

Investigation of Phytoconstituents in *Curcuma caesia* Rhizomes: Understanding their Potential for treating Urolithiasis through the Molecular Docking Study

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Abstract

Urolithiasis (kidney stone disease) affects millions of people globally and often requires costly surgical intervention. Conventional treatments carry risks of recurrence and side-effects. So, the medicinal plants with anti-urolithiatic properties are of great interest due to their safety, accessibility, and multi-target mechanisms. *Curcuma caesia* (black turmeric) is widely used in traditional medicine for its anti-inflammatory, antioxidant, diuretic, and litholytic properties. Several phytoconstituents present *Curcuma caesia* include curcuminoids, essential oils (e.g., α -turmerone, β -caryophyllene), and phenolics that may act on biochemical pathways involved in stone formation. For this study, the rhizomes of *Curcuma caesia* were collected, dried, powdered and extracted with ethanol using Soxhlet apparatus. The presence of phytoconstituents in *Curcuma caesia* were identified and characterized by phytochemical analysis and spectral studies respectively. Further molecular docking study was conducted to assess therapeutic potential of phytochemicals towards urolithiatic property in comparison to standard

drug (Hydrochlorothiazide). Hydrochlorothiazide is a thiazide diuretic commonly used to treat hypertension and edema. Interestingly, it also has a role in reducing calcium excretion, which can help prevent calcium-based stone formation in the urinary tract.

Keywords: Phytoconstituents, *Curcuma caesia*, Rhizomes, Extraction, Urolithiasis, Docking.

Introduction

The development of stones (calculi) in the kidneys, ureters, bladder, or urethra is the hallmark of the illness known as urolithiasis (1). These stones form due to the supersaturation of urine with crystalline substances, leading to nucleation, growth, aggregation, and retention of crystals (2). In order to effectively treat urolithiasis, a combination of minimally invasive stone removal techniques, symptomatic alleviation, and recurrence prevention measures are required (3). Treatment options are customized according to patient circumstances and stone characteristics. Therefore, natural therapies, dietary supplements, and herbal medicines are used to minimize stone formation, ease symptoms, and expedite stone passage (4). Because

of its many therapeutic potentials, including as its anti-inflammatory and antibacterial actions, *Curcuma caesia* is used extensively in traditional medicine (5). The anti-urolithiatic property of *Curcuma caesia* is due to presence of several phytochemicals such as flavonoids, phenols, and terpenoids etc. 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 Soxhlet apparatus (6). The presence of phytoconstituents in *Curcuma caesia* extract were identified by phytochemical screening. Further, the phytochemical were characterized by several analytical methods such as chromatography and spectroscopy (TLC, FT-IR, and GC-MS). Furthermore, the molecular docking study was performed to determine the binding

interactions of phytochemicals with target sodium-chloride cotransporter (PDB ID: 7Y6I). Hydrochlorothiazide (HCTZ) is a diuretic that acts by inhibiting the sodium-chloride cotransporter (NCC) in the kidneys and is also used clinically for the treatment of kidney stone (7-10).

Materials and Methods

Plant material collection and extraction

The rhizomes of *Curcuma caesia* Roxb were collected. The collected rhizome were cut into thin slices, cleaned, dried under shade. The dried rhizome were finely powered and then it was employed for extraction with ethanol using 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) (12).



Fig 1. Collection of Plant (*Curcuma caesia*) material.

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 (13).

Characterization of phytochemicals

The presence of several phytochemical in *Curcuma caesia* extract were characterized by several analytical methods such as chromatography and spectroscopy (TLC, FT-IR, LC-MS and GC-MS). Thin Layer Chromatography (TLC) of *Curcuma caesia* Roxb extract involves running the plant extract on a TLC plate with a solvent system to separate individual com-

pounds, which are then visualized under UV 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. Fourier transform infrared spectrophotometer (FT-IR) is one of the most efficient methods for determining the types of chemical bonds or functional groups that are present in phytoconstituents. The dried powders of *Curcuma caesia* extract was used for FT-IR analysis. The powdered extract was placed into FT-IR spectroscopy instrument (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} . The plant extract was further analyzed through Gas Chromatography/

Mass Spectrometry (GC-MS) and HPLC//Mass Spectrometry (LC-MS). Helium gas (99.999%) was utilized as the carrier gas at a steady flow rate of 1 ml/min and an injection volume of 2 μ l was employed (14).

