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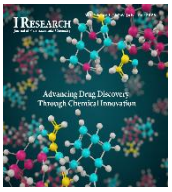

In-Silico Docking and *In-Vitro* Antiurolithiatic Activity of Hydroalcoholic Extract of *Corallocarpus epigaeus* Rhizome

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ABSTRACT

Corallocarpus epigaeus (Rottler) C.B. Clarke (Cucurbitaceae) is a traditionally used medicinal plant whose tuberous rhizome has long been applied for inflammatory, hepatic and urinary complaints. The present study evaluated the phytochemical composition and antiurolithiatic potential of a hydroalcoholic extract of C. epigaeus rhizome (HAECER) through preliminary phytochemical screening, GC-MS profiling, in silico molecular docking and an *in vitro* calcium oxalate dissolution assay. Preliminary screening revealed the presence of alkaloids, flavonoids, sterols, carbohydrates, proteins, amino acids, gum and mucilage, terpenoids, saponins and phenolic compounds. GC-MS analysis identified γ-sitosterol, α-tocopherol, hexadecanoic acid methyl ester (methyl palmitate) and 2,4-di-tert-butylphenol as the major bioactive constituents. Molecular docking of these four compounds against AKT1 protein showed favourable predicted binding affinities, with γ-sitosterol showing the strongest interaction (−10.991 kcal/mol), followed by α-tocopherol (−9.529 kcal/mol), 2,4-di-tert-butylphenol (−7.135 kcal/mol) and hexadecanoic acid methyl ester (−6.048 kcal/mol). The *in vitro* calcium oxalate dissolution assay demonstrated a concentration-dependent antiurolithiatic effect, with percentage inhibition rising from 16.47% at 10 mg/mL to 57.65% at 50 mg/mL and an IC_{50} of approximately 44.09 mg/mL. Taken together, these phytochemical, computational and experimental findings provide preliminary evidence supporting the antiurolithiatic potential of *Corallocarpus epigaeus* rhizome extract, warranting further *in vivo* and mechanistic investigation.

Introduction

Medicinal Plants in Traditional and Modern Therapeutics

Since antiquity, plants have served as a dependable source of therapeutic agents, and they remain central to healthcare systems around the world today. Plant-derived natural products supply a wide array of bioactive molecules with varied pharmacological effects. The World Health Organization (WHO) has noted that a substantial share of the world's population still depends on traditional herbal remedies for their primary healthcare needs. Rising rates of chronic illness, side effects linked to synthetic drugs, and a renewed interest in natural alternatives have all pushed researchers toward a closer study of medicinal plants and their phytoconstituents. Validating these traditional plants scientifically has therefore become a major focus of pharmaceutical research, paving the way for new therapeutic agents and safer alternative medicines.[1]

Ethnomedicine — the traditional knowledge of how plants are used to treat disease — has played a major part in shaping modern drug discovery. A number of widely used drugs can trace their origins back to plants first employed in traditional healing practices. Studying medicinal plants through the lens of their ethnomedicinal use is therefore a valuable strategy for uncovering bioactive compounds of therapeutic value. *Corallocarpus epigaeus* is one such plant from traditional Indian medicine that has drawn considerable attention for its varied pharmacological properties and its long-standing place in folk medicine.

Corallocarpus epigaeus: Botanical and Ethnomedicinal Background

Corallocarpus epigaeus (Rottler) C.B. Clarke, a perennial herb of the family Cucurbitaceae, is found across large parts of India, Sri Lanka and other tropical regions. It can be recognised by its large underground tuber, climbing stem, lobed leaves and yellow flowers. In Ayurvedic, Siddha and various tribal systems of medicine, different parts of the plant — especially the tuberous root — are traditionally used to treat inflammation, skin disorders, rheumatism, fever, snakebite, ulcers and urinary tract complaints. Its phytochemical makeup, which includes flavonoids, alkaloids, triterpenoids, glycosides, phenolic compounds, steroids and saponins, is thought to underlie these therapeutic effects.[5]

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More recent studies have documented a range of pharmacological activities for *Corallocarpus epigaeus*, among them antioxidant, anti-inflammatory, antimicrobial, analgesic, hepatoprotective and nephroprotective effects. These results lend scientific support to its traditional uses and point to its promise as a source of drug-worthy bioactive compounds. Of its many therapeutic properties, the plant's anti-urolithiatic activity is especially noteworthy given how common kidney stone disease has become worldwide.

Urolithiasis: Epidemiology and Therapeutic Challenges

Urolithiasis, better known as kidney stone disease, is a widespread urinary system disorder marked by the formation of solid crystalline masses in the kidneys, ureters, bladder or urinary tract. It ranks among the most frequently encountered urological conditions across all age groups, and both its incidence and recurrence have risen steadily over the past few decades as a result of dietary shifts, sedentary living, insufficient water intake, obesity, metabolic disturbances and environmental influences. Calcium oxalate stones alone make up roughly 70–80% of all urinary calculi, making them by far the most common stone type.[7]

Stone formation is a multi-step process that begins with the supersaturation of urine with stone-forming substances and proceeds through nucleation, crystal growth, aggregation and eventual retention of crystals within kidney tissue. When calcium, oxalate, phosphate, uric acid and cystine build up excessively in the urine, crystallisation is favoured and stones begin to form. Patients affected by urolithiasis typically present with severe flank pain, blood in the urine, urinary obstruction, nausea, vomiting, recurring urinary tract infections and, in some cases, compromised kidney function complications that can become serious if the condition is left unmanaged.

