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PHYTOCHEMICAL PROFILE AND *IN VITRO* ANTIUROLITHIATIC ACTIVITY OF *Senna alata* (L.) Roxb. FLOWER EXTRACT

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ABSTRACT

Plants are a primary source for producing cost-effective medicine. Even though various artificial methods are used in the treatment of kidney stones, they have a major drawback because of their severe side effects. Nature is producing several valuable medicines through the plants with no side effects. Hence, plant-based therapy received worldwide attention. The herbal drugs against kidney stones are studied and formulated from the medicinal plants. In the aim of the present study, the phytochemical profile and *in vitro* antiurolithiatic activity of *Senna alata* (L.) Roxb. flower extract. *Senna alata* flower extract showed a tannin, saponin, flavonoids, steroids, terpenoids, alkaloids, anthraquinone, polyphenol, glycoside, and coumarins in both extracts of aqueous and ethanol, while a significant amount of total phenol (296.74±5.97 mg/g) and flavonoids (23.00±1.75 mg/g) were evaluated. UV-visible spectroscopic techniques for the detection of flavonoids, carotenoids, anthocyanins, and chlorophylls; phytochemicals; and functional groups of *Senna alata* flowers in ethanolic extract confirmed the presence of secondary metabolites. Histochemical and fluorescence techniques further confirmed the presence of phytochemicals in *Senna alata* flowers. *Senna alata* flowers ethanolic extract showed a significant inhibitory effect on CaOx crystal aggregation and nucleation assay. Overall, it can be concluded from the present study that *Senna alata* flowers contain a rich source of phytochemicals and possess antiurolithiatic activity.

Keywords: *Senna alata* flower, antiurolithiatic activity, CaOx crystal aggregation, CaOx crystal nucleation.

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INTRODUCTION

Kidney stone disease, also known as nephrolithiasis or urolithiasis, is a disorder in which urinary solutes precipitate to form aggregates of crystalline material in the urinary space. The incidence of nephrolithiasis has been increasing, and the demographics have been evolving. Once viewed as a limited disease with intermittent exacerbations that are simply managed by urologists, nephrolithiasis is now recognized as a complex condition requiring thorough evaluation and multifaceted care. Kidney stones are frequently manifestations of

underlying systemic medical conditions such as the metabolic syndrome, genetic disorders, or endocrinopathies (Shastri et al., 2023). Urinary stones affect a large proportion of the population. Approximately 85% of urinary stones are calcium stones, which consist of oxalate and phosphate, either alone or in combination. The mechanisms involved in the formation of urinary stones are not fully understood but it is generally agreed that urinary lithiasis is a multifaceted process involving events leading to crystal nucleation, aggregation and growth of insoluble particles. Crystal growth and agglomeration may be due to super saturation with respect to stone forming constituents or the presence of various inhibitory or stimulatory biomolecules or even pH (Fan *et al*., 1999). Urine is always supersaturated with common stone forming minerals, however, the crystallization inhibiting capacity of urine does not allow urolithiasis to happen in most of the individuals, whereas this natural inhibition is impaired in stone formers (Tiselius *et al*., 2001).

In recent years, numerous studies describing the therapeutic properties of extracts from different parts of various medicinal plants have been developed. Indeed, the use of such extracts as complementary and alternative medicine has lately increased, and also serves as an interesting source of drug candidates for the pharmaceutical industrial research (Newman and Cragg, 2007).

Herbs and herbal drugs have created interest among the people by its clinically proven effects like immunomodulation, adaptogenic and antimutagenic. Also, the overuse of synthetic drugs, which results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies. The problem of urinary stones or calculi is a very ancient one and many remedies have been employed during the ages these stones are found in all parts of the urinary tract, the kidney, the ureters and the urinary bladder and may vary considerably in size. In the keep view, an attempt has been made to emphasis on Medicinal plants option for urinary stone.

