of the Apocynaceae family [3].

Thevetia peruviana belongs to the Apocynaceae family; it is also referred to by several common names, including fortunate nut and yellow oleander. This plant is mostly found in South America, Central America, and Mexico, although it is currently spreading across tropical and semi-tropical parts of the world. The plant is a lovely, evergreen tree that grows to a height of 11 to 16 feet. [2,4].

Common name: Lucky nut, yellow oleander

Habitat: beside roads used as soil gripper, gardens

Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Thevetia

Species: peruviana [5].

MATERIAL AND METHODS

Plant Collection and Authentication

Thevetia peruviana bark extract was gathered in December 2023, at 27 °C and 75% relative humidity, next to Jaipur National University in Jagatpura, Jaipur. A specimen of Thevetia Peruviana was deposited in the herbarium of Department of Botany, University of Rajasthan, Jaipur (voucher- RUBL21696).

Plant Extraction

Bark was collected from the plant and shade-dried at 25°C. The dried bark was crushed and ground into a powder. Plant material that had been shade-dried was extracted using 400 milliliters of acetone in a "Soxhlet Extraction Apparatus (Fig. 1)" [6]. Following extraction, the extracts were allowed to cool at room temperature before being filtered and sometimes evaporated to achieve concentration or dryness. The result was a semi-solid, dark greenish extract that was refrigerated for later use.Thevetia Peruviana acetonic extract is used as a test sample medication to evaluate its antifungal and antihelmintic properties [7].

Thevetia Peruviana bark extract made with acetone was more effective than extracts made using other solvents. Thus, the Thevetia Peruviana plant was analyzed using acetonic bark extract for the biochemical assessment in the lab.

Fig: 1. Soxhlet Apparatus.

Worms Description

Due of their morphological and physiological resemblance to human intestinal roundworm parasites, adult earthworms (Eisenia fetida) were employed [8]. Earthworms have been widely employed for the primary evaluation of anthelmintic medications due to their accessibility. There are other names for red worms, including red wigglers, dung worms, tiger worms, composting worms, and trout worms. Farm land was the source of the earth worms.

Species: Eisenia fetida

Diet type: Vegetarian

Test-microorganism

For the investigation, the normal strain of yeast was used. The stock culture was kept at 4°C while the organism was cultivated on Sabouraud's Dextrose Agar at 37°C for 48 hours.

Antihelmintic Assay

The anthelmintic test was carried out with little modification in accordance with the protocols of Agrahari A. K. et al. (2011) and Moghal, Md. et al. (2013). Adult-grown earthworms were used for the in vitro experiment. (*Eisenia fetida*) [9,10].

Eisenia fetida (earth worms) of almost identical size (6±1 cm) were used to test the antihelmintic efficacy of Thevetia Peruviana bark extract.

Before being evaluated, worms were modified to fit the laboratory setting. The earthworms were separated into six groups, each of which contained three to four earthworms in petri dishes with the extracted solutions and reference medications (Fig. 2 & 3.a, b, c, d), as described below:

Group 1: As a control, they were given regular saline

Group 2: Standard albendazole suspension (20 mg/ml) was administered at a dosage of 200 mg/10 ml.

Group 3: 200 mg/10 ml (20 mg/ml) of acetonic extract was administered.

Group 4: 400 mg/10 ml (40 mg/ml) of acetonic extract was administered.

Group 5: Acetonic extract was given at a dosage of 600 mg/10 ml (60 mg/ml).

Group 6: 800 mg/10 ml (80 mg/ml) of acetonic extract was administered.

Petri dishes were stored in a typical room. The time it took for each worm to reach paralysis (PT) and death (DT) was tracked while the worms were under careful observation. Every worm was routinely examined using an external prod, which, if the earthworm is alive, stimulates and prompts movement.

It was believed that paralysis had occurred, and the worms were deemed dead when they stopped moving and concurrently lost their body color, even if none of them survived in regular saline. The immobile worms were moved to a temperature of 40º C to ensure they were dead [11].

