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Assessment of In-Vitro Anti-Urolithiatic Activity of Guilandina Bonduc Extract

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ABSTRACT

Urolithiasis, a formation of urinary stones, is a prevalent condition affecting millions worldwide. Traditional medicinal practices often utilize herbal remedies to prevent or treat urolithiasis due to their perceived efficacy and fewer adverse effects. Guilandina bonduc, a traditional medicinal plant, has been explored for its potential anti-urolithiatic activity, aiming to address the growing prevalence of urolithiasis, or kidney stone formation. This study investigates the bioactive compounds in Guilandina bonduc responsible for its potential therapeutic effects and evaluates the plant's efficacy in preventing and treating urolithiasis. Additionally, it underscores the importance of standardized research methodologies and clinical trials to validate the efficacy and safety of herbal remedies in managing urolithiasis.

***Keywords**: Urolithiasis, Herbal extract, Anti-urolithiatic, Crystallization inhibition, Urinary stones.*

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INTRODUCTION

Overview Of Kidney Stone

Renal calculi, or nephrolithiasis, are other names for the hard deposits of minerals and salts that develop inside the kidneys. They can affect any region of the urinary tract, including the bladder and kidneys, and come in a variety of sizes. Calcium Particles The most prevalent kind, which is often calcium oxalate but can also be calcium phosphate. form as a reaction to an infection in the urinary system. They have a quick growth rate and can get rather big. form due to specific hereditary factors, excessively acidic urine, and gout. cystine stones uncommon and appear in patients with a genetic illness that leads to the kidneys excreting an excessive amount of cystine. Frequently referred to as one of the worst aches, back discomfort typically initiates the agony.[1]

The Urinary System & Stones: The kidneys, or renal system, are in charge of eliminating waste from the body and preserving the proper balance of fluids and minerals. These are the parts that make it up. bean-shaped organs that are situated on the spine's sides. To create and remove waste products, they filter blood. Urine travels from the kidneys to the bladder through two tiny tubes. an organ that is muscular and hollow that holds pee until the body eliminates it. The bloodstream is replenished with necessary materials such as glucose, water, and electrolytes. Urine contains extra ions and waste materials that are secreted. Kidney stones can occur anywhere in the urinary tract and vary in size and content. They are also referred to as renal calculi or nephrolithiasis.[2]

Types

Kidney stones can vary in composition, with different types forming based on the substances that crystallize and aggregate within the urinary tract.[3]

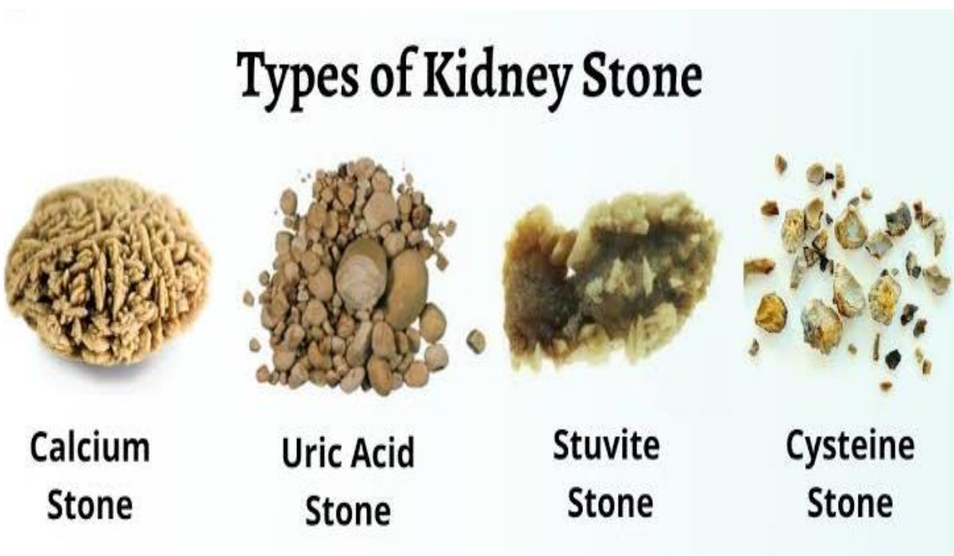


Fig. No.1: Varieties of kidney stones

Eighty percent of kidney stone cases are calcium stones, making them the most frequent form. Calcium mixed with either Diabetic Stones They are frequently referred to as infection stones and have a rapid growth rate. Rare cases of cystinuria, a genetic condition in which the kidneys discharge an excessive amount of the amino acid cystine, result in the formation of cystine stones.[4]

Mechanism of Calcium Oxalate Renal Stone's Formation.