We surveyed the approaches utilized by different researchers to prepare herbal extracts and deliver them to rats with experimentally induced hyperoxaluria and CaOx urolithiasis. Most studies involved induction of hyperoxaluria by delivering ethylene glycol (EG) to male rats. Aqueous, alcoholic, or hydroalcoholic extracts of various plant parts including leaves, stems, fruits, seeds, or a combination thereof were given to determine their efficacy in reducing renal crystal deposition and urinary, oxalate, crystals etc. All studies did not study same anti-urolithic activities of the herbal treatments. Changes in lithogenic factors such as change in urinary pH, urinary excretion of calcium, oxalate and reduction in CaOx crystal deposition in the kidneys were however, considered satisfactory outcomes. Few studies examined the antioxidant and diuretic actions of the herbal extracts and considered them as the basis for herbal treatments' anti-urolithic activities (Khan and Khan, 2022). In the aim of the present study, phytochemical profile and *in vitro* antiurolithiatic activity of *Senna alata* (L.) Roxb. flower extract

MATERIALS AND METHODS

Collection and extraction of seaweed

Senna alata (L.) Roxb. flowers, was obtained from Poovanam, Thanjavur district, Tamil Nadu, India in 2025. After cleaning and drying, it was ground into a fine powder and stored in a refrigerator. 2 gram of the powder of flowers were transferred in to two conical flask (250ml). The conical flask containing 50ml of ethanol and 50ml of aqueous solvent. The conical flask containing *Senna alata* flowers were shake it well for 45 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper no.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973). Total phenols estimated by the method of Edeoga *et al*., (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Histochemical tests (John Peter Paul, 2014) and Fluorescence behavior (Rao *et al.,* 2011). Functional groups were detection by the method of Ahluwalia and Dhingra, (2004).

In vitro Antiurolithiatic Activity

In vitro Nucleation and Aggregation assay were followed by Patel *et al.,* (2012).

RESULTS AND DISCUSSION

Phytochemical profile

The phytoconstituents present in the *Senna alata* flower extract showed a tannin, saponin, flavonoids, steroids, terpenoids, alkaloids, antroquinone, polyphenol, glycoside and coumarins in both extracts of aqueous and ethanol (Table 1). Table 2 shows the significant amount of total phenol (296.74±5.97 mg/g) and flavonoids (23.00±1.75 mg/g) in *Senna alata* flower, respectively. Medicinal plants used in different diseases and ailments are the richest bioreservoirs of various phytochemicals. The present *Senna alata* flowers ethanolic extract deals with the UV-visible spectroscopic techniques and detection of maximum possible qualitative phytochemicals of flavonoids (360 nm), carotenoids (410 nm), anthocyanins (530 nm), and chlorophylls (630 nm), represented in figure 3. The histochemical results revealed the presence of tannin, flavonoids, polyphenol, and terpenoids in *Senna alata* flowers (Table 3 and Figure 4). Fluorescence analysis of flower powder of *Senna alata* flower powder was observed in normal daylight and under short (245 nm) and long UV light (365 nm), and the results were presented in Table 4 and Figure 5. In this, the results further confirmed the presence of phytochemicals in *Senna alata* flowers. The functional groups are investigated and summarized in Table 5 and Figure 5. The functional groups are involved in metal-reducing and capping agents, leading to the formation of nanoparticle synthesis and revealing the presence of secondary metabolites (Karnan *et al.*, 2023b and 2023c). The medicinal properties of the plants are determined by the phytochemical constituents (Ezeonu and Ejikeme, 2016). Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc., which are distributed in various parts of the plants (Sheel et al., 2014). Nature is a unique source of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%), and alkaloids (18%) as major groups of phytochemicals (Saxena et al., 2013). Plant extract are reported to be the major phytochemicals involved in antiurolithiatic activity (Kaviraj et al., 2022).

