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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF *Curcuma caesia* RHIZOME EXTRACT

S. K. Sahoo[1], B. M. Sahoo[1,✉], A. Nanda[1], and B. Singh[2]
[1]School of Pharmacy and Life Sciences, Centurion University of Technology and Management, Bhubaneswar, Khurda-752050, Odisha, India
[2]Department of Pharmacy, S. N. Medical College, Agra-282002, India and Sharda University, Greater Noida-201310, Uttar Pradesh, India
✉Corresponding author: drbiswamohansahoo@gmail.com

ABSTRACT
The crystallisation of minerals in the urinary tract is a major cause of kidney stone formation, also known as urolithiasis. It is characterised by the formation of stones, also known as calculi or uroliths, within the urinary tract. These stones may accumulate in the bladder, urethra, ureters, or kidneys, resulting in several symptoms with potential complications. Kidney stones can cause severe discomfort, blood in the urine, nausea, vomiting, fever, and chills. Treatment of urolithiasis depends on the size, location, and content of the stones, as well as the degree of signs and symptoms. The primary objectives of the management are to relieve pain, enable stone passage, prevent recurrence, and protect kidney function. So, plant-based treatments are frequently used for the management of urolithiasis in traditional medicine. *Curcuma caesia*, also referred to as black turmeric, is well-known for its antiurolithiatic property in traditional medicine. The high levels of flavonoids, phenols, and other antioxidant phytochemicals in *Curcuma caesia* are mainly responsible for its anti-urolithiasis effects. The rhizomes of *Curcuma caesia* were collected, dried, powered and extracted with ethanol using a Soxhlet apparatus. The presence of chemical constituents in *Curcuma caesia* was identified and characterised by phytochemical screening and spectral studies. Further, the antiurolithiatic property of *Curcuma caesia* was assessed through an *in vitro* study. The evaluation results revealed that the ethanolic extract of *Curcuma caesia* exhibited promising antiurolithiatic activity in comparison with the marketed formulation (Cystone).
Keywords: *Curcuma caesia*, Extraction, Phytoconstituents, FT-IR, GC-MS, Urolithiasis, Evaluation.
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INTRODUCTION

Urolithiasis refers to the presence of stones in the urinary system, resulting from the crystallisation of mineral and organic substances in urine. It is a common urological condition that can cause significant discomfort and complications if untreated. The effective management of urolithiasis involves a combination of symptomatic relief, minimally invasive procedures for stone removal, and preventive strategies to reduce recurrence.[1] The choice of treatment is individualised based on stone characteristics and patient factors. So, herbal remedies, dietary supplements, and natural therapies are employed to facilitate stone passage, reduce stone formation, and alleviate symptoms.[2] Because of its numerous medicinal properties, including anti-inflammatory and antibacterial activities, *Curcuma caesia* is used extensively in traditional medicine. The anti-urolithiatic property of *Curcuma caesia* is due to the presence of several phytochemicals, such as flavonoids, phenols, and terpenoids, etc. It is a medicinal plant also known as kali haldi in India.[3] It is characterised by its bluish-black rhizomes due to the presence of anthocyanins. It contains essential oils like camphor, eucalyptol, ar-turmerone, and 1,8-cineole and borneol that contribute to its aromatic odor and medicinal properties. In the current study, the rhizomes of *Curcuma caesia* were collected, dried, powered and extracted with ethanol using a Soxhlet apparatus.[4] The presence of chemical constituents in *Curcuma caesia* extract was identified by phytochemical screening. Further, the phytochemicals were characterised by several analytical tools such as chromatography (TLC) and spectroscopy (FT-IR and GC-MS).[5] Finally, the *Curcuma caesia* extract was subjected to evaluation of antiurolithiatic activity using an *in vitro* method. The screening results

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demonstrated that the ethanolic extract of this plant displayed promising antiurolithiatic activity in comparison with the marketed formulation, Cystone.[6]

EXPERIMENTAL

Collection of Plant Material and Extraction

Curcuma caesia's rhizomes were gathered. The collected rhizomes were cut into thin slices, cleaned, and dried in the shade. The dried rhizomes were finely powered and then they was employed for extraction with ethanol using a Soxhlet apparatus for 8 hours.[11] The obtained extract was evaporated with a rotary evaporator under reduced temperature and pressure until a viscous extract was obtained. The extract was stored in the desiccator till to employ for phytochemical screening (Fig.-1).[7]