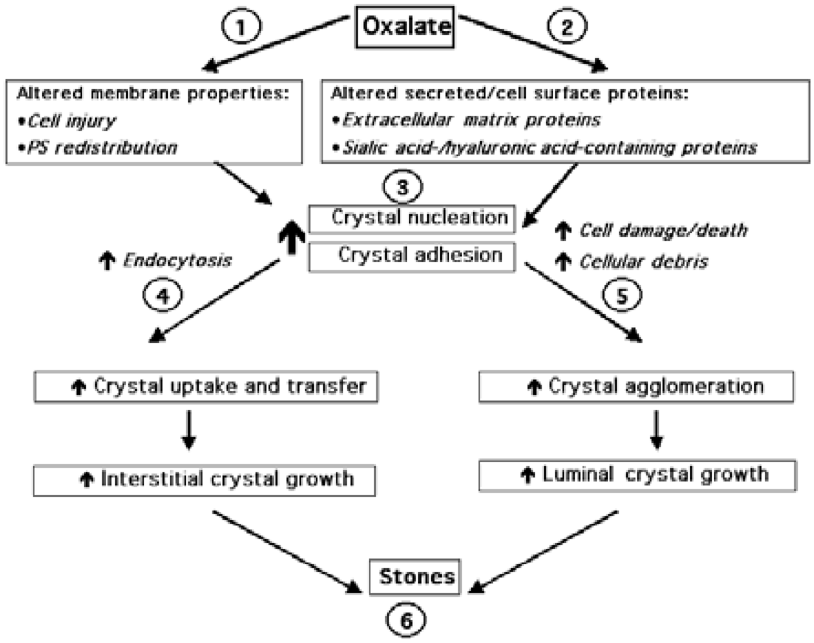


Fig. No. 2.: Pathway of Renal Stone Formation

Pathophysiology of Urolithiasis Diseases

The pathogenesis of kidney stones, or renal calculi, involves the formation of solid mineral deposits within the kidneys. These deposits can cause pain, urinary tract obstruction, and other complications. The pathogenesis is a complex process influenced by various factors, including urinary supersaturation, crystal formation, and retention. Kidney stone formation begins when urine becomes supersaturated with certain minerals or compounds, exceeding their solubility. This supersaturation creates conditions favorable for crystallization.[5]

Diagnosis of urolithiasis diseases

Ultrasound: This non-invasive method is often the first choice, especially for pregnant women and children. It can detect stones in the kidneys, ureters, and bladder and assess urinary tract obstruction. X-ray (KUB) However, it may not be effective for smaller or radiolucent stones. Intravenous Pyelogram (IVP): This procedure, which is less used these days, uses contrast dye injected into the bloodstream to see the urinary system and identify stones.[6]

Treatment and prevention of urolithiasis diseases

Hydration: Patients are advised to drink plenty of fluids to increase urine volume, which helps flush out smaller stones.

Surgical Intervention Open Surgery: Although rare, this needed "staghorn" complex cases where minimally invasive techniques are insufficient.

Prevention

Increased Hydration: Drinking sufficient water.

Dietary Changes: The recommendation to patients is to minimize sodium, cut back on foods high in oxalate, and continue to get a healthy amount of calcium. It can be advantageous to steer clear of too much animal protein.

Regular Exercise: Physical activity can improve overall health.[7]

PLANT PROFILE

Fig. No. 3.: Guilandina Bonduc

Guilandina bonduc, also known as Caesalpinia bonduc or Bonducella. It is commonly referred to as "Gray Nicker" or "Nickernut," among other names. This literature review provides an overview of the key aspects of Guilandina bonduc, including its botanical characteristics, traditional uses, phytochemical composition, and pharmacological activities.Guilandina bonduc is a perennial climbing shrub characterized by thorny branches and bipinnate leaves. Its flowers are small, yellow, and arranged in racemes. The plant produces hard, round seeds with a glossy grayish-green appearance. These seeds, often called "nickernuts," are a notable feature and are used in various traditional contexts[8]. Guilandina bonduc has been studied for its diverse range of phytochemicals, including alkaloids, flavonoids, glycosides, tannins, saponins, and terpenoids. The seeds contain significant levels of oil and protein, making them a potential source for nutritional applications. Researchers have identified specific compounds like bonducin, a bitter glycoside, as well as various amino acids and fatty acids. In Ayurveda, the seeds are used to treat fever, arthritis, diabetes, and gastrointestinal disorders.The seeds have also been used to make decorative items, such as necklaces and bracelets.[9]

