

## Assessment of the antimicrobial and mosquito larvicidal efficacy of medicinal herb *Elytraria acaulis*

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### Abstract

Antimicrobial resistance was an important issue that needed to be addressed due to the indiscriminate use of antibiotics in humans and animals. This investigation seeks to explore the antimicrobial and larvicidal properties of *Elytraria acaulis* herb extracts. The methanol extract exhibited significant antibacterial activity, with the highest zone of inhibition recorded at 20.0±0.3 mm against *Escherichia coli* and 12.0±0.5 mm against *Klebsiella pneumoniae*. In antifungal assays, the methanol extract showed strong effects against *C. albicans* (16±0.4 mm) and other species, while acetone and chloroform extracts displayed moderate activity. The larvicidal effectiveness of the methanol extract was notable, achieving the highest mortality rates at 150 ppm after 48 hours across mosquito larvae. LC50 values ranged from 9-125 ppm for *Culex quinquefasciatus* and 33-120 ppm for *Aedes aegypti*. These findings align with previous research highlighting the antibacterial and antifungal potential of plant extracts, emphasizing the role of phytochemicals such as phenolics and flavonoids. Overall, *Elytraria acaulis* demonstrates promise as a natural source for developing antimicrobial and larvicidal agents.

**Keywords:** Antimicrobial resistance, larvicidal efficacy, plant extract, zone of inhibition

### Introduction

The Acanthaceae family is one of the large group of flowering plants that includes approximately 210 genera and around 4,000 species. These plants are primarily found in tropical and subtropical regions, with a few species occurring in temperate areas worldwide (Britannica, 2018) [1]. *Elytraria acaulis* L.f. Lindau, a member of the Acanthaceae family, was a diminutive herb that flourished in hidden arid environments. The whole herb was employed for therapeutic applications (Ravikanth *et al.*, 2001) [2]. The antibacterial efficacy of *E. acaulis* against urinary tract infections was analyzed, demonstrating maximum effectiveness (Manigandan and Kolanjinathan, 2016) [3]. The unregulated application of antimicrobials in healthcare environments, especially by those engaging in self-medication, may have resulted in vulnerable patients contracting infections from multidrug-resistant microorganisms. The rise of antibiotic resistance and related side effects, such as toxicity, has restricted the application of these medications and sparked renewed interest in phytotherapy studies (Nakamura *et al.*, 2014) [4]. The increasing prevalence of antibiotic resistance worldwide is linked to the extensive application of antibiotics in medical, veterinary, and agricultural sectors. In India, there is a notable prevalence of  $\beta$ -lactam resistance among animal populations. Conversely, resistance to quinolones is predominantly observed in human subjects. In environmental contexts, resistance to aminoglycosides and  $\beta$ -lactams has also been documented (Gunjan *et al.*, 2024) [5]. Antimicrobial resistance threats was majorly addressed and undertaken by the WHO and numerous other researchers and organizations globally (Antimicrobial Resistance Threats report, 2019) [6]. Mosquitoes constituted

a significant public health issue due to their role as vectors for severe human illnesses, such as malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, and yellow fever. These diseases resulted in significant mortality and morbidity among populations living in tropical and subtropical areas (Govindarajan and Karuppanan, 2011) [7]. The present investigation aimed to explore the antimicrobial properties and mosquito control efficacy of herbal extracts under laboratory conditions. The extracts of *Elytraria acaulis* have been reported to exhibit mosquitocidal effects against all targeted species.

### Materials and Methods

#### Collection of Plant

The healthy and well-grown leaves of the indigenous plant variety *Elytraria acaulis*, belonging to the Acanthaceae family, were collected from local areas in and around Bhuvanagiri, located at latitude N 11°27'51 and longitude E 79°40'5 in the Cuddalore district of Tamil Nadu, India. The plant was recognized through morphological and taxonomical analysis, and the specimens were later submitted to the Department of Botany at Annamalai University.

#### Preparation of Plant Extracts

The gathered plant material underwent a meticulous washing process 2-3 times with tap water and was subsequently air-dried in the shade at room temperature (25°C) for a duration of 3-4 weeks until achieving a brittle texture. After thorough drying, the plant material was meticulously ground into a fine powder utilizing an electric blender. A total of fifty grams of the dried powder was introduced into a Soxhlet apparatus containing 300 ml of

solvents, including methanol, acetone, chloroform, and hexane, and extraction was conducted until the solution achieved clarity. The extracts' solvents were subsequently evaporated with a rotary vacuum evaporator, and the resultant extract was preserved in a refrigerator for later use (Chessbrough, 2000) [8]. A crude test solution of *Elytraria acaulis* was formulated by dissolving a specified weight of the crude extracts or fractions in 5% Dimethyl sulfoxide (DMSO). Sterile discs (Himedia Ltd., Mumbai, 6 mm) were treated with 20 µL of each extract (hexane, chloroform, acetone, and methanol) to assess their antimicrobial properties.

