



EVALUATION OF IN VITRO ANTI- UROLITHIATIC POTENTIAL OF *Sonchus arvensis*

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ABSTRACT

Background: Phytochemicals found in medicinal plants are used to treat a variety of diseases, including kidney stones, due to their ease of use, low toxicity, and effectiveness. In current research, we are using *Sonchus arvensis* for the treatment of urolithiasis. It means kidney disease. A common medicinal plant that can be found in many different locations is *Sonchus arvensis* (Linn.), sometimes known as tempuyung. *Sonchus arvensis* is a member of the Asteraceae family and is widely dispersed in Indonesia. In Indonesia, this plant is typically eaten raw (as salad) as an inexpensive and widely available source of vitamins, minerals, and proteins.

Method: The essential phytochemicals in the root aqueous and ethanolic extracts were estimated, and their in vitro anti-urolithiasis status was assessed. To the best of our knowledge, there have been multiple reports of tests and comparative analyses utilising these herbs. In vitro, anti-lithiatic research employed by a titration technique.

Result: Calcium oxalate by titrimetry method, the ethanolic extracts of *S. arvensis* roots have capability to dissolve calcium oxalate as foremost element for stone forming in the urinary tract. Percentage dissolution of calcium oxalate crystals was found to be 51.50% for cystone, 30.33% for 20 mg drug concentration, 29.68% for 40 mg drug concentration, 35.62% for 60 mg drug concentration and 32.22% for 80 mg drug concentration respectively.

Conclusion: According to the study's findings, *Sonchus arvensis*, one of the medicinal weeds chosen for this investigation, has the greatest concentration of bioactive chemicals and, as a result, the strongest in vitro anti-urolithic activity.

Keywords: Urolithiasis, Calcium Oxalate, Kidney stone.

INTRODUCTION

Nephrolithiasis, sometimes referred to as urolithiasis or kidney stone disease, is among the earliest illnesses recognized by modern medicine. An estimated 1–15% of people may have kidney stones at some point in their lives, and the incidence and prevalence of kidney stones are said to be rising globally [1]. A crystal concretion that typically forms inside the kidneys is known as kidney stone disease. It is a growing urological condition affecting human health. The most prevalent kind of kidney stone is calcium oxalate, which forms at Randall's plaque on the renal papillary surfaces. The genesis of kidney stones is complex. A number of physicochemical processes, including as supersaturation, nucleation, development, aggregation, and retention of urinary stone ingredients within tubular cells, contribute to the intricate process of stone formation [2]. Nephrolithiasis, or kidney stone disease, is a frequent condition that can be linked to changes in the composition of urine solutes, such as hypercalciuria [3]. There are three different ways kidney stones can occur. Underlying the first pathway is overgrowth on interstitial apatite plaque, which is observed in stone formers with brushite, ileostomy, primary hyperparathyroidism, and idiopathic calcium oxalate abnormalities. With the exception of the idiopathic calcium oxalate stone formers, all groups that form stones have crystal deposits in their renal tubules along the second pathway. Crystallization of free solutions is the third pathway. Patients with cystinuria or hyperoxaluria linked to obesity bypass surgery are prime instances of this pathway [4]. Based on the mineralogical composition, there are five primary forms of kidney stones: brushite (1.7%), calcium oxalate (65.9%), carbapatite (15.6%), urate (12.4%), struvite [(magnesium ammonium phosphate), 2.7%] (9,10). The two main types of kidney stones are non-calcareous and calcareous, or containing calcium. The most prevalent forms of kidney stones in humans are calcareous and radio-opaque stones called CaOx and calcium phosphate (CaP), either isolated or in combination [5].

A common medicinal plant that can be found in many different locations is *Sonchus arvensis* (Linn.), sometimes known as tempuyung. The anti-urolithiasis properties of tempuyung leaves, which have been employed in Indonesian traditional medicine, have been demonstrated by science. Empirically, it is used as a water infusion and to decrease blood pressure [6].

Sonchus arvensis is a member of the Asteraceae family and is widely dispersed in Indonesia. In Indonesia, this plant is typically eaten raw (as salad) as an inexpensive and widely available source of vitamins, minerals, and proteins. It is thought that consuming this plant, particularly in its raw form, can help with a number of health issues, including oxidative stress, asthma, brain dysfunction, adrenal dysfunction, hepatotoxicity, and nephrotoxicity [7].

