Assessment of In Vitro Antiurolithiatic Activity of Methanolic Extract of Clitoria Ternatea Flower by Titrimetric Method

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ABSTRACT

It is also called blue butterfly pea, is a medicinal plant widely used in traditional remedies. Kidney stones are a frequent health issue, and there is growing interest in finding safer, natural treatment options. Extracts prepared from dried flowers were tested to check their ability to prevent stone formation. The plant is rich in natural compounds such as flavonoids, tannins, and antioxidants that support overall health. The findings showed that the flower extracts can reduce or slow down the formation and growth of kidney stone crystals, especially at higher concentrations. The methanolic extract was found to be more effective than the aqueous extract.

KEY WORDS

Clitoria ternatea, Blue Butterfly Pea, Antiurolithiatic, Calcium oxalate, Urolithiasis, Diuretic, Urinary stones.

INTRODUCTION

Overview of Urinary Stones

Urinary stones are small, hard lumps that form in the kidneys when waste materials in the urine stick together, usually because of not drinking enough water. They can cause strong pain in the lower back or side, pain while passing urine, blood in the urine, and sometimes nausea or vomiting. They may happen due to poor diet, being overweight, infections, or family history. Small stones can often pass on their own by drinking plenty of water and taking medicine, while bigger ones may need treatment. Drinking enough water can help prevent them [1].

Kidney Stones Epidemiology

Urinary stones are a common health problem. Many people experience at some point in their lives. Cases have been increasing due to unhealthy eating habits, less physical activity, and rising obesity. They are more common in men than women and usually affect people between the ages of 30 and 50. People living in hot and dry climates are at a greater risk, as excessive sweating and not stay well-hydrated, which promotes stone formation. Another concern is that kidney stones often come back, and many people may face the problem again within a few years, making it a repeated health issue [2].

Kidney Stones and the Urinary System

The urinary system is made up of the kidneys, ureters, urinary bladder, and urethra, and its main job is to remove waste and extra minerals from the body through urine. Kidney stones are hard particles that form in the kidneys when urine becomes too concentrated with minerals. These stones may remain in the kidneys or travel through the urinary tract toward the bladder, often causing intense pain and obstruction of urine flow. Small stones are usually expelled naturally through urination, whereas larger stones can result in complications such as urinary infections or kidney damage. The pain becomes especially severe when the stones move through the ureters. If the condition is ignored or untreated for an extended period, it can gradually impair kidney function and may contribute to chronic kidney-related problems [3].

Types of Urinary Stones

Classified by what they are made of:

Calcium oxalate stones:

These are the most common type. They form when calcium combines with oxalate in the urine, often due to low water intake or eating foods like spinach, nuts, and chocolate.

Uric acid stones:

These occur when the urine becomes too acidic. They are often linked to diets high in red meat and conditions like obesity or diabetes.

Struvite stones:

These are usually caused by urinary tract infections. They can grow quickly and may become quite large, sometimes affecting a large part of the kidney.

Cystine stones:

These are rare and occur due to a genetic condition where excess cystine to be present in the urine.

