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In-Vitro Analysis of Ethanol Extract of *Saponaria Officinalis* for Antiurolithiatic Activity

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Abstract

The goal of the current study was to assess *Saponaria officinalis* ethanol extracts' in-vitro anti-urolithiatic effectiveness as a potential natural therapy to combat adverse effects brought on by the use of contemporary synthetic medications. *S. officinalis* extracted with ethanol was put to the test for its ability to treat urolithiasis utilizing in vitro studies (nucleation, aggregation assays). This extract was evaluated and contrasted with the reference medication cystone at increasing concentrations of 200, 400, 600, 800, and 1000µg/ml. The extract's ability to suppress the growth of calcium oxalate crystals in preformed forms was evaluated. The results of the *S. officinalis* extract on the nucleation assay were significant and showed considerable inhibition 64.2%±0.5 compared to standard cystone drug 67.7%±0.8 at 1000µg/ml concentration. Additionally, the ethanol extract of *S. officinalis* showed significant inhibition in the aggregation assay with a difference of 74.2%±0.08 compared to cystone 76.7%±0.09 at 1000µg/ml concentration. At a dose level of 1000µg/ml, the ethanol extract of *S. officinalis* possesses anti-urolithiatic potentials in vitro. It is possible to further exploit the ethanol extract of *S. officinalis* as a potential anti-urolithiasis medication.

Keywords: Urolithiasis, Nucleation, Aggregation, Calcium Chloride, Sodium Oxalate, *Saponaria officinalis*.

Introduction

Urolithiasis is a complex urologic illness that necessitates frequent trips to the emergency room and prompt urological management [1]. Kidney stones are tiny, hard deposits of mineral and acid salts formed in the urinary tract by crystals that separate from the urine. oxalate or phosphate, along with calcium, make up the most prevalent form of stone [2]. The issue of calculi production is seen and reported in all regions of the urinary tract, including the kidney, ureter, and urinary bladder, all of which can have a wide range of sizes [3].

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The physiochemical process that results in crystal nucleation, aggregation, and growth of calcified renal stones is aided by a number of biological processes, including urine volume, pH, elevated calcium oxalate or sodium oxalate, and urates [4]. Currently, 68 to 86% of patients with upper urinary tract stones have been successfully treated based on the size, nature, and position of the stones [5]. Kidney stone development rates have been linked to higher dietary protein intake, and calcium oxalate and/or calcium phosphate make up about 75% of all renal stones [6]. Kidney stone treatment has made significant strides, however they come with side effects like hypertension. Recurring hemorrhages, tubular nephrosis, etc. [7, 8]. Therefore, using complementary or alternative medicines with few adverse effects may be beneficial. Because of its extensive biological and therapeutic values, low toxicity, and lower cost, herbal medicines are becoming more and more popular among the general people for the treatment of urolithiasis in both developed and developing countries [9]. The majority of plant-based medications were utilized to treat kidney disorders [10]. A review of the literature suggests that kidney stones in Nepal have been successfully treated with herbal formulations of plant-based medicine [11, 12]. Although these herbal remedies are well-known in folklore, it is unclear why they are effective at treating urolithiasis on a pharmacological and physiochemical level. By using an aggregation model and nucleation test, the in-vitro antiurolithiatic properties of an ethanol extract of *Saponaria officinalis* were to be assessed in this work.

Materials and Methods

In-Vitro Antiurolithiatic Activity of EESO

Nucleation assay

The effect of EESO on the formation of calcium oxalate (CaOx) crystals was evaluated using a nucleation experiment. Calcium chloride (CaCl₂) (5 mmol/l) and sodium oxalate (Na₂C₂O₄) solution (7.5 mmol/l) solution were prepared to have a pH of 6.5 in Tris-HCl (0.05 mol/l) and NaCl (0.15 mol/l) buffer. EESO dilutions of 100 to 1500g/ml were produced in distill water. One milliliter of each EESO concentration was added to three milliliters of CaCl₂ solution and three milliliters of Na₂C₂O₄ solution. The final mixtures were cooked for 30 minutes at 370C. The optical density (OD) of the mixtures was calculated at a wavelength of 620 nm. The proportion of nucleation inhibition brought on by EESO was calculated using the formula below and contrasted with the percentage of inhibition brought on by cystone, the gold standard concentration [13].

