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International Journal of Pharmacy and Herbal Technology (Online)

Home Page: https://www.ijprdjournal.com/

ASSESSMENT OF IN VITRO ANTI-UROLITHIATIC ACTIVITY OF VACHELLIA EBURNEA

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ABSTRACT

Urolithiasis, affects millions worldwide, causing severe pain and potential renal damage. Traditional treatments include invasive surgical procedures and pharmacological therapies, which often have limitations such as high costs, side effects, and recurrence of stones. Consequently, there is a growing interest in exploring natural remedies with fewer side effects and cost-effective properties. Vachellia eburnea, a plant known for its medicinal properties in traditional medicine, particularly in treating urinary disorders, offers a promising alternative Urolithiasis, a significant global health problem, involves the formation of stones in the urinary system, primarily composed of calcium oxalate. The condition can cause severe pain, urinary obstruction, and potential renal damage. Conventional treatments include surgical intervention and pharmacotherapy, which often carry risks of recurrence, high costs, and adverse side effects. Therefore, there is an increasing demand for alternative therapies derived from natural sources. Vachellia eburnea, traditionally used in folk medicine for treating various ailments, including urinary disorders, presents a promising candidate for exploring its anti-urolithiatic potential.

Keywords: *Urolithiasis, anti urolithiatic activity, calcium oxalate crystal, Phytochemical screening, ethanol extract, kidney stones, herbal medicine, crystal growth inhibition.*

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Received on 06 July, 2024, Accepted 15 July, 2024

Please cite this article as: Kadam Vaishnavi et.al Assessment of In Vitro Anti-Urolithiatic Activity of Vachellia Eburnea
International Journal of Pharmacy And Herbal Technology 2024.

INTRODUCTION

Urolithiasis, commonly known as kidney stones, is a urinary problem caused by the formation of stones in the kidneys, bladder or urethra. These stones are usually made of calcium oxalate, calcium phosphate, uric acid, struvite or cysteine. The formation of these stones is a complex process that includes supersaturation, nucleation, growth, aggregation, and storage in the kidney. One such tree is Vachellia eburnea (formerly Acacia eburnea), a member of the legume family. However, its ability to treat urinary stones has not yet been studied. This study aims to evaluate the anti-urolytic activity of ebony leaf extract in vitro, focusing on its ability to inhibit crystallization of calcium oxalate, a component of kidney stones.
Leaves of Vachellia eburnea (formerly Acacia eburnea) have been examined for their urolytic activity; This refers to their ability to prevent or treat urolithiasis (urolithiasis).[1] Research shows that extracts from these leaves contain compounds that prevent kidney stone formation and promote the dissolution of kidney stones. These products may be due to the presence of bioactive substances such as flavonoids, tannins and saponins, which interact with the stone formation process and reduce crystallization, thus offering a natural option for the treatment of urolithiasis. Urolithiasis refers to the ability of products to prevent or treat urinary stones, diseases caused by the formation of stones (dental calculus) in the urine, including the kidneys, bladder and urethra. These stones often consist of mineral salts and can cause severe pain, urinary tract infections, and infections.
Natural plant extracts have attracted interest due to their bioactive compounds due to their potential urolytic properties[2]. Vachellia eburnea, a plant known for its medicinal properties, is an example. Research on ebony leaves has yielded positive results, showing that it contains substances that prevent stone formation and promote the dissolution of existing stones. This effect is mainly due to plant flavonoids, tannins and saponins and can be achieved by various processes such as reducing supersaturation of rock formation salts, modifying crystal aggregates and modifying concrete support. Therefore, anti-inflammatory leaf extract can be used as medicine for the treatment and prevention of urolithiasis.