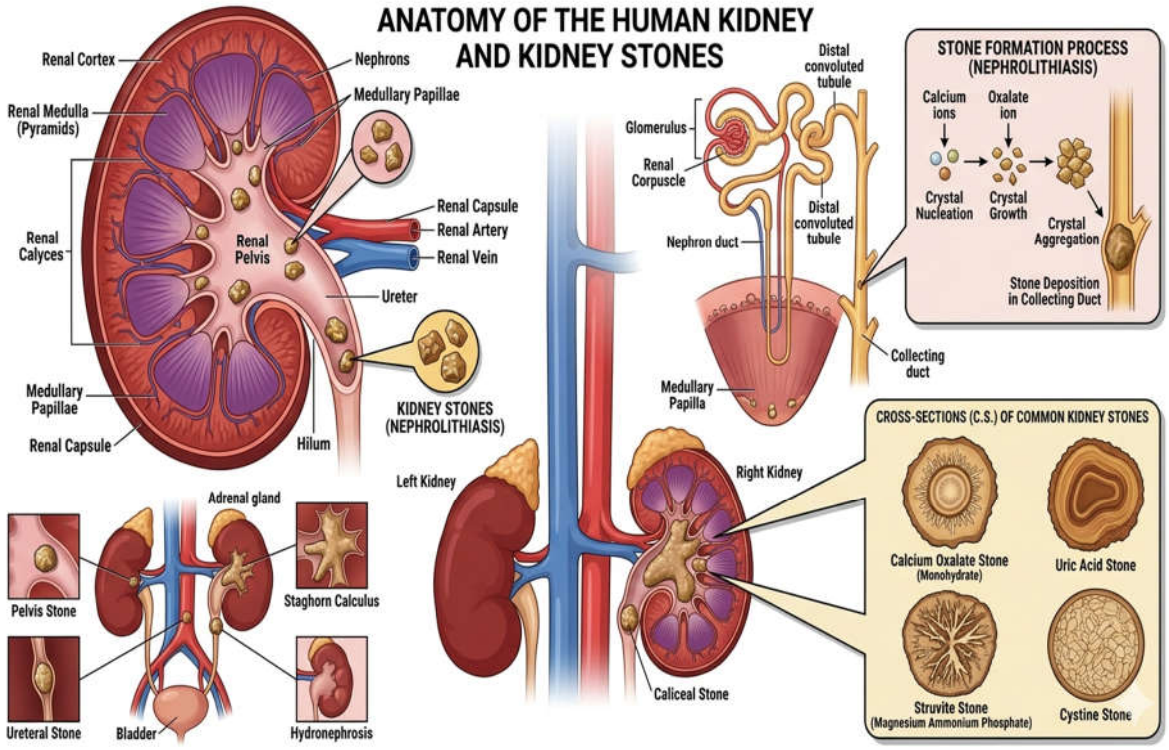

***Figure 1*:** *Anatomy of the Human Kidney and Urinary Stones*

Formation of Kidney Stones

Normally, the body prevents these substances from sticking together, but when this balance is disrupted, tiny crystals start to develop. This commonly happens due to low water intake. Over time, these small crystals join together and grow into hard stones. Factors like diet, family history, infections, and certain health conditions can increase the chances of this process. The stone may stay in the kidney or move through the urinary tract, causing severe, cramping pain [4].