$$\% \text{ Inhibition} = (1 - \text{OD}_{\text{TEST}} / \text{OD}_{\text{CONTROL}}) \times 100$$

Aggregation assay

The effect of EESO on CaOx crystal aggregation was evaluated using an aggregate test. A mixture of CaCl₂ and Na₂C₂O₄ solutions (50 mmol/l each) was heated to 600C in a water bath for one hour, then incubated at 370C overnight to produce calcium oxalate crystals visible. After drying at pH 6.5, CaOx crystal solution (0.8 mg/ml) was created in the buffer of 0.05 mol/l Tris-HCl and 0.15 mol/l NaCl. 3 ml of CaOx solution received aliquots of EESO in the range of 100-1500 g/ml, which were then added, vortexed, and incubated for 30 minutes at 370C. At a wavelength of 620 nm, the final mixture's optical density (OD) was measured, and the percent inhibition of aggregation was calculated similarly to how it was done for the nucleation experiment.

$$\% \text{ Inhibition} = (1 - \text{OD Test} / \text{OD Control}) \times 100$$

Result and Discussion

Effect of Inhibition in Nucleation Assay

The effects of extract concentrations ranging from 200 to 1000µg/ml on nucleation activities. The % inhibition of the standard and EESO is shown by the data in table 1. The extract's 1000 µg/ml concentration produced a nucleation activity inhibition of 64.2%±0.5, which was higher than the benchmark (cystone's)

67.7%±0.8. The extract has nucleation-preventing chemicals that will stop kidney stones from growing, according to the results of the nucleation test.

Table 1: Effect of inhibition in nucleation assay of EESO

Sl.No	Concentration (µg/ml)	Nucleation assay	
		STANDARD (Cystone)	EESO
1	200	38.0%±0.9*	32.9%±1.2*
2	400	48.5%±1.6***	39.3%±0.7**
3	600	53.0%±0.6*	48.9%±0.7*
4	800	60.1%±0.3*	55.5%±1.0***
5	1000	67.7%±0.8*	64.2%±0.5**