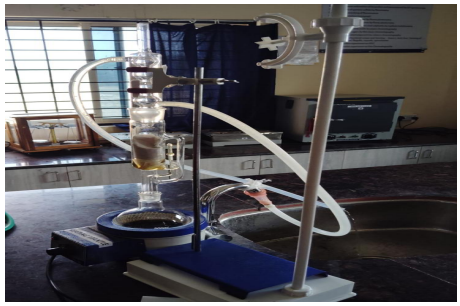

Fig.-1: Extraction of *Curcuma caesia* rhizomes

Preliminary Phytochemical Screening

Preliminary phytochemical evaluation was performed for qualitative estimation of phytoconstituents (alkaloids, terpenoids, glycosides, flavonoids, tannins, saponins, and steroids) present in *Curcuma caesia* extract.[8]

Characterization ofPhytochemicals

The presence of several phytochemicals in *Curcuma caesia* extract was characterized by several analytical methods, such as chromatography and spectroscopy. Thin Layer Chromatography of *Curcuma caesia* rhizome extract involves running the plant extract on a TLC plate with a solvent system to separate individual compounds, which are then visualised under ultraviolet light to identify several components. Studies use solvent systems like chloroform: ethyl acetate (7:3) to achieve optimal separation of flavonoids, phenolics, terpenoids, and other compounds etc. One of the best tools for determining the types of functional groups or chemical bonds present in phytoconstituents is a Fourier transform infrared spectrophotometer. The dried powders of *Curcuma caesia* extract were used for FT-IR analysis. The powdered extract was analyzed using an FT-IR spectrophotometer (Shimadzu, IR Affinity1, Japan) with a scan range of 400 to 4000 cm^{-1}. The plant extract was further analyzed through Gas Chromatography/Mass Spectrometry.[9]

In vitro Anti-Urolithiatic Activity Study

The anti-urolithiatic activity was assessed using calcium oxalate crystals and a turbidimetric technique.[10] This method is employed to study how the plant extract of *Curcuma caesia* inhibits the formation of calcium oxalate crystals, a key component of kidney stones.[11] By calculating the optical density of solutions made by combining dehydrated calcium chloride (solution A) (7 mmol/L) and sodium oxalate (solution B) (2 mmol/L), including 200 mmol/L sodium chloride, the inhibition of calcium oxalate crystallization was examined. The ultraviolet-visible spectrophotometer was used to evaluate the optical density at 620 nm in the presence and absence of plant extract. Every test was conducted at ambient temperature.[12] The % inhibition of crystallization was calculated using the following formula:

Percentage inhibition of crystallization = [(Absorbance of control- Absorption of sample)/ Absorbance of control] X 100

Whereas the absorbance of the sample represents absorbance in the presence of the inhibitor (plant extract/Cystone), and the absorbance of the control refers to absorbance without the inhibitor.

RESULTS AND DISCUSSION

The current study uses ethanolic extract from *Curcuma caesia* for phytochemical screening to reveal the presence of phytochemicals with proven therapeutic potential, including flavonoids, steroids, phenols, tannins, terpenoids, and saponins. Hence, this plant extract can be applied pharmacologically to generate novel molecules that have health benefits (Table-1).

Table-1: Phytochemical Analysis of *Curcuma caesia* Extract

Tests performed	Phytochemicals	*Curcuma caesia* extract
Ferric Chloride Test	Tannin	+
Liebermann-Burchard test	Terpenoid	+
Shinoda Test	Flavonoid	+
Ferric Chloride Test	Phenol	+
Salkowski's test	Phytosterol	+
Foam test	Saponin	+

Based on the peak values in the IR spectrum, the FT-IR analysis was carried out to identify the functional groups present in the active components of the *Curcuma caesia* extract. The functional groups of individual components were separated according to their peak ratios when the extract was allowed to run through the FT-IR. The FT-IR analysis results verified the presence of multiple functional groups, including N-H, O-H, C=C, C-H, C-O, and CH_3 (Fig.-2, Table-2).

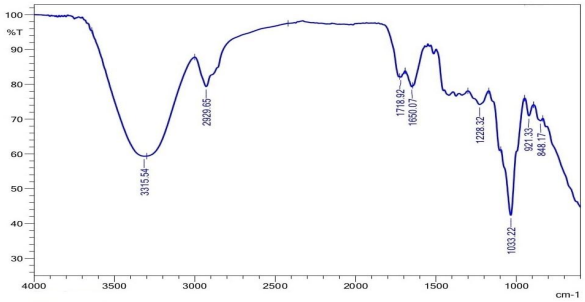

Fig.-2: FT-IR Analysis of *Curcuma caesia* Extract

Table-2: Functional Groups Determination by FT-IR Analysis for *Curcuma caesia* Extract

S. No.	Peak values	Functional groups
1	3315.54	N-H
2	2929.65	O-H
3	1718.92	C=C
4	1228.32	C-H
5	1033.32	C-O
6	921.33	CH_3

GC-MS analysis was employed to detect the phytochemicals found in the extract of *Curcuma caesia* (Fig.-3). Table-3 lists the active constituents along with their molecular formula, retention duration, molecular weight, and concentration (%). The key phytoconstituents present in the *Curcuma caesia* were found to be α-Santalol, Isoborneol, Ar-tumerone, (+)-Curcumenol, etc. These phytochemicals are responsible for the antiurolithiatic property of *Curcuma caesia*.

