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IN VITRO ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF CLITORIA TERNATEA AND CLEOME GYNANDRA

Meghana B P*, Sinchana D M, Tejas S U, Varsha C N

Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara , Maddur taluk, Mandya district, Karnataka, India-571422

***Corresponding author** E- mail id: meghanamaadu5@gmail.com

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ABSTRACT

The present investigation aimed to assess and compare the *in vitro* anti-inflammatory and antioxidant activities of ethanolic extracts of *Clitoria ternatea* and *Cleome gynandra*. The extracts were tested at different concentrations ranging from 200 to 1000 µg/mL. Anti-inflammatory activity was evaluated by the inhibition of protein denaturation method, and antioxidant potential was determined using the DPPH radical scavenging assay. The results revealed that both plant extracts exhibited a concentration-dependent increase in activity; however, *Clitoria ternatea* showed a significantly higher percentage of inhibition than *Cleome gynandra* in both assays. The lower IC_{50} value obtained for *Clitoria ternatea* indicates its stronger free radical scavenging ability and higher efficacy in preventing inflammation. The potent biological activities observed may be due to the presence of various phytoconstituents such as flavonoids, phenolic compounds, tannins, and alkaloids, which are well-known for their antioxidant and anti-inflammatory roles. These findings suggest that *Clitoria ternatea* could serve as a valuable natural source for developing safe and effective anti-inflammatory and antioxidant agents. Further research focusing on isolation, purification, and structural characterization of the bioactive compounds, along with *in vivo* pharmacological studies, is recommended to validate and enhance its therapeutic potential.

INTRODUCTION:

Antioxidant activity: Antioxidants are substances that slow down or prevent oxidative reactions responsible for generating free radicals. Such oxidative processes can cause the deterioration of organic molecules and damage living tissues. In both plants and animals, antioxidants help neutralize these reactive species, protecting cells from oxidative harm. Even at low concentrations, they effectively suppress oxidation and play vital physiological roles in maintaining cellular integrity. The natural antioxidant compounds present in plants act as radical scavengers, transforming reactive molecules into more stable and less harmful forms.(1) Antioxidants primarily act on the oxidative stress pathway, which is significantly involved in various disease mechanisms, including those triggered by coronavirus infection. It is important to recognize that their activity extends beyond simple free radical scavenging. Antioxidants also function by binding transition metal ions, breaking down hydrogen peroxide and hydroperoxides, neutralizing pro-oxidant molecules, strengthening the body's natural antioxidant defense systems, and aiding in

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the repair of cellular damage resulting from oxidative stress. (2)

Anti-inflammatory: Inflammation is a multifaceted biological reaction of vascular tissues that occurs in response to harmful stimuli such as pathogens, irritants, or cellular injury. It is generally divided into acute and chronic types and involves a series of complex biochemical interactions between the vascular system, immune components, and various cells at the affected site. Acute inflammation represents the body's immediate defense mechanism, characterized by the rapid movement of plasma and immune cells particularly neutrophils and macrophages from the bloodstream to the injured tissue. In contrast, chronic inflammation develops gradually and is marked by continuous cellular infiltration, leading to both tissue damage and repair occurring simultaneously.(3) In the initial phase of inflammation, signaling molecules such as leukotrienes, prostaglandins, and histamine interact with receptors on endothelial cells, leading to vasodilation, endothelial cell retraction, and increased vascular permeability. Histamine further stimulates the production of P-selectin and platelet-activating factor (PAF), facilitating leukocyte attachment and migration across the vessel wall. Activated macrophages and endothelial cells then secrete cytokines like TNF-α and IL-1, which prolong the inflammatory response by enhancing the expression of E-selectin and sustaining P-selectin levels. These adhesion molecules, in turn, direct leukocytes toward chemokines such as IL-8 and MCP-1, enabling their recruitment to the site of infection or tissue injury.(4,5)

