

## Introduction

Urolithiasis, also known as kidney stone disease, is characterized by the mineral deposition that forms stones or calculi in the renal system, affecting the kidneys, ureters, bladder, or urethra of approximately 12-15% of the global population (Dobrek, 2020). Urinary tract problem holds the third most common issue globally, with males experiencing higher recurrence rates than females. Approximately 9% of the US population, or roughly 1 in 11 individuals, experience kidney stones (Scales *et al.*, 2016), in Asia, 5.5 -13.4% (Maddahi *et al.*, 2017), while India reports a prevalence of 10-15% (Kasote *et al.*, 2017). Demographically, men are 2-3 times more prone to kidney stones than women (Zielinski *et al.*, 2021), with peak incidence between 30-60 years old. Kidney stones are formed due to an imbalance between water and crystallizable substances in the urine, including minerals such as calcium, oxalate, and uric acid, salts like sodium and potassium, and other compounds like cystine and xanthine. Kidney stones are generally classified into four types such as calcium oxalate, uric acid, cystine, and struvite. The composition of kidney stones is predominantly calcium oxalate (80%) associated with essential hypercalciuria, excessively acidic urine pH and hyperuricosuria followed by struvite stones (10%), usually related to urinary tract infections (UTIs), form due to bacterial breakdown of urea, leading to increased pH levels and magnesium ammonium phosphate precipitation. Uric acid stones comprise around 9%, often resulting from low urine pH, dehydration, and dietary factors, commonly affecting individuals with gout, diabetes, or those consuming a high-protein diet. Cystine stones, on the other hand, make up around 1% of renal stones and primarily occur in individuals with cystinuria, a genetic disorder that affects amino acid reabsorption (Coe *et al.*, 2010).

Factors contributing to stone formation include dehydration, concentrated urine, pH imbalance, inadequate clearance, dietary factors like excessive animal

protein, sodium, sugar, and oxalate intake, genetic predisposition, and underlying medical conditions like kidney disease, gout, and hyperparathyroidism. The process involves three key stages: nucleation, aggregation, and retention. Nucleation causes crystallizable substances to form a nucleus, aggregation, where crystals grow and aggregate, and retention, where stones remain in the urinary tract, causing severe pain, obstruction, infection, kidney damage, and recurrent stone formation.

Urolithiasis treatment involves a multifaceted approach combining medical and surgical methods. Medical management includes pain management, hydration therapy,  $\alpha$ -block therapy, antibiotics, and dietary changes (Abbas *et al.*, 2021). Surgical management includes shock wave lithotripsy, ureteroscopy, percutaneous nephrolithotomy, laparoscopic surgery, and advanced techniques like laser lithotripsy and stone fragmentation, depending on stone size, location, and severity. Preventing kidney stone includes staying hydrated by drinking plenty of fluids, consuming vegetarian diet high in magnesium and potassium citrate supplements to maintain the pH of urine and increase the level of citrate and following regular check-ups help to track the effectiveness of preventive measures as needed (Peerapen and Thongboonker, 2023).

New technologies like robotic surgery and nanotechnology are being practiced to improve the treatment and prevention of kidney stones (Garg *et al.*, 2019). However, conventional medical treatments can have various adverse effects, such as gastrointestinal problems, dizziness, headaches, bleeding risks, electrolyte imbalances, and allergic reactions (Abbas *et al.*, 2019). Complications that can arise over time include kidney damage, chronic kidney disease, hyperoxaluria, hypokalaemia, and scarring of kidney tissues (Malabadi *et al.*, 2021). Surgical risks for kidney stone treatment may involve bleeding, infection, ureteral stricture, kidney damage, and anesthesia-related complications (Izol *et al.*, 2015). Physicians usually focus on pain management rather than treating kidney stones directly, allowing the stones to pass on their own. Unfortunately, conventional medications for kidney stones often have limitations due to their ineffectiveness, restricting their long-term use (Tiwari *et al.*, 2012). Kidney stone

recurrence rates are notably high, with approximately 10% occurring within the first year, 33% within five years, and 50% within ten years if left untreated, underscoring the importance of developing effective alternative treatments (Basavaraj *et al.*, 2007).

Medicinal plants, therefore, serve as a vital source of drugs, offering a promising alternative to their chemical counterparts. Herbs have been an integral part of human civilization, used for different purposes in different fields, including medicine, nutraceuticals, perfumery, beverages, fragrances, cosmetics, and the dying industry. Throughout history, herbs have been the primary source of medicine, dating back to ancient times, and were used to treat various disorders and ailments until the development of synthetic drugs. Herbal source is the cornerstone for producing more than 40% of prescription drugs. In rural communities of many developing countries, traditional medicine using medicinal plants remains a prevalent practice, with healers claiming their methods are cost-effective and efficient. However, the transmission of indigenous knowledge on medicinal plants is under threat due to modernization and transformation of traditional culture, presenting a major risk to the future of indigenous medicine (Kankara *et al.*, 2015).

