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Study of Anti Urolithic Effect of the Chloroform Extract on the Leaves of Orthosiphon Stamineus Benth

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ABSTRACT:
Extensive research has been carried out on Orthosiphon stamineus Benth. (lamiaceae) since the 1930s. This plant is used in several countries (especially in Indonesia, Malaysia, Thailand, Vietnam and Myanmar) as traditional medicine .Orthosiphon stamineus used in India to treat cytotoxic, diabetic, anti-inflammatory and hypertensive. Orthosiphon stamineus is used to treat diabetes, hypertension, oedema, epilepsy, fever, influenza and jaundice. Urolithiasis is a type of disease characterized by the crystallization of mineral substances within the urinary tract or kidney which commonly known as kidney stone or renal calculi. The occurrence of kidney stone disease has account for 15% of world population suffered and ranked the third most common urinary tract problems following the infection of urinary tract and prostate diseases. The presence of renal calculi was initiated by the interruption in the salt solubility and precipitation balance in individual's urinary tract and kidney.

Keywords: Orthosiphon stamineus,Antiurolithic,Kidney stone,Treatment.

I. INTRODUCTION:

Orthosiphon stamineus Benth. (Family Lamiaceae) is a widely distributed plant in Africa and South-eastern Asia. The word Orthosiphon is derived from two Latin words, orthos and siphon, which mean straight while cylindrical, respectively. O. stamineus is a perennial herb. Its height is 0.3–1 m. The stem is four-angled, while the leaves are simple, lanceolate-like, elliptical or rhomboid, 2–4 cm wide and 4– 7 cm long, and the flowers are white or pale lilac. They have stamens that extend from the corolla-tube with a length of more than 2 cm. The herb grows in temperate and tropical areas such as India, Malaysia, China, Australia, and the Pacific.According to both the floral and calyx colours.

Orthosiphon sp. is classified into one of two varieties:6 one with white flowers (white variety) and the other with light purple flowers (purple variety). The purple variety has more bioactive compounds than the white one. However, most scientific investigations have used the white variety. There is substantial interest in O. stamineus, as evidenced by the volume of research devoted to it. Therefore, it is interesting to perform an up-to-date comprehensive review that correlates the phytochemical content of this plant with its traditional and folk medical uses.

II. MATERIAL AND METHOD

Plant Collection And Authenication

The cat whisker's plant (orthosiphon stamineus) plant was collected from an organic farm in Perambalur district, Tamil Nadu, India.

PLANT PROFILE:

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SCIENTIFIC CLASSIFICATION:
Kingdom: Plantae
Division : Tracheophytae
Subdivision: Spermatophytina
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Orthosiphon
Species: Orthosiphon stamineus

SYNONYMS OF PLANT:
- Orthosiphon aristatusvar.aristatus
- Orthosiphon grandiflorus Bold.
- Orthosiphon spicatus(thunb.)Backer,Bakh.f. &steenis

LOCAL NAMES OF PLANT:
Tamil: Poonaimeesai
Malayalam: Poochameesa
Telugu: Pillimeesalu
Kannada: Bekkinameesai
Hindi: Mutritulsi

COMMON NAMES OF PLANT:
- Cat's whisker
- Java tea
- Kidney tea plant
- Misaikucing

SAMPLE PREPARATION

The samples were dried in oven at 37˚c for 5hours, to ensure the moisture content was around 8 to 10%. This step is crucial to avoid fungal and mould contamination.

Preparation Of Chloroform Extract Of Orthosiphon Stamineus

The samples were extracted by maceration method using chloroform extract. Plant sample (50g) was weighed separately in different beakers and later was added with solvent (150ml) respectively. It was properly sealed with aluminium foil to reduce the loss of solvent by evaporation and was left for 72hours at room temperature with frequent agitation, until plant sample become homogenized. Next, the mixture was filtered using filter paper and the filtered extracts were concentrated in the oven at a temperature of 40˚c to a solid form.

PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

Phytochemical screening confirmed the presence of phyto-constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, anthraquinone, and reducing sugar. Methanol, chloroform and ethanol extracts exhibited higher phenolic content as compare to aqueous extract.

Test for alkaloids

The test for alkaloids was carried out on 5 g ground plant material that had been extracted with 10 mL ammonical chloroform and 5mL chloroform. After filtration, the supernatant was shaken with drops of 0.5 M sulphuric acid. The appearance of a creamy precipitate indicated the presence of alkaloid.

Test for saponin

The test for saponin was carried out by adding 15 mL methanol to 5 g of the powdered plant extract. After evaporation, the residue was shaken vigorously with ethyl ether and 5 mL 2N HCL. The appearance of a precipitate indicated the presence of saponin.

Test for flavanoids

The alcoholic extract (15 mL) corresponding to 3 g of plant material was treated with a few drops of concentrated HCL and 0.5 g

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magnesium ribbon. The appearance of a pink-red color indicated the presence of flavanoids.

Test for tannins

One gram of the ground plant sample was boiled in 20 mL of 70 % ethanol for 2 min. The mixture was filtered and a portion of the filtrate was diluted with sterile distilled water in a ratio of 1:4. Three drops of 10% ferric chloride solution was then added. The appearance of a blue to black precipitate indicated the presence of tannins.

Test for steroids and terpenoids

Steroids and terpenoids tests Steroids and terpenoids were detected using the Liebermann-Burchard reaction. Plant material (200 mg) was boiled in chloroform before being filtered. The filtrate (2 mL) was added to 2 mL acetic anhydride and 50% concentrated H2SO4. A blue-green ring indicated the presence of steroids and red colour indicated the presence of terpenoids.

Test for glycosides

The plant extract (2mL) was diluted in chloroform (2mL), followed by the addition of a few drops of concentrated H2SO4. The mixture was observed for the formation of a reddish-brown color to indicate the presence of glycosides in the extract.

Test for phenolic compounds

The plant extract (50 mg) was dissolved in d.H2O (5mL), followed by the addition of a few drops of ferric chloride (5%). The mixture was monitored for the formation of a bluish-black colour to indicate the presence of phenolics in the extract.

Table no: 2 Preliminary phytochemical screening of Orthosiphon stamineus powder

S.No	PHYTOCONSTITUENTS	REPORT
1.	Alkaloids	+
2.	Flavanoids	+
3.	Steroids and Terpenoids	+
4.	Tannins	+
5.	Saponin	+
6.	Phenolic compounds	+
7.	Cardiac glycosides	+

EVALUATION TEST FOR UROLITHIATIC ACTIVITY

Materials and Instruments

Analytical grade of anhydrous methanol, ammonium dihydrogen ortho phosphate, magnesium acetate, sodium metasilicate were all purchased from sigma-aldrich, New Delhi, India.

Growth of Struvite crystals

The single diffusion reaction technique was employed. The crystal growth of sample is used reactants are 0.5M ammonium dihydrogne phosphate (ADP) and added to sodium meta silicate solution with the side of test tube and the density of 1.04g/cm3 and it contains the PH level is 9.4 then the mixture pH level are maintained at range of 6. The gel is formed and closed with airtight stoppers and the gel is undistrubed 4 to 5 days. After few days the gelation process, the 1 molarity of magnesium acetate was added in gel test tubes without disturbing gel. After the added solution, its kept at room temperature at (37c) carried out the full experiment.

The nomenclature of different additive solution on the growth of struvite crystals

To study the effect of sample S at a concentrations 1, 2, 3,4 and 5 % of each sample S were added in equal amounts in supernatant solutions (magnesium acetate) by using gel method. The supernatant solutions are given in table 1 were added to the formed gels and left undisturbed for 7 days. The average weight of the grown crystals was measured.

.Percentage of inhibition were calculated based on the formulae.

