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In-Vitro Anti-Urolithiatic Properties and GC-MS profiling of *Salvia rosmarinus* Spenn. leaf extract

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Abstract

A frequent urological condition caused by the crystallization of minerals and acid salts in the urinary tract is nephrolithiasis. Calcium oxalate (CaOx) calculi are the predominant kind of renal calculi. Larger kidney stones may require medication or surgery, but smaller ones are usually treated conservatively. This present research involves an *in-vitro* nucleation and dissolution study to investigate the anti-urolithiatic properties of leaf extracts from *Salvia rosmarinus* Spenn. The leaves were shade dried, and powder samples were extracted using a soxhlet apparatus by *n*-hexane and further fractionated successively with sodium bicarbonate, and sodium hydroxide solution. The inhibitory effect of the leaf extracts (50–800 µg/mL) on calcium oxalate (CaOx) crystal formation was evaluated using UV spectrophotometry at 620 nm. Dissolution studies were conducted by titrimetric method. The results demonstrated that different extracts of *Salvia rosmarinus* Spenn. effectively inhibited CaOx crystal formation and facilitated the dissolution of kidney stones. This study indicates the antiurolithiatic potential of extracts from *Salvia rosmarinus* Spenn. as an effective replacement for kidney stone treatment.

Keywords: Calcium oxalate crystal, dissolution study, GC-MS, nucleation assay, *Rosmarinus officinalis.*

Introduction

Urolithiasis was a disorder characterized by the formation or presence of urinary calculi within the urinary system, encompassing the kidneys, bladder, and ureters, as well as the accumulation of hard, solid, non-metallic minerals in the urinary tract (1, 2). The factors influencing urolithiasis include heredity, age, and gender, which are associated with differing risks for stone disease (3). Men are typically more affected by nephrolithiasis, with a ratio of approximately 2:1, however, the incidence of this condition in women had risen in recent decades (4). Diets low in calcium and high in animal protein, salt, oxalates, or sugar along with insufficient magnesium or potassium could have contribute to this condition (5). Urinary tract infections (UTIs) lead to the formation of stones comprised of struvite (magnesium ammonium phosphate) and varying quantities of calcium phosphate or calcium oxalate (6). The production of kidney stones was facilitated by an imbalance in urine between lithogenic chemicals and inhibitors of crystal formation. Key variables that facilitate stone formation include calcium, oxalate, phosphate, bacterial byproducts, cystine, diminished urine volume, uric acid, and acidic urine pH, whereas citrate, magnesium, and increased urine dilution mitigate the chance of crystallization (7).

The primary urinary stones consisted of cystine, silica, calcium oxalate, urate, and struvite (magnesium ammonium phosphate) (8). The prevalence and risk factors of urolithiasis varied among geographic locations and ethnic groups (9). The global prevalence of urolithiasis varied between 2% and 20%. The percentages are 1% to 5% in Asia, 5% to 9% in Europe, and 13% in North America. Moreover, elevated prevalence rates have been noted in Caucasians relative to African-Americans and Asians. The prevalence of kidney stones in the Middle East was between 20% and 25% of the population, whereas Greenland and Japan had the lowest incidence rates (10). Medical intervention employed in the management of urinary system stone disease is contingent upon the type of stone and was effective in only a minority of cases. Alkalinization was effective in managing uric acid stones and, when combined with thiol agents, also helped treat cystine stones. In cases with infectious stones, urine acidity served as an additional medical therapy strategy (11). In all urinary stone disorders, the first general instruction was substantial fluid consumption. With an abundant fluid load, the urine output increased, leading to a reduction in the concentration of insoluble compounds in urine and supersaturation (12). The rising incidence of urolithiasis, along with the emergence of advanced technology, had significantly elevated global healthcare expenditures for its treatment (13). Alternatively, numerous herbal remedies had been developed since ancient times that may be useful in treating renal calculi. Numerous herbal folk medications have anti-urolithiatic properties and were essential for preventing illnesses (14).

