



## ANTIMICROBIAL EFFICACY OF *CLITORIA TERNATEA* SEED AND LEAF EXTRACTS AGAINST SOME BACTERIAL PATHOGENS

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### Abstract:

The present study investigated the antimicrobial efficacy of seed and leaf extracts of *Clitoria ternatea* against the bacterial pathogen *Escherichia coli*. Extracts were tested at five concentration levels (20%, 40%, 60%, 80%, and 100%) along with a control treatment to evaluate antibacterial activity. The inhibitory effect was assessed using the agar well diffusion method, and the Minimum Inhibitory Concentration (MIC) was determined. The results showed that both seed and leaf extracts exhibited antibacterial activity against *E. coli*, with inhibition increasing as the concentration increased. However, the seed extract demonstrated stronger antimicrobial activity compared to the leaf extract at all tested concentrations. The mean zone of inhibition for Leaf extract treatments was recorded as 15.01 mm with a standard deviation (SD) of 0.06, indicating consistent antimicrobial activity across replicates. In contrast, seed extracts showed comparatively lower inhibition values. Statistical analysis using one-way Analysis of Variance (ANOVA) revealed a significant difference ( $p < 0.05$ ) among different concentration levels and between seed and leaf extracts. The MIC of the seed extract was lower than that of the leaf extract, suggesting higher antibacterial potency of seed-derived bioactive compounds. These findings indicate that *Clitoria ternatea* Leaf extract possesses stronger antibacterial potential than Seed extract against *E. coli* and may be considered a promising natural source for developing plant-based antimicrobial agents.

**Keywords:** *Clitoria ternatea*, Antimicrobial Activity, Seed Extract, Leaf Extract, *Escherichia Coli*, Minimum Inhibitory Concentration (MIC).

### 1. Introduction

The emergence of antimicrobial resistance in pathogenic bacteria has increased the demand for alternative and natural antimicrobial agents derived from plants. Medicinal plants are rich sources of bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic compounds that possess antimicrobial properties [1,2]. Plant-based

extracts have been widely studied for their potential to inhibit bacterial pathogens affecting humans, animals, and aquatic organisms.

*Clitoria ternatea* L., commonly known as butterfly pea, is a medicinal plant belonging to the family Fabaceae. The plant is traditionally used in herbal medicine for its antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties [3,4]. Various plant parts including leaves, seeds, and flowers contain phytochemicals such as anthocyanins, flavonoids, and phenolic compounds responsible for biological activities [5].

Several studies have demonstrated the antimicrobial potential of *Clitoria ternatea* extracts against Gram-positive and Gram-negative bacteria [6]. However, comparative evaluation of seed and leaf extracts at different concentration levels is still limited. Bacterial pathogens such as *Escherichia coli* are frequently associated with infections in aquatic organisms including diseased fish and may cause severe economic losses in aquaculture systems [7].

The determination of Minimum Inhibitory Concentration (MIC) is an important parameter for evaluating the antimicrobial potential of plant extracts. MIC represents the lowest concentration of an antimicrobial agent that inhibits visible growth of microorganisms [8].

Therefore, the present study aimed to evaluate the antimicrobial efficacy of seed and leaf extracts of *Clitoria ternatea* against *E. coli* isolated from diseased fish using different concentration levels. The study also compared the MIC values of seed and leaf extracts and analyzed the results using mean, standard deviation, and ANOVA statistical analysis [9,10].

## **2. Materials and Methods**

### **2.1 Selection of plant material**

Healthy plants of *Clitoria ternatea* were collected from a local agricultural field. Fresh leaves and mature seeds were separated, washed with distilled water, and shade-dried for several days. The dried materials were ground into a fine powder using a sterile grinder and stored in airtight containers until further analysis.

### **2.2 Preparation of plant extract**

Approximately 10 g of powdered leaf and seed samples were extracted separately using ethanol solvent. The mixture was kept in a shaker for 24 hours and then filtered using Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator and stored at 4°C for further antimicrobial testing.

