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EVALUATION OF ANTI-UROLITHIATIC AND ANTI-NEPHROLITHIATIC ACTIVITY OF COMBINED EXTRACTS OF PHYLLANTHUS FRATERNUS AND CORN SILK

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ABSTRACT

Urolithiasis is a prevalent urinary disorder characterized by the formation of calcium oxalate crystals in the urinary tract, leading to significant morbidity worldwide. The present study investigates the anti-urolithiatic and anti-nephrolithiatic potential of combined extracts of *Phyllanthus fraternus* and corn silk (*Zea mays* L.), both widely recognized in traditional medicine. The extracts were prepared using Soxhlet extraction and subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, tannins, saponins, alkaloids, and terpenoids. HPTLC analysis further confirmed the presence of key phytoconstituents. The *in vitro* antiurolithiatic activity was evaluated using calcium oxalate crystal nucleation, aggregation, and growth inhibition assays at concentrations ranging from 250–5000 µg/mL, with sodium citrate as the standard. The combined extracts demonstrated significant concentration-dependent inhibition across all assays. At 5000 µg/mL, the formulation showed 87.21% inhibition of nucleation, 75.81% inhibition of aggregation, and 82.81% inhibition of crystal growth, comparable to the standard drug. Individual extracts showed moderate effects, indicating a synergistic interaction in combination. These findings highlight the potential of the combined extract as a promising natural therapeutic candidate for managing urolithiasis.

Keywords: *Anti-urolithiatic activity, Phyllanthus fraternus, Corn silk, Calcium oxalate crystals, Phytochemicals.*

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INTRODUCTION

Urolithiasis refers to the formation of stones within the urinary system. Nephrolithiasis is a condition characterized by the formation of crystalline stones, also known as renal calculi, within the kidneys or ureters. These stones consist of both inorganic crystalline substances and organic matrix materials. Herbal remedies are the least expensive, safest, and have no negative side effects; they date back thousands of years [1]. There have also been numerous reports of plants all over the world that can prevent kidney stones. These plants are also useful for their ability to produce terpines and terpenoid metabolites, and aqueous extracts of their various plant parts—roots, stems, leaves, fruit, etc are used to treat various illnesses. The World Health Organization estimates that 80% of the world's nations rely on medicinal plants to manage illness. A substantial body of research has demonstrated the potential of medicinal plants utilized in a variety of traditional systems [2].

Phyllanthus fraternus (Bhumyamlaki or "Jaundice plant") is a small, erect, annual herb (30–60 cm tall) native to India and Pakistan, commonly found as a weed in tropical regions. Known for its medicinal properties, it is widely used to treat liver ailments, bruises, and edema. It is characterized by numerous small, elliptic-oblong, pale green leaves arranged in two rows on branching stems. *Phyllanthus fraternus* is widely used in traditional medicine for various ailments, most notably for treating liver disorders (hepatoprotective) and urolithiasis (kidney stones). It is commonly known as "stone breaker" or "shatterstone" in many regions due to its historical use in eliminating urinary and gall bladder stones. Corn silk (*Zea mays* L.) consists of the elongated, thread-like stigmas and styles of the female

corn flower, acting as the receiving end for pollen to fertilize kernels. Known for its medicinal, antioxidant, and anti-inflammatory properties, this often-discarded agricultural byproduct is rich in flavonoids, polyphenols, vitamins, and minerals Corn silk is widely used in traditional medicine as a complementary remedy for nephrolithiasis (kidney stones), primarily due to its diuretic, anti-inflammatory, and antioxidant properties. It helps manage symptoms and may reduce the risk of stone formation, but it does not chemically decompose existing stones [3].

MATERIALS AND METHODS

Collection of Plants

Procurement of leaf extract of *Phyllanthus fraternus* from ARK Wild Herbs, India is a cultivator, manufacturer & exporter of Ayurvedic herbal products under the Herbal Hill Brand.

Extraction by Soxhlet apparatus

100 grams of *Phyllanthus fraternus* powder was taken in a Soxhlet apparatus and extracted with 70% solvent ethanol by continuous percolation.

Preliminary phytochemical analysis

Preliminary phytochemical screening was carried out using Khandelwal method, standard protocol to detect the presence of phenols, flavonoids, alkaloids, sterols, saponins, carbohydrates, terpenes, tannins, glycosides and reducing sugars in the individual and mixture [4].