1% = [(TSI-TAI)] / TSI X 100

TSI are denotes the crystal numbers without inhibitors

TAI is denotes the crystal number after the addition of inhibitors.

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Table 1: Supernatant solutions added to the set gels for struvite crystals by using sample S separately.

Supernatant solution (SS) (Groups and treatment)	Composition
A (Control)	10 ml of 1M magnesium acetate
B (Distilled water)	5ml of 1 M magnesium acetate + 5ml of distilled water
C(Control+methanol)	5ml of 1 M magnesium acetate + 5ml of methanol
D (1% sample)	5ml of 1 M magnesium acetate + 5ml of 1% sample of separately
E (2% sample)	5ml of 1 M magnesium acetate + 5ml of 2% sample separately
F (3% sample)	5ml of 1 M magnesium acetate + 5ml of 3 % of sample separately
G (4% sample)	5ml of 1 M magnesium acetate + 5ml of 4% of sample separately
H (5% sample)	5ml of 1 M magnesium acetate + 5ml of 5% of sample Sseparately

III. RESULTS FOR SAMPLE

Table-1: Percentage of inhibition for harvested Sample struvite crystals:

CRYSTALS	GROUP	TREATMENTS	HARVESTED CRYSTALS (GRAM)	PERCENTAGE OF INHIBITION
Struvite	A	Control	1.886	0%
	B	Control+Distilled water	1.643	12.88%
	C	Control+methanol	1.712	9.22%
	D	Control+1%	0.732	61.18%
	E	Control+2%	0.656	65.21%
	F	Control+3%	0.514	72.74%
	G	Control+4%	0.478	74.65%
	H	Control+5%	0.326	82.71%

FIGURE 1: The effect of sample Snanoparticles on struvite crystals (a) with magnesium acetate (b) with distilled water (c) with methanol (d) with 1%, (e) with 2%, (f) with 3%, (g) with 4% (h) with 5% after 15th days.