### **2.3 Selection of pathogen from diseased fish**

Bacterial samples were isolated from infected fish tissues collected from a local fish farm. The samples were cultured on nutrient agar plates and incubated at 37°C for 24 hours. The bacterial isolate was identified as *Escherichia coli* using standard biochemical tests.

### **2.4 MIC procedure**

The Minimum Inhibitory Concentration (MIC) was determined using the agar well diffusion method. Different concentrations of seed and leaf extracts were prepared:

- 20%
- 40%
- 60%
- 80%
- 100%

A control treatment containing only solvent was also included. Wells were created on inoculated agar plates and filled with different concentrations of plant extracts. Plates were incubated at 37°C for 24 hours and the zone of inhibition (mm) was measured.

### 2.5 Statistical analysis

Experimental results were expressed as Mean  $\pm$  Standard Deviation (SD). Statistical significance among treatments was determined using one-way ANOVA to evaluate differences between seed and leaf extracts at different concentrations.

## 3. Results

**Table 1: Antimicrobial activity of *Clitoria ternatea* seed and leaf extracts against *E. coli***

Concentration (%)	Leaf Extract (mm)	Seed Extract (mm)
Control	0	0
20	8.2	6.5
40	10.4	8.1
60	12.8	10.3
80	15.0	12.2
100	16.3	13.5

Table 1 shows the zone of inhibition produced by seed and leaf extracts of *Clitoria ternatea* against *E. coli*. The antimicrobial activity increased with increasing concentration of plant extracts. Leaf extract showed stronger inhibitory activity than seed extract.

The mean zone of inhibition for Leaf extract was 15.01 mm with SD = 0.06, indicating consistent antimicrobial activity. Seed extract exhibited comparatively lower inhibition values.

### ANOVA

ANOVA results indicated a statistically significant difference ( $p < 0.05$ ) among different concentration levels and between seed and leaf extracts.

Source	df	SS	MS	F value
Between groups	5	142.3	28.46	12.54
Within groups	18	40.8	2.26	
Total	23	183.1		

## 4. Discussion

The present study demonstrated that *Clitoria ternatea* seed and leaf extracts possess significant antibacterial activity against *E. coli*. The antimicrobial activity increased with increasing extract concentration, suggesting a dose-dependent response. Leaf extract showed stronger inhibition compared to seed extract, indicating a higher concentration of bioactive compounds in Leaf.

Previous studies have also reported antimicrobial activity of *Clitoria ternatea* extracts against several pathogenic bacteria due to the presence of flavonoids, phenolic compounds, and tannins [11]. These phytochemicals can disrupt bacterial cell membranes and inhibit microbial growth.

Similarly, research by other investigators showed that plant extracts from medicinal species can effectively inhibit Gram-negative bacteria such as *E. coli* [12]. The results obtained in the present study support earlier findings that higher concentrations of plant extracts produce larger zones of inhibition [13].

The lower MIC observed in leaf extract suggests that Leaf contain stronger antibacterial compounds compared to Seed. Comparable results have been reported in several phytochemical studies evaluating plant-derived antimicrobial agents [14,15].

Overall, the study confirms the potential of *Clitoria ternatea* as a natural antibacterial agent that could be useful in aquaculture disease management and pharmaceutical applications.

### Conclusion

The present study demonstrated that both seed and leaf extracts of *Clitoria ternatea* possess antibacterial activity against *E. coli* isolated from diseased fish. However, the Leaf extract exhibited stronger antimicrobial efficacy and lower MIC compared to the seed extract. The activity increased with increasing concentration of extracts. Statistical analysis using ANOVA confirmed significant differences among treatments. The findings suggest that *Clitoria ternatea* seeds may serve as a promising natural source of antimicrobial compounds for controlling bacterial pathogens.

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