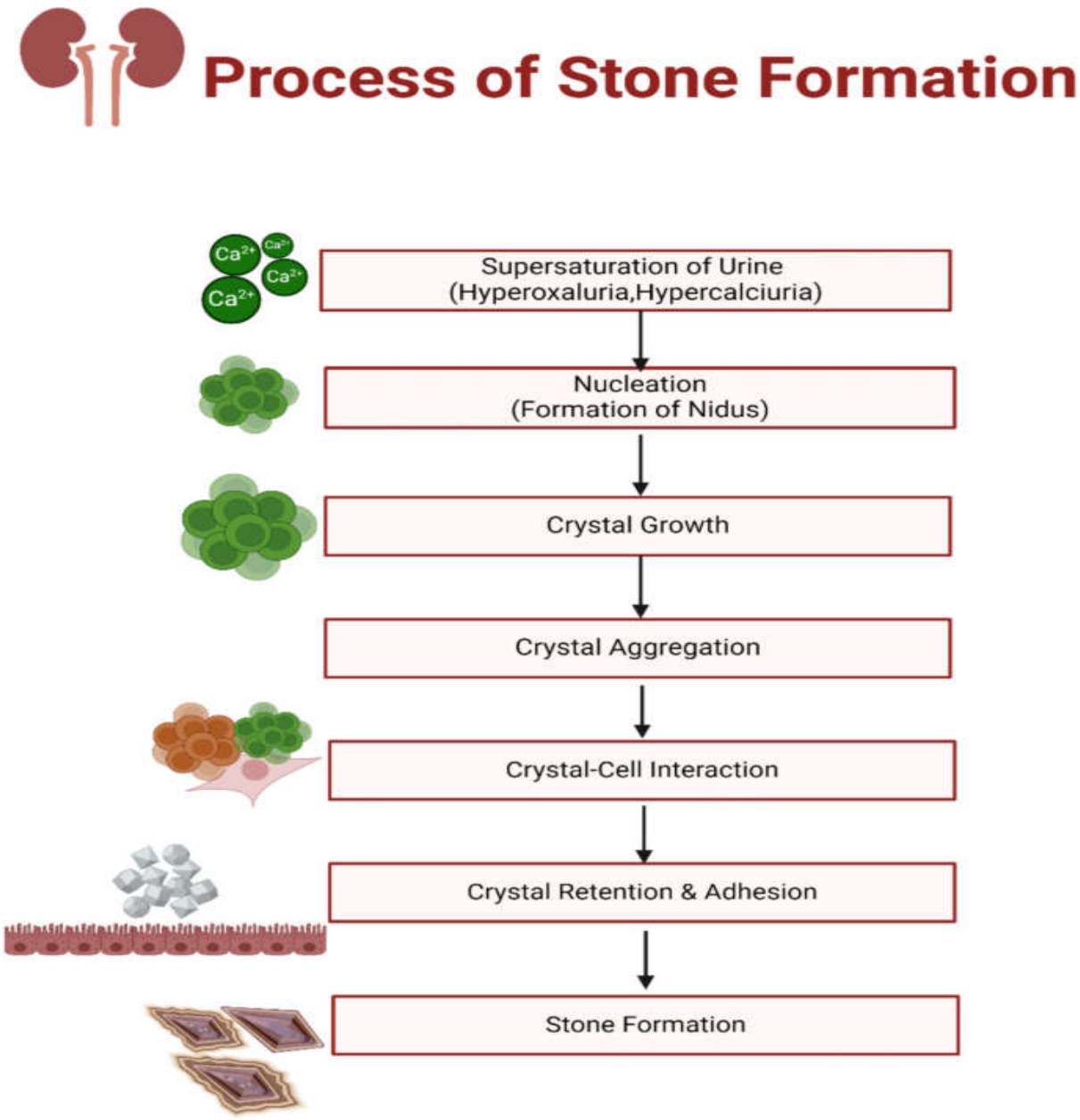

Figure 2*: *Process of Stone Formation

MATERIAL AND METHODOLOGY

Plant Collection and Authentication

Flowers of Clitoria ternatea were collected in April 2026 from Sangola in Solapur district, Maharashtra. The plant material was properly identified and authenticated to confirm its botanical identity. They were then dried in shade at room temperature to help preserve their active compounds. Once completely dried, the material was considered suitable for further extraction and evaluation of antiurolithiatic activity [5,6].

Plant Profile

Figure 3*: *Blue Butterfly Pea

Introduction

Clitoria ternatea, also known as butterfly pea, is a common medicinal plant from the Fabaceae family. It has been used for a long time to help improve memory and reduce stress. Nowadays, it is getting more attention because it contains natural compounds like anthocyanins and flavonoids, which have strong antioxidant and anti-inflammatory effects. It is also used as a natural food color and is known to have antibacterial properties and help control blood sugar levels. One of its less studied uses is in treating kidney stones [7,8].

Scientific Classification of Clitoria ternatea

Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Genus: Clitoria
Species: Clitoria Ternatea [9].

Synonyms of Plant

English: Butterfly Pea / Blue Pea
Sanskrit: Gokarna / Aparajita
Hindi: Aparajita / Koyal Phool
Marathi: Gokarna / Aparajita
Kannada: Shankha Pushpa / Gokarna [10].

Morphological Characteristics of Blue Butterfly Pea

Root: Deep main root with many side branches; light brown in color.
Leaves: Arranged alternately, made up of 5–7 small oval or slightly long leaflets with smooth edges and a green surface.
Flower: Single flowers that grow from the leaf axils; large, pea-shaped, and attractive, usually deep blue with a white or yellow center.
Calyx: Green, tube-like structure that may be slightly hairy or smooth, formed by 5 sepals joined at the base.
Seeds: Hard and smooth seeds, brown to black in color; usually 6–10 seeds present in each pod.
Parts Used: Flowers, roots, and leaves [11].

PROPAGATION

Clitoria ternatea can be grown using seeds, stem cuttings, or layering. The easiest and most common method is growing it from seeds. The seeds are usually soaked before planting in well-drained soil, and they start to sprout within one to two weeks. It can also be grown from healthy stem cuttings placed in moist soil, where they form roots. Another method is layering, where a branch is bent into the soil until it develops roots. This plant grows well in warm conditions with good sunlight and moderate watering [12].

USES

Clitoria ternatea is commonly used as a decorative plant, a medicinal herb, a natural food color, and even as animal feed. In traditional medicine, it is used to improve memory, reduce stress, and help with problems like inflammation, fever, skin conditions, and urinary issues. It also shows antioxidant, antimicrobial, antidiabetic, and antiurolithiatic effects. In addition, the plant helps improve soil quality by fixing nitrogen in the soil [13,14].