Characterization of compounds in *Phyllanthus fraternus* by HPTLC method

HPTLC analysis was performed using silica gel 60 F254 aluminium-backed plates (10 × 10 cm). The plates were pretreated by dipping them in a 4% sodium acetate solution prepared in methanol–water (3:2) for approximately 5 seconds. After treatment, the plates were dried at room temperature for 1 hour before sample application. The chromatographic separation was carried out in a CAMAG twin trough chamber using the mobile phase Toluene: Chloroform: Ethanol (4:4:1). The chamber was pre-saturated with the mobile phase for 20 minutes before development. The plates were developed up to a distance of approximately 62.9 mm. After development, the plates were dried and heated at 110°C for 1 hour. The developed plates were analyzed using a CAMAG TLC Scanner 3 controlled by WinCATS software. The densitometric scanning was performed at 254 nm, 366 nm, 540 nm [4,5].

In Vitro Assays-Anti-urolithiatic and Anti-nephrolithiatic activity

Preparation of an extract concentration of *P. fraternus* and corn silk in a ratio of 2.1, respectively, as the stock solution extract.

i. Crystal Nucleation assay of *P. fraternus* and Corn silk.

Solutions of 4 mM sodium oxalate and 4mM calcium chloride were prepared using a buffer consisting of 0.05 M/L Tris-aminomethane hydrochloride (Tris-HCl) and 0.15 M sodium chloride at pH 6.5. An 8 ml calcium chloride solution was mixed simultaneously with 1 ml of extract at concentrations of 250, 500, 1000, 2000, and 5000µg/ml. Crystallisation was stimulated with 1 ml of sodium oxalate solution, and the absorbance shift was recorded at 620 nm in a UV spectrophotometer for 30 minutes at 37°C. The procedure was followed for the control, substituting distilled water instead of the extract. All samples were inspected in triplicate. Standard drug Sodium citrate was used as a positive control for comparison at distinct concentrations, including 250, 500, 1000, 2000 and 5000µg/ml [6,7]. The percentage inhibition of nucleation rate was then accomplished by comparing the turbidity slope of the varying concentrations of Sodium citrate or extract with the control by the following formula.

% inhibition of nucleation = [(ΔA_control – ΔA_sample)/ΔA_control] × 100,

where ΔA = final A – initial A (over a fixed time)

ii. Crystal Aggregation assay of P. Fraternus and Corn silk.

The severity of the crystal aggregation of CaOx was determined. The COM crystals were produced by combining 50 mM solutions of sodium oxalate and calcium chloride. The solutions were warmed at 60°C in a water bath, cooled to 37°C and held overnight. The solution was then centrifuged and evaporated at 37°C. CaOx crystals were used at a desired concentration of 0.8 mg/ml, articulated with a buffer containing 0.05 M of Tris-HCl and 0.15M of sodium chloride at pH 6.5. The test was tracked at 37°C in the existence and absence of extract at distinct concentrations of 250, 500, 1000, 2000 and 5000 µg/ml. The absorbance was determined for one hour for every 10 minutes time duration at 620 nm. All experiments were performed in triplicates. Sodium citrate was used as a positive control [7,8]. Percentage inhibition of aggregation intensity was then computed by contrasting the turbidity slope of varied concentrations of extract or Sodium citrate with the turbidity slope of the control ensuing formula.

Calculation: % inhibition of nucleation = [(ΔA_control – ΔA_sample)/ΔA_control] × 100,

where ΔA = final A – initial A (over a fixed time)

iii. Crystal Growth assay of P. fraternus and Corn silk

The crystal growth assay is presented on the basis of the frame work documented COM stone slurry 0.2 mg/ml was processed with 50 mM sodium acetate buffer of pH 5.7. Calcium chloride 1 mM and sodium oxalate 1 mM were formulated with a buffer comprising 10M of Tris-HCl and 90 mM of NaCl was regulated to pH7.2. COM crystal seed (0.2µl) was subjected to the solution typically consisting of 1 mM of calcium chloride and 1 mM of sodium oxalate. The abundance of free oxalate reduced with the advent of COM slurry owing to the initiation of the consumption of oxalate. The deterioration in free oxalate was gauged by spectrophotometry at a wavelength of 214 nm. In order to examine the inhibitory effect of the extract on CaOx crystal growth, one ml at distinct

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concentrations of 250, 500, 1000, 2000 and 5000µg/ml was applied to the above-described COM slurry containing calcium chloride and sodium oxalate and Sodium citrate was used as a positive control. A similar procedure was repeated for the control using distilled water in place of the Sodium citrate or extract. All experiments were inspected in triplicate. The relative reduction rate of free oxalate was determined using the baseline value and the value after 30 seconds in gestation with or without Sodium citrate or extract [9,10]. The relative percentage inhibition of crystal growth was computed as follows.