Scientific Classification

Kingdom: Plantae
Division: Magnoliophyta (Angiosperms)
Subdivision: Angiospermae
Class: Magnoliopsida (Eudicots)
Order: Fabales
Family: Fabaceae
Subfamily: Caesalpinioideae
Genus: Guilandina

MORPHOLOGICAL CHARACTERISTICS

Stems: The stems are woody and covered with sharp thorns or spines, which can grow in pairs along the branches. These thorns provide the plant with a form of defense against herbivores and also aid in climbing.[10]

Leaves: The leaves are bipinnate, meaning they consist of multiple pairs of leaflets arranged on a central stem. The leaflets are ovate to oblong in shape, with a slightly pointed tip, and are typically dark green in color. Each leaflet has a smooth margin.[11]

Flowers: Developed from flattened segments of subapical marginal areoles, the evening nocturnal emerging flowers are solitary, sessile, limp and drooping after anthesis, and possess a powerful fragrance (25-30 cm long, 12-17 cm broad).

It is distinguished by short bracteoles (sevales) and a naked, green pericarpel that slopes gently. 13–18 cm long, brownish, curved receptacle tube with distant, narrow triangular to lanceolate scales that are 90 degrees bent forward and vary in length from 3 to 10 mm. There are several 8–10 cm long, linear to oblanceolate, sharp, often recurved outer perianth segments that are colored pale green, pinkish red, reddish, or amber. Several pale cream 3-3.5 mm long anthers with short, weak filaments that are either white or greenish white in color are attached to the throat-inserted stamens. The 15–2 stigma lobes are narrowly linear, white or cream in color, 1.6–1.8 mm long overall, curved, 20–22 cm long, 4 mm thick, and equal in length to the inner tepals. Style: white or greenish white (or reddish-orange). Ovules on the parietal placenta of the inferior, unilocular ovary. Usually abandoned following anthesis.[12]

Leaves: The leaves are compound, usually bipinnate, with several pairs of pinnae. Each pinna has several pairs of smaller leaflets, typically oval or elliptical in shape, with smooth edges.

Fruits: The fruit is a large, round to elliptical pod with a hard and spiny outer covering. The spines are sharp and protect the seeds within. The pod splits open when mature, releasing the seeds.

Seeds: The seeds are large, hard, and round, resembling marbles. They are commonly known as "nickernuts" and can vary in color from gray to brown. Due to their hardness, they are sometimes used in traditional games or as beads.[13]

Roots: Guilandina bonduc has a robust root system that helps it anchor in sandy or loose soils.

Blooming season: The exact blooming seasons can vary depending on factors such as local climate, rainfall patterns, and specific environmental conditions. In some regions, Guilandina bonduc may exhibit peak flowering during the wet season when conditions are more favorable for growth and reproduction. However, it can also produce flowers sporadically during other times of the year, depending on localized conditions. Overall, its ability to bloom year-round contributes to its resilience and adaptability in various ecosystems.

Pharmacognostic characteristics of Guilandina bonduc:

Pharmacognostic characteristics of Guilandina bonduc encompass a range of botanical, chemical, and physical traits that are relevant to its medicinal properties and identification.

Organoleptic Characteristics:

The organoleptic characteristics of Guilandina bonduc refer to the sensory properties that can be perceived by the human senses, particularly taste, odor, and appearance.

Parts Used:

Leaves, Stem, Flowers, Seeds.

Propagation:

Seeds are the most common method of propagation. They have a hard coat that needs to be scarified (nicked with a file or soaked in hot water) to improve germination. Soak seeds overnight before planting them at a depth of 1-2 inches in pots or directly in the ground. Seeds germinate in 3-4 weeks. Take stem cuttings from mature plants during the growing season. Choose cuttings that are 6-8 inches long and have a few nodes. Plant the cuttings in pots filled with moist potting mix and keep them in a warm, humid location. Stem cuttings should root in 4-6 weeks.[13]

Benefits & Uses:

The seeds and leaves of G. bonduc have been used in traditional medicine to relieve colic, fever, hydrocele, diarrhea, and rheumatism.