PREPARATION OF PLANT EXTRACT CLITORIA TERNATEA

Fresh flowers of Clitoria ternatea are first collected, cleaned, and dried in shade. After drying, the flowers are powdered and used for extraction with suitable solvents to obtain active compounds.

Preparation of Methanolic Extract

About 20 g of dried and powdered Clitoria ternatea flowers are carefully weighed and placed in a filter paper thimble, which is then set inside a Soxhlet apparatus. Around 200 mL of methanol is added to a clean round-bottom flask attached to the setup. The mixture is heated gently so the methanol boils, evaporates, condenses, and continuously passes through the powder for extraction. This process is carried out for about 6 to 8 hours or until the solvent in the siphon tube becomes almost colorless. After extraction is complete, the methanol extract is collected and the solvent is removed by gentle heating or using a rotary evaporator. The dried extract is then weighed, stored in an airtight container [15,16].

Figure 4*: *Extraction Process

PHYTOCHEMICAL INVESTIGATION

The prepared methanolic extract was collected and concentrated. It was then used for phytochemical investigation.

Sr. No.	Name of the Tests	Observations	Inference
1.	**Test for Saponins (Foam test):** Take 2ml extract + 5ml distilled water, shake well form 2min.	Stable froth forms.	Saponins Present
2.	**Test for Phenols:** Take 2 mL extract + add 2–3 drops $FeCl_3$ solution.	Deep blue or green colour appears.	Phenols Present
3.	**Test for Tannins:** Take 2 mL extract + add 2–3 drops 5% ferric chloride.	Blue-black or green colour appears.	Tannins Present
4.	**Test for Terpenoids (Salkowski test):** Take 2 mL extract + 2 mL chloroform, add 1 mL conc. H_2SO_4 carefully.	Reddish-brown layer forms.	Terpenoids Present
5.	**Test for Flavonoids:** Take 2 mL extract + add 2 mL 10% NaOH solution. Then add few drops dilute HCl.	Intense yellow colour appears and disappears after adding acid.	Flavonoids Present

6.	**Test of Carbohydrates (Molisch's test):** Take 2 mL extract + 2 drops Molisch reagent, then 1 mL conc. H_2SO_4 carefully.	Violet ring forms.	Carbohydrates Present
7.	**Test for Proteins:** Take 2 mL extract + 2 mL NaOH + 2–3 drops $CuSO_4$	Violet colour appears.	Proteins Present
8.	**Test for Alkaloids:** **1) Dragendorff's test:** Take 2 mL extract + add 2–3 drops Dragendorff's reagent.	Orange or reddish precipitate forms.	Alkaloids Present
	2) Mayer's test: Take 2 mL extract + add 2–3 drops Mayer's reagent.	Cream or white precipitate forms.	Alkaloids Present
	3) Hager's test: Take 2 mL extract + add 2–3 drops Hager's reagent.	Yellow precipitate forms.	Alkaloids Present
	4) Wagner's test: Take 2 mL extract + add 2–3 drops Wagner's reagent.	Brown or reddish-brown precipitate forms	Alkaloids Present

Table No. 1*: *The Phytochemical components of the extract of Clitoria Ternatea [17,18]

Figure 5*: *Chemical Tests of Clitoria Ternatea

EXPERIMENTAL WORK

Apparatus

Beakers, Conical flask, Measuring cylinder, electronic balance, Water bath, pH meter, Incubator, Microscope, Soxhlet apparatus.

Chemicals Used

Calcium chloride, Sodium oxalate, Sulfuric acid, Potassium permanganate, Ammonia solution, Distilled water, Test sample / Plant extract, Egg membrane, Stonil.

Using Egg to Prepare a Semi-Permeable Membrane

A fresh hen's egg is used to obtain the inner membrane, which acts as a natural semi-permeable membrane in the titrimetric method for antiurolithiatic activity. First, the outer shell is carefully removed without damaging the inner membrane. A small hole is made to remove the contents of the egg, and the membrane is then washed properly with distilled water. After cleaning, the empty membrane is filled with calcium oxalate crystals along with the test sample or plant extract. It is then tied securely and placed in distilled water or a buffer solution for incubation. After the incubation period, the contents are taken out, and the remaining calcium oxalate is measured by titration to evaluate the antiurolithiatic activity [19,20].

