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Original Research Article

A pharmacological investigation for therapeutic potential of Callistemon citrinus as an anthelmintic agent (Bottle-Brush Plant)

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A B S T R A C T

The growing resistance of parasitic helminths to standard anthelmintic medications has sparked increased interest in exploring alternative treatments, particularly those derived from herbal sources. This research focuses on the anthelmintic properties of selected herbal plants, assessing their ability to inhibit and eliminate parasitic worms. Standard extraction techniques were employed to prepare various plant extracts, which were then tested using both in vitro and in vivo assays to determine their effectiveness. The findings revealed that several plant extracts showed significant anthelmintic effects, comparable to those of conventional medications. Phytochemical analysis identified a high concentration of alkaloids, tannins, saponins, and flavonoids, which are thought to be the primary contributors to the observed activity. These results indicate that these herbal plants may serve as promising natural anthelmintic agents, providing a sustainable and environmentally friendly alternative to synthetic drugs. Further research is necessary to isolate and identify the specific bioactive compounds, clarify their mechanisms of action, and assess their safety and efficacy in clinical applications. This study highlights the potential of herbal medicine in developing novel anthelmintic treatments and encourages the integration of traditional knowledge with modern scientific research.

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1. Introduction

Anthelmintic activity is defined as the capability of a substance to expel or eliminate parasitic worms (helminths) from a host organism. Helminth infections, which are caused by parasites such as roundworms, flukes, and tapeworms, impact millions of individuals and animals globally, particularly in tropical and subtropical regions. Due to the growing problem of resistance to conventional anthelmintic drugs, there is an increasing focus on discovering new, effective treatments, especially those derived from herbal sources.[1,2]

1.1. Mechanisms of anthelmintic activity

Anthelmintic agents employ various mechanisms to target and eliminate helminths:

1. Neuromuscular Blockade: Some anthelmintics interfere with the worms' neuromuscular systems, causing paralysis and subsequent expulsion from the host body.[3]
2. Metabolic Disruption: Certain agents disrupt critical metabolic functions in the worms, such as glucose uptake, which is essential for their survival.
3. Structural Damage: Some compounds inflict direct harm on the worms' outer surface (tegument), resulting in their death.

Exploring herbal plants for their anthelmintic properties offers a promising alternative to traditional drugs,

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particularly given the increasing challenge of drug resistance. Ongoing research aimed at identifying, isolating, and characterizing active compounds, along with thorough safety and efficacy testing, will be crucial in developing effective and sustainable anthelmintic treatments. Combining traditional knowledge with modern scientific methodologies has the potential to significantly improve global health outcomes in the fight against helminth infections.

Figure 1: A red bottlebrush plant.

Callistemon citrinus, commonly referred to as the common red bottlebrush, crimson bottlebrush, or lemon bottlebrush, is a member of the myrtle family and is native to eastern Australia. Traditionally, this plant has been used for its diuretic properties and to address urinary tract issues, including urinary incontinence and bed-wetting in children. Melaleuca citrina, a closely related shrub, can grow up to 20 feet in height and features hard, fibrous or papery bark along with soft, silky hairs on its new growth. The leaves are narrow, egg-shaped with pointed tips, and are alternately arranged along the stem. Its distinctive red flowers are arranged in spikes at the tips of branches, each containing 30 to 45 stamens. The plant produces woody, cup-shaped capsules as fruit, which remain closed until the plant dies.[4]

Due to its adaptability and hardiness, Callistemon citrinus is a popular choice for landscaping. Its vibrant flowers attract a variety of native nectar-feeding wildlife, including insects, butterflies, and birds. Although the genus Callistemon, part of the Myrtaceae family, includes nearly 40 species, it is closely related to the Melaleuca genus. While Callistemon species are endemic to Australia, they are widely cultivated in other parts of the world and have naturalized in some areas. These plants thrive in temperate regions, particularly along the east coast of Australia, and prefer moist environments, benefiting from regular watering when grown in gardens. Notably, Melaleuca citrina (or Callistemon citrinus) has shown potential anthelmintic activity, indicating its usefulness in treating parasitic worm infections.