Calculation: % inhibition of nucleation = [(ΔA_control – ΔA_sample)/ΔA_control] × 100,

where ΔA = final A – initial A (over a fixed time)

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Phyllanthus fraternus and corn silk have been utilized in traditional systems of medicine for the treatment of various diseases including urolithiasis. The study aimed to perform phytochemical studies and anti-urolithiatic potential of leaf extracts of *P. fraternus* and corn silk. Sequential extraction was performed using ethanol and hydroalcohol. The preliminary phytochemical analysis showed the presence of tannins, saponin, alkaloids and flavonoids. The extracts of the plant were then subjected to quantitative tests for phytochemical analysis and the results are given in table 1.

Table 1: Preliminary phytochemical screening of P. fraternus and Corn silk

S.No	*Phyllanthus fraternus*		Corn silk	
	Methanol extract	Hydroalcoholic extract	Methanol extract	Hydroalcoholic extract
Alkaloids	-	+	-	+
Tannis	+	+	+	+
Saponis	+	+	+	+
Flavanoids	+	+	+	+
Terpenoids	+	+	+	-
Glycosides	-	-	+	+

Characterization of compounds in *P. fraternus* by HPTLC method

At wavelengths 254 nm and 366 nm, only chlorophyll bands were visible. However, after heating the plate at 110°C for 1 hour, an orange-brown band appeared at 540 nm, indicating the presence of alkaloid compounds in the extract.

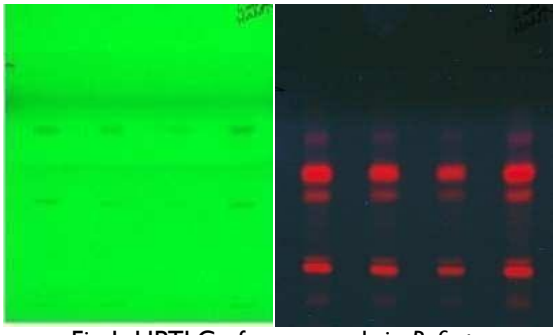

Fig 1: HPTLC of compounds in *P. fraternus*

Anti-urolithiatic and Anti-nephrolithiatic activity

An anti-urolithiatic activity was investigated via *in vitro* nucleation and aggregation assay using spectrophotometer. In the nucleation assay the extract exhibit maximum activity i.e %inhibitory at concentration of 5000ug/ml and sodium citrate exhibited 88.42% inhibition while the combined extracts showed 87.21% inhibition the individual plant extracts also shown inhibitory activity where *P. fraternus* demonstrated 51.31% inhibition and corn silk at 48.21% inhibition. In the crystal growth assay the inhibitory activity also increased with increasing concentration of the extract at 5000ug/ml, sodium citrate showed 85.26% inhibition while the combined extract showed 75.12% and 73.46% inhibition respectively.

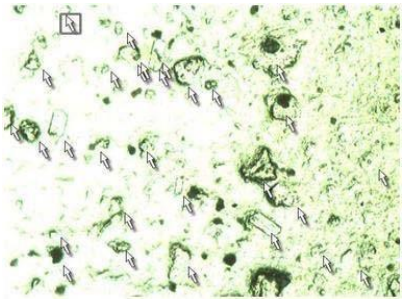

Fig 2: Control-Group-I

The huge number of calcium oxalate crystals creation were seen and scored [11,12].

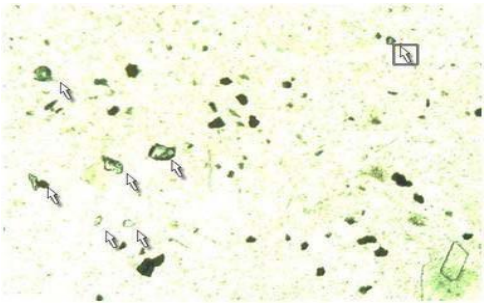

Fig 3: Furosemide Group-II

The huge number of calcium oxalate crystals creation were not dissolved by the drug and scored.

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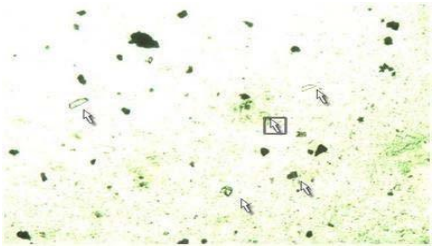

Fig 4: Spiranolactone Group-III

The number of calcium oxalate crystals dissolutions was less by the drug and scored as 2.