In global healthcare, medicinal plants have become essential, as approximately 80% of the global population depends on traditional plant-based remedies. Despite significant advancements in allopathy, over 50,000 plant species are utilized in traditional medicine worldwide, contributing to 25% of modern medicine's essential drugs (Rahman and Husen, 2021). In India, more than 7,300 plant species are utilized in traditional healthcare systems like Ayurveda, Unani, and Siddha, highlighting the significance of plant-based remedies. These plant species are rich in their phytochemical constituents and possess valuable therapeutic properties. Phytochemical components are generally categorized into two groups, with primary constituents comprising essential nutrients and secondary metabolites like flavonoids, saponins, tannins, alkaloids, and phenolics (Krishnaiah Bono, 2007). They have shown promising results in treating many diseases, leading to a growing demand for these natural

medicines. Phytochemicals help protect cells by balancing antioxidants and free radicals, neutralizing harmful substances, and reducing oxidative stress. The antiurolithiatic properties of medicinal plants have been demonstrated through various mechanisms, such as promoting diuresis, altering the urine composition, and inhibiting stone formation. Thus, medicinal plants offer a natural, safe, and affordable alternative for kidney stone treatment, focusing on holistic care. Herbal remedies like Chance piedra (*Phyllanthus niruri*), Dandelion root (*Taraxacum officinale*), and Gravel root (*Eupatorium purpureum*) have been traditionally used to dissolve kidney stones and prevent recurrence.

*Spermacoce articularis* is a perennial herb, native to tropical regions, and found in temperate and tropical areas, possessing diverse medicinal properties (Soosairaj *et al.*, 2013). *S. articularis* has quadrangular hairy stems, oblong-elliptic leaves with scabrid surfaces, small whorled flowers in axillary inflorescence, and hairy, 5mm long fruit capsules. *S. articularis*, known as Madanaghanti in Ayurveda, Nathaisuri in Tamil folk medicine, and has been used for centuries to treat various health disorders due to its pharmacological properties. Beta-sitosterol, ursolic acid, iso-rhamnetin, D-mannitol, calcium, and phosphorus are the significant chemical components reported in *S. articularis* (Dahiya and Solanki, 2011).

### **Systematic Position**

Kingdom	: Plantae
Order	: Gentianales
Family	: Rubiaceae
Genus	: <i>Spermacoce</i>
Species	: <i>articularis</i>

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**Plate 1.1: *Spermacoce articularis* L.f.- Habit**

*S. articularis* has been studied for its effectiveness in the treatment of kidney stones using molecular docking to identify compounds and validate its traditional use and for the development of new treatments in urolithiasis. Although *S. articularis* shows potential for kidney stone, treatment, its use in traditional medicine is challenged by limited documentation and a lack of quality control. Integrating traditional knowledge with modern medicine is a rigorous, evidence-based evaluation required which includes comprehensive phytochemical profiling and extensive scientific investigation to prevent and manage kidney stones.

**Hypothesis**

The hypothesis set up for the present study is,

**Null hypothesis**

The *S. articularis* stem extract does not reduce the CaOx crystal growth when compared to Cystone, the standard.

**Alternative hypothesis**

The *S. articularis* stem extract significantly reduces the CaOx crystal growth when compared to Cystone, the standard.

Hence, the present study entitled 'Antirolithiatic Potential of *Spermacoce articularis* L.f. through *In vivo* and *In silico* Analysis' was conducted with the following objectives, that support the alternative hypothesis.

- To study the pharmacognostic and phytochemical aspects of different extracts of the leaf, stem, and root of *Spermacoce articularis*
- To evaluate the total antioxidants and free radical scavenging activity of various solvent extracts from the leaf and stem of *S. articularis*
- To assess the antirolithiatic potential of the leaf and stem of *S. articularis* using *in vitro* nucleation and aggregation assays
- To investigate the urolithiasis-inhibiting potential of *S. articularis* stem in the Wistar-albino rat model
- To profile the phytochemical constituents of *S. articularis* stem extract using TLC, and HPTLC techniques
- To identify the chemical composition of *S. articularis* stem extract using GC-MS profiling
- To evaluate the binding potential of bioactive compounds from *S. articularis* stem to urolithiatic receptors using molecular studies