The leaves and roots are used as an astringent, febrifuge (reduces fever), and vermifuge.

Scientific studies have shown that G. bonduc has potential anti-cancer, anti-malarial, antimicrobial, and anti-inflammatory properties. However, it is important to note that these studies have been conducted in laboratories.

The hard seeds of G. bonduc are sometimes used to make jewelry, beads, and good luck charms. The plant can also be used as a live fence or hedge.[14]

MATERAILS & METHODOLOGY

Plant Collection and Authentication.

Indian state of Maharashtra's Solapur District is home to Sangola, where leaves of Guilandina bonduc were collected in March 2024. Dr. R. R. Tembhurne of the Department of Botany and Plant Physiology confirmed the legitimacy of the plant. After washing with tap water, the leaves were dried in the shade.[15]

Preparation Of Plant Extracts Guilandina bonduc.

The fresh stem and leaves were ground into a coarse powder in a machine grinder, dried, and cleaned under running water.

Preparation of Ethanolic Extracts.

A few changes were made to the extract preparation techniques from those outlined in. After being cleansed with ordinary water and dried, the leaf sample was placed in a blender to be ground into a powder. Ethanol is ulised in a range of ratios as a solvent for the Soxhlet extraction procedure. After being collected for 6 to 8 hours, the extract should be combined with a muslin cloth, transferred to 50 ml tubes, and centrifuged for 15 minutes at 4,000 rpm and 25 °C. Once collected, the supernatant was set aside for drying.[16]

PHYTOCHEMICAL INVESTIGATION [17]

Preliminary phytoconstituents detected in the ethanol extract of Guilandina bonduc were based on the following chemical analyses.

Sr. No.	Name of Test	Observation	Inference
1	Test for Saponins: A test tube containing the extract was filled and shake vigorously.	Formation of stable foam.	Saponins Present.
2	Test for Phenols: Mixture of extract and 2 milliliters of 2% Fecl3 solution.	Blue/green colour	Phenols Absent.
3	Test for Tannis: Mixture of extract and 2 milliliters of 2% Fecl3 solution.	No Black Colour	Tannins Present.
4	Test for Terpenoids: Chloroform (2 ml) was combined with the extract. After that, 2 milliliters of concentrated sulfuric acid were added and gently shaken.	Reddish-brown colour seen during the transition.	Terpenoids Present.
5	Test for Flavonoids: A few drops of sodium hydroxide solution were added to the extract.	A strong yellow tint that fades to colorlessness when diluted acid is added.	Flavonoids Present.

6	Test for Carbohydrates: 2ml of glacial acetic acid with a few drops of 2% FeCl3 were combined with the extract and then transferred into a second tube that had two milliliters of concentrated sulfuric acid.	A brown ring at the inter-phase.	Carbohydrates Present.
7	Test for Protein: A little amount of strong nitric acid was added to the extract.	The formation of yellow colour.	Protein Absent.
8 A)	Test for Alkaloids: Dragendroff's Test: One or two milliliters of Dragendroff's reagent—potassium bismuth iodide solution—were added to a few milliliters of extract.	Orange brown coloured ppt.	Alkaloids present.
B)	Mayer's Test: Two drops of Mayer's reagent (potassium mercuric iodide solution) were added to a few milliliters of extract.	Cream Coloured ppt.	Alkaloids present.
C)	Hager's Test: One or two milliliters of Hager's reagent (a saturated solution of picric acid) were added to a few milliliters of extract.	Yellow coloured ppt.	Alkaloids present.
D)	Wagner's Test: To a few ml of the extract, few drops of Wagner'sreagent (iodine in potassium iodide) were added.	Reddish Brown coloured ppt.	Alkaloids present.

Table No.1: The phytochemical components of the extract of Guilandina bonduc.

INVESTIGATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY TEST BY TITRIMETRY:

Fig. No. 4: Decalcification of egg shell in H_2SO_4 overnight

PROCEDURE:

The lab produced calcium oxalate (CaOx) experimental kidney stones by mixing an equimolar solution of sodium oxalate in 10 ml of 2N H2SO4 with calcium chloride dehydrate in distilled water. The result of giving both enough distilled water to react in a beaker was calcium oxalate precipitate. The precipitate was cleaned with distilled water and dried at 60°C after any remaining sulfuric acid was removed using an ammonia solution. As seen in the model created below, 1 mg of the extract and 10 mg of calcium oxalate were carefully mixed in an egg's semi-permeable membrane to measure the percentage of calcium oxalate that dissolved. This was left to float in 100 milliliters of 0.1M Tris's buffer within a conical flask. Just one milligram of calcium oxalate was given to the first group as a control. The second group functioned as a positive control and contained 1 mg of calcium oxalate and 10 mg of Cystone. In addition to 1 mg of calcium oxalate, the third and fourth groups contain ethanolic extracts. Each group's conical flasks were kept in an incubator for two hours after it had been heated to 37 degrees Celsius. Fill separate test tubes with the contents of the semi-permeable membranes from each group. Put two milliliters of IN sulfuric acid into each test tube. After that, titrate with 1N KMnO4 until the end point is reached and the color turns a vivid pink. The total amount of calcium oxalate dissolved by various solvent extracts is calculated by deducting the amount of undissolved calcium oxalate that remained after the experiment's first run.[18]

TITRATION

Sample	Burette reading			Mean
	First	Second	Third	
Blank	0	0	0	0
Ethanolic Extract	8.4	9.0	8.9	8.7

Table No. 2: Burette Reading

Percentage purity by titrimetric method,

$$\% \textbf{ Purity} = \frac{\textbf{Burette reading} \times \textbf{Factor} \times \textbf{Mole (cal)}}{\textbf{Wt. of sample} \times \textbf{Mole (known)}} \times \mathbf{100}$$

$$= \frac{8.7 \times 0.094 \times 2}{1 \times 1} \times 100$$

$$= 1.6356 \times 100$$

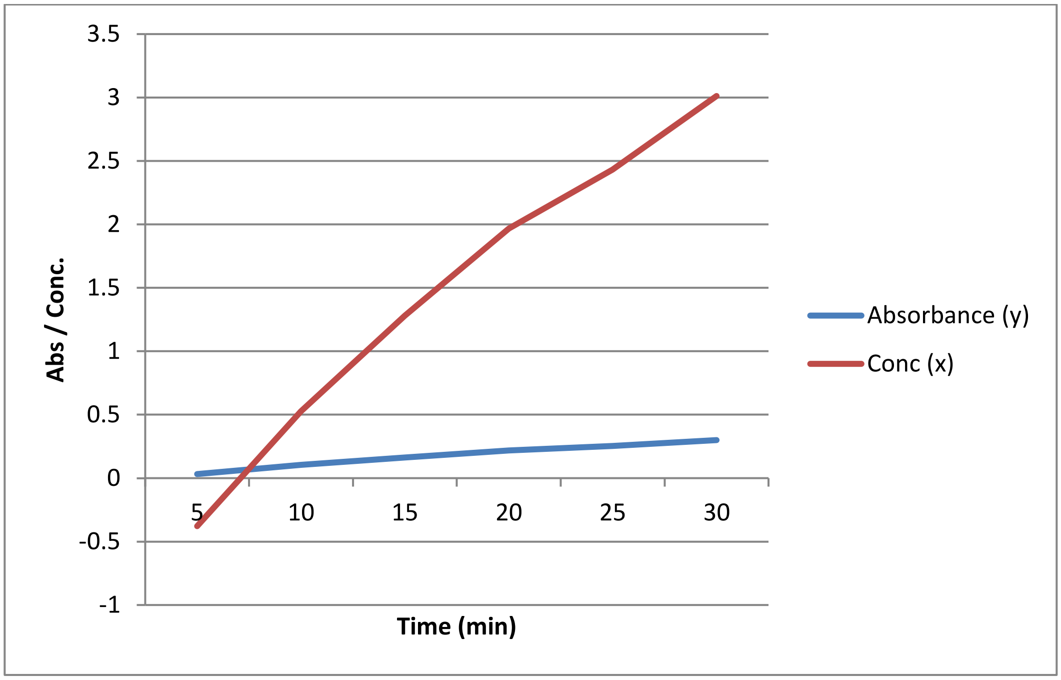
$$\% \text{ Purity} = 163.56\%$$

UV SPECTROSCOPY:

Highest peak at 3.00 Abs at 337 nm,

Sr. No.	Time (min)	Absorbance (y)	Conc. (x)
1	5	0.0321	- 0.3784
2	10	0.1036	0.526
3	15	0.1633	1.282
4	20	0.2175	1.968
5	25	0.2542	2.432
6	30	0.3000	3.012

Table No. 3: Measurement of Absorbance of Sample.