Overview of kidney stones

Kidney stones, also known as nephrolithiasis, are substances deposited in the kidneys by substances often found in urine. It caused by chemicals; calcium oxalate. Other types include calcium phosphate, uric acid and struvite. Factors that contribute to this deficiency include dehydration, dietary factors (such as consuming foods high in oxalates or consuming too much salt), metabolic disorders, certain medications, and the following conditions.[3] vary in size and location. Small stones may pass through the urine unnoticed or cause discomfort; Large stones can cause severe pain, often described as a sharp or stabbing pain, from the back spine or both sides towards the stomach and groin. Other symptoms may include blood in the urine, frequent urination, urgency, nausea and vomiting.

Epidemiology of kidney stones

Epidemiology of kidney stones concerns the study of their formation, distribution, and resolution in humans. Here are some important facts about the spread of kidney stones.[4] Prevalence: Kidney stones are a common disease worldwide. Epidemic rates vary between populations and regions. For example, it is estimated that approximately 10-15% of adults will develop kidney stones at some point in their lives . However, this gap is closing and rates are increasing for women.

Urinary system of stones

The urinary tract, also known as the renal system. The body contains bladder and urethra. Kidney stones, also known as nephrolithiasis or nephrolithiasis, can form anywhere in the urine and can vary in size and composition. They play an important role in filtering waste and waste products from the blood to produce urine. Kidney stones form in the kidneys when substances such as calcium, oxalates, uric acid or cystine crystallize and accumulate. When kidney stones travel from the kidneys to the bladder, the stones can lodge in the ureters and cause severe pain and discomfort. The ureter determines the severity of symptoms

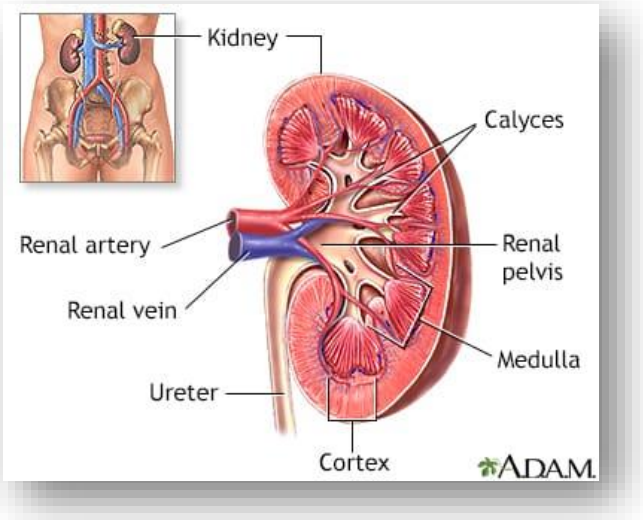


Figure No.1: Location of Stone

Types of kidney stones

Kidney stones vary in composition and form different types depending on the material that crystallizes and accumulates in the urine. kidney stones include:

Calcium stones

The constitute approximately 80% of kidney stones. Calcium stones can be divided into two types:

Calcium oxalate stones: These stones form when calcium combines with oxalate, a byproduct of the liver, found in some foods. High oxalate levels in urine increase.[5] Calcium phosphate stones: These stones are rare and usually occur in people with diseases that make the urine alkaline.

Uric acid stones

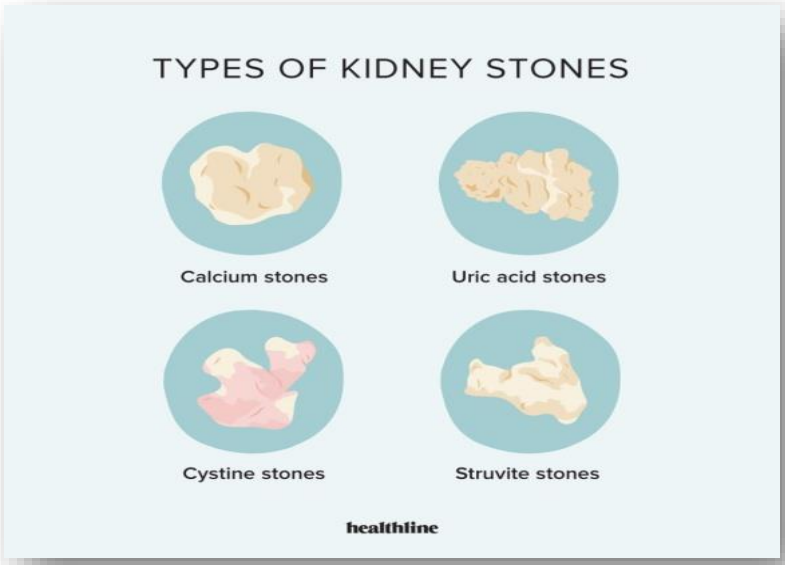
When the uric acid is high in the urine, it can cause urate crystals to form. Factors that may increase the risk of uric acid stone formation include a high-purine diet, gout, and conditions that cause excess uric acid or low urine pH.