Clitorea ternatea: *Clitoria ternatea*, commonly known as Asian pigeonwings, bluebell, blue pea, butterfly pea, cordofan pea, and Darwin's pea, is a plant species belonging to the *Fabaceae* family. A known synonym is *Clitoria principissae*. This plant is available in two flower colours: blue and white**.**(6) In southern India, the plant is known as 'Shankhapuspi.' Although Aparajita is commonly found in Maharashtra, its use as a *Medhya* (nootropic) drug remains underexplored.(7) The plant *Clitoria ternatea* included proteins, alkaloids, anthraquinone, anthocyanins, cardiac glycosides, phenols, tannins, phlobatannin, carbohydrates, saponins, triterpenoids, phenols, flavanoids, flavonol glycosides, and volatile oils in addition to steroids.(8) Various parts of *Clitoria ternatea* have been used as active ingredients in numerous Ayurvedic formulations for the treatment of a wide range of disorders. Several traditional Ayurvedic 'medha' (cognitive-enhancing) preparations include *C. ternatea* in combination with other medicinal plants. (9) It has been traditionally utilized for centuries in Ayurveda for its wide range of benefits, including enhancing memory, acting as a nootropic, relieving stress, reduce anxiety, treating depression, preventing seizures, and serving as a natural sedative and tranquilizer.(10) *Clitoria ternatea* was selected for this study because it is a well-known medicinal plant traditionally used in Ayurveda for treating various ailments. It is rich in bioactive compounds such as flavonoids, tannins, and phenolic compounds, which are known to possess strong antioxidant and anti-inflammatory properties. These phytochemicals make the plant a promising natural source for exploring therapeutic effects against oxidative stress and inflammation.

Cleoma gynandra : *Cleome gynandra (Capparaceae)* is also known as spider plant. *Cleome gynandra* is an erect, annual or perennial herbs. It is used as a medicinal plant and can be found in all over world .It grows as a weed in paddy fields and also in road sides and in open grass lands.(11) *C. gynandra* plant occurring throughout the tropics and subtropics of Africa. In South Africa, it is found in agricultural land and near human settlements. It is less common in areas with highly humid climate.(12) Spider plant has also been used as traditional medicine to heal a range of ailments in different communities which include food poisoning, rheumatism, inflammation, toothache, headache, bacterial infections, snake bites. These uses as traditional medicine hint at secondary plant metabolites that are health beneficial.(13) It grows from seed which are dispersed and germinate during the rainy season. Although it is

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considered an agricultural weed, most local use it as a valuable easily grown agricultural crop. Seeds can be collected and distributed before or after tilling the soil. The plant does not require any maintenance. (14) Nearly all parts of this plant are utilized in traditional medicine for treating various conditions and It is rich in bioactive compounds such as flavonoids, tannins, and phenolic compounds, which are known to possess strong antioxidant and anti-inflammatory properties.

METHODOLOGY:

Collection of plant and authentication: The Dry seeds of plant *Clitorea ternatea* and whole plant of *Cleome gynandra* were collected from doddarasinakere village, maddur tq, mandya dist, Karnataka and authenticated by Biologist Dr. Thejesh Kumar M P, Head of the Botany Department, Bharathi College Post-graduation Research Centre, Bharathinagara, maddur tq, mandya dist, Karnataka.

Preparation of Extract: The seeds of plant *Clitorea ternatea* were used to prepare extracts for the study. The fully dried seeds collected and seeds were powderd using mechanical mixer. The powered sample is stored in a clean glass container. The extraction was done by soxhlet apparatus using solvent Ethanol (70% with water) and the extract stored in low temperature until needed for analysis. The collected *Cleome gynandra* plant was washed, shade-dried at room temperature, and coarsely powdered using a mixer grinder. About 65 g of coarse powder was macerated with hydroalcoholic solvent (70% ethanol:30% water) for 48hr with intermittent shaking and magnetic stirring. The mixture was filtered, and the filtrate was dried at room temperature to yield a semi-solid extract.

ANTI-OXIDANT ACTIVITY: [15] The antioxidant potential of the extracts was evaluated based on their hydrogen-donating or free radical scavenging ability using the stable DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical method, with slight modifications. Different concentrations of the sample extract were prepared, and the volume was adjusted to 1 mL using dimethyl sulfoxide (DMSO). To this, 2 mL of 0.1 mM DPPH solution in methanol was added and mixed thoroughly. The mixture was then incubated in the dark at room temperature for 20 minutes. The absorbance was recorded at 517 nm. The percentage of DPPH radical inhibition was determined using the formula, % inhibition = Absorbance of control- Absorbance of test sample/ Absorbance of Control× 100. Where Abs control represents the absorbance of DPPH with DMSO, and Abs sample represents the absorbance of DPPH with the extract. Appropriate blanks were used to correct for background absorbance. The IC_{50} value, indicating the concentration required to achieve 50% inhibition, was subsequently determined.