Fig: 2. Albendazole 20mg/ml

Fig: 3.a. Test Sample: 20mg/ml

Fig: b. Test Sample: 40mg/ml

Fig: c. Test Sample: 80mg/ml

Fig: d. Test Sample: 60mg/ml

ANTI-FUNGAL ACTIVITY

Test-microorganism

In this investigation, the normal strain of yeast was employed. The organism was cultured on Sabouraud's Dextrose Agar at 37º C for 48 hours, and the stock culture was kept at 4º C.

Preparation of Sabouraud's Dextrose Agar Media (SDA)

Both pathogenic and non-pathogenic yeasts and fungi can be cultivated, isolated, and maintained on Sabouraud's Dextrose Agar (SDA). Sabouraud created SDA in 1892 to cultivate dermatophytes. The pH is kept close to 5.6 in order to promote the development of fungus [12].

Composition of Sabouraud's Dextrose Agar Media

i. Dextrose (Glucose) - 40 gm/L
ii. Peptone - 10 gm/L
iii. Agar - 15 gm/L
iv. Distilled Water - 1000 ml

- Combine all ingredients in ~900 ml of distilled water.
- Adjust the pH 5.6 with HCL and make up the volume to 1L.
- Heat to boiling to dissolve the medium completely.
- Autoclave the media at 121º C for 15 min.
- Cool the media to ~45 to 50º C and transfer into petri dishes or tubes for slants.

Screening for Antifungal activity

Cup plate diffusion method

The antifungal activity of Thevetia peruviana bark extract was evaluated using the cup plate diffusion technique. Pipette out 0.1 ml of the suitable fungal inoculum dilution and spread it over the entire agar plate surface to inoculate it. Using a sterile spreader, evenly distribute the inoculum throughout the agar plate's surface. Next, using a sterile cork borer, create an aseptic well-like hole with a diameter of around 6 to 8 mm. Fill the well with the extract solution or antifungal agent at a volume (20–100 ml) of the appropriate concentration. The agar plates are next incubated in the necessary conditions based on the test microorganism. The studied fungal strain's development is inhibited or slowed down by the antifungal drugs' appropriate diffusion in the agar medium (13).

Antifungal Assay

Petri plates were filled with 18 milliliters of Sabouraud's dextrose agar for yeast. To achieve a suitable confluent growth, 100 microliters of inoculum were dispersed on the designated media plates. Wells or cups with a diameter of 8 mm were created. The cups of the infected agar plates were filled with 100 microliters of extract, allowed to stand for 10 minutes for adequate diffusion, and then incubated at 37º C for 24 to 48 hours. To assure uniformity, the experiment was conducted three or four times. Zone of inhibition was used to assess the antifungal activity of the agar plates following incubation. Vernier calipers were used to measure the zone of inhibition, which was expressed in millimeters (14–16).

RESULTS AND DISCUSSION

Antihelmintic activity-

Earth heats were used to test the anthelmintic properties of Thevetia Peruviana bark extract. The study employed acetonic extracts at various concentrations. Table 1 shows how long it took for earthworms to become paralyzed and die. The data analysis reveals that the acetonic extract at concentrations of 20 mg, 40 mg, 60 mg, and 80 mg/ml caused paralysis and death in 56, 43, 31, 24, and 69, 54, 42, and 52 minutes. correspondingly. In normal saline, there was no paralysis or death and the impact grew with concentration. Fig. 4-A, B demonstrates that albendazole suspension at a concentration of 20 mg/ml caused paralysis and death in 34 and 46 minutes, respectively. At every tested dosage concentration, the extract paralyzed the worms before killing them. Thevetia peruviana's acetonic extract was shown to be a more effective medication

Table 1: Antihelmintic activity of *Thevetia Peruviana* bark extract in earth worms.