Rosemary, or *Rosmarinus officinalis L.,* is a member of the Lamiaceae family. In a recent phylogenetic analysis, the genus Rosmarinus was integrated into the genus Salvia. *Rosmarinus officinalis* was no longer the appropriate designation for the species under investigation. This species required a new specific epithet in Salvia, as the name *Salvia officinalis* was already in use at the time of the merger. Consequently, it is now referred to as *Salvia Rosmarinus* Spenn (15). Rosemary is among the most common shrubs in the Mediterranean region, with its original habitat spanning the Mediterranean areas of Asia, Africa, and Europe, particularly in its principal islands, including Sicily, the Balearic Islands, Sardinia, Elba, and several smaller islands. (16). It is an aromatic shrub with acicular leaves that is grown globally. Rosemary possesses therapeutic characteristics and has been utilized in traditional medicine as an oral remedy to alleviate renal colic, dysmenorrhea, and muscle spasms (15). This plant attracted attention due to its biological activities, which include antibacterial, hepatoprotective, antithrombotic, anticancer, hypoglycaemic, antioxidant, and anti-inflammatory effects. The biological characteristics of *S. rosmarinus* are chiefly ascribed to the presence of phenolic diterpenes, notably carnosol, and polyphenols, such as rosmarinic acid and carnosic acid (19, 20, 21).

Salvia subg*. rosmarinus* is a genus of three aromatic plant species: *Salvia jordanii* J.B.Walker (synonymous *with Rosmarinus eriocalyx Jord. & Fourr.), Salvia granatensis* B.T. Drew (synonymous with *Rosmarinus tomentosus* Hub.-Mor. & Maire*), and Salvia rosmarinus* Spenn. *(*synonymous with *Rosmarinus officinalis L.* and isonymous with *Salvia rosmarinus Schleid).* The initial two species were the most frequently utilized in traditional medical and culinary applications. (22, 23, 24, 25). The hexane and aqueous layers of the ethanol extract of *Salvia rosmarinus* Spenn. dried leaves exhibited an inhibitory effect on urease activity. However, the potential of the extract against urolithiasis has not been well documented. The objective of this study is to assess the various amounts of sodium hydroxide fraction, sodium bicarbonate fraction extract, and *n*-hexane extract from the leaves of *Salvia rosmarinus* Spenn., with cystone serving as a positive control for *the in-vitro* investigation of urolithiasis activity.

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Materials and methods

Collection and authentication

Leaves of *Salvia rosmarinus* Spenn. (*Rosmarinus officinalis L.*) were obtained from A. M. herbal store at Maraimalai Nagar, Tamil Nadu. The plant material was recognized and verified by Dr. KN Sunil Kumar and Dr. P. Elankani at Siddha Central Research Institute (SCRI), Arumbakkam, Chennai.

Extraction

About 500 g of dried, powdered leaves were extracted with 1200ml of *n*-hexane using a Soxhlet apparatus. The extract was then concentrated; the non-volatile compounds were dissolved in 50ml of ether and washed with 20ml of water. The solution was successively extracted with 30ml of saturated sodium bicarbonate ($NaHCO_3$) and 30 ml of sodium hydroxide (NaOH) to separate the strongly acidic and weakly acidic fractions, respectively (26). These fractions were used for further evaluation, and their percentage yields were determined. The effect of *Salvia rosmarinus* Spenn. extract on calcium oxalate crystal formation was determined by a nucleation assay according to the method described by Dulce María González Mosquera et al. (2020) with modifications. To about two milliliters of Tris-buffer solution (pH 7.4), one milliliter of total *n*-hexane extract, $NaHCO_3$ fraction, and NaOH fraction at varying concentrations (50, 100, 200, 400, and 800 µg/ml), and one milliliter of 0.025 M calcium chloride dihydrate were added. About 1 milliliter of a sodium oxalate solution at a concentration of 0.025 M was added and absorbance was measured at 620 nm. Cystone was served as the standard drug, and experiment was carried out in triplicates. The antiurolithiatic activity was calculated by the formula (29).

$$\text{Percentage yield (\%)} = \frac{\text{(Weight of extract obtained}}{\text{Weight of plant material used)}} \times 100$$

Phytochemical screening

A preliminary phytochemical analysis was conducted using established protocols to determine the chemical compounds available in the *n*-hexane rosemary extract. To confirm the presence of phytochemicals, chemical test like Dragendorff's test for alkaloids, Lead acetate test for tannins, Libermann-burchard test for steroids, triterpenoids and diterpenoids, Keller-killiani test for glycosides, concentrated H2SO4 test for flavonoids, Fehling's test for reducing sugar, Millon's test for proteins, Ninhydrin test for amino acids, and Barfoed's test for monosaccharides were performed (27).