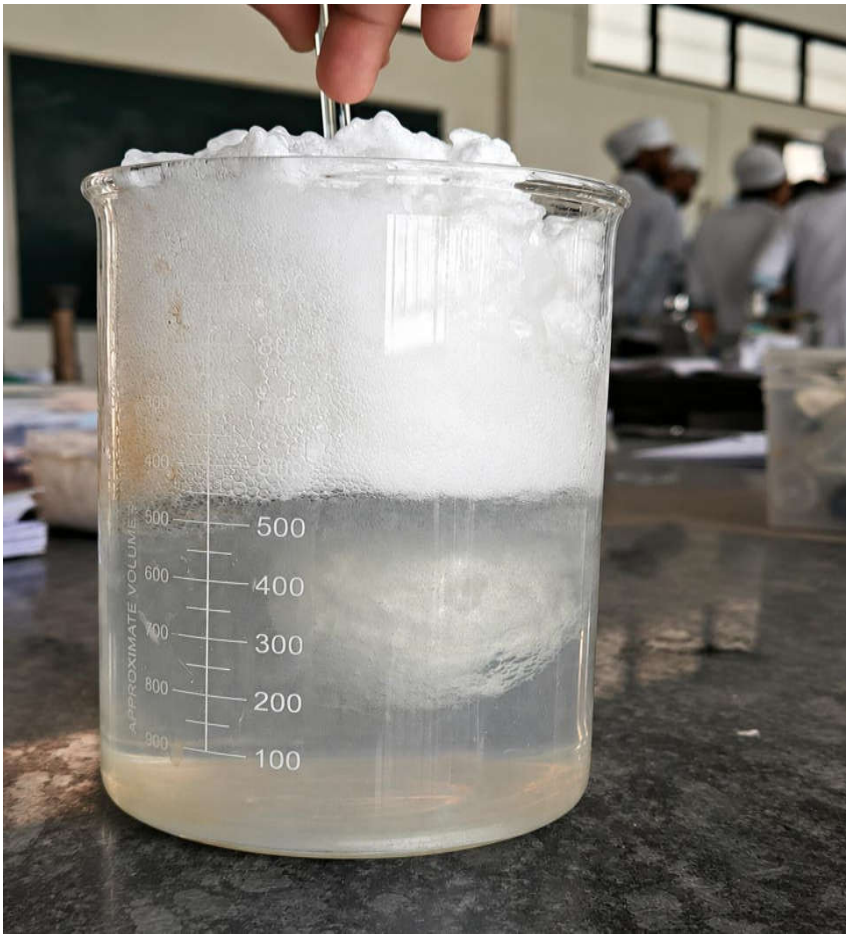

Figure 6*: *Preparation of Semi-permeable Membrane

EXAMINING THE INVITRO ANTIUROLITHIATIC ACTIVITY TEST USING TITRATION

In this in vitro antiurolithiatic study using a titrimetric method, calcium oxalate crystals are first prepared by mixing 50 mL of 0.1 M calcium chloride with 50 mL of 0.1 M sodium oxalate. The solid formed is collected and washed well with distilled water. A fresh hen's egg is then used to obtain the inner membrane by carefully removing the hard outer shell. This membrane is filled with 10 mg of kidney stone crystals and 100 mg of the test sample or plant extract, and then tied properly with a thread.

The prepared membrane is placed in a beaker containing 100 mL of distilled water or a suitable buffer solution and kept at 37°C for about 2 hours. After this, the contents are transferred into a conical flask, and 10 mL of 1 N sulfuric acid is added to dissolve the remaining calcium oxalate. The solution is then titrated with $KMnO_4$ until a light pink color appears and stays for a few seconds. The amount of calcium oxalate dissolved is used to determine the antiurolithiatic activity of the sample [21,22].