Struvite stones

It also known as bacterial stones, form in presence of urease bacteria. These enzymes hydrolyze urea to Ammonia, which causes alkaline urine pH and the formation of struvite crystals.[6] Struvite stones are frequently associated with urinary tract infections, especially infections caused.

Cystine stones

Cystine stones are rare and develop due to a genetic disease called cystinuria. People with cystinuria have kidney deficiency of the amino acid cystine, which causes more cystine to be found in the urine.[7] At the same time, cystine can crystallize and form stones.



FigureNo.2: Type Of Kidney Stones

Mechanism of Action: To elucidate the possible mechanisms by which Vachellia eburnea extracts inhibit stone formation, focusing on parameters like crystal size reduction and prevention of aggregation.

Various steps for stone formation

Supersaturation

A solution is that contains more dissolve solute that could be dissolved under normal conditions is called supersaturation. Under these circumstances, solutes precipitate in urine, leading to nucleation and crystal formation.

Nucleation

Nucleation is the transformation from liquid to a solid phase in a supersaturated solution. The beginning of this process consists of coalescence of stone salts in clusters which can increase in size with the addition of new components.[8] Nucleation can be homogeneous if it consists only if solute molecules, or heterogeneous if nucleating agents (such as cellular debris, epithelial cell, red blood cells, urinary casts and proteins) are present.

Crystal Growth

The process whereby crystal components are added to the crystal growth.

Crystal Aggregation

Crystal aggregation consists of crystal in solution combining to form lager particles.

Crystal-Cell interaction

Crystal -cell interaction may occur during progressive crystal growth.[9] These interactions including adhesion and endocytosis appear to be essential for the development of urinary stones. Crystal can also be transported to the interstitium.

These interactions can be inducing the epithelial cell injury, which causes increased expression and the synthesis of proinflammatory macromolecules. This process is a predisposing factor for the formation of stones.

Stone Formation

The aggregation of further crystal and organic components leads to the formation of concretion called kidney stones.

Pathophysiology of Urolithiasis

The pathogenesis of nephrolithiasis or nephrolithiasis is the formation of stones in the kidneys. These deposits can cause pain, urinary tract obstruction, and other problems. Its pathogenesis turns into a complex process influenced by many factors, such as urine supersaturation, crystal formation and processing. [10] Kidney stones begin to form when urine becomes saturated with certain foods or compounds and exceeds their solubility. This supersaturation creates favorable conditions for crystallization.

Urolithiasis can have the infectious cause such as Urolithiasis, or kidney stone formation, can have an infectious cause. This type of kidney stone is often referred to as a struvite stone or infection stone.[11] Struvite stones form in response to an upper urinary tract infection with urease-producing bacteria Management of struvite stones often requires treating the underlying infection with antibiotics, as well as potentially removing the stones through surgical means to prevent recurrence. Medications used to manage and prevent kidney stone formation depend on the type of stones and their underlying causes.

Calcium Stones (Calcium Oxalate or Calcium Phosphate)

Thiazide Diuretics (e.g., hydrochlorothiazide, chlorthalidone): Reduce calcium excretion in the urine.

Potassium Citrate: Helps to alkalinize the urine and bind calcium, reducing stone formation.