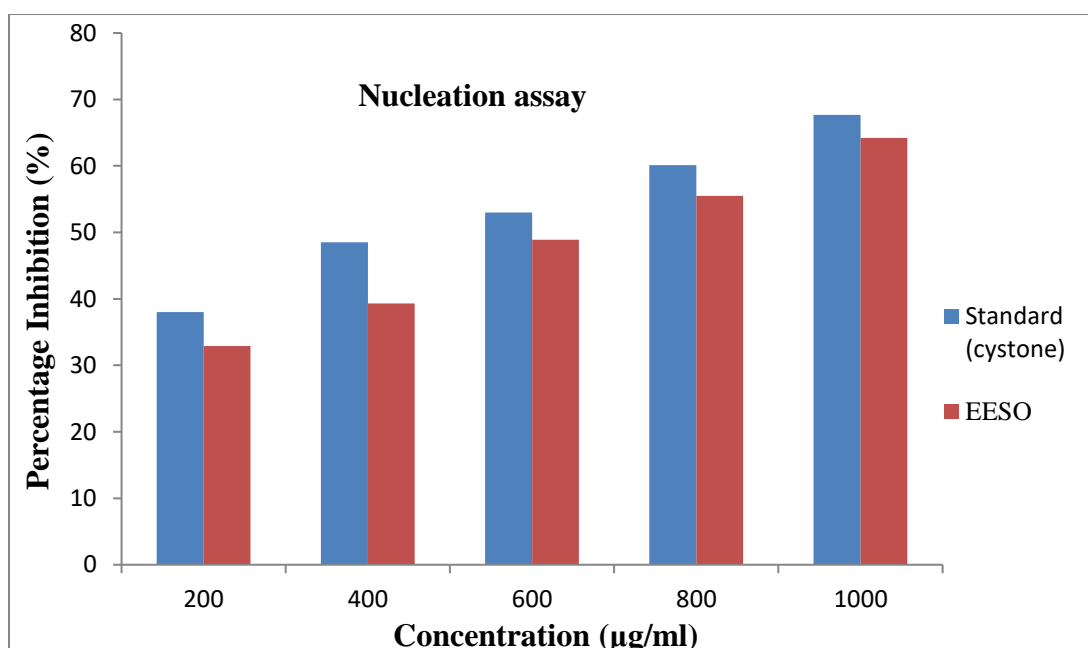


Figure 1: Effect of inhibition in nucleation assay of EESO

Effect of Inhibition in Aggregation Assay

The results of inhibiting aggregation activities at various concentrations of extract and standard were shown; the largest inhibition was at 1000µg/ml for the extract, 74.2%±0.08 which was inhibited, while the standard (cystone) was 76.7%±0.09 inhibited. Both in the extract and the standard, the inhibition was discovered to be enhanced from 200-1000µg/ml. The results of the aggregation experiment indicate that the extract includes nucleation-preventing compounds that will prevent kidney stone growth.

Table 2: Effect of inhibition in aggregation assay of EESO

Sl. No	Concentration (µg/ml)	Aggregation assay	
		STANDARD (Cystone)	EESO
1	200	46.4%±0.07***	41.0%±0.00***
2	400	56.0%±0.01***	53.2%±0.10**
3	600	67.8%±0.08***	61.0%±0.13**
4	800	71.7%±0.07***	68.2%±0.76*
5	1000	76.7%±0.09***	74.2%±0.08**