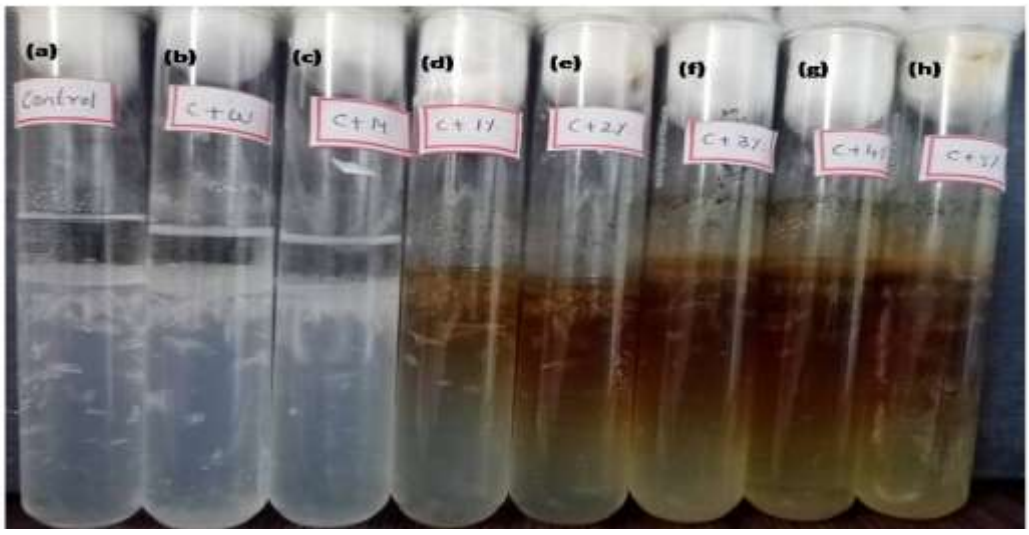

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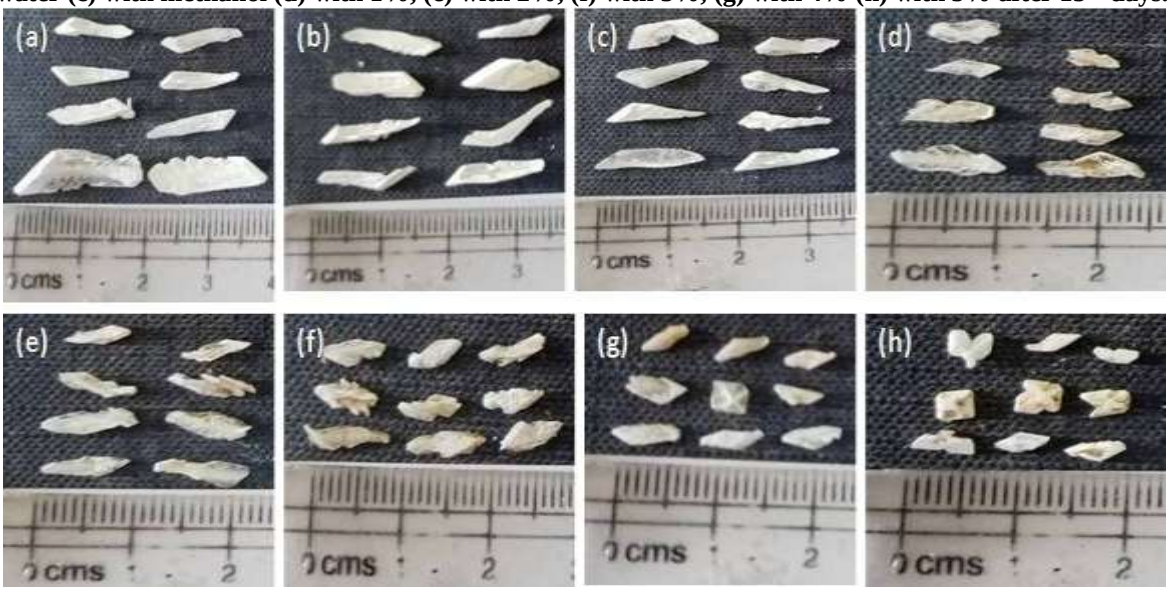
Figure 2: The harvested struvite crystals (a) with magnesium acetate (b) with distilled water (c) with methanol (d) with 1%, (e) with 2%, (f) with 3%, (g) with 4% (h) with 5% after 15th days.

Figure 3:Scale measurement of harvested of struvite crystals(a) with magnesium acetate (b) with distilled water (c) with methanol (d) with 1%, (e) with 2%, (f) with 3%, (g) with 4% (h) with 5% after 15th days.