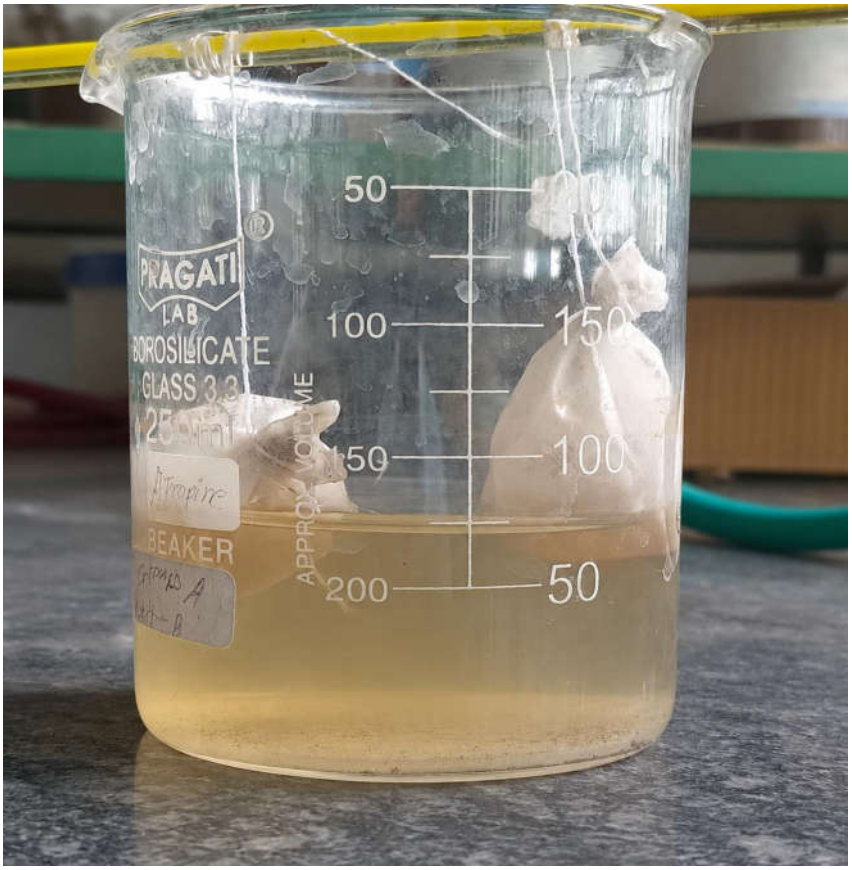

Figure 7*: *Incubation of Semi-permeable Membrane

TITRATION

Figure 8*: *Titrimetric Analysis

Sample	Burette Reading			Mean
	First	Second	Third	
Control (Blank)	10	10.5	9.5	10
Stonil	3.1	3.2	3	3.1
Methanolic Extract	3.8	4	3.6	3.8

Table No. 2*: *Burette Reading

Calculation

$$\% \textbf{ Inhibition} = \frac{\textbf{Control} - \textbf{test}}{\textbf{control}} \times \textbf{100}$$

Were,

Control = Vc

Test = Vt

% Inhibition of Stonil

$$\% \text{ Inhibition} = \frac{\text{Vc} - \text{Vt}}{\text{Vt}} \times 100$$

$$= \frac{10-3.1}{10} \times 100$$

$$= \frac{6.9}{10} \times 100$$

% Inhibition = 69%

% Inhibition of Methanolic Extract of Clitoria Ternatea

$$\% \text{ Inhibition} = \frac{\text{Vc} - \text{Vt}}{\text{Vt}} \times 100$$

$$= \frac{10-3.8}{10} \times 100$$

$$= \frac{6.2}{10} \times 100$$

$$\% \text{ Inhibition} = 62\%$$

Results

Sr. No.	Chemical Constituents in Methanolic Extract	Observations
1.	Saponins	+
2.	Phenols	+
3.	Tannins	+
4.	Terpenoids	+
5.	Flavonoids	+
6.	Carbohydrates	+
7.	Proteins	+
8.	Alkaloids	+

(+) Indicates the Presence of Compounds.
(-) Indicates the Absence of Compounds.