Gas chromatography - mass spectroscopy (gc-ms) analysis of n-hexane extract of salvia rosmarinus spenn.

The gas chromatography-mass spectroscopy (GC-MS) analysis was conducted utilizing an Agilent 7890B-5977A apparatus, featuring an HP-5MS 5% phenyl methyl silox capillary column (30 m × 0.25 mm × 0.25 µm) and an Agilent 5977A mass spectrometer detector. One microliter of the material was fed into the GC-MS in splitless mode. Helium served as the carrier gas at a purge flow rate of 1 mL/min. The injector temperature was 280 degrees Celsius. The procedure was conducted at a column temperature of 50°C for 2 minutes, thereafter increasing to 270°C at a rate of 5°C/min, and ultimately maintaining that temperature for 10 minutes. All data were acquired by collecting full-scan mass spectra within the range of 40–600 amu, and the findings were analyzed using the NIST 2011 library (28).

Assessment of the *in-vitro* anti-urolithiatic efficacy

Nucleation assay

The effect of *Salvia rosmarinus* Spenn. extract on calcium oxalate crystal formation was determined by a nucleation assay according to the method described by Dulce María González Mosquera et al. (2020) with modifications. To about two milliliters of Tris-buffer solution (pH 7.4), one milliliter of total *n*-hexane extract, $NaHCO_3$ fraction, and NaOH fraction at varying concentrations (50, 100, 200, 400, and 800 µg/ml), and one milliliter of 0.025 M calcium chloride dihydrate were added. About 1 milliliter of a sodium oxalate solution at a concentration

of 0.025 M was added and absorbance was measured at 620 nm. Cystone was served as the standard drug, and experiment was carried out in triplicates. The antiurolithiatic activity was calculated by the formula (29).

Titrimetric analysis of calcium oxalate

The titrimetric method was one of the dissolution methods, and the method was carried out according to V. Anu et al. (2020). The calcium oxalate crystals were encapsulated in a semi-permeable egg membrane, serving as a control, to which various fractions of extract were added, with cystone utilized as a positive control. The semi-permeable membrane was then incubated for two hours at 37°C while suspended in 0.1M Tris buffer. After removing the contents from the membrane, 2 ml of 2N sulphuric acid was added to the separate test tube and titrated using 0.9494N potassium permanganate until a pale pink colour was formed (30).

Development of calcium oxalate crystals via homogeneous precipitation

In 100 ml of 2N H_2SO_4, 1.34 grams of sodium oxalate and 1.47 grams of calcium chloride dihydrate were dissolved, separately. The two solutions were combined in equal proportions in the beaker to precipitate calcium oxalate. Ammonia was added to the solution until the solution becomes basic (9-11) for effective precipitation and free from traces of sulfuric acid. The precipitate was rinsed with distilled water and dried at 60°C for four hours.

Making of semi-permeable membrane utilizing eggs

The eggs were cleaned, and the top of the egg was punctured, and the entire content was squeezed out of the egg. The empty egg was rinsed with distilled water, and the empty eggshell was suspended in the beaker containing 2M HCl overnight for decalcification of eggs. Following decalcification, the membrane was rinsed with distilled water and was immersed it in ammonia solution to neutralize any residual acid while maintaining a moist state for a brief period. It was rinsed again with distilled water and stored in the refrigerator at a pH of 7-7.4.