### Antimicrobial activity

A culture of bacterial pathogens, including *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Staphylococcus aureus*, as well as fungal pathogens such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Candida krusei*, *Candida tropicalis*, and *Candida glabrata*, was prepared after twenty-four hours. This culture was then mixed with physiological saline, and the turbidity was adjusted by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (106 CFU/mL) was achieved. The antimicrobial activity of the chosen plant extract was assessed using the disc diffusion method established by Bauer *et al.* (1966) [9]. The Gentamicin (10 µg) was used as positive control for bacteria and Fluconazole (25 µg) for fungi. The DMSO 5% was used as negative control. Twenty microliters (for a concentration of 100 microliters) of the plant extract was applied to sterile empty discs measuring 6 mm in diameter and permitted to dry under sterile conditions (Manigandan, 2016) [10].

### Larvicidal activity of the plant extract

The eggs of *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti* were obtained from the vector control research center located in Pondicherry, India. The eggs were adequately preserved for maturation into the larval stage and were utilized during their third instar stage (Aarthi and Murugan, 2010) [11]. The larvicidal activity of *E. acaulis* plant extract was evaluated against three mosquito species: *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*, under *in-vitro* conditions. In order to determine the LC50 value, groups of 20 late third instar larvae were placed into 200 ml plastic cups containing 100 ml of dechlorinated water with different concentrations of plant extracts, specifically 50 µg/ml, 100 µg/ml, and 150 µg/ml. Following a 24-hour exposure period, mortality was assessed by delicately contacting each larva with a wooden toothpick. Larvae that exhibited no reaction to this stimulus were deemed deceased. The outcomes of concentration – mortality assays underwent logit regression analysis. To validate the reproducibility of the findings, groups of 20 late third instars were subjected to dechlorinated water (serving as the control) or a specific concentration of plant extract, corresponding to the LC50 concentration determined in the earlier experiment. The mortality percentage was determined utilizing Abbott's formula (Abbott, 1925) [12]. Abbott's corrected mortality,

$$\text{Percentage mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

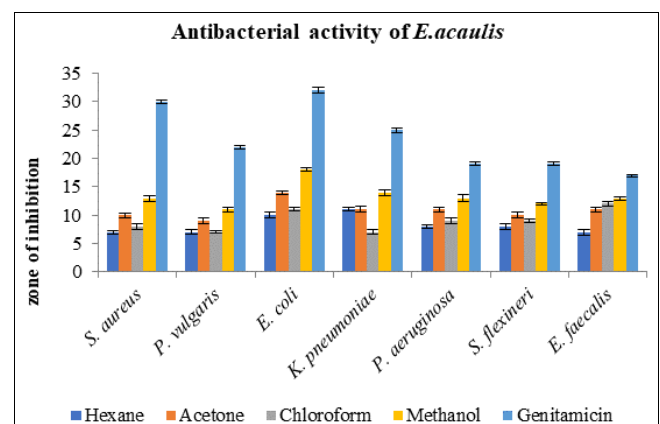
The statistical assessment of the LC50 and LC90 regression equations, along with the 95% confidence limits, including the lower and upper confidence limits, was conducted based on the data, which were analyzed at a 5% probability level for significant outcomes ( $P = 0.05$ ).

## Result and Discussion

### Antimicrobial activity

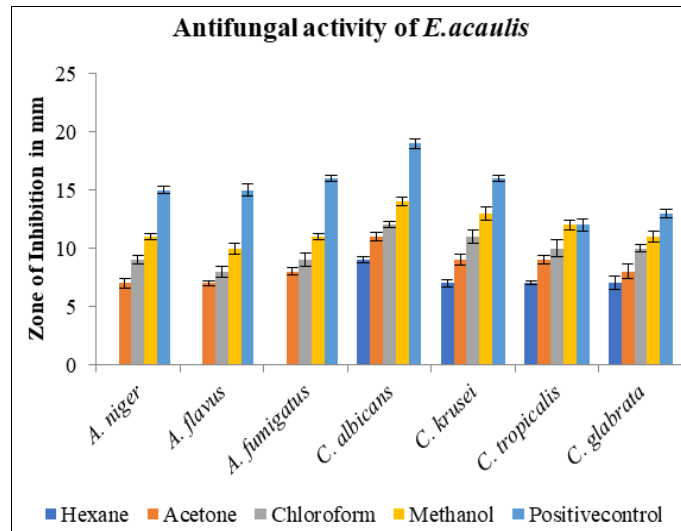
The crude extracts of *E. acaulis* exhibited significant antibacterial activity against all tested bacterial pathogens. Among the four extracts, the methanol extract demonstrated the highest antibacterial efficacy, with a maximum zone of inhibition measured at  $20.0 \pm 0.3$  mm against *E. coli*, followed by a zone of  $12.0 \pm 0.5$  mm against *Klebsiella pneumoniae*. The chloroform extract displayed a moderate zone of inhibition of  $14.0 \pm 0.3$  mm against *E. coli*, while the hexane extract showed the least antibacterial activity. In comparison, the positive control, Gentamicin, produced a maximum inhibition zone of  $31.0 \pm 0.5$  mm against *E. coli*. In terms of antifungal activity, all extracts showed potential against the tested pathogens, with methanol again exhibiting the strongest effect. At a concentration of 100 µg, the maximum zone of inhibition was recorded at  $16 \pm 0.4$  mm against *Candida albicans*, followed by  $14 \pm 0.57$  mm against *C. krusei* and  $11 \pm 0.42$  mm against *C. tropicalis*. Moderate to low zones of inhibition were observed against *Aspergillus* species when compared to the *Candida* species. The chloroform and acetone extracts showed moderate antifungal activity across all pathogens, whereas the hexane extracts exhibited minimal activity. The positive control, Fluconazole, demonstrated a zone of inhibition of  $19 \pm 0.43$  mm against *C. albicans*.