Molecular docking study

Selection of phytoconstituents for the study

Four phytoconstituents namely Camphor, Curcumin, Quercetin and Turmerone were selected for the molecular docking study. The structural details of the compounds obtained from PubChem database. The chemical structures of the compounds were drawn using ChemDraw tool and saved in .pdb format for further study (15, 16).

Target identification

Here, the target protein is Solute carrier family 12 member 3 (sodium-chloride cotransporter, PDB ID: 7Y6I). From UniProt database (3) the details of this gene got identified having the entry id P55017, with gene name SLC12A3 and target organism *Homo sapiens* (Human). From Protein Data Bank database, the verified structure of the protein got downloaded with PDB id 7Y6I with 2.85 Å. Here, the 3D structure of the target protein being visualized in Biovia Discovery studio tool and other impurities like water molecules, and attached ligands got removed. The pure form of the Solute carrier family 12-member 3 protein structure taken for further study (17).

Active site prediction and molecular docking study

The active sites of Solute carrier family 12 member 3 protein structure are detected by CastpFold server (5). Employing the Auto Dock 4.2 tool (6) the grid box the protein got determined basing upon the predicted active sites. And molecular docking interaction study of the selected compounds performed by the Auto dock vina tool at its detected active sites and the consequence results being obtained. Hydrochlorothiazide, a standard drug was taken for the comparison study (18-21).

Results and Discussion

In the current study, the exploration of phytochemical screening with ethanolic extract of *Curcuma caesia* demonstrated the presence of phytochemicals such as flavonoid, steroid, phenol, tannin, terpenoid, and saponin which are known to have therapeutic potentials. Hence, this plant extract can be applied pharmacologically to generate novel molecules that have health benefits (Table 1).

Table 1. Results of the phytochemical test for *Curcuma caesia* extract.

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

The FT-IR analysis was performed to identify the functional groups present in the active constituents of *Curcuma caesia* extract based on the peaks values in the IR spectrum. 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 presence of several functional groups (N-H, O-H, C=C, C-H, C-O, and CH₃) were confirmed by the FT-IR study results (Fig. 2, Table 2).

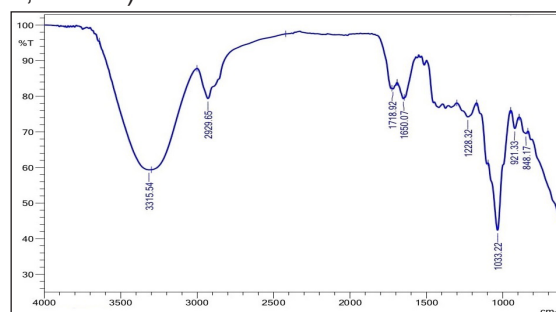


Fig 2. FT-IR analysis of *Curcuma caesia* Roxb.

Table 2. Functional groups determination by FT-IR analysis for *Curcuma caesia* extract.

Sl. 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 and LC-MS analysis were used to identify the chemical constituents found in the ethanolic extract of *Curcuma caesia*. The active constituents with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) were listed in Table 3 and 4. The major phytoconstituents present in the *Curcuma caesia* were found to be α -Santalol, Isoborneol, Ar-tumerone, (+)-Curcumenol etc (Fig. 3 and 4). These phytochemicals are responsible for several pharmacological effects, including antibacterial, anti-oxidant, and anti-inflammatory properties.

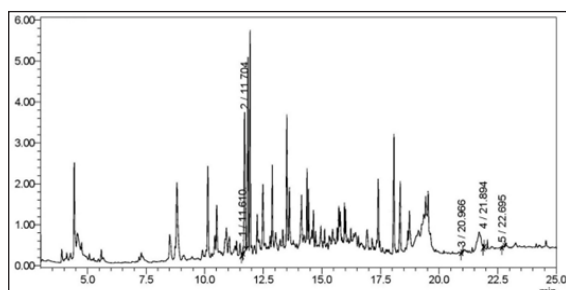


Fig 3. GC-MS analysis of ethanolic extract of *Curcuma caesia*.