Determination of calcium oxalate via titrimetric analysis

1 mg of calcium oxalate crystals, 10 mg of plant extract, $NaHCO_3$ fraction, NaOH fraction of the extract, and 10 mg of cystone were encapsulated within a semi-permeable membrane. The membrane was submerged in a beaker containing 100 ml of 0.1 M Tris buffer. Merely 1 mg of calcium oxalate within the membrane functioned as a control. All beakers with semi-permeable membranes were maintained in an incubator at 37ºC for two hours. The contents were removed from the membrane into a separate test tube, 2 ml of 2N sulfuric acid was added, and the solution was titrated with 0.9494N potassium permanganate until a pale pink colour was achieved as the endpoint. To determine the total amount of dissolved calcium oxalate by different extracts, the amount of calcium oxalate that is remained was deducted from the total amount that was utilized at the start of experiment. Each millilitre of 0.9494 N potassium permanganate was equivalent to 0.1898 milligrams of calcium oxalate.

The percentage of dissolution was calculated by using the formula, Undissolved Calcium oxalate (mg) = Volume of $KMnO_4$ used (mL) × 0.1898 mg/mL

Dissolved Calcium oxalate crystals = Initial weight of calcium oxalate (mg) – Undissolved calcium oxalate crystals

Percentage dissolution = Dissolved Calcium oxalate crystals × 100

Microscopic assessment

The quantity and shape of CaOX crystals produced with and without extract and Cystone were assessed using a Labomed CLX monocular microscope at 40× magnification ***(31).*** lar microscope at 40× magnification (31).

Statistical assessment

All data are expressed as the mean ±

Table 1: Characterization and percentage yield of *Salvia rosmarinus* Spenn extract

Plant	Method of extraction	Solvent used	Physical nature	Colour	Yield (% W/W)
Leaves of *Salvia rosmarinus Spenn.*	Successive soxhlet extraction	*n*-hexane	Solid	Dark green	7.8 % w/w
		Sodium bicarbonate	Solid	White	4.9 % w/w
		Sodium hydroxide	Solid	Ash brown	2.5 % w/w

standard error of the mean (SEM). Experiments were performed in triplicate. Comparisons were evaluated using 2-way ANOVA multiple comparisons in GraphPad Prism 7 software, with a significance threshold of $P < 0.05$.

Results and Discussion

Extraction

The dried and powdered leaves of *Salvia rosmarinus* Spenn were subjected to Soxhlet extraction using *n*-hexane and further fractionated with sodium bicarbonate and sodium hydroxide, and their percentage yields were determined and descripted in Table 1.

Phytochemical screening

The qualitative phytochemical investigation of the leaf extract of *Salvia rosmarinus* Spenn yielded results as illustrated in Table 2 and Figure 1.

The *n*-hexane extract of *Salvia rosmarinus* Spenn showed the presence of alkaloids, tannins, steroids, diterpenes, glycosides, flavanes, proteins, and reducing sugar, which could contribute to the biological properties of the extract. A study by Sharifi-Rad et al. discussed that the presence of flavonoids and tannins exhibited strong antioxidant and anti-inflammatory properties that may inhibit CaOX crystallisation and stone formation (32).

Figure 1: Results of Phytochemical Screening

Table 2: Results of phytochemical screening of *n*-hexane *S. rosmarinus* Spenn.

Test	Procedure	Observations	Results
Test for alkaloids	2 ml extract + few drops of Dil.HCl + 1 ml of dragendorff's reagent.	Appearance of orange to red colour	+
Test for tannins	2 ml extract + few drops 10% lead acetate	Appearance of white precipitate	+
Test for saponins	1 ml of extract in a measuring jar combined with 9 ml of distilled water, shaken violently for 15 seconds, and allowed to stand for 10 minutes.	Formation of unstable foam	-

Test for steroids	2 ml extract + 10 ml chloroform + 1 ml acetic anhydride + 2 ml of Con. H_2SO_4 along sides of test tube	Appearance of blue green colour at junction	+
Test for triter-penes	2 ml extract + 10 ml chloroform + 1 ml acetic anhydride + 2 ml of Con. H_2SO_4 along sides of test tube	Appearance of blue green colour at junction	-
Test for diter-penes	2 ml extract + 10 ml chloroform + 1 ml acetic anhydride + 2 ml of Con. H_2SO_4 along sides of test tube	Appearance of green colour	+
Test for glyco-sides	1 ml extract + few drops of glacial acetic acid + FeCl3 + 3-4 drops of Con.H_2SO_4	Appearance of blue green colour	+
Test for flavo-noids	1 ml extract + Con. H_2SO_4	Appearance of orange to red colour (Flavanes)	+
Test for reducing sugar	1 ml of extract + 1 ml water + 5-8 drops of Fehling's solution A&B and heat it	Appearance of brick red pre-cipitate	+
Test for proteins	3 ml extract + 5 ml millon's reagent and heat it	Appearance of Brick red	+
Test for Amino acids	3 ml sample + 3 drops of Ninhy-drin solution. Heat the solution for 10min	Appearance of brown colour	-
Test for Mono-saccharides	1 ml sample + Barfoed reagent. Heat the solution	Appearance of blue precipi-tate	-