Measuring the phytochemical components and in vitro anti urolithiatic capabilities of *S. arvensis* can help establish its medicinal potential. As a result, it becomes necessary to quantify and examine the phytochemical components of the leaves of this medicinal plant, which are primarily in charge of its anti urolithiatic action. The present study to measure the phytochemical components and assess the in-vitro anti urolithiatic properties.

Method

Collection and Authentication of the Plant Material: The fresh plant of *Sonchus arvensis* was collected from the College Campus (College of Pharmacy, Shivalik), Central Hope Town, Dehradun, Uttarakhand. The specimen of the dried plant with herbarium file has been submitted to the The Botanical Survey of India, Dehradun, for the authentication. The authentication of plant material is under process.



Figure 1: Collection of *S. arvensis*

Preparation of Extract

A mechanical grinder is used to ground the plant's dried roots into a fine powder. Soxhlet extraction is used to powder material using different polarity solvents, such as petroleum ether, chloroform, ethanol and water. The powdered material is dried in a hot air oven below 50°C prior to each extraction. To obtain the aqueous extract, marc is finally digested for 24 hours at 50°C with distilled water. The extracts are concentrated in a rotary vacuum evaporator set at 40°C, and they are then stored at 4°C in the refrigerator until needed again. The extract was weighed, and the yield was determined.

Phytochemical Analysis of *S. arvensis*

The crude extracts of *S. arvensis* roots was screened for various phytochemicals such as alkaloids, glycosides, tannins, flavonoids, saponins etc.

Test for Alkaloids- Alkaloid was identified by extract of *S. arvensis* roots based on was Dragendorff's test and Mayer's test.

(a) Dragendorff's test. By adding 1 mL of Dragendorff's reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

(b) Mayer's test. Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

Test for Flavonoids- Flavonoid was identified by dissolving methanol, and 0.5 grams of magnesium, and 5 drops of HCl into extract of *S. arvensis* roots.

Test for Glycosides- A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract. Later, 1 mL of concentrated H₂SO₄, was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides.

Test for Terpenoids- Terpenoid were identified by adding 0.5 mL of anhydrous acetic acid, 0.5 mL of chloroform, and 2 mL of concentrated sulfuric acid into extract of *S. arvensis* roots.

Test for Saponins- A drop of Na₂CO₃ solution was added to 5 mL of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins.

Test for Tannins- Tannis was identified by added with 2 mL of 1% FeCl₃ into extract of *S. arvensis* roots.

✓ Estimation of Calcium oxalate by titrimetry Method

- Preparation of Calcium Oxalate by homogenous precipitation:** Exactly 2.940 g of calcium chloride dihydrate was dissolve in 100 ml distilled water and 2.68 g of calcium oxalate was dissolve in 100 ml of 2N sulphuric acid. Equimolar prepared solutions of calcium chloride dihydrate and sodium oxalate were allowed to reach in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate precipitate was freed from traces of sulfuric acid by ammonia solution, washed with distilled water, and dried at 60°C for 2 h [8].



Figure 2: Homogenous precipitation of Calcium Oxalate

Estimation of Calcium oxalate by titrimetry Method

- Preparation of Calcium Oxalate by homogenous precipitation:** The calcium oxalate homogenous precipitation prepared by 2.940 g of calcium chloride dihydrate was dissolve in 100 ml distilled water and 2.68 g of calcium oxalate was dissolve in 100 ml of 2N sulphuric acid after stirring we found the precipitation.
- Preparation of the semi permeable membrane from farm eggs:** Apex of eggs was punctured to remove out the entire content, and empty shells were washed thoroughly with distilled water and then placed in a beaker containing 4ml of concentrated HCl in 200 ml distilled water. It

was kept overnight for decalcification

process; on the next day, semipermeable membrane was removed carefully, washed thoroughly with distilled water, and placed it in ammonia solution for neutralization of acid traces. Finally, semi-permeable membrane was rinsed with distilled water, stored in refrigerator, and used for calcium oxalate dissolution method [9].

