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ASSESSMENT OF IN-VITRO ANTI-UROLITHIATIC ACTIVITY OF ZORNIA GIBBOSA SPAN EXTRACT

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ABSTRACT

This study evaluated Zornia gibbosa span extract's anti-urolithiatic efficacy in vitro. Flavonoids, alkaloids, and tannins were detected in the extract, which was made with ethanol as the solvent, according to a qualitative phytochemical examination. In-vitro assays were conducted to evaluate the extract's potential in inhibiting urinary crystal formation, preventing crystal aggregation, and reducing oxalate levels. Additionally, antioxidant activity was assessed using DPPH scavenging assay. The extract exhibited significant inhibition of crystal formation and aggregation, along with notable antioxidant activity. This research highlights the potential of Zornia gibbosa span as a natural anti-urolithiatic agent, contributing to the development of novel therapeutic strategies for urolithiasis. In-vitro assays were conducted to assess its ability to inhibit urinary crystal formation and aggregation, reduce oxalate levels, and scavenge free radicals. The extract demonstrated significant inhibition of crystal formation and aggregation, accompanied by notable antioxidant activity. These findings suggest the potential of Zornia gibbosa span as a natural remedy for urolithiasis. Its mechanism of action and in vivo efficacy need to be further investigated in order to facilitate the creation of innovative urolithiasis treatment strategies.

Keywords: *kidney stone, anti urolithiatic activity, Cystone, Anti-urolithiatic activity, Zornia gibbosa span.*

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INTRODUCTION

overview of kidney stones:

A kidney stone, also known as renal calculi or nephrolithiasis in medical terminology, is a solid mass composed of small crystals. Many materials found in the urine, including calcium, oxalate, uric acid, and cysteine, may crystallize into these formations. Kidney stones can be any size from the size of a golf ball to a grain of sand. These can cause excruciating pain and other problems. They might appear in one or both kidneys, stay there, or move down the urinary system. Urinary tract infections, kidney damage, and urinary tract obstruction are among the issues that kidney stones can cause and need immediate medical intervention.[1]

Types of kidney stones:

The composition of kidney stones can vary, with several varieties developing depending on the materials found in the urine. Kidney stones most frequently occur in the following types:

One of the most prevalent types of kidney stones is a calcium stone, which is mainly made up of calcium phosphate or calcium oxalate. Urinary tract stones may develop as a result of elevated calcium and oxalate levels. Calcium stone development can be exacerbated by specific dietary circumstances, such as eating meals high in oxalate or calcium.[2]

High quantities of uric acid in the urine can result from a high-purine diet, dehydration, or certain medical diseases like gout. When this happens, uric acid stones can develop. People whose urine has an acidic pH are more likely to develop these stones.

Struvite Stones, they tend to develop in people who have urinary tract infections (UTIs) caused by certain bacteria that produce urease, an enzyme that increases urine pH and promotes stone formation. Struvite stones can grow rapidly and become quite large.[3]

Rarely occurring, cystine stones are made of the amino acid cystine. People with a genetic condition known as cystinuria, in which the kidneys discharge an excessive amount of cystine into the urine, are susceptible to developing them. Cystine stones tend to be larger and more difficult to treat than other types of kidney stones.[4]