Treatment	Concentration (mg/ml)	Time taken for Paralysis (min)	Time taken for Death (min)
Normal Saline	-	-	-
Albendazole Suspension	20mg/ml	34	46
Test (Acetonic Extract)	20mg/ml	56	69
	40mg/ml	43	54
	60mg/ml	31	42
	80mg/ml	24	35

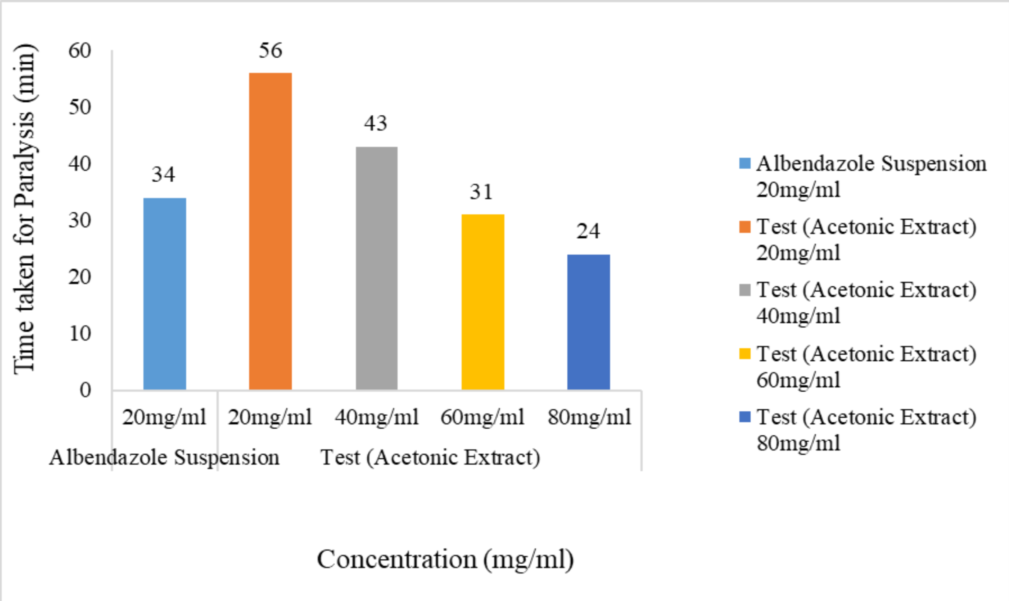

Fig. 4-A: Graphical representation of antihelmintic activity of *Thevetia Peruviana* acetonic extract compared to standard drug (Paralysis).

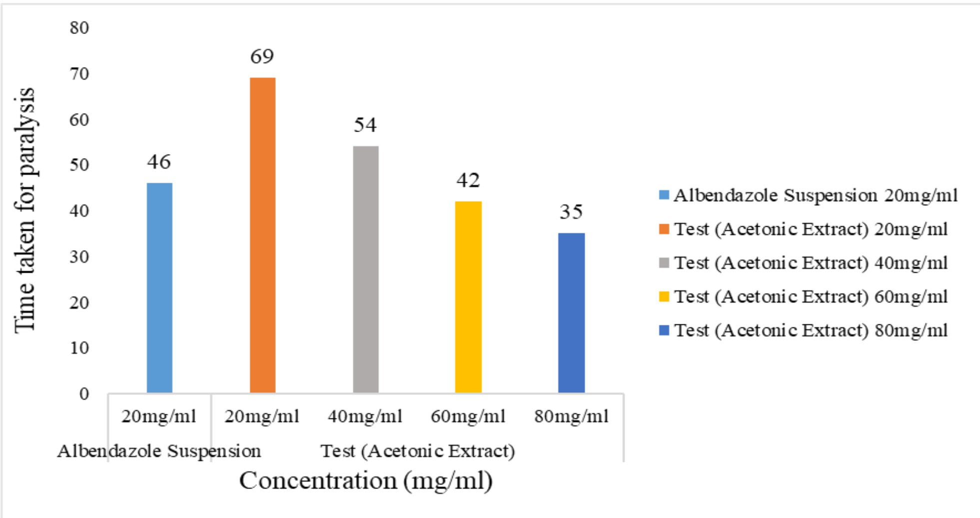

Fig. 4-B: Graphical representation of antihelmintic activity of *Thevetia Peruviana* acetonic extract compared to standard drug (Death).

Antifungal activity-

According to the antifungal activity data, the antifungal drug exhibited outstanding effectiveness against the tested yeast, with zones of inhibition growing as concentrations increased. There was no zone of inhibition in the control sample (Table 2), the biggest zone of inhibition (26 mm) was found at 500 µg/mL, and the lowest inhibition was found at concentrations of 100 µg/mL and below.