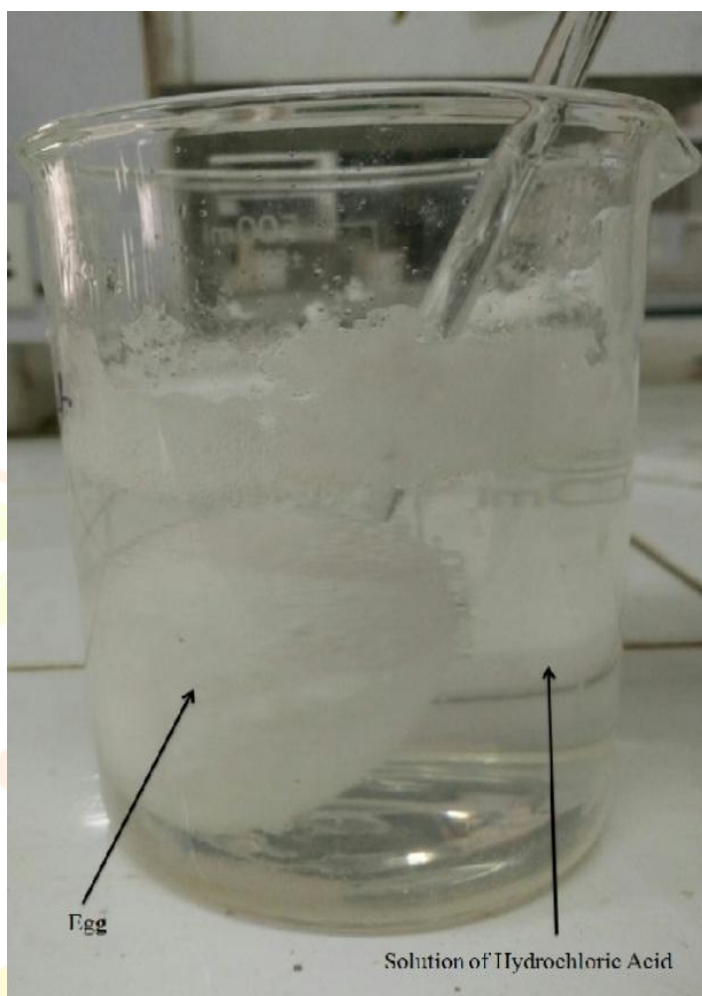


Figure 3: Preparation of Semi Permeable Membrane from Egg

Procedure: As per experimental design, dissolution studies of calcium oxalate were carried out in six groups;

one group served as negative control (Group I) which consists of 1 mg of calcium oxalate alone, weighed exactly 1 mg of calcium oxalate and packed along with 10 mg of Cystone (removing color coating and crushed into powder form) as standard group (Group II). In test groups (Groups III-VI), 20 mg, 40 mg, 60 mg, 80 mg of test were packed along with 1 mg of the calcium oxalate. All groups were packed as above in separate egg-based semipermeable membranes tied with thread by suturing at one end. Now, the materials packed in semipermeable membranes were allowed to suspend in separate conical flasks containing 100 ml of 0.1M TRIS buffer. Another end of the thread was tied on the mouth of conical flasks and covered with aluminum foil. The conical flasks of all groups were kept in an incubator, preheated to at $37 \pm 1^\circ\text{C}$ for 7 h. After 7 h, flasks with membrane were kept in magnetic stirrer for 5 min.



Figure 4: An Incubator, Heated to 37°C

Finally, all the membranes were taken out of the flask and content of each membrane was collected in different test tubes by removing the sutures. 2 ml of 1 N sulfuric acid was added to each test tube and titrated with 0.9494 N KMnO_4 till a dark pink color end point obtained. 1 ml of 0.9494 N KMnO_4 is equivalent to 0.2103 mg of calcium. The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate was dissolved by the test substances. There is inverse relationship between the dissolution of calcium oxalate and concentration of calcium ions in solutions [10]. Each treatment was repeated 3 times and volume of standard KMnO_4 was expressed as mean $\pm$ SD. The percentage dissolution of calcium oxalate in various groups was calculated.