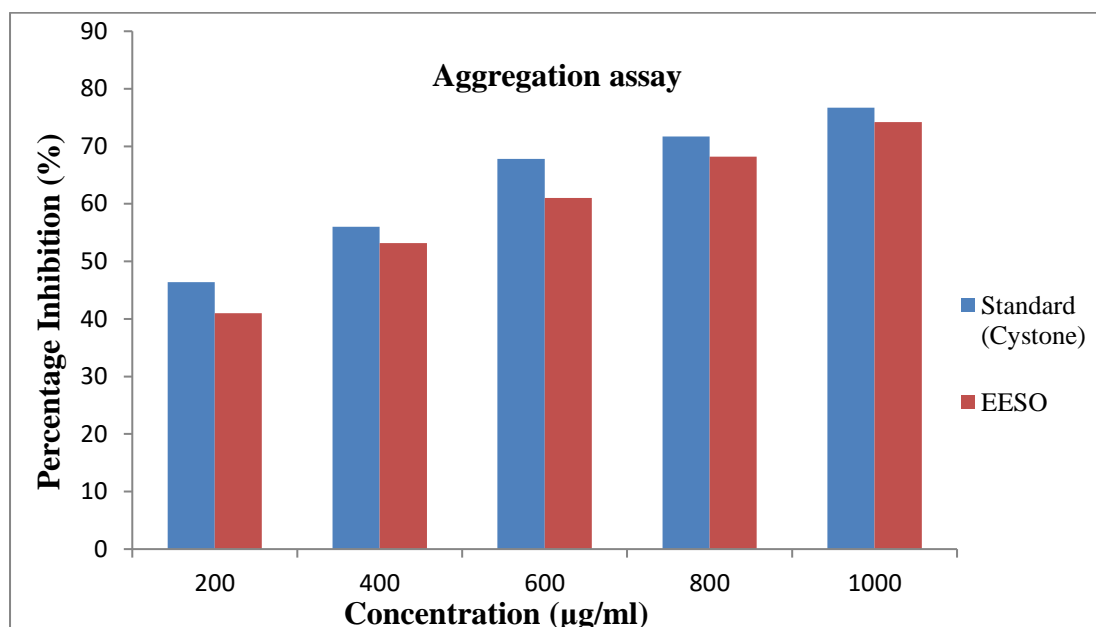


Figure 2: Effect of inhibition in aggregation assay of EESO

Discussion

Since ancient times, traditional medicine has made extensive use of all *Saponaria officinalis* sections [14]. A tentative scientific finding of *Saponaria officinalis* extracts lithotripsy characteristics has been made by this investigation. Although the precise mechanism of stone formation is not entirely known, it is most frequently stated that the development happens as a result of a sequence of physiochemical events including nucleation and aggregation in renal tubules [4]. There are two forms of nucleation: homogeneous nucleation and heterogeneous or secondary nucleation. Nucleation is the first stage of the creation of kidney stones and establishes the smallest unit lattice of a crystal species [15]. In order to compare the inhibition of the activities of nucleation and aggregation, cystone was used as a positive control in this investigation. Cystone, a formulation of many herbs including *Didymocarpus pedicellata*, *Saxifraga ligulata*, and *Gokshura*, was created and developed to treat urolithiasis and renal calculi illness [16]. Similar to Sharma et al. [17] and Chaudhary et al. [18], these researchers used cystone as a control in their tests to examine the leaf extracts' ability to suppress the formation of calcium oxalate. Crystals must initially undergo nucleation in order to begin growing and forming aggregates. In order to test the potential of several *Saponaria officinalis* extracts to inhibit calcium oxalate, an in vitro crystallization research was conducted. The extract contains substances that prevented both nucleation and aggregation, according to the results of the nucleation experiment. The saponin in the *Saponaria officinalis* extract may be able to prevent the formation of CaOx crystals [19]. Additionally, it was discovered that a saponin-rich fraction of *Saponaria officinalis* was a strong in vitro inhibitor of CaOx crystal formation [20]. Other phytochemicals may have contributed to the stated actions, though this cannot be ruled out. The best strategy to prevent and treat urolithiasis is to control the crystallization process, which is crucial for the production of stones. In order to cure kidney stones, plant extracts like *Saponaria officinalis* have been used extensively in folk and Ayurvedic medicine. Plant extracts often include a complex mixture of several bioactive chemicals that can be extracted using a variety of extraction methods [21]. Using a formula, the extract's % suppression of calcium oxalate crystallization was determined. The current study clearly demonstrates that, as compared to this extract, the ethanolic extract of *Saponaria officinalis* entire plant had better calcium oxalate crystallization inhibition ($64.2\% \pm 0.5$) by nucleation assay at concentrations of $1000\mu\text{g/ml}$. Cystone demonstrated the greatest inhibition ($67.7\% \pm 0.8$). In the aggregation assay, the extract inhibited by $74.2\% \pm 0.08$ and the standard by $76.7\% \pm 0.09$, respectively. Therefore, the nucleation and aggregation assays of the EESO indicated that it may prevent crystallization and had antiurolithiatic activity.

Conclusion

The findings lead us to the conclusion that the ethanol extract of *S. officinalis* can serve as the sole source of cutting-edge antiurolithiatic medications. Future research will focus on the isolation of these species' active ingredients as well as the underlying mechanisms that underlie their antiurolithiatic effects.

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Conflict of Interest: The authors declare that there are no conflicts of interests.

