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Research Article
Antiurolithiatic Activity of Hydro-Alcoholic Extract of *Ocimum sanctum* Using Nucleation Assay

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Abstract

Background and Objective: *Ocimum sanctum*, Holy basil or Tulsi is an aromatic plant with a number of properties. *Ocimum sanctum* leaves have been utilised for their preventive and therapeutic qualities for a variety of ailments in the traditional Indian medical system since ancient times. Therefore, this study explored the antiurolithiatic activity of hydro-alcoholic extract obtained from *Ocimum sanctum* by nucleation assay. **Materials and Methods:** Preparation of extraction by maceration method and storing the extract in a cool and dry place. The phytochemical screening of the extract is done to know the chemicals present in the extract. The antiurolithiatic activity is done by nucleation assay to show percentage inhibition for preventing stone formation in the kidney. **Results:** The presence of antiurolithiatic activity by nucleation assay indicated that percentage of inhibition was higher in hydro-alcoholic extract at 100 µg/mL when compared to standard drug Cystone tablet. The hydro-alcoholic extracts of *Ocimum sanctum* leaves have an inhibitory effect on CaOx crystallization and thus may be beneficial in the treatment of urolithiasis. **Conclusion:** *Ocimum sanctum* leaves hydro-alcoholic extracts block the crystallization of CaOx, which suggests that they could be useful in the treatment of urolithiasis.

Key words: *Ocimum sanctum*, antiurolithiatic activity, nucleation assay, urolithiasis, hydro-alcoholic extract

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural products are compounds found in nature or derived from natural sources that have excellent biological and physiological qualities and can be used in drug design and discovery[1]. Medicinal plants, often known as plant materials or herbalism, are used whole or in part to cure wounds or illnesses[2]. Any portion of plants, marine organisms or fermentation by microorganisms can be extracted to produce these compounds. Researchers and scientists are paying close attention to substances derived from plants since they have a wide range of uses. All of these plants have therapeutic qualities, making them a rich supply of pharmaceutical excipients, chemical entities for synthetic pharmaceuticals, food nutrients and contemporary and traditional medicines[3]. Tulsi or *Ocimum tenuiflorum*, is a multipurpose, aromatic herb that is also known by the names *Ocimum sanctum* and holy basil. Native to the tropics and eastern regions of the planet, it is widely grown worldwide. There are about 35 species of aromatic annual and perennial shrubs and herbs in the genus *Ocimum*[4]. Some species include *Ocimum basilicum* or Thai basil; *O. gratissimum* or African Basil, *O. campechianum* or Amazonian basil: *O. tenuiflorum* or *O. sanctum* or Tulsi, with purple or crimson subquadrangular branches, *O. sanctum* can reach a height of 60 cm. Simple and hairy are the leaves[5]. *Ocimum sanctum* shows activation to reduce disease of the head and neck, swelling, pain and headache. *Ocimum sanctum* exhibits activation to lessen headache, neck pain and edema. Purple flowers are seen in nature. When fruits get wet, they become smooth to the touch and do not become mucilaginous. It is made using seeds. Direct sowing of seeds into the earth occurs. When young plants reach a height of 8 to 10 cm and develop skin problems, they are transplanted to the field. Additionally, *O. sanctum* leaves are highly beneficial for lowering stress, blood sugar and cholesterol[6].

One common urinary tract disorder is urolithiasis, which is characterized by the accumulation of sediment in the urinary tract that contains one or more extremely poorly soluble urine crystalloids[7].

This illness is rapidly spreading throughout numerous nations, including South Africa, India, South Asia and the United States. The renal stone disease affects about 2% of the global population, with a male-to-female ratio of 2:1. Hematuria indicates that the patient is experiencing severe discomfort (renal colic) due to kidney stones. The primary causes of these stones in the urinary tract are nephrolithiasis and inadequate microbial infections, urinary drainage, high-oxalate and high-calcium diets, vitamin abnormalities (e.g., excess vitamin D, deficiency of vitamin A), metabolic diseases (e.g., hyperparathyroidism), intestinal dysfunction (e.g., cystinuria, gout) and environmental factors (e.g., dry and hot climate conditions)[8]. A disorder known as urolithiasis is the formation of material, primarily salt, in any area of the body that takes the shape of a stone. As a result of metabolism, oxalate is a primary constituent of most renal stones[9].