GC-MS analysis of n-hexane extract

Figure 2 displays the GC-MS chromatogram of the *n*-hexane extract derived from leaves of *S. rosmarinus* Spenn. The compounds detected in the GC-MS spectra of the *n*-hexane extract of *S. rosmarinus* Spenn. leaves, along with their retention times and peak areas, were detailed in Table 3. A total of 33 components were detected in the n-hexane extract, accounting for 99.78% of the total peak areas of the components. Eucalyptol was found to be the predominant constituents, is known to possess anti-inflammatory, antioxidant, and antimicrobial properties. These biological effects may help to protect against urolithiasis by reducing the inflammation and preventing urinary tract infection, both of which are common factors in the kidney stone formation (33). Additionally, the potential of *Salvia rosmarinus* to prevent kidney stones is further supported by presence of squalene, a well-known antioxidant, which may aid in the reduction of oxidative stress (34). Other compounds, including camphene and α-terpineol, have been linked to nephroprotective effects and may contribute to the prevention of kidney stone formation (Erkan et al., 2008) (21).

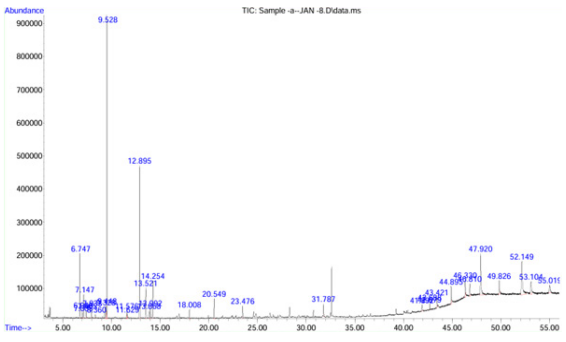

Figure 2: Gas Chromatography – Mass Spectrometry of the phytochemicals in the n-hexane extract of *S. rosmarinus* Spenn.

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Table 3: Compounds detected in the GC-MS profile of the n-hexane extract from *S. rosmarinus* Spenn. leaves

S.No	Retention time	Name of the compounds	Molecular formula	Molecular weight	Peak area %
1.	6.681	Hyocholic acid	$C_{24}H_{40}O_5$	408	4.775
2.	6.996	Nickel tetracarbonyl	C_4NiO_4	170	0.119
3.	7.005	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.057
4.	7.147	Camphene	$C_{10}H_{16}$	136	2.123
5.	7.400	Hydroperoxide, 1-methylpentyl	$C_6H_{14}O_2$	118	0.612
6.	7.942	β-Terpinene	$C_{10}H_{16}$	136	0.779
7.	8.346	β-Pinene	$C_{10}H_{16}$	136	0.248
8.	9.332	o-Cymene	$C_{10}H_{14}$	134	0.908
9.	9.448	D-Limonene	$C_{10}H_{16}$	136	1.178
10.	9.532	Eucalyptol	$C_{10}H_{18}O$	154	24.778
11.	11.576	β-Linalool	$C_{10}H_{18}O$	154	0.759
12.	11.629	Decane	$C_{10}H_{22}$	142	0.307
13.	12.890	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)	$C_{10}H_{16}O$	152	13.579
14.	13.521	endo-Borneol	$C_{10}H_{18}O$	154	3.262
15.	13.859	Terpinen-4-ol	$C_{10}H_{18}O$	154	0.698
16.	13.988	1H-Indene, 1-methylene	$C_{10}H_8$	128	1.026
17.	14.254	α-Terpineol	$C_{10}H_{18}O$	154	3.292
18.	17.999	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212	0.834
19.	20.544	Caryophyllene	$C_{15}H_{24}$	204	1.990
20.	23.511	Molybdenum, dicarbonylbis(η-4-2-methylenecyclo-heptanone)	$C_{18}H_{24}MoO_4$	402	1.112
21.	31.760	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	1.168
22.	41.906	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366	0.876
23.	42.679	1,4-Thiazine, perhydro-1-benzoylimino-4-(3-chloro-5-trifluoromethyl-2-pyridyl)	$C_{17}H_{15}ClF_3N_3OS$	401	0.059
24.	42.688	Androst-4-en-11-ol-3,17-dione, 9-thiocyanato	$C_{20}H_{25}NO_3S$	359	0.072
25.	43.434	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366	1.430
26.	44.873	7,7-Diethylheptadecane	$C_{21}H_{44}$	296	2.785
27.	46.374	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	2.473
28.	46.814	Squalene	$C_{30}H_{50}$	410	1.720
29.	47.938	Tetratriacontane	$C_{34}H_{70}$	478	8.360
30.	49.799	Tetratriacontane	$C_{34}H_{70}$	478	2.654