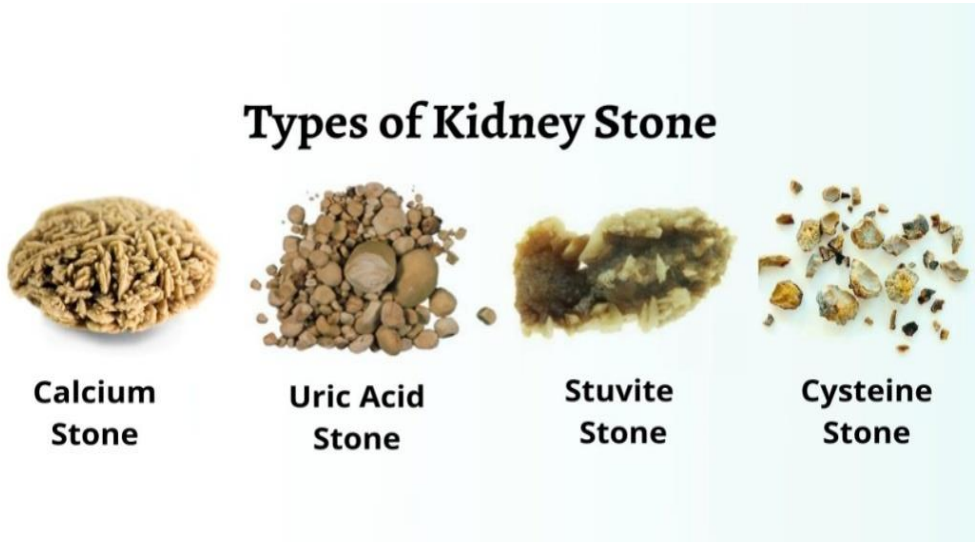

Figure No.1: Types of Kidney stone

PLANT PROFILE

Figure No.2: Zornia Gibbosa Span

Span Plant, also called Zornia gibbosa, is a species of flowering plant in the Fabaceae family. Originating in tropical parts of Asia and Africa, Zornia gibbosa is prized for its eye-catching growth pattern and decorative qualities. The plant typically grows as a low-growing herbaceous perennial, forming dense mats or clumps of foliage along the ground. Zornia gibbosa produces small, pea-like flowers that can vary in color from white to pink or purple, adding a splash of color to its surroundings.[5] In addition to its aesthetic appeal, Zornia gibbosa is valued for its ecological role, providing habitat and forage for various wildlife species. Moreover, certain traditional medicinal uses have been attributed to Zornia gibbosa in some regions, although scientific research on its medicinal properties is limited. With its charming appearance and ecological significance, Zornia gibbosa is a noteworthy species deserving of attention in botanical studies and horticultural practices.[6]

Taxonomy:

The scientific classification of Zornia gibbosa, commonly known as Span Plant, is as follows:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Equisetopsida C. Agardh
Subclass: Magnoliidae
Order: Fabales
Family: Fabaceae
Genus: Zornia
Species: Zornia gibbosa Span [7]

Pharmacognostic Characteristics

Pharmacognostic characteristics of Zornia gibbosa include compound leaves with elliptical leaflets, slender reddish-brown stems, small pea-like flowers varying in color, and flattened pods containing seeds. These features aid in the identification and authentication of the plant for medicinal purposes.[8]

MATERIALS & METHODOLOGY

Plant Collection and Authentication:

In February 2024, Zornia gibbosa Span leaves were gathered from Sangola in the Solapur District of Maharashtra, India. Dr. Tembhurne R.R. from the Sangola College Botany Department verified the authenticity of the plant. After being cleaned with tap water, the leaves were allowed to dry in the shade.[9]

Preparation Of Plant Extracts of *Zornia gibbosa Span*:

The fresh stem and leaves were ground into a coarse powder in a machine grinder, dried, and cleaned under running water.[10]

Preparation Of Ethanolic Extracts:

The extract prepared using a somewhat different procedure from what was stated in. A blender was used to pulverize the leaf sample after it had been cleansed with ordinary water, dried, and placed in it. Different ratios of ethanol are used as a solvent for the Soxhlet extraction method. Place the extract in 50 ml tubes and centrifuge them for 15 minutes at 4,000 rpm and 25 °C after it has been collected for 6 to 8 hours. The extract can also be filtered using a cotton cloth. The supernatant was collected and then preserved for drying.[11]

PHYTOCHEMICAL INVESTIGATION

Based on the following chemical analyses, preliminary phytoconstituents found in the ethanol extract of *Zornia gibbosa Span* were identified.[12]

Sr No.	Name of Test	Observation	Inference
1.	**Test for Saponins:** Shake the sample with water vigorously for about 30 seconds. Persistent froth that lasts for 10-15 minutes indicates the presence of saponins.	The creation of sturdy foam	Saponins present
2.	**Test for Phenols:** Add a few drops of ferric chloride solution to the sample.	Blue/green color	Phenols present
3.	**Test for Tannins:** Add a few drops of ferric chloride to the sample. Formation of a blue-black or greenish-black precipitate indicates the presence of tannins.	No Black color	Tannins present
4.	**Test for Terpenoids:** Mix the sample with chloroform and concentrated sulfuric acid	colors seen in the interphase that are reddish-brown	Terpenoids present
5.	**Test for Flavonoids:** A few drops of sodium hydroxide solution were added to the extract.	creation of a strong yellow tint. It turns colorless when weak acid is added.	Flavonoids present
6.	**Test for Glycosides:** The combination of the extract and two milliliters of glacial acetic acid, which included a few drops of 2% FeCl3, was transferred into a second tube that held two milliliters of concentrated sulfuric acid.	A brown ring at the inter-phase.	Glycosides Absent