ANTI-INFLAMMATORY ACTIVITY: [16]

The anti-inflammatory activity was assessed by evaluating the inhibition of protein denaturation using egg albumin as a protein source, with minor modifications to the standard procedure. In this method, 0.2 mL of 1–2% egg albumin solution (prepared from fresh hen's egg or commercially available powder), 2 mL of the test extract or standard drug (Aspirin) at various concentrations, and 2.8 mL of phosphate-buffered saline (pH 6.4) were combined to obtain a total reaction volume of 5 mL. The control was prepared by mixing 2 mL of distilled water, 0.2 mL of egg albumin solution, and 2.8 mL of phosphate-buffered saline. All reaction mixtures were incubated at 37 ± 2°C for 30 minutes, followed by heating at 70 ± 2°C for 5 minutes in a water bath. After cooling, absorbance was measured at 280 nm using a UV-Vis spectrophotometer, with triple-distilled water as the blank. The percentage inhibition of protein denaturation was calculated using the formula: % inhibition = Absorbance of control- Absorbance of test sample/ Absorbance of Control × 100**.** The concentration of the extract required to produce 50% inhibition (IC_{50}) was determined by plotting the percentage inhibition against the corresponding concentrations of the extract.

RESULT:

Assessment of *in-vitro* anti-oxidant activity: Estimation of Radical Scavenging Activity Using DPPH Assay: Medicinal plants serve as valuable sources of natural antioxidants.

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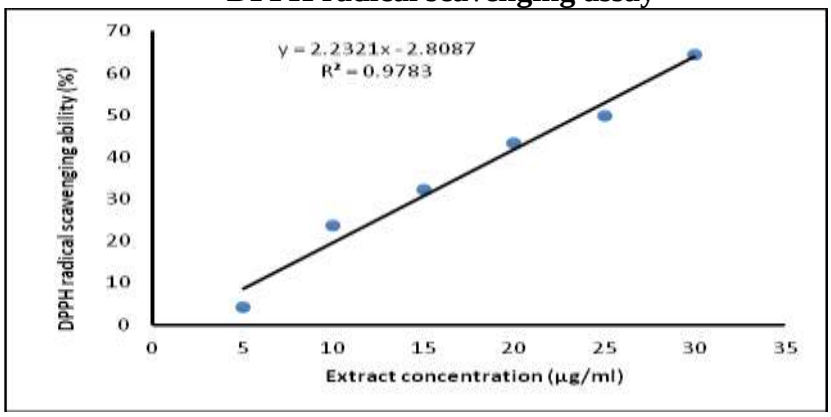
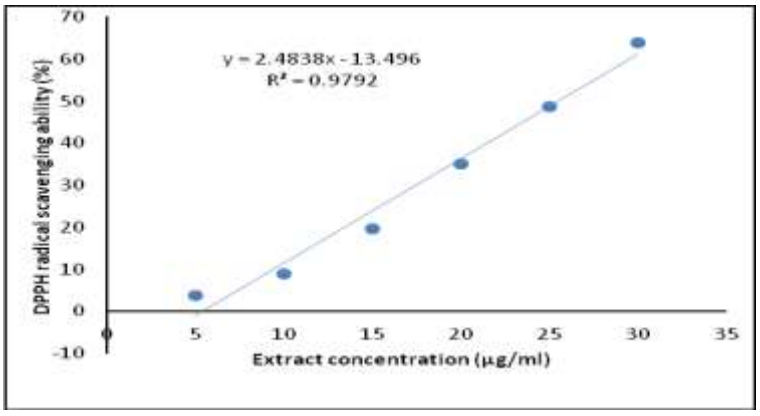
In-vitro anti-oxidant activity of _Clitorea ternatea_ seeds extract by DPPH radical scavenging assay



Percentage inhibition of antioxidant activity by DPPH Assay.