31.	52.180	Triacontane	$C_{30}H_{62}$	422	10.492
32.	53.157	Propane-1,3-dione, 1,3-bis(2,4-dimethoxyphenyl)	$C_{19}H_{20}O_6$	344	3.361
33.	55.019	Hexadecanoic acid, 2-(octadecyloxy)ethyl ester	$C_{36}H_{72}O_3$	552	2.114

Nucleation assay

The results of the assay were depicted in Figure 3 and Table 4**.** Sodium oxalate solution was added to a reaction mixture containing calcium chloride ($CaCl_2$); it led to the formation of calcium oxalate (CaOx) crystals. The nucleation assay results demonstrated a concentration-dependent suppression of CaOx crystal formation. The sodium hydroxide fraction demonstrated the most significant inhibitory effect (88.66% at 800 µg/mL), followed by the n-hexane extract (87.24%) and the sodium bicarbonate fraction (84.44%). The inhibitory effect of these extracts was similar to that of the standard drug Cystone (86.83%). In the study conducted by Rajeshwari et al., inhibitory effects of herbal extracts were identified, indicating that phytochemicals such as polyphenols and flavonoids diminish the aggregation and proliferation of CaOx crystals (1). The strong inhibition at elevated doses suggests a possible dose-dependent mechanism, which is essential for future formulation research to ascertain the appropriate dosage for therapeutic use.

Table 4: Effects of extract of *S.rosmarinus* Spenn. on Calcium oxalate crystals

S. No	Extract	Concentration					IC50 Value
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	
1.	*n*-hexane Extract	46.08±0.05 ***	59.45±0.06 ***	75.37±0.14 ***	79.49±0.13 *	87.24±0.23	52.71
2.	Sodium bicarbonate fraction	25.50±2.24 ***	61.02±0.02 ***	66.35±0.80	75.37±0.14 ***	84.44±0.09	85.96
3.	Sodium hydroxide fraction	63.11±0.29	66.39±0.17	76.86±0.32 ***	86.50±0.09	88.66±1.58	25.08
4.	Cystone	65.68±0.32	68.72±0.18	69.93±0.56	79.23±0.11	86.83±1.14	16.44

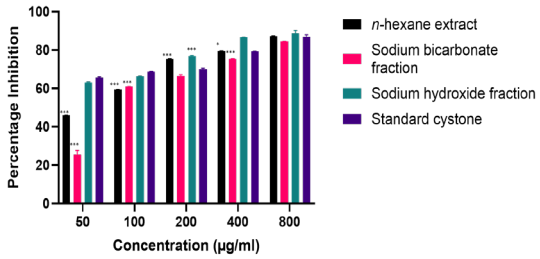

Figure 3: Effect of n-hexane extract of *S. rosmarinus* Spenn. and standard cystone by nucleation assay. Data are presented as mean ± SEM of triplicate measurements.