Graph No. 1. Absorbance and concentration of Sample

Time (min)	Abs.	Conc.	Dil.	Conc. (5%)	Cumulative (5%)	Conc. (900%)	Cumulative (900ml)ug	Cumulative (mg)	%
5	0.0321	-0.372	-3.78	-18.92	-18.92	-3405.6	-3405.6	-3.4056	6.8
10	0.1036	0.526	5.26	26.3	7.38	4734	4715.08	4.71508	9.4
15	0.1632	1.282	12.82	64.1	71.48	11538	11545.38	11.5453	23
20	0.2175	1.968	19.68	98.4	169.88	17712	17783.48	17.7834	35.56
25	0.2542	2.432	24.32	121.6	291.48	21888	22057.88	22.0578	44.11
30	0.3000	3.012	30.12	150.6	442.08	27108	27399.48	27.3994	54.79

Table No.4: Calculation of % dissolution rate

% Dissolution rate for the Ethanolic Extract,
For 30 min,

$$\% \text{ dissolution rate} = \frac{27.3994}{50} \times 100$$

$$\textbf{\% dissolution rate} = \textbf{54.79\%}$$

RESULTS

Sr. No.	Constituents in Ethanolic Extract	Observation
1	Saponins	+
2	Phenols	-
3	Tannins	+
4	Terpenoids	+
5	Flavonoids	+
6	Glycosides	+
7	Proteins	-
8	Alkaloids	+

Table No.5 Result of preliminary phytochemical of *Gunlandina Bonduc.*

Percentage Purity of Ethanolic Extract by Titrimetric method was found to be 163.56%

This study evaluates the ethanolic extract of Guilandina Bonduc antiurolithiatic activity. The calcium oxalate, or "CaOx," dissolved at the fastest rate (54.79%) in the thanolic extract. Hanolic extracts from Guilandina bonducwere found to be more effective in dissolving calcium oxalate.

Sr. No.	Groups	Guilandina Bonduc
1	Blank	0
2	Positive Control	73%
3	Ethanolic extract	54.79%

Table No. 6: Shows % dissolution of calcium oxalate (CaOx) by Guilandina Bonduc leaves extracts

DISCUSSION

Alternative medications that have less side effects than existing therapies are needed for kidney stones, commonly known as urolithiasis, which is a serious global health concern. The possibility of bioactive components and historical use in folk medicine make Guilandina Boduc a promising candidate for this sort of study.

After preparation in accordance with the experimental design, guilandina boduc extract is subjected to in-vitro models that imitate conditions relevant to urolithiasis. Details such kidney stone type, concentration of extract, and duration of treatment are taken into account. To determine how effective the extract is, measurements of crystal growth inhibition, nucleation time, aggregation, and crystal form are required.

The extract's ability to alter urine pH, facilitate the transit of stones through the urinary system, inhibit crystal formation, and possess anti-inflammatory and antioxidant qualities are all investigated through mechanistic studies, which look at the underlying processes of action.

It is possible to compare the extract's effectiveness against existing treatments by including positive control groups that were treated with traditional anti-urolithiatic drugs.
Data analysis and interpretation, which also emphasize trends, correlations, and statistically significant findings, demonstrate the extract's capacity to prevent or dissolve kidney stones. For the creation of novel anti-urolithiatic medications, the study is important. Along with suggesting areas for more study, it also lists clinical trials to determine the treatment's effectiveness in humans as well as safety profiles and any side effects.
To sum up, studies on the in-vitro anti-urolithiatic effectiveness of Guilandina Boduc extract offer valuable new insights into natural kidney stone therapy approaches. Based on thorough research into its efficacy and mechanisms of action, guilandina boduc extract exhibits promise as a potential natural alternative or supplemental therapy for kidney stone prevention and treatment.

CONCLUSION

In conclusion, the research on Guilandina Boduc extract's in-vitro anti-urolithiatic activity concludes that there is considerable potential for kidney stone prevention and therapy. The extract has been shown via methodical investigation to be effective in preventing crystal formation and encouraging disintegration. These results open the door for more investigation and clinical validation of Guilandina Boduc's therapeutic effects as a useful natural treatment for urolithiasis.

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