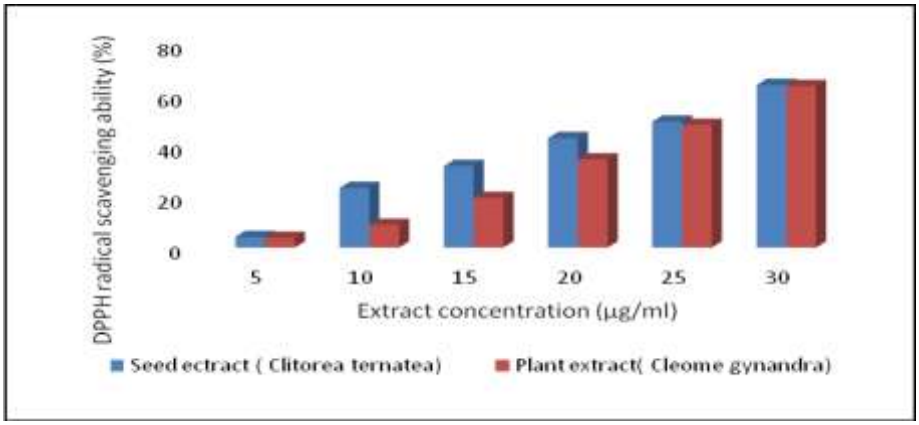
S. No	Concentration (µg/ml)	Absorbance	Percentage inhibition %	IC_{50} Value
1	5	0.674	4.2	**25.56 µg**
2	10	0.538	23.57	
3	15	0.477	32.24	
4	20	0.399	43.32	
5	25	0.353	49.85	
6	30	0.251	64.34	

In-vitro anti-oxidant activity of _Cleome gynandra_ plant extract by DPPH radical scavenging assay.



Percentage inhibition of antioxidant activity by DPPH Assay.

Comparison of standard drug, Plant and Seed extracts of anti-oxidant activity:



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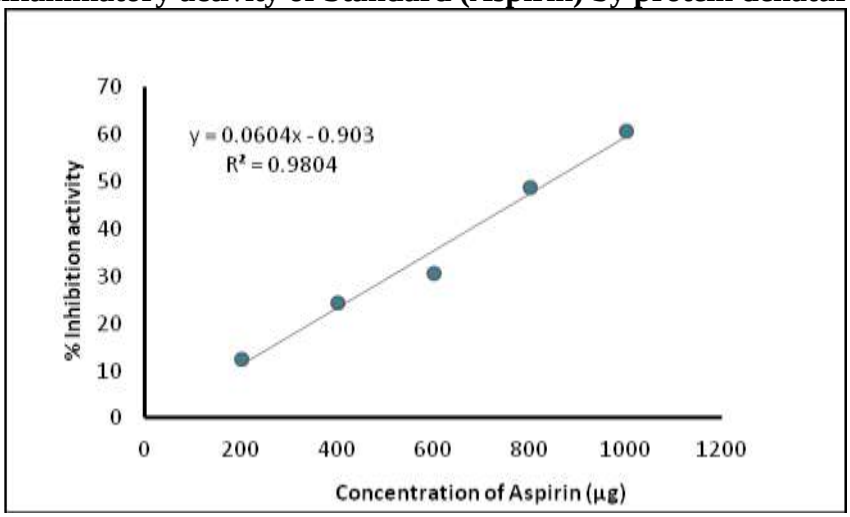
Sl. No	Concentration (µg/ml)	Absorbance	Percentage inhibition %	IC_{50} Value
1	200	0.580	12.39	**986.14µg**
2	400	0.500	24.47	
3	600	0.460	30.51	
4	800	0.340	48.64	
5	1000	0.260	60.72	

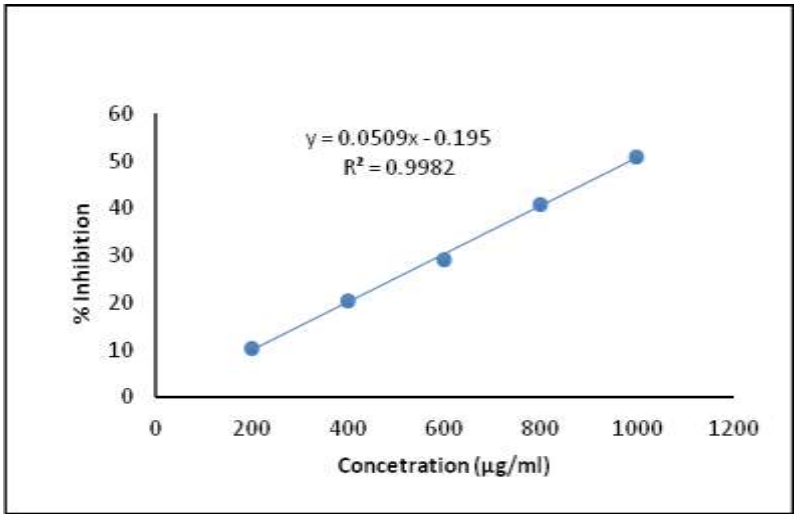
***In vitro* anti-inflammatory activity of Standard (Aspirin) by protein denaturation method.**



Standard graph of Aspirin by protein denaturation method.