Titrimetric analysis of calcium oxalate

The percentage dissolution of the *n*-hexane extract, sodium bicarbonate, and sodium hydroxide fractions of *Salvia rosmarinus* Spenn. was determined, using cystone as the standard. The dissolution percentages of the extract and its fractions are presented in Table 5. The titrimetric analysis further confirmed the anti-urolithiatic efficacy of *S. rosmarinus* extracts. The *n*-hexane extract demonstrated the highest dissolving percentage (90.51%), succeeded by sodium bicarbonate (86.71%) and sodium hydroxide (79.12%), all exceeding the standard Cystone (82.91%). This indicates that *S. rosmarinus* extracts not only inhibit stone formation but also promote the dissolution of existing stones. Sharma et al. have shown that polyphenolic chemicals improve the dissolution of CaOx by destabilizing crystal lattice integrity (33). The capacity of the extracts to dissolve pre-

existing stones underscores their potential as therapeutic agents capable of both preventing and treating kidney stones, offering a dual strategy for managing urolithiasis.

Table 5: Percentage dissolution of calcium oxalate

Extract	Dissolved Calcium oxalate (mg)	Percentage Dissolution
n-hexane extract	0.9051	90.51%
Sodium bicarbonate fraction	0.8671	86.71%
Sodium hydroxide fraction	0.7912	79.12%
Cystone	0.8291	82.91%

Microscopic analysis

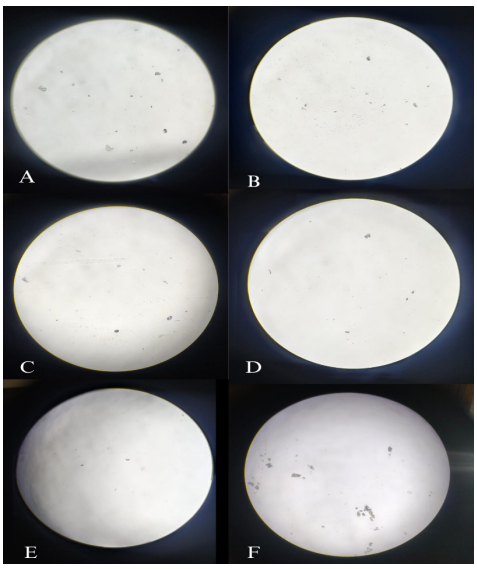

Figure 5: Microscopic analysis of CaOX crystals in the in the presence of extract (A) 50 µg/ml, (B) 100 µg/ml, (C) 200 µg/ml, (D) 400 µg/ml, (E) 800 µg/ml, and (F) absence of extract.

Microscopic examination validated the decrease in both the number and dimensions of CaOx crystals when *S. rosmarinus* extracts were present. At elevated concentrations, the extracts modified the shape of CaOx crystals, resulting in diminished aggregation, a critical element in the production of kidney stones. The crystals in the control group were big and densely aggregated, while those subjected to *S. rosmarinus* extracts were diminutive and more scattered. This structural transformation is essential since it inhibits the development of larger, clinically relevant stones that may result in considerable urinary blockage (35).

Conclusion

In the present study, the preliminary phytochemical screening, GC-MS analysis, and anti-urolithiatic activity of the n-hexane extract of *Salvia rosmarinus* leaves and its fractions were investigated. Phytochemical screening confirmed the presence of alkaloids, tannins, steroids, diterpenes, glycosides, flavonoids, and proteins. GC-MS analysis identified 33 components included β-Terpinene, β-Linalool, Camphene present in the n-hexane extract. The anti-urolithiatic activity was assessed through nucleation and titrimetric assays. At a higher concentration of 800 µg/ml, both the sodium hydroxide (NaOH) fraction and the n-hexane extract significantly inhibited the formation of calcium oxalate (CaOx) crystals in the nucleation assay. Additionally, the titrimetric assay demonstrated the effective dissolution of CaOx crystals by the n-hexane extract than fractions. The strong inhibitory effects observed in nucleation and dissolution assays suggest that these extracts hold potential for developing effective herbal therapeutics for managing urolithiasis. This study provides a foundation for further research and development in urolithiasis treatment.

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