2. History

Callistemon citrinus is native to eastern Australia, specifically in the states of New South Wales and Queensland. It can thrive in a wide range of environments, from coastal areas to inland forests. This plant's ability to adapt to different soil types and climatic conditions has contributed to its extensive distribution within its native habitat. In the 19th and 20th centuries, Melaleuca citrina gained popularity in horticulture beyond Australia. It's striking red flower spikes and resilience made it a popular choice for ornamental gardens in various parts of the world, especially in regions with Mediterranean climates. The plant was introduced to Europe, North America, and other global regions, where it became a favored ornamental species. Although Callistemon citrinus is not classified as endangered, its popularity as a garden plant has led to its introduction in non-native areas, where it sometimes becomes invasive. Therefore, managing its spread in these regions is important to prevent potential ecological imbalances.[5]

2.1. Significance

The anthelmintic properties of Callistemon citrinus are primarily due to its chemical composition, which includes compounds such as 1,8-cineole, α-terpineol, and other terpenoids. These compounds are thought to disrupt the nervous system of parasitic worms, leading to paralysis and eventually death. Traditionally, Callistemon citrinus has been used in folk medicine to treat a range of ailments, including intestinal worm infections. However, more research is needed to thoroughly evaluate its effectiveness and safety as an anthelmintic agent. In addition to its medicinal uses, Callistemon citrinus is valued for its ornamental appeal, ecological role.[6]

3. Materials and Methods

1. **Plant material:** Leaves, flowers and bark of Callistemon citrinus obtained from campus of Himgiri Zee University,Sherpur, chakrata road, Dehradun, India. The Barks were taken out in such a way that there shouldn't be much damage to the plant.
2. **Chemicals:** Normal., saline, Piperazine citrate and distilled water was taken from the Laboratory of Himgiri zee university.
3. **Collection of test subjects:** The Earthworms along with vermicompost were collection from Laxmi Nursery, Sudhowala Dehradun.
4. **Glassware's and other materials:** Glassware taken were 5 petri dishes of same size, 3 beakers of 500ml, 3 glass rods, one test tube, a hot plate, Digital., weighing

balance and 3 conical., flask. Lastly for observing the time of death and paralysis of earthworms a stopwatch is also used.

3.1. Methodology

3.1.1. Collection of plant material

The plant material was collected from the university premises including bark, flowers and leaves.

3.1.2. Drying and blending

The plant materials were washed properly and shade dried at room temperature for 5 to 6 days, and after that it is dried further inside the tray dryer at 60°C to eliminate the remaining moisture on the leaves, flower and the bark. Now the dried flower, leaves and bark were powdered into coarse grain particles using blender machine and were stored separately in compartments respectively.

Figure 2: Collection and drying of plant material in a tray dryer.

4. Extraction of Leaf, Flower and Bark

Maceration: Put the crushed dry leaf, flower and bark powder (50gm) in a conical. iodine flask and add 250ml methanol in 1^{st} flask and 250ml Distilled water in 2^{nd} flask (250ml) respectively.[7]

Agitation: Some of the protocols may require daily re-shaking of the maceration mixture to ensure better extraction performance. To accomplish it, use low-speed motion like the shaking or stirring. Or incubate at 37^{o} C for 24hr at 120 rpm.

Filtration: Maceration period is over after that, apply a filter to differentiate liquid extract from the solid plant material.Apply the fine-mesh filter or whattman filter paper to capture all particles that are coming out from solution.

5. Collection of Extract and Concentration

The Extract was collected in a China dish/petri dish and left 24 hrs for evapouration of the filtered solvent in wax form

a b c

Figure 3: **a)** Filtration of leaf. **b)** Filtration of Flower. **c)** Filtration of Bark.

and stored in a dark place away from sunlight for further use. A suitable option will be concentration of the extract, if superior activity is desired of the active compounds. This stage can be achieved by either distillation under atmospheric pressure or using gentle heating so that the significant components do not degrade.[8,9]

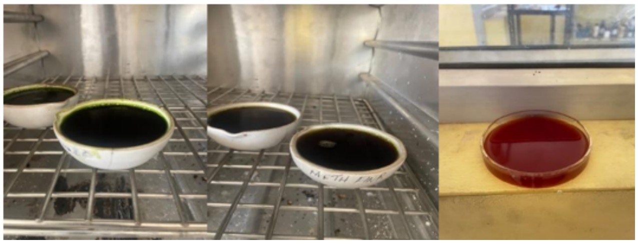

Figure 4: **a)** Collected extract of leaf **b)** Collected extract of flower. **c)** Collected extract of Bark.