Sl. No	Concentration (µg)	Absorbance	Percentage inhibition	IC_{50} Value
1	200	0.594	10.27	**986.14µg**
2	400	0.526	20.50	
3	600	0.469	29.10	
4	800	0.391	40.85	
5	1000	0.325	50.95	

In vitro anti-inflammatory activity of Clitorea ternatea seed extract by protein denaturation method.



Graph of seed extract of *Clitorea ternatea* by protein denaturation method.

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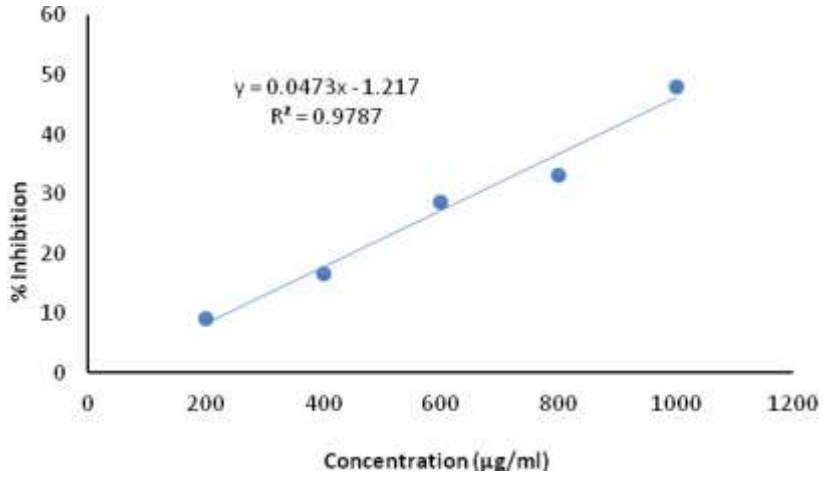
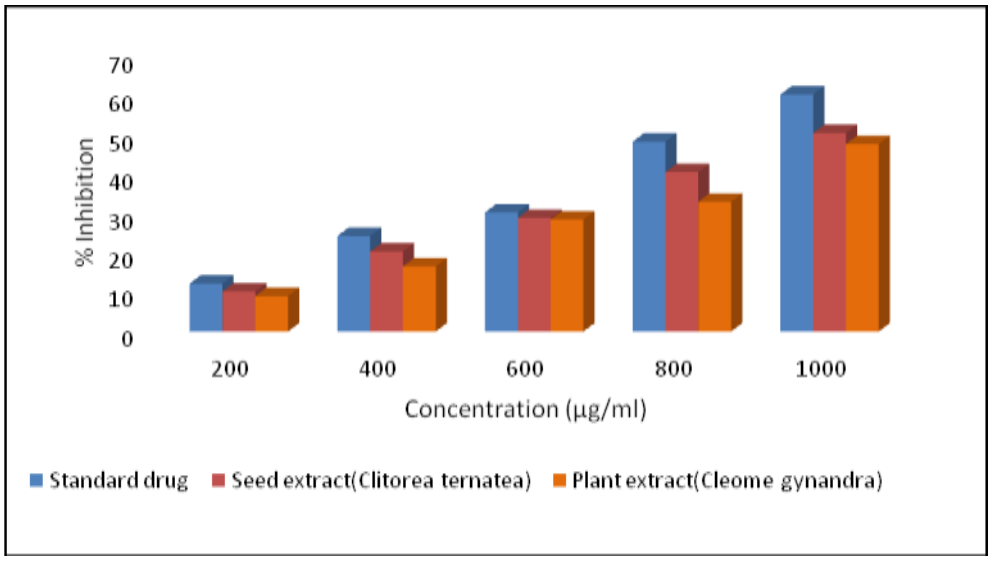
Sl. No	Concentration (µg)	Absorbance	Percentage inhibition	IC_{50} Value
1	200	0.602	9.06	**1082.81 µg**
2	400	0.552	16.66	
3	600	0.472	28.70	
4	800	0.402	33.23	
5	1000	0.344	48.03	

***In vitro* anti-inflammatory activity of *Cleome gynandra* plant extract by protein denaturation method**



Graph of plant extract of *Cleome gynandra* by protein denaturation method.