7.	**Test for Protein:** Using a few drops of strong nitric acid, the extract was treated.	The formation of yellow color.	Protein Absent
8.	**Test for Alkaloids:** **Dragendroff's Test:** A few milliliters of extract were mixed with one or two milliliters of Dragendroff's reagent (potassium bismuth iodide solution).	Orange brown coloured ppt.	Alkaloids Absent
	Mayer's Test: Two drops of potassium mercuric iodide solution, often known as Mayer's reagent, were added to a few milliliters of extract.	Cream coloured ppt	Alkaloids present
	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
	Wagner's Test: Wagner's reagent (iodine in potassium iodide) was added in little amounts to several milliliters of the extract.	Reddish Brown coloured ppt.	Alkaloids present

Table No. 1: Phytochemical Constituents of *Zornia gibbosa Span* Extract

EXPERIMENTAL WORK

The homogenous precipitation technique is used to create calcium oxalate crystals:

Soluble sodium oxalate (4.02g) in 2N sulfuric acid and dissolved calcium chloride dihydrate (4.41g) in distilled water were placed in two different beakers. After that, the two solutions were mixed and agitated until a precipitate of calcium oxalate formed. Washing with pure water and ammonia solution, respectively, eliminated the excess sulfuric acid. It was left to dry for four hours at 60 °C.[13]

Making semi-permeable membranes out of farm eggs:

A glass rod was used to puncture the egg's apex and extract the entire contents. After completely cleaning with distilled water, empty egg shells were set aside in a beaker with 2M HCl for an entire night. The beaker was then submerged in an ammonia solution to neutralize any remaining acid traces while it was moist. Following a distilled water rinse, they were refrigerated at a pH of 7.4–7.4[14]

Figure No.3: Decalcification of Egg Shell

Titrimetric analysis is used to assess anti-urolithiatic activity:

There were nine semi-permeable membranes created. Five milligrams of calcium oxalate crystals were carefully sutured into each membrane along with four different extract concentrations (10 mg, 20 mg, 30 mg, and 40 mg) and a standard (Positive control). [15][16] One sample containing just crystals of calcium oxalate served as the negative control. The 100 ml of 0.1M tris buffer solution in each individual conical flask was allowed to float. Each conical flask was incubated at 37 °C for seven hours. After that, two milliliters of IN sulfuric acid were introduced to the semi-permeable membrane's contents in a test tube. After the mixture became pale pink, it was titrated against the standard KMnO4 solution. To obtain reliable findings, this entire process was repeated three times. For each sample, the percentage of calcium oxalate crystals that dissolved was computed in order to assess the activity.[17]

RESULTS

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

Table No. 2: Results of Preliminary Phytochemical Screening of *Zornia gibbosa Span*

Percentage purity by titrimetric method was found to be 151.52%.

This study evaluates the antiurolithiatic activity of Zornia gibbosa Span's ethanoic extract. The ethenoic extract demonstrated a high 64% dissolution rate of calcium oxalate, or "CaOx." It was discovered that Zornia gibbosa Span ethanoic extract was more effective in dissolving calcium oxalate.

Sr. No.	Group	Zornia gibbosa Span
1	Blank	0
2	Positive Control	73%
3	Ethanolic extract	64%

Table No3: Shows % dissolution of calcium oxalate (Ca Ox) by Zornia gibbosa Span extracts

DISCUSSION

Urolithiasis, or the formation of kidney stones, presents a significant health concern globally, necessitating alternative therapies with fewer side effects than existing treatments. Zornia gibbosa Span, chosen for its traditional use in folk medicine and potential bioactive compounds, emerges as a candidate for such investigation.

The experimental design involves preparing Zornia gibbosa Span extract and subjecting it to in-vitro models mimicking conditions relevant to urolithiasis. Parameters such as the type of kidney stone model, extract concentration, and treatment duration are carefully considered. Assessing the extract's efficacy entails measuring crystal growth inhibition, nucleation time, aggregation, and crystal morphology.