Uric Acid Stones

Allopurinol: Decrease uric acid production.

Potassium Citrate: Alkalinizes the urine to prevent uric acid stone formation.

Struvite Stones (Infection Stones)

Antibiotics: To treat the underlying urinary tract infection caused by urease-producing bacteria.

Acetohydroxamic Acid (AHA): An enzyme inhibitor that can reduce stone formation in some cases.[12]

Cystine Stones

Potassium Citrate: Helps to alkalinize the urine.

Penicillamine or Tiopronin: These drugs help bind cystine, making it more soluble and less likely to form stones.

General Preventive Measures

Pain Relief: NSAIDs stronger pain relief like opioids, may be prescribed for pain management during stone passage.

Hydration

Encouraging high fluid intake to dilute the urine

Dietary Modifications

Depending on the type of stone, dietary changes may be recommended, such as reducing sodium, oxalate-rich foods, and purines.[13]

Medications& Prevention

Medications for kidney stones aim to manage pain, facilitate the passage of the stones, or prevent future stones. Here are common medications used:

Pain Relievers

NSAIDs: Ibuprofen, naproxen.[14]

Opioids: For severe ache, but typically used with caution due to the risk of addiction.

Medications to Prevent Stone Formation

Thiazide Diuretics: Hydrochlorothiazide reduces calcium in the urine.

Potassium Citrate: Helps prevent the uric acid stones.

Allopurinol: Used by reducing uric acid levels in the blood and urine.

Sodium Bicarbonate or Sodium Citrate: Helps to alkalize the urine, making it less acidic and less likely to form certain types of stones.[15]

Antibiotics

If there is a urinary tract infection associated with the stones

Home Remedies of kidney stones

Hydration

Aim for 8-12 glasses a day to help flush out the urinary system and potentially pass smaller stones.

Citrus Juice

Lemon Juice: Contains citrate, which can help prevent stone formation.

Orange Juice: Also contains citrate and can help in reducing the risk of stones.

Dietary Adjustments

Limit Sodium Intake: High sodium can increase the amount of calcium in your urine.

Reduce Oxalate-Rich Foods: Spinach, nuts, and tea are high in oxalates, which can contribute to stone formation.[16]

Moderate Protein Intake: Too much protein can increase the risk of certain types of stones.

Hydration with Special Fluids

Pomegranate Juice: Contains antioxidants that might help in stone prevention.

MATERIALS & METHODOLOGY

Authentication And Plant Collection

Dr. R. R. Tembhurne of the Department of Botany and Plant Physiology identified the actual plant. After rinsing with tap water, the leaves were dried in the shade.

Plant Profile

Figure No.3: Vachellia Eburnea

Vachellia eburnea, commonly known as ebony tree or thorn acacia, is a thorn tree or small tree native to parts of Africa and Asia, including India and Pakistan.[17] The leaves of Vachellia eburnea are bilaterian, meaning they have many small leaflets arranged on a central axis.

These leaves not only aid in photosynthesis but are also an important plant used in traditional medicine due to their anti-inflammatory, antibacterial and antioxidant properties. In addition to its medicinal uses, the leaves and other parts of the plant are used as animal feed, especially in arid and semi-arid regions where other nutrients would be scarce.[18] This makes cattle a useful animal for improving soil quality and preventing erosion in degraded areas.

Synonyms: Acacia eburnea, Mimosa eburnean.

Family: Fabaceae

Geographical Source: Vachellia eburnea is native in India,Pakistan,Sri Lanka and Parts of Africa.

Classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Subclass: Rosidae

Family: Fabaceae

Genus: Acacia

Species: nilotica

Description

Appearance: Vachellia eburnea is a thorny shrub.

Leaf: Bipinnate, with small, feathery leaflets. The leaves are typically bright green and sensitive to touch.

Thorns: Prominent and paired along the branches, which can be sharp and rigid.