Table No. 3*: *Results of Preliminary Phytochemical Screening of Clitoria Ternatea

% Inhibition

Sr. No.	Sample	% Inhibition
1.	Blank	00
2.	Stonil	69
3.	Methanolic Extract of Clitoria Ternatea	62

Table No. 4*: *Result of % Inhibition

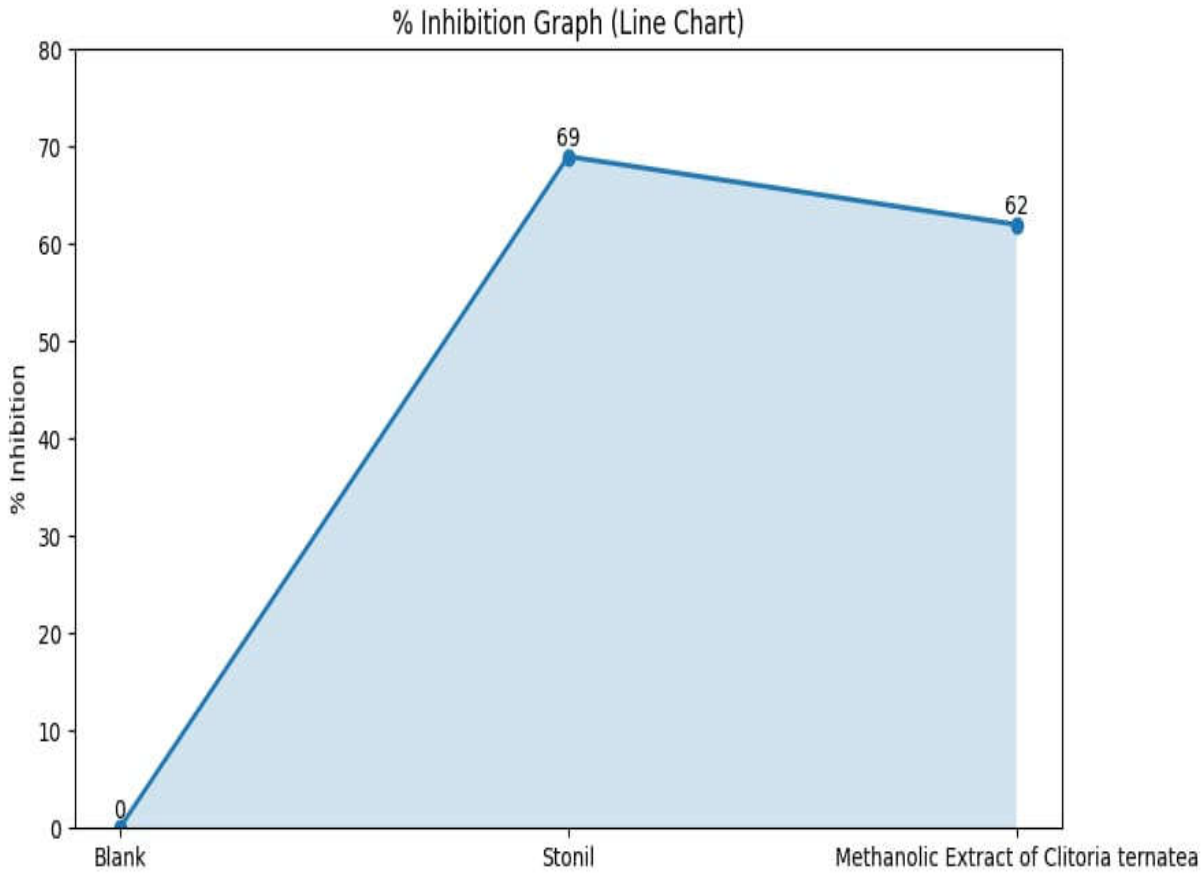

Figure 9**:** *% Inhibition of Blank, Stonil and Methanolic Extract of Clitoria Ternatea*

Discussion

The methanolic extract of Clitoria ternatea showed good anti-urolithiatic activity with 62% inhibition of calcium oxalate crystals, while Stonil showed 69%. This suggests the plant has strong potential in preventing kidney stones, but slightly less effective than the standard. The plant contains compounds like flavonoids, tannins, and saponins, which help reduce crystal formation, protect kidney tissues, and provide diuretic effects. Overall, Clitoria ternatea may be a useful natural option for kidney stone prevention, but more studies are needed to confirm its safety and effectiveness.

Conclusion

The findings of this study indicate that the methanolic extract of Clitoria ternatea possesses considerable antiurolithiatic activity against calcium oxalate crystal formation in vitro. The extract exhibited 62% inhibition, which was comparatively close to the 69% inhibition produced by the standard formulation Stonil, suggesting its potential role in the prevention of kidney stone formation. Overall, Clitoria ternatea shows promise as a natural option for preventing and managing kidney stones, but more studies are needed to confirm its safety and effectiveness.

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