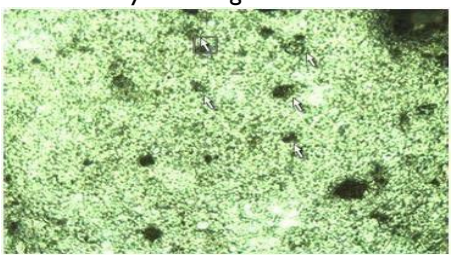

Fig 5: Cystone Group-IV

The number of calcium oxalate crystals dissolution more the poly-herbal formulation and scored as 1.

Table 2: Percentage Inhibition of Crystal Nucleation by test extracts

Percentage Inhibition of Crystal Nucleation				
Concentration (µg/ml)	Sodium Citrate	Combined extract	*P. fraternus*	Corn silk
250	28.62	27.28	20.31	18.25
500	54.38	53.31	27.86	25.81
1000	65.52	64.16	39.46	37.51
2000	84.31	82.26	46.12	34.31
5000	88.42	87.21	51.31	48.21

Fig 6: Percentage Inhibition of Crystal Nucleation by test extracts

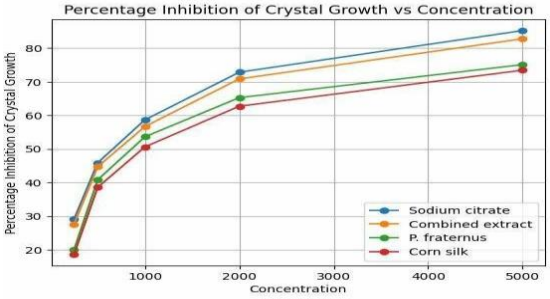

Table 3: Percentage Inhibition of Crystal Aggregration by test extracts

Percentage Inhibition of Crystal Aggregration				
Concentration (µg/ml)	Sodium Citrate	Combined extract	*P.fraternus*	Corn silk
250	26.58	25.62	24.01	22.62
500	40.87	38.72	36.82	35.51
1000	55.67	53.56	50.62	48.61
2000	68.86	60.87	58.28	55.23
5000	80.62	75.81	60.12	58.46

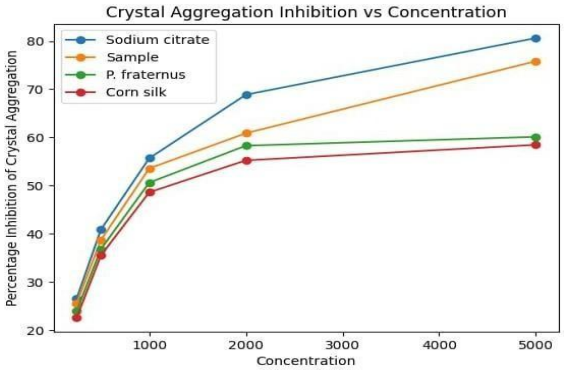

Fig 7: Percentage Inhibition of Crystal Aggregration by test extracts

Table 4: Percentage Inhibition of Crystal Growth by test extracts

Percentage Inhibition of Crystal Growth						
Concentration (µg/ml)	Sodium Citrate	Combined extract	*P. fraternus*	Corn silk	Concentration (µg/ml)	Sodium Citrate
250	29.08	27.62	20.01	18.62	250	29.08
500	45.87	44.72	40.81	38.57	500	45.87
1000	58.67	56.67	53.62	50.61	1000	58.67

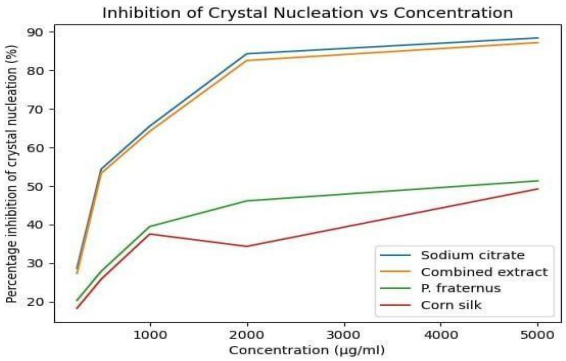

Fig 8: Percentage Inhibition of Crystal Growth by test extracts

CONCLUSION

Kidney stones (urolithiasis or nephrolithiasis) are a common urinary disorder caused mainly by the formation and deposition of calcium oxalate or calcium phosphate crystals in the urinary tract [13]. Phytochemicals such as flavonoids, saponins, tannins, and phenolics contribute to antiurolithiatic activity In addition, some plant extracts have demonstrated the ability to dissolve existing calcium oxalate stones and improve renal function. However, further clinical trials, pharmacological investigations, and standardization of herbal formulations are necessary to confirm their therapeutic In addition, some plant extracts have demonstrated the ability to dissolve existing calcium oxalate stones and improve renal function.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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