Table 3. Phytocomponents identified in ethanolic extract of *Curcuma caesia* by GC-MS.

Compound Name	Molecular formula	Molecular weight	Retention time
Isoborneol	C ₁₀ H ₁₈ O	154	4.39
trans-Sesquibinene 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

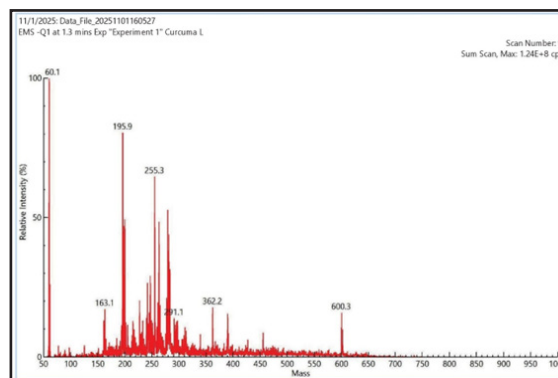


Fig 4. LC-MS analysis of ethanolic extract of *Curcuma caesia*.

Table 4. List of possible identified compounds in LC-MS profiling of ethanolic extract of *Curcuma caesia*.

Compound Name	Molecular Mass	Molecular Formula
Phylloquinone	450.3	C ₃₁ H ₄₆ O ₂
Trimethobenzamide	388.1	C ₂₁ H ₂₈ N ₂ O ₅
Perphenazine	403.1	C ₂₁ H ₂₆ ClN ₃ OS
Threoninyl-Lysine	255.3	C ₁₀ H ₂₁ N ₃ O ₄
Metochlopramide	291.1	C ₁₄ H ₂₂ ClN ₃ O ₂
A-Nor-5alpha-cholestan-2-one	362.2	C ₂₆ H ₄₄ O

Molecular docking analysis

The predicted actives for the Solute carrier family 12 member 3 protein are Arg145, Leu148, Ile192, Lys197, Glu214, Leu221, Ala228, Thr241, Leu245, Glu248, and Gln327. Looking into the active sites, the grid box was made up of the following dimensions, size_x = 70.00, size_y = 72.00, and size_z = 98.00. After determining the grid box, the molecular docking was performed. Here, all the four compounds along with the standard drug Hydrochlorothiazide got docked with Solute carrier family 12 member 3 protein. Here, it was obtained that, Quercetin scored the highest binding affinity of -9.0 Kcal/Mol with Solute carrier family 12 mem-

ber 3, followed by Turmerone and Curcumene having docking scores of -7.1 Kcal/Mol and -6.6 Kcal/Mol respectively, where as the standard drug Hydrochlorothiazide scored only -6.0 Kcal/ Mol which is lower than the high scoring compounds. This defines that, the high scoring compounds have more potential effect than the standard drug. Table 5 represents all the docking results. Figure 1 shows three-dimensional structure of Solute carrier family 12 member 3 (PDB ID- 7Y6I). Figures 5 and 6 displayed the two- and three-dimensional interactions of protein -ligands complexes respectively.

Table 5. Molecular docking scores of selected phytocompounds against Solute carrier family 12 member 3 (PDB ID: 7Y6I).