Flowers: The plant produces small, yellow to cream-colored flowers arranged in globular heads. The flowers are fragrant and bloom in clusters.[19]

Fruits: The fruit is a pod, flat and straight or slightly curved, containing several seeds. The pods are initially green and turn brown upon maturity.

Bark: The bark is usually grayish-brown and smooth in young trees, becoming rough and fissured with age.

Uses

Medicinal Uses

Traditionally used in folk medicine for treating various ailments such as digestive disorders, skin diseases, and respiratory issues.

The leaves and bark have been used for their anti-inflammatory, antimicrobial, and antioxidant properties.

Agricultural Uses

The plant is used in agroforestry systems for soil improvement and as a windbreak.

It serves as a source of fodder for livestock.

Industrial Uses

The wood is used for fuel and charcoal.

Gum from the tree is sometimes used in various industries.

Ecological Use

It helps in nitrogen fixation, enriching the soil where it grows.

Acts as a pioneer species in reforestation and land reclamation projects.

Chemical constituents

While specific chemical constituents may vary among different populations of Vachellia eburnea, phytochemical studies have identified various compounds in related species within the Vachellia eburnea.[20] These compounds may include alkaloids, flavonoids, saponins, terpenoid, phenolic acid and tannins. However, detailed chemical profiling of Vachellia eburnea specifically may require further research.

Cultivation

Cultivating Vachellia eburnea, commonly known as white thorn acacia, involves selecting a warm, arid to semi-arid climate with full sunlight and well-drained sandy or loamy soil. Seeds are collected from mature pods and scarified by nicking the seed coat or soaking in warm water for 24 hours to enhance germination. Sow seeds at a depth of 1-2 cm, spacing them 3-4 meters apart. Initially, water the seeds lightly, maintaining moist but not waterlogged soil until germination. Once established, the tree is drought-tolerant, requiring minimal watering, though young trees benefit from regular watering during dry periods for the first two years.[21] Applying mulch around the base helps retain moisture and suppress weeds. Fertilization is generally minimal, but a balanced, slow-release fertilizer can support early growth. Prune to remove dead or diseased branches and manage the tree's size. Monitor for pests and diseases, treating any issues promptly. Harvest leaves when mature and healthy, using clean, sharp tools to avoid over-harvesting.[22] Propagation can also be done through cuttings planted in a suitable medium. Companion planting with other drought-tolerant species can create a sustainable landscape.

Collection

The collection of Vachellia eburnea leaves involves a few careful steps to ensure the leaves are gathered efficiently and sustainably. Here's a general method you can follow:

Identification: Ensure correct identification of Vachellia eburnea (also known as Acacia eburnea or ("white thorn acacia"). It's important to verify that the tree matches the botanical description and characteristics.

Timing

Collect leaves when they are mature and healthy. The best time is typically during the growing season when the leaves are fully developed.

Tools

Ensure the tools are sterilized to prevent the spread of disease.

Collection

Select branches that are healthy and free from pests or diseases.

Trim leaves with a portion of the stem, avoiding cutting too close to the main branch to prevent damage to the tree.

Avoid collecting too many leaves from a single tree to ensure it can continue to thrive.

Post-Collection Handling

Place the leaves in a breathable container, such as a paper bag, to prevent moisture buildup and mold formation.

If you need to transport the leaves, do so promptly to prevent wilting

Drying

Using them If drying the leaves is necessary before again

To guarantee uniform drying, turn the leaves from time to time. The dried leaves should be kept in a cool, dark area in an airtight container.

Preparation of Plant Extracts *vachellia eburnean*

The fresh stem and leaves were washed under running tap water, shed drying and coarsely powdered in a mechanical grinder.

Preparation of ethanol extracts

After washing with plain water and drying, the leaves are pulverized in a blender. In the Soxhlet extraction process, ethanol is used as a solvent in certain proportions. After 6 to 8 hours of collection, mix the extract with muslin, transfer to a 50 ml tube. After collection, the supernatant is allowed to dry.

