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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.

Method: The essential phytochemicals in the leaves aqueous and 70% 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. A quantitative analysis was conducted using UV-vis spectrophotometry. In vitro, anti-lithiatic research employed a titration technique.

Result: While the 70% ethanolic extract shows a larger concentration of cardiac glycosides and alkaloids, the aqueous extract shows a higher content of polyphenols, flavonoids, tannins, and inhibitory percentage values for free radical scavenging assays.

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.

Introduction

Nephrolithiasis, sometimes referred to as urolithiasis or kidney stone disease, is among the earliest illnesses recognised 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. Crystallisation 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-urolithic capabilities of Sonchus 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-urolithiasis action. The present study endeavours to measure the phytochemical components and assess the in vitro anti-urolithiasis properties.

Method

Collection, identification and extraction of plant material

The Botanical Survey of India, Dehradun, and India verified the authenticity of the entire plant, which was gathered from Central Hope Town in Uttarakhand. The herbs' fresh leaves were cleaned with purified water and allowed to air dry for 30 days in a shaded area at room temperature. Powdered dried leaves were extracted with either 70%

ethanol or double-distilled water (one gramme of powdered leaf was extracted with 50 millilitres of solvent). Filter paper was then used to clarify each extract's solution, which was then kept at 4°C. The extracts were suitably diluted in order to facilitate additional research.

Chemicals and reagents

Every chemical and reagent utilised in the studies was obtained from institutionally authorised chemical providers and was of analytical quality. A spectrophotometer was used to measure the specific optical density for quantitative testing.

Evaluation of in vitro anti-lithiatic activity (percentage dissolution of calcium oxalates).

By calculating the % dissolution of calcium oxalates, the conventional procedure was applied in triplicate, but with some alterations, to assess the in vitro anti-lithiatic activity. The entire process consists of three distinct steps: (i) Preparing experimental kidney stones (calcium oxalate stones); (ii) Making semi-permeable membrane from chicken eggs; and (iii) Titration technique estimation of calcium oxalate [8].

Experimental kidney stone (calcium oxalate stones) preparation

In a beaker, distilled water was left to react with an equimolar (100 mM) solution of calcium chloride dehydrate in double-distilled water and disodium oxalate in 10 ml of 2 N H_2SO_4. Calcium oxalate precipitated as a result. After removing any remaining H_2SO_4 residue from the precipitate with a 10% ammonia solution, it was cleaned with double-distilled water and allowed to dry for five hours at 70°C.

Preparation of semi-permeable membrane from chicken eggs

The chicken eggs, which were purchased from a nearby store, were chemically shelled by immersing them in 10% Glacial acetic acid for 48 hours, resulting in total decalcification. The egg membrane was properly cleaned with distilled water, placed in a 10% ammonia solution, and kept at a pH of 7.4 in a refrigerator.

Estimation of calcium oxalate by titration method

By precisely combining 10 mg of calcium oxalate and 10 mg of lyophilized extracts in a typical 1:1 ratio and packing them both inside the egg's semi-permeable membrane, the dissolving percentage of calcium oxalate was determined. This was left to suspend in 100 millilitres of 0.1 M Tris buffer in a conical flask. The first group had only 10 mg of calcium oxalate and was designated as a blank.
As a positive control, a second group was created and given 10 mg of calcium oxalate and 10 mg of the common medication cystone. The third and fourth groups had aqueous and 70% ethanol extracts, respectively, and 10 mg of calcium oxalate. All of the groups' conical flasks were preheated to 37°C for three hours in an incubator. Each group's semi-permeable membrane contents were taken out and placed into a different test tube. Next, 2 ml of 1 N H_2SO_4 was added to each test tube, and the mixture was titrated with 0.9494 N $KMnO_4$ until a pale pink hue was obtained.
One millilitre of 0.9494 N $KMnO_4$ is equal to 0.1898 milligrammes of 4 calcium in the computation. To determine the total amount of calcium oxalate dissolved by different solvent extracts, the amount of undissolved calcium oxalate that remains is deducted from the total amount utilised in the experiment at the beginning [9].

Result

Titration was employed in the study to evaluate the in vitro anti-lithiatic properties of 70% ethanolic and aqueous extracts using a calcium oxalate dissolving assay. The study's findings indicate that the greatest percentage of calcium oxalate that dissolved in aqueous and 70% ethanolic extracts, respectively, was 79.98±0.95% and 74.41±4.43%. The lowest is 10.45±1.78% for 70% ethanolic extract and 46.37±3.22% for aqueous extract, respectively. At a concentration of 1 mg/ml, the dissolution percentage of calcium oxalate for the standard medication cystone was 86.42±3.57%

Discussion

To the best of our knowledge, the current study demonstrated the leaves of Sonchus arvensis extracts' long-standing in vitro anti-urolithiasis effectiveness. The researchers found that mildly elevated calcium or phosphate can encourage the formation of calcium phosphate crystals in the renal interstitium, leading to localised swellings and collagen deposition that produce Randall's plaque. Alternatively, it's possible that mildly elevated calcium and phosphate or oxalate, along with low levels of citrate or magnesium in the urine, can cause crystallisation in the kidney's collecting ducts that are injured and under stress from oxidative processes [10]. According to the current study, *Sonchus arvensis* leaf extracts that are 70% ethanolic and aqueous have a stronger capacity to dissolve calcium oxalate stones than other leaf extracts. This investigation is consistent with the study's conclusion that aqueous extracts are essential for the treatment of renal stones.

Conclusion

It is determined that the finest sources of phytochemicals and healing agents for a variety of illnesses are medicinal plants. The current study discovered that Sonchus arvensis extracts were abundant in secondary plant metabolites, which had notable anti-urolithiasis properties in vitro [11]. Despite the in vitro trials, more research is advised to determine and assess the pharmacological efficacy, potential, and mode of action. The maturity of the leaves, fertility, pest exposure, moisture, relative water content, pH, solubility, solvent polarity, and environmental factors like pollution, solar reflectance, rainfall, precipitation, location, and temperature could all be contributing factors to the reasonable variation in phytochemical contents [9],[12].

The work has so demonstrated that the route that has historically employed readily accessible weeds can be a low-cost source of significant bioactive compounds with potential for the creation of herbal drugs.

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