Zone of inhibition measurements

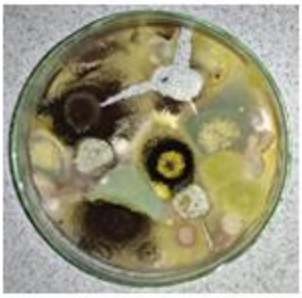

Fig: 5-A. 500, 400, 300 µg/ml

Fig: 5-B. 200, 100, 0 µg/ml

Table 2: Zone of Inhibition.

Antifungal Conc. (µg/ml)	Zone of Inhibition (mm)
Control	0
100	3
200	8
300	14
400	20
500	26

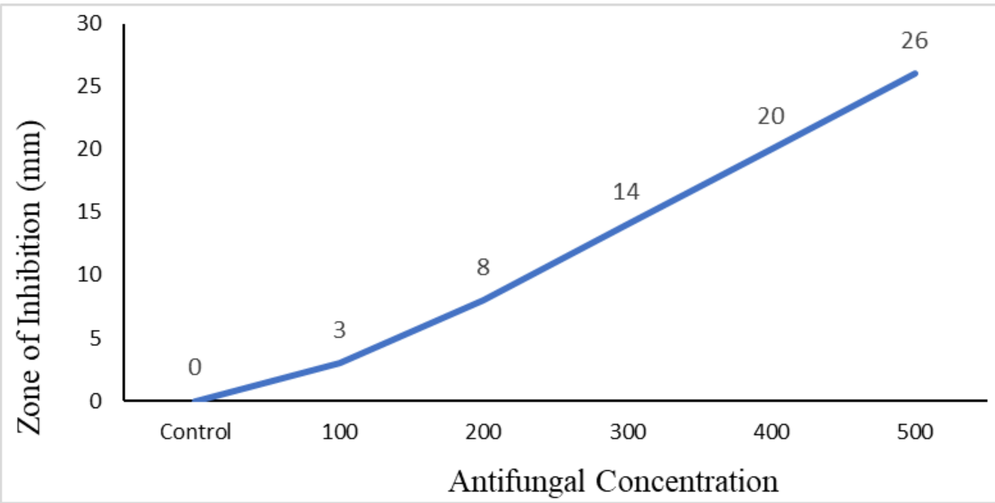

Fig. 6: Graphical representation for antifungal concentration against the zone of inhibition to visualize the relationship between concentration and antifungal activity.

Determination of Minimum Inhibitory Concentration (MIC):

The MIC is the lower concentration able to inhibit the growth. MIC was determined by broth dilution (using test tube). The tubes of broth medium, having different Conc. of test compound inoculated with the test organisms. After incubation, growth will occur in those test tubes (table-3) (14).

Table 3: Minimum Inhibitory Concentration

Test Tube	Antifungal Conc. (µg/ml)	Fungal Growth
1	1000	No
2	500	No
3	250	No
4	125	No
5	62.5	Yes
6	31.25	Yes
7	0	(Control)

Table 3 displays the minimal inhibitory concentration determination. The absence of fungal growth in test tubes 1 through 4 indicates that the antifungal drug is effective at these doses. Fungal growth is evident in test tubes 5 and lower, suggesting that these concentrations are insufficient to prevent fungal development.

Consequently, the minimum inhibitory concentration (MIC) is the concentration at which no growth is seen. The MIC in this instance would be 125 µg/ml. These results align with the antifungal properties of plant extracts documented in earlier research.

(Sharma & Tripathi, 2008; Patel *et al.,* 2010).

CONCLUSION

The study provides compelling evidence of Thevetia peruviana acetonic extracts' anthelmintic and antifungal properties. When compared to conventional medications, the extracts exhibit considerable anthelmintic activity, which makes them a viable option for the development of herbal anthelmintic medicines. Additionally, Thevetia peruviana may be a useful source of antifungal chemicals due to its strong antifungal action against yeast. However, further investigation is required to identify the active ingredients and create appropriate therapeutic dosages due to the recognized toxicity of Thevetia peruviana, particularly its cardiac glycosides. To fully alter Thevetia peruviana's medicinal potential while lowering its hazards, future research should concentrate on in-vivo evaluations, toxicological analyses, and the identification of synergistic effects with contemporary medications.

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