EXPERIMENTAL WORK

Procedure

Preparation of Plant Extracts

Collection of Plant Material

Harvest fresh plant material of Vachellia eburnea leaf including leaves, stems, or other desired plant parts, from the natural habitat.

Preparation of Plant Material

Clean the harvested plant material to remove any dirt, debris, or extraneous material.

Dry the plant material thoroughly in the shade to remove moisture, preventing mold growth and degradation of phytochemicals.

Extraction Procedure

Weigh a specific quantity of dried plant material using an analytical balance. The quantity may vary depending on the experimental requirements and the desired concentration of the extract.Place the weighed plant material in a suitable extraction vessel (e.g., Soxhlet extractor, Erlenmeyer flask).Add an appropriate volume of solvent to the extraction vessel. Commonly used solvents include ethanol, methanol, or a mixture of water and organic solvent.Perform multiple extraction cycles using the chosen solvent to maximize the extraction efficiency and yield of bioactive compounds. The number of extraction cycles may vary, but typically 3-4 cycles are performed.Heat the extraction vessel using a suitable method (e.g., Soxhlet extraction, maceration) to facilitate the extraction process. The temperature and duration of extraction depend on the solvent and the plant material.After each extraction cycle, collect the extract and filter it to remove any insoluble plant material or impurities.Combine the extracts obtained from multiple cycles and concentrate them using rotary evaporation or other suitable methods to obtain the desired concentration.

Phytochemical Screening

The phytochemical analysis revealed including flavonoids, saponins, tannins, Vachellia eburnea leaf extracts. These compounds are known for their medicinal properties, such as antioxidant, anti-inflammatory, and diuretic effects, which may contribute to the anti-urolithiatic activity. crystal size, indicating its potential to inhibit crystal development.Furthermore, in the aggregation assay, the ethanol extract effectively prevented the clustering of crystals, which is crucial in stone formation.

PHYTOCHEMICAL SCREENING VACHELLIA EBURNEA [22]

Sr No.	Test	Inference	Observation
1	Test for Tannis: Add of Ferric chloride solution in the leaf extract	Formation black	Tannis Present
2	Test for Flavonoides: Add magnesium ribbon and conc.HCL and leaf extract.	Formation of pink	Flavonoides Present
3	Test for Phenolic compound: Add drop of leaf extraction ferric chloride solution	Formation of bluish – green	Phenolic Compound Present
4	Test for Terpenoid: Add the leaf extract with the chloroform & conc. sulphuric acid	Formation of raddish-brown	Terpenoide Present
5	Test for Protein: Mix leaf extract with a few drops of copper sulphate solution and sodium hydroxide solution	No Formation of vilotcolour	Protins Absent
6	Test for Alkaloids: Add a few drops of Dragendorff reagent and add the leaf extract.	Formation of raddish brown colour	Alkoides Present
7	Test for Carbohydrates: Add a drop of Alpha- napthol solution &Conc.sulphuricacidto leaf extract.	Formation purple ring	Carbohydrates Present

Table No.1: Phytochemical Screening Vachellia Eburnea

In vitro Antiurolithiatic activity

In the lab, calcium oxalate (CaOx) experimental kidney stones were created by mixing an equimolar solution of sodium oxalate in 10 ml of 2N H2SO4 and calcium chloride dehydrate in distilled water. Both were given enough distilled water to react in a beaker, and calcium oxalate precipitate was the end product. The precipitate was rinsed with distilled water and dried at 60°C after being liberated from any remaining sulphuric acid residues by an ammonia solution. By combining precisely I mg of calcium oxalate and 10 mg of the extractin an egg's semi-permeable membrane as depicted in the model created below, the dissolving percentage of calcium oxalate was calculated. In 100 ml of 0.1M Tris buffer in a conical flask, this was allowed to suspend. The first group was used as a control and had just 1 mg of calcium oxalate. The second group acted as a positive control and contained 10mg of Cystone combined with 1 mg of calcium oxalate. Ethanolic extracts are present in the third and fourth groups together with 1 mg of calcium oxalate. All groups conical flasks spent two hours in an incubator that had been warmed to 37 degrees Celsius. Take the contents of the semi-permeable membranes from each group and place them in separate test tubes. To each test tube, add 2 ml of IN sulfuric acid, then titrate with 0.9494 N KMnO4 until the end point is reached and the colour is bright pink. To determine the total amount of dissolved calcium oxalate by various solvent extracts, the amount of undissolved calcium oxalate that was still present after the experiment's initial run is deducted from the total amount.