Comparison of standard drug, Plant and Seed extracts of anti-inflammatory activity:



The antioxidant potential of these plants is largely attributed to their phenolic compounds, particularly phenolic acids and flavonoids. Variations in antioxidant activity among different plant extracts are often linked to differences in their total phenolic content. The DPPH assay, which utilizes the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), is a widely accepted method for evaluating the free radical scavenging efficiency of plant extracts. This method is based on the principle that antioxidants can reduce the DPPH radical, leading to a measurable decrease in its purple coloration. In the present study, the extract demonstrated a potent antioxidant effect with an IC_{50} value of 23.65 µg/mL against DPPH radicals.

IN VITRO ANTI-INFLAMMATORY ACTIVITY:

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Inhibition of Albumin Denaturation: The results of the protein denaturation inhibition assay for various concentrations of the extract are presented below. The percentage inhibition values for the seed extract at concentrations of 200, 400, 600, 800, and 1000 µg/mL were found to be 10.27, 20.50, 29.10, 40.85, and 50.95%, respectively. Similarly, the standard drug Aspirin exhibited percentage inhibition values of 12.39, 24.47, 30.51, 48.64, and 60.72% at the same concentrations. The IC_{50} value of the seed extract was calculated to be 986.14 µg/mL, whereas the IC_{50} for the standard Aspirin was 842.76 µg/mL. These findings indicate that the seed extract demonstrates notable anti-inflammatory potential by effectively inhibiting protein denaturation, although its activity is slightly lower compared to the standard drug. The comparative study of the ethanolic extracts of *Clitoria ternatea* and *Cleome gynandra* demonstrated notable differences in both antioxidant and anti-inflammatory activities. In the DPPH radical scavenging assay, *Clitoria ternatea* showed a higher antioxidant potential with an IC_{50} value of 23.65 µg/mL, whereas *Cleome gynandra* exhibited a comparatively weaker activity with an IC_{50} value of 25.56µg/mL. This indicates that *Clitoria ternatea* possesses stronger free radical scavenging constituents such as flavonoids and phenolic compounds. In the anti-inflammatory assay based on inhibition of protein denaturation, *Clitoria ternatea* displayed a maximum inhibition of 50.95% at 1000 µg/mL, while *Cleome gynandra* showed 48.03% inhibition at the same concentration. In the Protien denaturation assay, *Clitoria ternatea* showed a higher anti-inflammatory potential with an IC_{50} value of 986.14µg/mL, whereas *Cleome gynandra* exhibited a comparatively weaker activity with an IC_{50} value of 1082.81µg/mL. The enhanced anti-inflammatory activity of *Clitoria ternatea* may be attributed to the presence of bioactive compounds like flavonoids, tannins, and alkaloids that stabilize proteins and prevent inflammatory mediator release. Overall, the results clearly indicate that *Clitoria ternatea* exhibits superior antioxidant and anti-inflammatory potential compared to *Cleome gynandra*, suggesting its more promising therapeutic efficacy against oxidative stress and inflammation-related disorders.

CONCLUSION:

Based on the findings, it can be concluded that the ethanolic extracts of *Clitoria ternatea* and *Cleome gynandra* at various concentrations (200, 400, 600, 800, and 1000 µg/mL) demonstrated significant anti-inflammatory potential in a dose-dependent manner. Among these, *Clitoria ternatea* exhibited a more pronounced inhibitory effect compared to *Cleome gynandra*, indicating its stronger efficacy in reducing inflammation. The observed activity may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, tannins, and alkaloids, which are known for their anti-inflammatory properties. These results suggest that *Clitoria ternatea* could serve as a promising natural source for developing anti-inflammatory agents. However, further pharmacological and biochemical studies are needed to confirm its mechanism of action and to isolate and characterize the specific bioactive compounds responsible for the observed effects. Additionally, exploring different in vivo and in vitro models would help establish its therapeutic potential and safety profile for future medicinal applications.

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Conflict of interest: The authors declare no conflict of interest.

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