References

1. Harper JD, Cunitz BW, Dunmire B, Lee FC, Sorensen MD, Hsi RS, et al. First in human clinical trial of ultrasonic propulsion of kidney stones. *J Urol.* 2016; 195 (4):956-64.
2. Tyagi S, Patel C, Patel J, Tarun PS. Review on Kidney Stones. *Journal of Biomedical and Pharmaceutical Research.* 2012; 1(3):06-09.
3. Havagiray R, Chitme SA, Jain SK, Monika S. Herbal treatment for urinary stones. *IJPSR* 2010; 1:1-10.
4. Ratkalkar VN, Kleinman JG. Mechanisms of Stone Formation. *Clinical reviews in Bone and Mineral Metabolism.* 2011; 9(3-4):187-197.
5. Bahmani M, Baharvand-Ahmadi B, Tajeddini P, Rafieian-Kopaei M, Naghdi N. Identification of medicinal plants for the treatment of kidney and urinary stones. *Journal of Renal Injury Prevention.* 2016; 5(3):129-133.
6. Stamatelou KK, Francis ME, Jones CA, Nyberg Jr LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. *Kidney International.* 2003; 63(5):1817-1823.
7. Chaudhary A, Singla S, Tandon C. In vitro evaluation of *Terminalia arjuna* on calcium phosphate and calcium oxalate crystallization. *Indian Journal of Pharmaceutical Sciences.* 2010; 72(3):340.
8. Patel KM, Samir KS. Evaluation of Antiurolithiatic Activity of *Lawsonia inermis* Linn in Rats. *International Journal of Pharmaceutical Science and Nanotechnology.* 2017; 10(1):3728-3735.
9. Aggarwal A, Tandon S, Singla SK, Tandon C. Reduction of oxalate-induced renal tubular epithelial (NRK-52E) cell injury and inhibition of calcium oxalate crystallisation in vitro by aqueous extract of *Achyranthes aspera*. *International Journal of Green Pharmacy.* 2010; 4(3):480-489.
10. Bhattarai S, Chaudhary RP, Quave CL, Taylor RSL. The use of medicinal plants in the trans-Himalayan arid zone of Mustang district, Nepal. *Journal of Ethnobiology and Ethnomedicine.* 2010; 6:4-14.
11. Kumaran MS, Patki PS. Evaluation of an Ayurvedic formulation (Cystone), in urolithiasis: A double blind, placebo-controlled study. *European Journal of Integrative Medicine.* 2011; 3(1):23-28.
12. Brardi S, Imperiali P, Cevenini G, Verdacchi T, Ponchiotti R. Effects of the association of potassium citrate and *Agropyrum repens* in renal stone treatment: results of a prospective randomized comparison with potassium citrate. *Archivio Italiano di Urologia e Andrologia.* 2012; 84(2):61-67.
13. Patel PK, Patel MA, Vyas BA, Shah DR, Gandhi TR. Antiurolithiatic activity of saponin rich fraction from the fruits of *Solanum xanthocarpum* Schrad. And *Wendl.* (Solanaceae) against ethylene glycol induced urolithiasis in rats. *J Ethnopharmacol.* 2012; 144 (1), 160-70.
14. Sampath Kumar KP, Bhowmik D, Duraiavel S, Umadevi M. Traditional and medicinal uses of banana. *J Pharmacogn. Phytochem.* 2012; 1:53.
15. Vekilov PG. Nucleation. *Cryst Growth Des.* 2010; 10(12):5007-19.
16. Karamakar D, Patki P. Evaluation of efficacy and safety of a herbal formulation cystone in the management of urolithiasis: meta-analysis of 50 clinical studies. *Int J Altern Med.* 2013; 8(1):1-10.

17. Sharma D, Dey YN, Sikarwar I, Sijoria R, Wanjari MM, Jadhav AD. In vitro study of aqueous leaf extract of *Chenopodium album* for inhibition of calcium oxalate and brushite crystallization. *Egypt J Basic Appl Sci.* 2016; 3(2): 164–71.
18. Chaudhary D, Paudel S, Rana RM, Timsina S, Malla KP, Giri PM, Neupane BP. Inhibition of calcium oxalate crystallization in vitro by methanolic leaf extract of *Murraya koenigii* (L.) Spreng. *Int J Herb Med.* 2018; 6(2):13–5.
19. Onyema CT, Ofor CE, Okudo VC, Ogbuagu AS. Phytochemical and antimicrobial analysis of banana pseudo stem (*Musa acuminata*). *J Pharma Res Int.* 2016:1–9.
20. Fouada A, Yamina S, Nait MA, Mohammed B, Abdlekrim R. In vitro and in vivo antilithiatic effect of saponin rich fraction isolated from *Herniaria hirsuta*. *J Bras Nefrol.* 2006; 28: 199–203.
21. Sivaraman D. Evaluation of anti- urolithiasis potential of Indian medicinal herbs *Ficus hispida*, *Morinda tinctoria* and *Sapindus emarginatus* by struvite crystal growth inhibition assay. *Int J ChemTech Res.* 2017; 10 (10):436–48.

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