Therefore this study evaluated the effect of hydro-alcoholic extract of *Ocimum sanctum* on calcium oxalate by nucleation assay.

MATERIALS AND METHODS

Study area: The extraction of the plant materials was conducted in Pharmacognosy Laboratory at Haldia Institute of Pharmacy, West Bengal, India. The research was carried out from January-April, 2024.

Collection and preparation of test sample: A healthy and fresh leaves of *Ocimum sanctum* is collected from the local area of Haldia. Prepare a herbarium sheet and get the plant authorization from the Botanical Survey of India, Howrah, West Bengal. The leaves were mechanically powdered using an electric blender (Bajaj Pharmacognosy Laboratory in Haldia Institute of Pharmacy, West Bengal, India) after being air-dried in the shade for 21 days at 28±2°C. For further extraction, the powdered sample was stored in a container that was tightly closed[10].

Test extract

Maceration: One hundred grams of the dried, powdered leaves of *O. sanctum* were poured into 500 mL of petroleum ether for 24 hrs to reduce the fatty acid and filtered. Dried the filtrate and macerated in a mixture of distilled water and methanol (1:1) for 7 days. To ensure complete extraction, the sample was periodically shaken. The extract was filtered and dried. Repeat the above step to produce the appropriate amount of extract. Phytochemical tests were performed[11,12].

Simple distillation: The filtered extract is transferred into a distillation apparatus and heated the mixture gently to evaporate the solvent (alcohol and water) using simple distillation. The solvent vapors will rise through the distillation setup and condense back into liquid form in a condenser, leaving behind a concentrated extract in the distillation flask. Collected the extraction and weight. Store the extraction in a cool and dark place[13].

Nucleation assay method: For evaluating antiurolithiatic activity, nucleation assay performed, with minor modifications. The following was prepared in a pH 6.5 Tris buffer: 0.05 mol/L Tris and 0.15 mol/L NaCl. The solutions of calcium chloride (4 mmol/L) and sodium oxalate (7.5 mmol/L). To the extracts, a certain amount of calcium chloride solution was added. After that, sodium oxalate was added to the mixture to cause the crystallisation reaction. The mixture was kept at 37°C. By contrasting the optical density in the presence of distilled water with that of the negative control, the nucleation activity was calculated. Positive control was provided by Cystone. The absorbance of 620 nm was used to measure the inhibitory reaction[14,15].

Statistical analysis: Each experiment was performed in triplicates (n = 3) and results were presented as Mean±SD. The statistical significance of data was assessed by analysis of by Graphpad prism significance was considered at $p<0.05$, the percentage of inhibition.

RESULTS AND DISCUSSION

Results: The results of phytochemical tests are shown in Table 1. Hydro-alcoholic extract of *Ocimum sanctum* shows positive responses in the presence of carbohydrates, phenol, tannin, flavonoid, saponin, glycoside, alkaloid and fixed oil. It also shows negative response on protein, steroid, terpenoid, anthraquinone and lactone.

The values of Table 2 represent the percentage of inhibition of standard (Cystone tablet) and test (*Ocimum sanctum*) in concentration 100, 200, 400, 600 and 800 µg.

Figure 1 states that the standard (Cystone) at different concentrations show maximum absorbance at 100 µg/mL i.e., 0.066.

From the above date it is observed that the percentage of inhibition of extract gradually decreases as concentration increases. But at 800 µg/mL the percentage of inhibition increases. When compared to normal medicine Cystone tablets, the hydro-alcoholic extract at 100 µg/mL showed a higher percentage of inhibition due to the presence of antiurolithiatic activity by nucleation assay (Fig. 2).

Discussion: The present study demonstrates the significant antiurolithiatic activity of *Ocimum sanctum* hydro-alcoholic extract, evidenced by its inhibition of calcium oxalate nucleation *in vitro*. This finding supports the traditional use of *Ocimum sanctum* in Ayurvedic medicine for urinary disorders.