Table 1: Qualitative screening of secondary metabolites of *Senna alata* flowers extract

S. No	Phytochemicals	Aqueous	Ethanol
1	**Tannin**	+	+
2	**Saponin**	++	++
3	**Flavonoids**	++	++
4	**Steroids**	+	++
5	**Terpenoids**	++	++
6	**Alkaloids**	+	++
7	**Antroquinone**	++	++
8	**Polyphenol**	++	++
9	**Glycoside**	+	++
10	**Coumarins**	++	++

(+) Presence, and (–) Absences

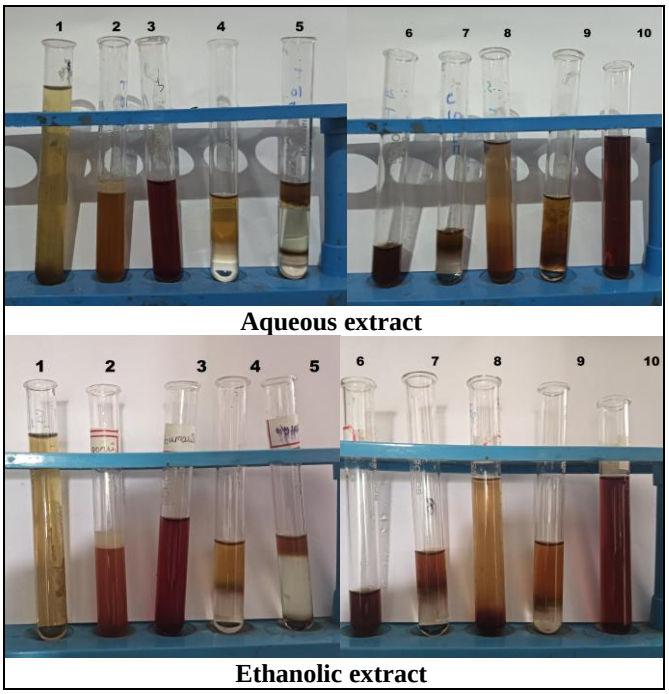

Figure 2: Qualitative screening of phytochemicals (Secondary metabolites) of *Senna alata* flowers extract