Mechanistic studies delve into the underlying mechanisms of action, exploring the extract's ability to inhibit crystal formation, enhance urinary stone passage, modulate urinary pH, and exert antioxidant and anti-inflammatory effects. Positive control groups treated with standard anti-urolithiatic drugs are included for comparison, enabling the evaluation of the extract's effectiveness relative to existing treatments.

Data analysis and interpretation reveal the extract's potential in inhibiting or dissolving kidney stones, highlighting trends, correlations, and statistically significant findings. The implications of the study extend to the development of novel anti-urolithiatic therapies, with identified areas for further research including clinical trials to validate efficacy in humans and investigations into safety profiles and potential adverse effects.

CONCLUSION

In conclusion, through systematic experimentation, the extract demonstrates efficacy in inhibiting crystal formation and promoting dissolution. These findings highlight Zornia gibbosa Span as a valuable natural remedy for urolithiasis, paving the way for further research and clinical validation of its therapeutic benefits.

REFERENCE

1. Durgawale P, Shariff A, Hendre A, Patil S, Sontakke A. Chemical analysis of stones and its significance in urolithiasis. Biomedical Research 2010; 21(3):305- 310.
2. Noonan SC, Savage GP. Oxalate content of foods and its effect on humans. Asia Pacific J Clin Nutr 1998; 8(1): 64-74.
3. Massey LK, Roman-Smith H, Sutton RA. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. J Am Diet Assoc 1993; 93(8):901-906.
4. Garimella TS, Jolly CI, Narayanan S. In vitro studies on antilithiatic activity of seeds of Dolichos biflorus Linn and rhizomes of Bergenia ligulata Wall. Phytother Res 2001; 15(4):351-355.
5. Aziz SA, See TL, Khuay LY, Osman K, Abu Bakar MA. In vitro effects of Plantago major extract on urolithiasis. Malays J Med Sci 2005; 12(2):22-26.
6. Verma P, Gauttam V, Kalia AN. Comparative Pharmacognosy of Pashanbhed. J Ayurveda Integr Med 2014; 5(2):104-108.
7. Shashi Alok, Sanjay Kumar Jain, Amita Verma, Mayank Kumar, Monika Sabharwal. Pathophysiology of kidney, gallbladder and urinary stones treatment with herbal and allopathic medicine: A review. Asian Pac J Trop Dis 2013; 3(6): 496-504.
8. Rivers K, Sheety S, Menon M. When and how to evaluate a patient with nephrolithiasis. Urol Clin Amer .2000;27(2): 203– 213.
9. Aslokar LV, Kakkar KK, Chakre OJ. Glossary,Indian medicinal plants with active principles. Part I, first edition, New Delhi, CSIR 1992; 5(6); 119-125.
10. Sivarajan VV, Balachandran I, eds. In: Ayurvedic drugs and their plant sources. 1st edition, New Delhi, CSIR 1992:8(2);119-130.
11. Bashir, S., Gilani, A.H., Antiurolithiatic effect Bergenia ligulata rhizome: an explanation of the underlying mechanisms. J. Ethnopharmacol, 2009; 12(2):106–116.
12. Mohansundari M; Sabesan M and Sethupathy S; Reno protective effect of grape seeds extract in ethylene glycol induced nephrotoxic mice. 2005; 43(3):365-359.
13. Ruckmani, K., Jaykar, B., Anandan, R., Diuretic activity of Moringa oleifera Lam. Indian Drugs.1997; 3(4): 289–291.
14. Sanjay K Bais, Amol V. Pore, Shreya Kamavaram, A comprehensive review on commercial collection and cultivation aspects of medical and aromatic plants, International Journal Of Pharmacy And Herbal Technology, 2023: 1(3): 297-308
15. Amol V. Pore, Sanjay K. Bais, Shravani A. Mundhe. Review on drug evaluation methods International Journal of Pharmacy and Herbal Technology, 2024;1(2): 240 – 250.
16. Amol V. Pore, Sanjay K. Bais, Sushant L. Pawar. The quality aspects of herbal drugs and its formulation. International Journal of Pharmacy and Herbal Technology, 2024: 2(1); 945-954.
17. Amol V. Pore, Sanjay K Bais, Sarfaraz M. Kazi, Akanksha A. Nikte, Assessment of in- anti urolithiatic activity epiphyllum oxypetalum, International Journal of Pharmacy and Herbal Technology, 2023: 1(2): 72-76.