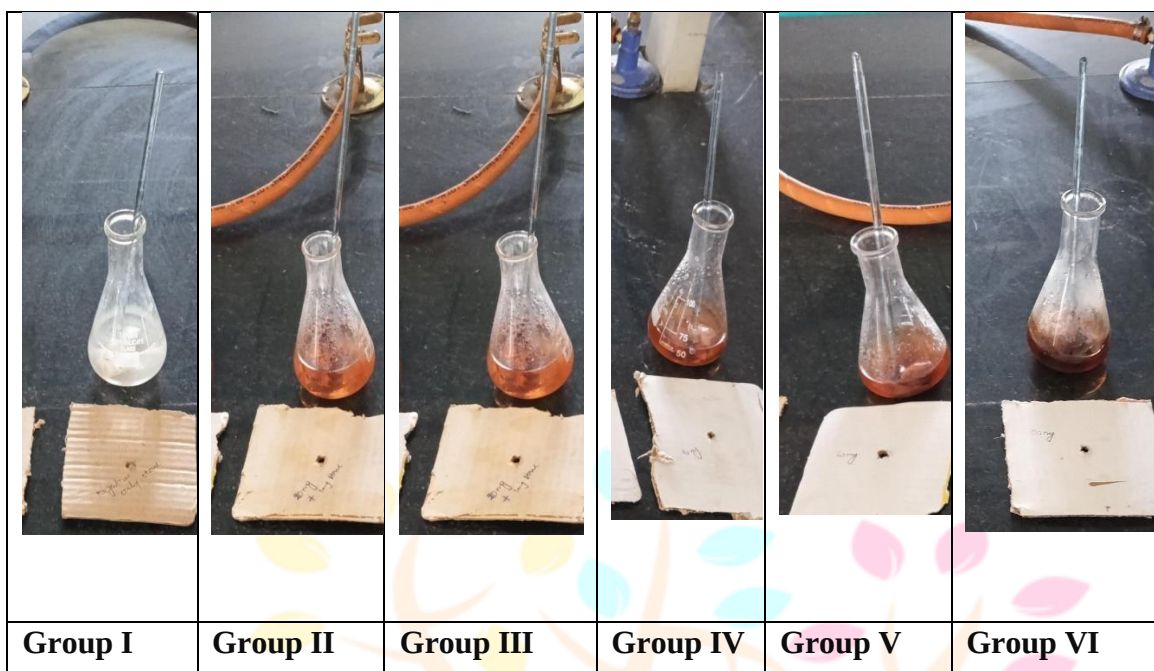


Figure 5: Samples after Incubating

Results

Preparation of Extraction: A mechanical grinder is used to ground the plant's dried roots into a fine powder. Soxhlet extraction is used to powder material using different polarity solvents, such as petroleum ether, chloroform, ethanol and water.

Table 1: Percentage yield of extracts.

S. No	Name of Extract	Yield (% w/w)
1.	Petroleum ether Extract	2.5%
2.	Chloroform Extract	2%
3.	Ethanol Extract	1.5%
4.	Water Extract	3.2%

Phytochemical screening of *S. arvensis*: The crude extracts of *S. arvensis* roots were screened for phytochemical analysis and the results are shown in Table 1.

Table 2: Phytochemical screening of *S. arvevsis* root

Experimental Design	Volume standard of KMnO ₄	Wt of calcium oxalate estimated (mg)	Wt of calcium reduced (mg)	Percentage Dissolution
Group I	0.52	0.7895	-	-
Group II	2.52	0.4342	0.3842	51.50
Group III	4.51	0.5674	0.5002	30.33
Group IV	3.22	0.6765	0.6124	29.68
Group V	2.57	0.5182	0.4675	35.62
Group VI	3.53	0.6810	0.6384	32.22

- Calcium oxalate by titrimetry method, the ethanolic extracts of *S. arvensis* roots have capability to

S. No.	Extract	Alkaloids	Flavonoids	Glycosides	Terpenoids	Saponins
1.	Petroleum Ether	+	-	-	+	-
2.	Chloroform	+	+	-	-	-
3.	Ethanol	-	+	-	+	-
4.	Water	-	-	+	-	+

dissolve calcium oxalate as foremost element for stone forming in the urinary tract. Percentage dissolution of calcium oxalate crystals was found to be 51.50% for cystone, 30.33% for 20 mg drug concentration, 29.68% for 40 mg drug concentration, 35.62% for 60 mg drug concentration and 32.22% for 80 mg drug concentration respectively (Table 3).

Table 3: Effect of *S arvensis* on percentage dissolution of calcium oxalate crystals

Conclusion:

The study's goal is to learn more about the beneficial effects of *Sonchus arvensis* plant, particularly roots, in preventing and treating medical issues such kidney stones, which are becoming more common in the younger population as a result of sedentary lifestyles and poor eating habits. This study show the *in vitro*

anti-urolithiatic potential. For further studies to check the potential of plant then *in vivo* study is required. The phytochemicals in this plant individually identify and work on isolation should be done in the future for identify which constituent is responsible for this study.

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