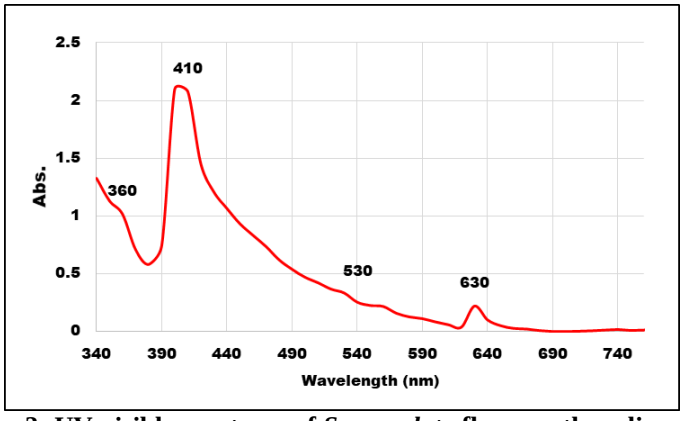

Figure 3: UV-visible spectrum of *Senna alata* flowers ethanolic extract

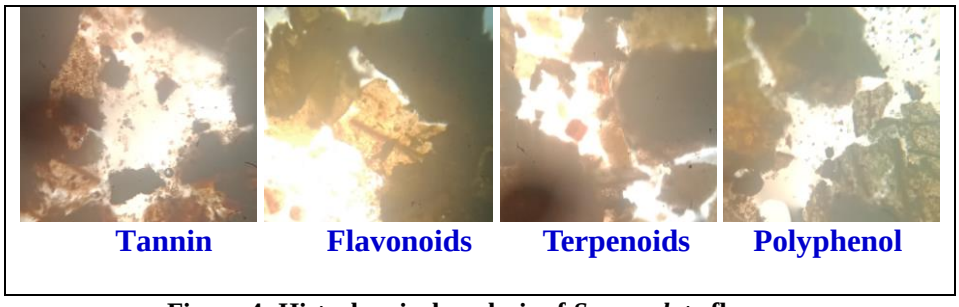

Figure 4: Histochemical analysis of *Senna alata* flowers

Table 4: Fluorescence analysis of *Senna alata* flowers

S. No	Test	Visible Light	Short UV Light (254)	Long UV Light (365)
1	Plant powder	Black	Black	Black
2	Plant powder treated with distilled water	Ash	Ash	Black
3	Plant powder treated with Hexane	Yellow	Black	Black
4	Plant powder treated with Chloroform	Black	Black	Black
5	Plant powder treated with Methanol	Ash	Brown	Black
6	Plant powder treated with Acetone	Black	Brown	Black
7	Plant powder treated with 1N Sodium Hydroxide	Blackish brown	Black	Black
8	Plant powder treated with 1N HCL	Brown	Black	Black
9	Plant powder treated with sulphuric acid with equal volume of water	Brown	Light green	Black
10	Plant powder treated with Nitric acid diluted with an equal volume of water	Light yellow	Light green	Black

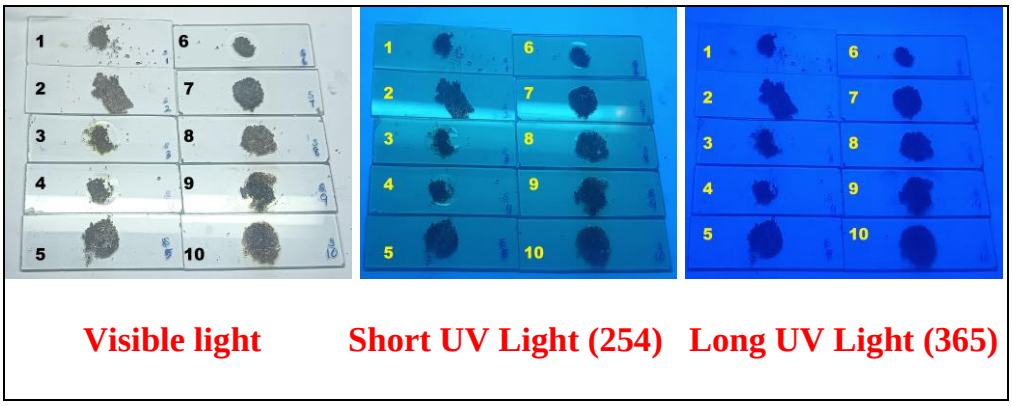

Figure 4: Fluorescence analysis of *Senna alata* flowers

Table 5: Qualitative functional groups analysis of *Senna alata* flowers ethanolic extract

S. No	Functional groups	Results
1	Alcohols	++
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	+
5	Ketones	+
6	Carboxylic acids	++

(+) Presence and (–) Absences

***In vitro* Antiurolithiatic activity of *Senna alata* flowers ethanolic extract**

Aggregation of crystals marks the process wherein numerous crystals in the solution come together and adhere, forming large crystal agglomerates. Aggregation is a key determinant of crystal retention, as large crystal agglomerates are the ones that produce renal tubular obstruction, thereby promoting stone formation. The half inhibition concentration (IC_{50}) of Cystone (237.67µg/ml) and *Senna alata* flowers ethanolic extract