Sl. No.	Drug Name	Chemical formula	Binding Energy scores (Kcal/ Mol)
1.	Camphor	$C_{10}H_{16}O$	-6.4
2.	Curcumene	$C_{15}H_{22}$	-6.6
3.	Quercetin	$C_{15}H_{10}O_7$	-9.0
4.	Turmerone	$C_{15}H_{22}O$	-7.1
5.	Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	-6.0

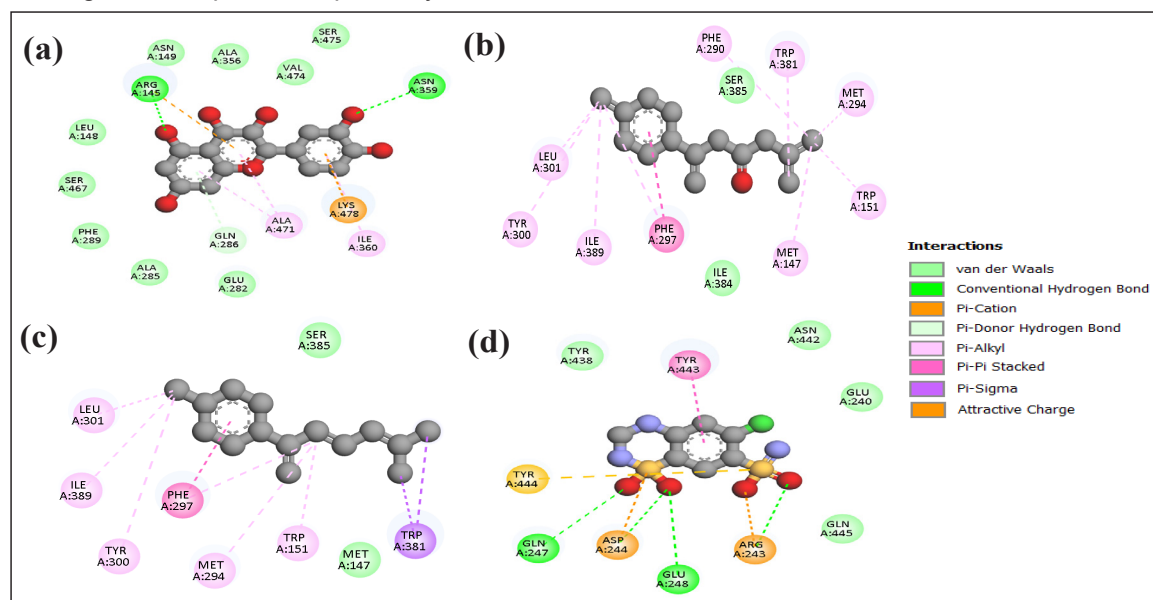


Fig 5. 2-Dimensional interaction of (a) Solute carrier family 12 member 3- Quercetin complex (b) Solute carrier family 12 member 3- Turmerone complex (c) Solute carrier family 12 member 3- Curcumene complex (d) Solute carrier family 12 member 3- Hydrochlorothiazide complex.

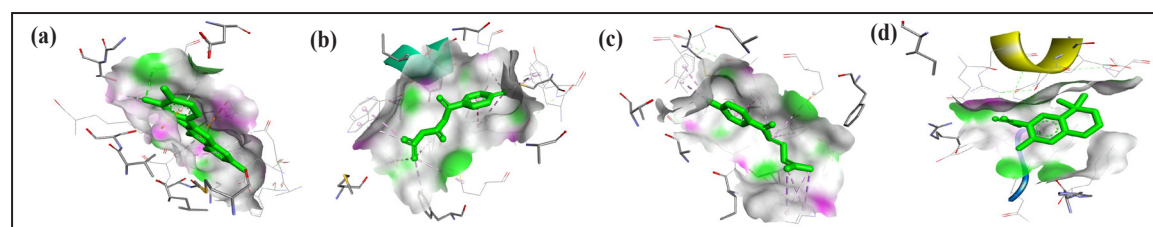


Fig 6. 3-Dimensional interaction of (a) Solute carrier family 12 member 3-Quercetin complex (b) Solute carrier family 12 member 3-Turmerone complex (c) Solute carrier family 12 member 3-Curcumene complex (d) Solute carrier family 12 member 3-Hydrochlorothiazide complex.

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 stone become high due to inefficient treatment. Several preclinical and clinical studies revealed that Hydrochlorothiazide is employed effectively for the prevention of recurrence of kidney stones. Molecular docking study revealed that most of the phytochemicals have good binding affinities towards target as compared to standard drug, Hydrochlorothiazide.

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