Ouattara *et al.* (2013) [13] investigated the antibacterial activity and phytochemical profile of various extracts from three species within the Acanthaceae family. All extracts and fractions demonstrated effective inhibition against bacteria and contained a high concentration of phenolic compounds. Among the tested strains, *E. coli* was most significantly inhibited, with the butanol fraction from *N. canescens* exhibiting the strongest antibacterial activity. The study also revealed that the fractions had the highest levels of total phenolics, flavonoids, flavonols, and tannins. Additionally, the authors highlighted the traditional uses of these plants in treating infectious diseases. Very few of the research works are carried out using methanol extracts of *Elytraria acaulis* (Aswini *et al.*, 2020) [14].



\*Positive control – Gentamicin – 10 µg/disc; Values represents mean ± SD of triplicates.

**Fig 1:** Antibacterial activity of various solvent extracts of *Elytraria acaulis*



\*Positive control - Flucanazole (25 µg); Values represents mean ± SD of triplicates.

**Fig 2:** Antifungal activity of various solvent extracts of *Elytraria acaulis*

**Mosquito larvicidal activity**

The larvicidal activity of *Elytraria acaulis* Methanolic extract was highly effective against the tested mosquito vectors and results were noted in the table. Highest mortality rate was recorded in the 150 ppm concentration

against all the larvae at 48 hours. The LC<sub>50</sub> values were observed in the ranges from 9 - 125 ppm against *Culex quinquefasciatus* and for *Aedes aegypti*, it ranged from 33 - 120 ppm and 59 - 107 ppm for *Anopheles stephensi*.

**Table 1:** Larvicidal activity *Elytraria acaulis* Methanol extract at various concentration against the Mosquito vector

Mosquito larvae	Time interval in hours	Number of dead larvae						
		50 µg/ml	Mortality %	100 µg/ml	Mortality %	150 µg/ml	Mortality %	LC <sub>50</sub> (ppm)
<i>Aedes aegypti</i>	24	04	20	09	45	12	60	120.46
	36	07	35	14	70	16	80	72.68
	48	12	60	16	80	19	95	33.20
<i>Anopheles stephensi</i>	24	05	25	10	50	13	65	107.18
	36	08	25	12	60	16	80	86.93
	48	12	40	16	80	18	90	59.51
<i>Culex quinquefasciatus</i>	24	05	25	09	45	11	55	125.66
	36	08	40	13	65	15	75	69.41
	48	13	65	15	75	18	90	9.84
Control	20	0	0	0	0	0	0	

The current investigation revealed that the methanol extract exhibited the most significant activity against mosquito vectors. In a similar vein, Batista *et al.* (2009) [15] documented that extracts from 60 species of higher plants belonging to 34 families were evaluated against Plasmodium strains, revealing that more than one hundred extracts exhibited inhibitory effects on the parasites. The methanol extracts demonstrated the highest levels of inhibitory activity in anti-malarial assays, significantly impacting both chloroquine-resistant and chloroquine-sensitive strains. Recently, numerous compounds extracted from various plants have been identified as effective antimalarial agents. Saxena *et al.* (2003) [16] identified a total of 18 quassinoids, 127 alkaloids, 23 terpenes, 27 triterpenoids, 21 flavonoids or xanthenes, 9 quinones, and 25 miscellaneous compounds that were either isolated or derived from plant extracts. The data indicates that ethnopharmacological approaches have been effective in identifying new candidates for antimalarial agents.

**Conclusion**

Our investigations provided important data regarding the utilization of *Elytraria acaulis*, a significant herbal medicine used in Ayurveda for treating various illnesses, while

exploring its antimicrobial and mosquito larvicidal efficacy. The Acanthaceae family of medicinal herbs and plants needed further research and exploration to reveal valuable bioactive compounds, as there were few publications on this topic.

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