(423.72 µg/ml) exhibited a significant dose-dependent inhibition of aggregation assay activity (Figure 6). Nucleation is a prerequisite in the pathogenesis of CaOx urolithiasis. Nucleation basically marks a thermodynamically driven event of phase change wherein dissolved substances in a supersaturated solution spontaneously crystallize. The half inhibition concentration (IC_{50}) of Cystone (226.09 µg/ml) and *Senna alata* flowers ethanolic extract (307.83 µg/ml) exhibited a significant dose-dependent inhibition of nucleation assay activity (Figure 7). The mechanisms involved in the formation of urinary stones are not fully understood, but it is generally agreed that urinary lithiasis is a multifaceted process involving events leading to crystal nucleation, aggregation, and growth of insoluble particles (Baumann, 1998). Calcium oxalate (CaOx) is the most common component of human kidney stones. Heterogeneous nucleation is regarded as the key mechanism in this process (Egan *et al.*, 2004). Plant extract can inhibit the nucleation and aggregation of CaOx crystallization, which responds to preventing kidney stones (Saha and Verma, 2013). *Senna alata* flowers ethanolic extract showed a significant inhibitory effect on CaOx crystal aggregation and nucleation assay.

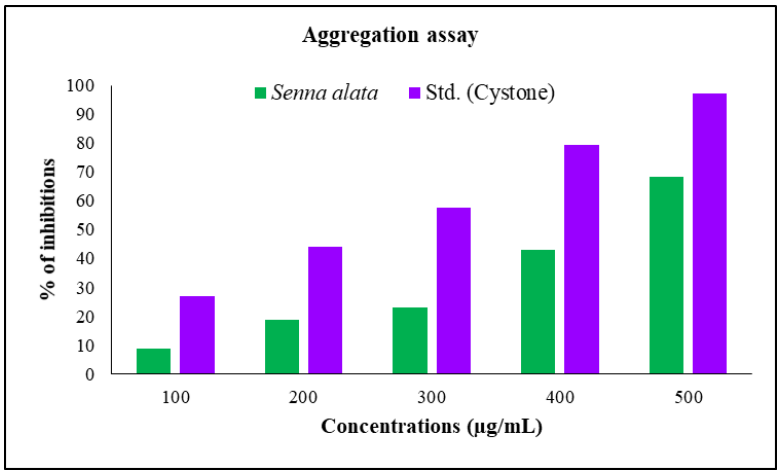

Figure 6: *In vitro* Antiurolithiatic activity of *Senna alata* flower extract, through inhibitions of Aggregation assay

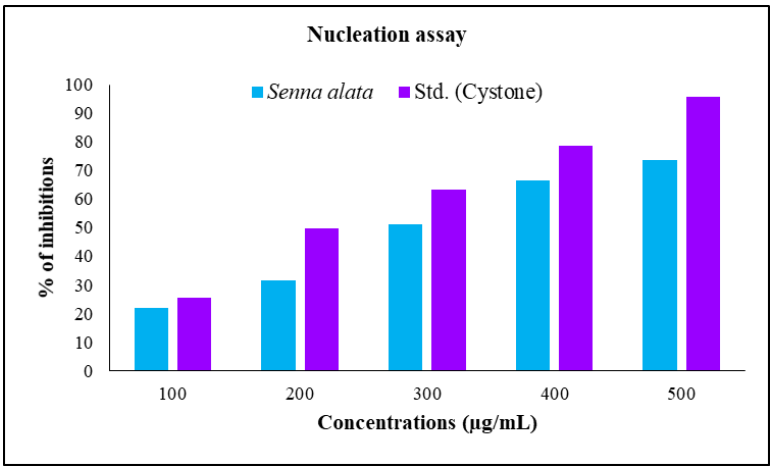

Figure 7: *In vitro* Antiurolithiatic activity of *Senna alata* flower extract, through inhibitions of Nucleation assay

CONCLUSION

Overall, it can be concluded from the present study that *Senna alata* flowers contain a rich source of phytochemicals and possess antiurolithiatic activity. This study is the scientific report that provides convincing phytochemicals and antiurolithiatic evidence for the relevance of *Senna alata* flowers ethanolic extract, thus providing scientific validity to its traditional consumption. As a study of *Senna alata* flowers can be used as an alternative for the treatment of kidney stones in this investigation.

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