6. Anthelmintic Activity of Callistemon Citrinus on Pheretima Posthuma Species of Earthworm

1. Five Petri dishes of same sizes were taken and washed thoroughly with water and dried eventually inside a hot air oven.
2. The dried wax of the extracts was scratched off from the china dish/petri dish and collected separately in a watch glass.
3. Each of the wax extracts of Leaf, Flower and fruit and the bark were weighed respectively and from each extract 2 grams and 1.5 gram were weighed respectively.
4. 2 grams of drug was dissolved in 100ml of normal., saline and concentration was taken 20% w/v. In another petri dish 1.5 grams of drug dissolved in 100ml and concentration was made upto 15% w/v.[10]
5. Piperazine citrate taken was 15 mg/ml concentration was taken in 30ml normal saline in a different petri dish.[11–13]

Table 1: Paralysis, and death time of leaf extract

Treatment group	Concentration (mg/ml)	Time taken (in minutes)	
		Paralysis time	Death Time
Methanolic extract	20	15.04 ± 2	19.33 to ± 4
	15	22.08 ±3	28.80 ±4
Distilled water extract	20	24.06 ±2	29.5 ±2
	15	26.30 ±2	33.23 ±1
Piperazine citrate	20	23.05 ±2	40.32 ±3
	15	27.35 ±2	57.32 ±2
Normal Saline	Not Required	No Paralysis	No Death

Table 2: Paralysis, and death time of flowers and fruit and flower extract [14]

Treatment group	Concentration (mg/ml)	Time taken (in minutes)	
		Paralysis time	Death Time
Methanolic extract	20	19.21 ±3	27.05 ± 2
	15	28.08 ±3	34.80 ±3
Distilled water extract	20	24.06 ±2	29.5 ±2
	15	26.30 ±2	33.23 ±2
Piperazine citrate	20	23.05 ±2	40.32 ±3
	15	27.35 ±2	57.32 ±2
Normal Saline	Not Required	No Paralysis	No Death

Table 3: Paralysis, and death time of bark.

Treatment group	Concentration (mg/ml)	Time taken (in minutes)	
		Paralysis time	Death Time
Methanolic extract	20	25.05±0.28	51.20 ±8
	15	32.08 ±25	64.80 ±7
Distilled water extract	20	37.06 ±17	63.05 ±6
	15	44.30 ±14	68.23 ±7
Piperazine citrate	20	23.05 ±2	40.32 ±3
	15	27.35 ±2	57.32 ±2
Normal Saline	Not Required	No Paralysis	No Death

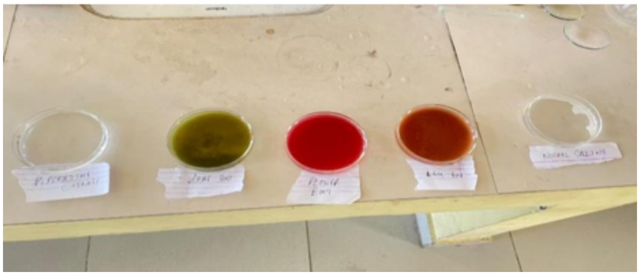

Figure 5: Standard drug, plant's materials and Normal. Saline are taken accordingly in different petri dishes.

Figure 6: In vitro anthelmintic assay.

6.1. For group I, 15% w/v drug concentration

1. 30 ml of the drug concentration was taken in petri dishes respectively.
2. Six healthy earthworms of same size were placed in each petri dish and time was recorded upto paralysis and death. [15]

7. For group II, 20% w/v drug concentration

1. 30 ml of the drug concentration was taken in petri dish respectively.
2. Six healthy earthworms of same size were placed in each petri dish and time was recorded upto paralysis and death. [16,17]

8. Results and Discussion

The paralysis and death time of the earthworms from leaf, flower and fruit and bark extract was recorded respectively as follows:

9. Conclusion

Helminth infections, caused by parasitic worms, pose significant health challenges, especially in tropical and subtropical regions with poor sanitation and limited access to healthcare. These infections can lead to a variety of diseases with substantial morbidity, affecting the gastrointestinal tract, liver, lungs, and other organs. Effective management and treatment of helminth infections rely on accurate diagnosis and the use of appropriate anthelmintic drugs.

The study of Calistemon citrinus revealed promising anthelmintic properties, supported by the presence of bioactive compounds identified in phytochemical screenings, such as alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids. In vitro testing confirmed the efficacy of Calistemon citrinus extracts, particularly the ethanol extract, in paralyzing and killing helminths.

The choice of anthelmintic drug depends on the type of helminth infection, the severity of the disease, and patient-specific factors. Proper diagnosis and treatment are essential to effectively manage helminth infections and prevent complications. Regular deworming programs and improved sanitation are critical components of public health strategies to control helminth infections in endemic regions.

10. Source of Funding

None.

11. Conflict of Interest

None.

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