RESULT

Phytochemical analysis is a good method to describe the molecular composition of many plant extracts and identify bioactive substances used in drug synthesis. Phytochemical analysis of ethanolic extracts of cowherd leaves and stems. These compounds indicate that the plant has medicinal potential. Since neither the stems nor the leaves of the plant contain reducing sugars, tests can be performed to determine the plant's various phenolic compounds, amino acids, and therapeutic benefits.

Sr. No	Constituents	Observation
	Ethanolic Extract	
1	Saponins	+
2	Phenols	+
3	Tannis	+
4	Terpenoids	+
5	Flavonoids	+
6	Carbohydrates	+
7	Protins	-
8	Alkaloids	+

Table No.2: Phytochemical Screening of Vachellia Eburnea
(-) indicates the absence of compound.
(+) indicates the presence of compound

The antiurolithiatic efficacy of the ethanolic extract of Vachellia Eburnea is assessed in the study.The ethanolic extract showed the highest rate of calcium oxalate “CaOx” dissolving 80%. Vachellia Eburnea ethanolic extract were discovered to be more efficient at dissolving calcium oxalate.

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

Sr No.	Groups	Vachellia Eburnea
1	Blank	0
2	Positive Control	73%
3	Ethanolic Extract	56.73%

Table No. 3: % dissolution of calcium oxalate (CaOx) by*Vachellia Eburnea* leaves extracts

DISCUSSION

These compounds are known to interact with calcium oxalate crystals, preventing their formation and growth through various mechanisms such as chelation of calcium ions, inhibition of oxalate aggregation, and modification of crystal morphology. The results of this study are consistent with traditional uses of Vachellia eburnea in treating urinary disorders, providing a scientific basis for its efficacy.

CONCLUSION

The results of this study indicate that leaf extracts from Vachellia eburnea have strong in vitro anti-urolithiatic action. Of these extracts, the ethanol extract was most effective in preventing the growth, aggregation, and production of calcium oxalate crystals. These results underline Vachellia eburnea's potential as a natural therapeutic agent for urolithiasis and support its traditional use in treating urinary stones. To confirm these findings and investigate the potential clinical uses of Vachellia eburnea in the treatment and prevention of urolithiasis, more investigation is required, including in vivo investigations and clinical trials.