Our results suggest that the phytochemical constituents, particularly flavonoids, phenolic acids and terpenoids, contribute to the observed antiurolithiatic activity. These compounds have been reported to possess antioxidant, antiinflammatory and crystal-inhibiting properties[16], which may synergistically prevent kidney stone formation.

Table 1: Phytochemical screening result of hydro-alcoholic extract of *Ocimum sanctum*

Phytochemicals tests	Result
Protein	-
Carbohydrate	+
Phenol	+
Tannin	+
Flavonoid	+
Saponin	+
Glycoside	+
Steroid	-
Terpenoid	-
Alkaloid	+
Anthraquinone	-
Fixed oil	+
Lactone	-

+: Present and -: Absent

Table 2: Percentage inhibition of standard and sample of hydro-alcoholic extract of *Ocimum sanctum*

Concentration of serial dilution (µg/mL)	Inhibition of standard (%)	Inhibition of sample (%)
100	53.84±3.12	94.40±4.25
200	57.34±2.95	68.53±3.91
400	71.32±3.88	71.32±4.22
600	76.92±3.33	76.22±4.92
800	87.41±3.89	90.20±5.00

±Significance was considered at $p<0.05$, the percentage of inhibition

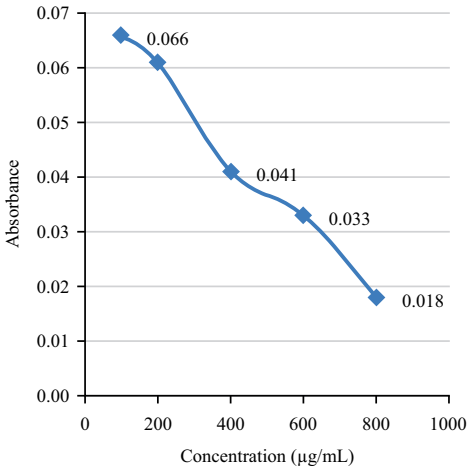

Fig. 1: Absorbance of Cystone as standard at 620 nm

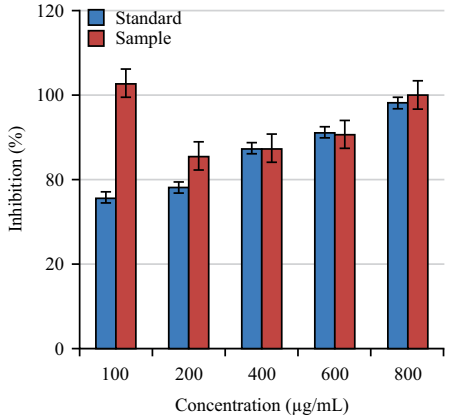

Fig. 2: Graphical comparison of standard and test values

The nucleation assay employed in this study reliably mimics the initial stages of kidney stone formation, validating its use in evaluating antiurolithiatic activity. Notably, our findings align with previous reports of Anand *et al.*[17], on the antiurolithiatic activity of other herbal extracts, such as *Tribulus terrestris* and Cystone.

Compared to previous studies, our research provides novel insights into the antiurolithiatic potential of *Ocimum sanctum* extract. While, Srinivasa *et al.*[18] reported similar findings, our study employs a more comprehensive nucleation assay and elucidates the role of phytochemical constituents. Furthermore, our results complement the *in vivo* studies on *Ocimum sanctum's* antioxidant and antiinflammatory effects.

CONCLUSION

This study demonstrates that, when compared to other currently available medications, the hydro-alcoholic extract of *Ocimum sanctum* leaves possesses very strong action against calcium stone inhibition activity. Patients with urolithiasis may benefit from new formulations made using the hydro-alcoholic extract of *Ocimum sanctum in vivo* leaves, which may then be further estimated and its active ingredients characterised.

SIGNIFICANCE STATEMENT

The hydroalcoholic extract of *Ocimum sanctum* exhibits significant antiurolithiatic activity, as evidenced by its ability to inhibit the nucleation of calcium oxalate crystals *in vitro*, suggesting its potential to prevent or treat kidney stone formation by reducing the likelihood of stone nucleation and growth, thereby offering a natural and complementary approach to managing urolithiasis. The future aspect of the work is the design of clinical trials to evaluate the safety and efficacy of *Ocimum sanctum* extract in preventing kidney stone recurrence in human.

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