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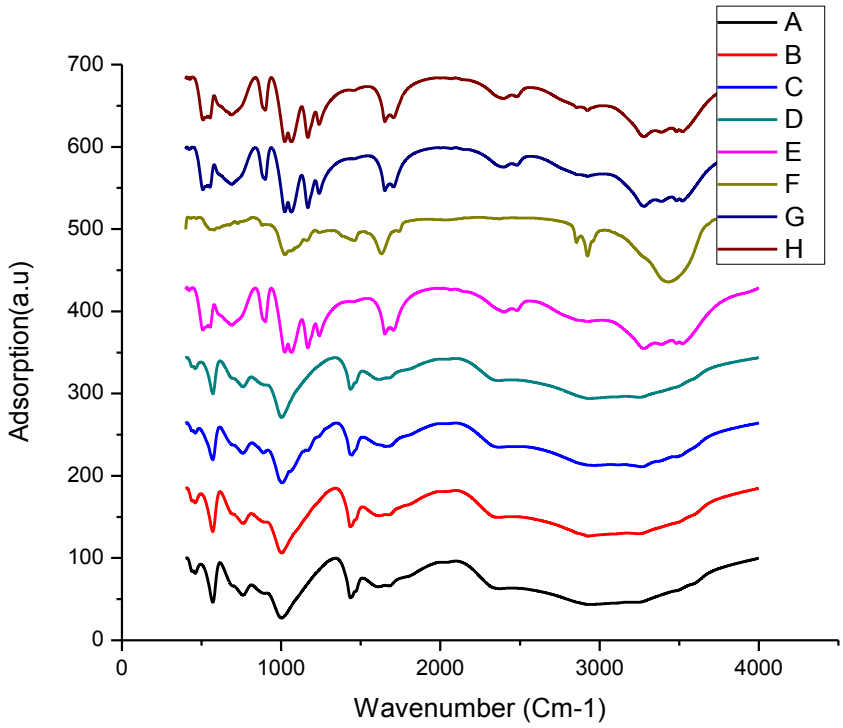
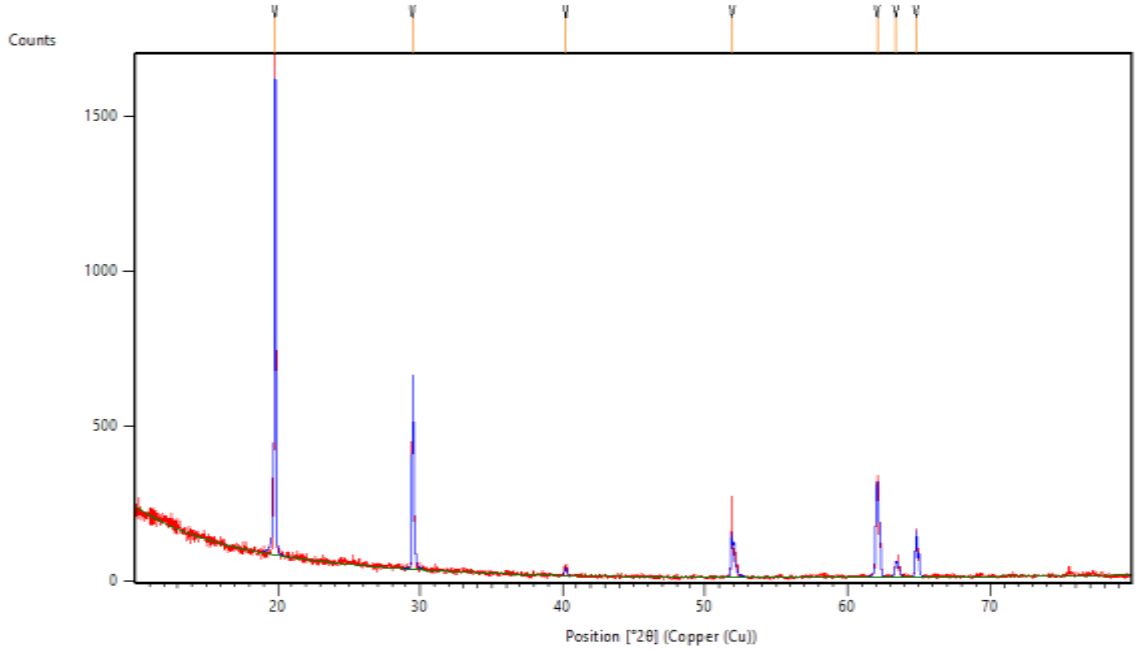
FT-IR RESULTS:



XRD RESULTS OF CONTROL (A) AND H:



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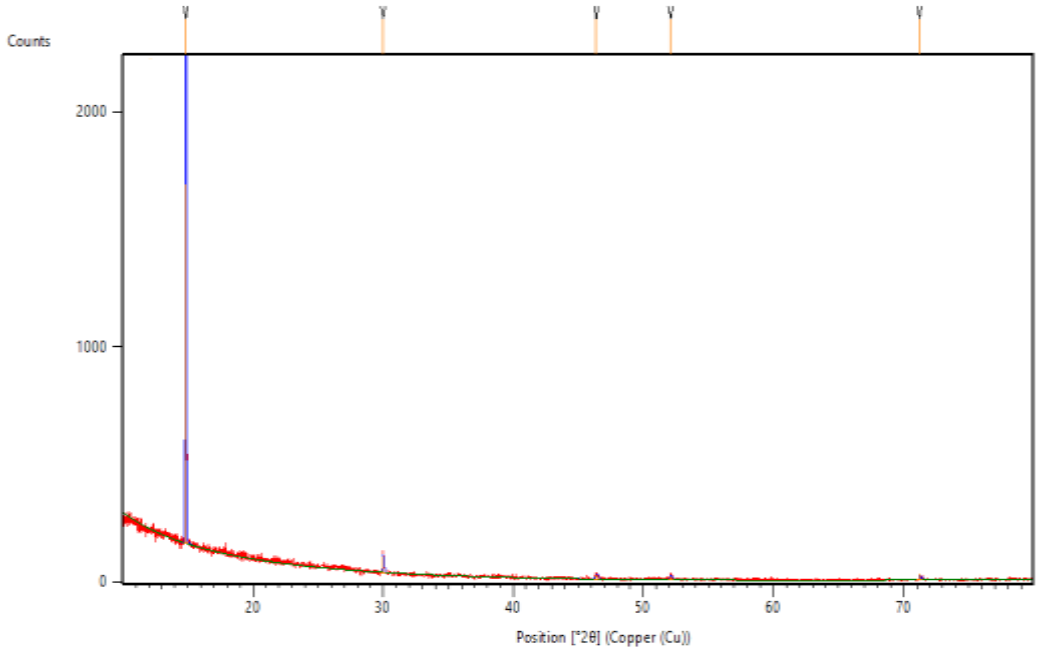
Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
19.8181	1281.84	0.0771	4.47627	100.00
29.4538	658.03	0.0666	3.03014	51.34
40.2163	23.23	0.1448	2.24059	1.81
51.8928	151.27	0.1335	1.76056	11.80
62.0820	291.69	0.2079	1.49384	22.76
63.4081	48.23	0.1892	1.46575	3.76



Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
14.8369	1528.80	0.0974	5.96599	100.00
29.9852	71.93	0.0765	2.97764	4.70
46.3961	15.65	0.2359	1.95552	1.02
52.1132	15.64	0.1456	1.75363	1.02
71.2296	26.63	0.0706	1.32278	1.74

IV. DISCUSSION

Phytochemical screening confirmed the presence of phyto-constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, anthraquinone, and reducing sugar. Chloroform extracts exhibited higher phenolic content.

The single diffusion reaction technique was employed for Struvite crystal growth.
0.5M ammonium dihydrogne phosphate (ADP) and sodium meta silicate solution are used for crystal growth and the pH level are maintained at range of 6. To study the effect of sample S at a concentrations 1, 2, 3,4 and 5 % of each sample S were added in equal amounts in supernatant solutions (magnesium acetate) by using gel method. The supernatant solutions are given in table 1 were added to the formed gels and left undisturbed for 7 days. The average weight of the grown crystals was measured.Percentage of inhibition were calculated based on the formulae. It reveals that the Percentage of inhibition is increased for harvested Sample Struvite crystals when increasing the % of solutions. From the

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report 5% solution of sample gives excellent inhibition against the crystal growth.

V. CONCLUSION

Orthosiphon Stamineus Benth is a traditional medicinal plant has more number of activities and growing well in many countries especially southeast asia countries. Nearly above 100 compounds have been isolated . Methylpariochromene has diuretic action and 2-o-decaacetylorthosiphonone showed antioxidant property. Orthosiphol A is used as a Vasodilator and Antimicrobial effect is due to Rosamarinic acid. No toxic effects were observed during toxicity studies. Systolic and Diastolic pressure were reduced by observation after 12 weeks of administration during clinical studies . finally we conclude the O.Stamineus meets its scientific evidence in aspects of phytochemical, pharmacological, toxicological as well as clinical.

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