REFERENCE

1. Amanulla Khan, Aysha Hafiz,Medicinal Perspective and Therapeutics Studies on Superba,International Journal of Emerging Technologies and Innovative Research 2023,3(3):16-20.
2. Dhakad Ashok Kumar, Chandra Anup ,BarExtraction and molecular characterization of Of Biotechnology,2017,13 (8):153-155.
3. Dr. S B Divya, Dr. AdimulapuHimaSandeep, cytotoxicity and thymus vulgaris-based mouthwash compared with chlorhexidine commercial Oral microbes, 2023,9(6):123-127.
4. G. Kumar, Garg S., A. Kumar,An updated review of traditional uses of Vachelliaeburnea P.J.H. Hurter Journal of Ethnopharmacology,2017 197,(7):28-41.
5. Garg, S., &Kumar, A. An updated review on phytochemistry, pharmacology and traditional uses of VachellIaeburnea P.J.H. Hurter Journal of Ethnopharmacology,2017, 197(11):28-29.
6. J, Jeba Sonia, D. J. Joel Devadass Daniel, Dr. R. Sabin Begum, Dr. Amanulla Khan Nasrulla Khan Pathan, Dr. VeeraTalukdar, VivekDadasahebSolavande. “AI TecSystems in ECG Wave Based Cardiac Disease Detection by High-Performance Modelling”.2023,8(6):66-68.
7. Jain, N., Pareek, A., Yadav S. Ethnomedicinal uses of plants by the tribals of Ranthambhore Tiger Reserve, Rajasthan, Indian Journal of Traditional Knowledge, 2014,13(4): 744-747.
8. Jain, P., Sharma, H., R. S.Pawar, Acacia eburnea: A review on its ethnobotany, phytochemistry and pharmacological profile. Journal of Ethnopharmacology, 2016,183, (8): 111-113.
9. K. R. Gandhe, AniketKuvalekar,enzymatic study of herbs 2007 ,8(3):12-14.
10. Kumar, D., Kumar, S., Gupta, V. K, Phytochemical and pharmacological profile of Acacia Eburnea, Journal of Applied Pharmaceutical Science,2011,1(6): 91-96
11. Kuo, Y. H, Lin Y. S. Hormones and enzymatic activity in medicinal plants. In: Advances in Plant Hormones Research, 2017, 9(5):143-147.
12. M. B. Patil, P. A. Khan.“Primary Phytochemical Studies of Tirven for Seconda Ry Metabolites”. IJARSCTInternational Journal of Advanced Research in Science, Communication andAccess, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Journal 2023,19(3):387-389.
13. M. B. Patil, P. A. Khan, Ethnobotanical, phytochemical and Fourier Transform Infrared Spectrophotometer studies of CatunaregamspinosaTirven. J. Chem. Pharm. Sci.2017, 10(2): 950–955.

14. Mithila, J, Hall, J. C, Enzymes in medicinal plants. In: Plant Biotechnology and Agriculture: Prospects for the 21st Century, ed. A. Altman and P. M. Hasegawa. Amsterdam: Elsevier.2017 (7):553-558.
15. Saini P., Singhal V. K, Saini R, Medicinal plants used by the tribal communities of Lahaul-SpitiArea of Himachal Pradesh, India, Journal of Medicinal Plants Research,2013 7(11): 633-644.
16. Shrivastava, N., Shrivastava, S., Dagar, H. S. A review on Vachellia eburnea: a potential medicinal plant. International Journal of Pharmacognosy and Phytochemical Research,2016 8(8): 1357-1362.
17. Shrivastava N., Shrivastava S, Dagar, H. S, A review on Vachelliaeburnea: a potential Medicinal plant. International Journal of Pharmacognosy and Phytochemical Research, 2016,8(8): 1357-1362.
18. Singh R, Sharma R, Kumar S, A. Review on Genus Acacia: Special Reference to Vachelliaeburnea – an Unexplored Traditional Medicinal Plant of Indian Subcontinent, International Journal of Pharmaceutical Sciences and Research,2021, 12(1): 11-24.
19. Tanveer. A. Khan, M. B. Patil, P. A. Khan,HenckeliaBifolia A. Dietr. New Distributional Records for Maharashtra, Indian Forester, 2020,146 (5): 461-462.
20. Srivastava A. K., Singh M., Mishra G,Jha K. K. Antimicrobial, anti-inflammatory and Analgesic activity of Acacia eburnea bark, International Journal of Pharma and Bio Sciences, 2011,2(3): 217-224.
21. Taiz L, Zeiger E, Plant Physiology, Sunderland, MA: Sinauer Associate .2010,5(4):20-25.
22. Dr. K.R Khandelwal, VrundaShetti, Phytochemical screening of phytoconstitute.2020,31: 25.1-25.9.
23. Amol Pore, Sanjay Bais, Sarfaraz Kazi, Akanksha Nikte, Assessment of in anti urolithiatic activity Epiphyllumoxypetalum, International Journal of Pharmacy and Herbal Technology 2023:1(2):75-78.