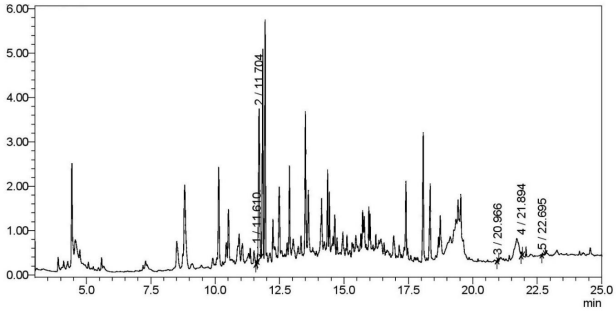

Fig.-3: GC-MS Chromatogram of *Curcuma caesia* Extract

Table-3: GC-MS Study of Phytocomponents of *Curcuma caesia* Extract

Name of phytochemicals	Molecular formula	Molecular weight	Retention time (min)
Isoborneol	$C_{10}H_{18}O$	154	4.39
trans-Sesquisabinene hydrate	$C_{15}H_{26}O$	222	9.71
α-Santalol	$C_{15}H_{24}O$	220	10.74
Ar-tumerone	$C_{15}H_{20}O$	216	11.46
(+)-Curcumenol	$C_{15}H_{22}O$	234	11.70

The effectiveness of the anti-urolithiatic property of the extract is assessed by measuring the ability to reduce the increase in turbidity, which signifies the inhibitory effect on crystallization. The evaluation results were used to compare the efficacy of several concentrations of *Curcuma caesia* extract in comparison with standard drugs like Cystone (Table-4 and Fig.-4**)**.

Table-4: Results of Anti-Urolithiatic Activity

Entry	Concentration (mg/mL)	Absorbance	% Inhibition
Blank	-	0.3264	0
Cystone (Standard)	10	0.2254	30.94
	25	0.1931	40.83
	50	0.1584	51.47
	75	0.1349	58.67
	100	0.1124	65.56
Plant Extract (*Curcuma caesia*)	10	0.2453	24.84
	25	0.2143	34.34
	50	0.1792	45.09
	75	0.1584	51.47
	100	0.1298	60.23

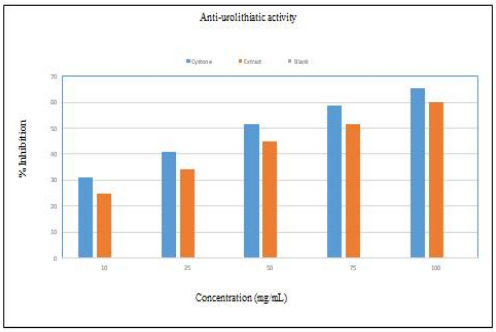

Fig.-4: Anti-urolithiatic activity of *Curcuma caesia* Extract

CONCLUSION

Kidney stones affect people of all ages, genders, and geographical locations, and their incidence and prevalence are rising worldwide. The majority of kidney stones are made of calcium phosphate, calcium oxalate, or a combination of these substances. The recurrence rate of kidney stones becomes high due to inefficient treatment. Several preclinical and clinical studies revealed that Hydrochlorothiazide is employed effectively for the prevention of recurrence of kidney stones. Further, natural products like turmeric are found to possess unique therapeutic properties that are beneficial to human health, instead of just adding spice to our diet. In the present study, the results of the in vitro evaluation clearly revealed that the nucleation, development, and aggregation of crystals were readily suppressed by *Curcuma caesia* extract. The plant's ethanolic extract significantly reduced the crystallisation process. It was observed that the ethanolic extract efficiently inhibits calcium oxalate crystallisation, which may be because the extract contains a variety of chemical components. In comparison to standard (Cystone), the study results demonstrated that the *Curcuma caesia* extract exhibits remarkable litholytic action on stones.

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CONFLICT OF INTERESTS

The author(s) declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

S.K. Sahoo https://orcid.org/0009-0001-6989-8950
B.M. Sahoo https://orcid.org/0000-0001-8822-0427
A. Nanda https://orcid.org/0000-0002-9820-0208
B. Singh https://orcid.org/0000-0002-9427-4242

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