A range of conventional treatments exist for managing urolithiasis, from potassium citrate therapy, thiazide diuretics and allopurinol to procedures such as extracorporeal shock wave lithotripsy, ureteroscopy and open surgery. While these approaches work well in many patients, they can also bring adverse effects, considerable expense, stone recurrence and general discomfort. This has fuelled interest in plant-based alternatives capable of preventing stone formation, breaking down existing stones, and protecting the kidneys with fewer side effects.

Plants with antioxidant, anti-inflammatory, diuretic and crystal-growth-inhibiting properties are increasingly being explored as alternatives for preventing and treating urolithiasis. *Corallocarpus epigaeus* fits well within this group, given its rich phytochemical composition and its established traditional use for urinary complaints. Experimental evidence indicates that the plant can inhibit calcium oxalate crystal formation, ease oxidative-stress-related renal injury, encourage urinary excretion and limit crystal aggregation mechanisms that together may explain its anti-urolithiatic action.[18]

Phytochemical Profiling and Computational Approaches in Drug Discovery

Pinpointing the phytochemicals behind these biological effects calls for sophisticated analytical tools. Gas Chromatography–Mass Spectrometry (GC-MS) stands out as one of the most reliable and widely applied methods for identifying and quantifying volatile and semi-volatile compounds in plant material. By separating individual constituents and matching their mass spectra to known structures, GC-MS makes it possible to characterise bioactive compounds in detail, making it an indispensable tool in phytochemical research aimed at discovering new therapeutic agents.[28]

Alongside phytochemical analysis, computational methods such as molecular docking have become key tools in contemporary drug discovery. This in-silico technique predicts how bioactive compounds interact with proteins implicated in disease, estimating binding affinities and interaction patterns that help clarify a phytoconstituent's probable mechanism of action and flag promising lead compounds for further study. Combining GC-MS profiling with molecular docking therefore offers a well-rounded strategy for probing the therapeutic potential of medicinal plants and putting their traditional uses to a scientific test.

Accordingly, this review sets out to present a comprehensive account of *Corallocarpus epigaeus* — its taxonomy, botanical features, phytochemical composition, ethnomedicinal importance, pharmacological activities and, in particular, its potential against urolithiasis. It also examines how GC-MS and molecular docking studies have been applied to identify and evaluate the bioactive compounds responsible for the plant's therapeutic effects on kidney stone disease. Findings of this kind can inform the development of effective plant-based interventions for managing urolithiasis and support the wider scientific use of such valuable medicinal plants.

Materials and Methods

Botanical Description

- It develops from a tuberous root and can extend up to around 4 m in length.
- Stems and Tendrils: The stems are angular or slightly ridged, typically smooth or with fine hairs, and bear simple, elongated tendrils.[13]

***Leaves*:** The leaves are palmately lobed into 3 to 5 segments, 2.5–9 cm long and 3.5–11.5 cm wide, with softly hairy surfaces, pointed tips, heart-shaped bases and

serrated margins. Petioles measure 1–3.5 cm and are lightly hairy.

***Flowers*:** The plant is monoecious. Male flowers are small and yellow, borne in racemes of 5–40 flowers on peduncles 1–15 cm long, each flower about 1–2 mm across with three erect stamens; female flowers are usually solitary or occasionally clustered on shorter pedicels, with an oblong, beaked ovary 5–6.5 mm long.

***Fruit*:** The fruit is an ovoid to ellipsoid berry, 10–21 mm long and 4–8 mm wide, with a 2.5–7 mm beak. It is smooth and hairless, ripening to bright red except for a green basal cup and beak, and splits open circumscissile at the base.

***Seeds*:** Pear-shaped, around 4 x 2.5 mm, smooth, and yellowish.

***Phenology*:** Flowering and fruiting occur from roughly December to March in its native range. [13]

***Family*:** Cucurbitaceae.

***Synonyms*:**

- Aechmandra epigaea
- Bryonia epigaea
- Corallocarpus gracilipes

Common names:

- Red fruit creeper
- Kollankova
- Aakhasha garudan
- A bastard

Taxonomical Classification

KINGDOM	**:** Plants
PHYLU	**:** Tracheophyta
SUBKINGDOMS	**:** Tracheobionta- Vascular plants
SUPERDIVISION	**:** Spermatophyta- Seed plants
DIVISION	**:**Magnoliophyta-Flowering plants
CLASS	**:** Magnoliopsida- Dicotyledons
SUBCLASS	**:** Dilleniidae
ORDER	**:** Violales
FAMILY	**:**Cucurbitaceae- Cucumber family
GENUS	**:** Corallocarpus
SPECIES	**:***Corallocarpus epigaeus*(Rottl) [12]

Vernacular Names

TAMIL	**:** My kilanku karutan.
TELUGU	**:** Murudonda, Nagadonda
MALAYALAM	**:** Kollam kova kizhang
KANNADA	**:** Akasha garudagadde
MARATHI	**:**Karunai,Kadavinai, Akashagurudi
HINDI	**:** Murchiakand, Kirakanda, Kadvi naahi, Naahi kand
SANSKRIT	**:** Kadamba, Katunahi, Mahamula

Collection and Authentication

Corallocarpus epigaeus plant collected from Nelpettai market, Madurai, Tamil Nadu in the month of June 2026. The species for the proposed study was identified & authenticated by Dr.D. Stephen, Professor Department of Botany (Retd), American College, Madurai-625002.

Figure 1: Corallocarpus epigaeus plant rhizome

Chemicals and Reagents

- Calcium chloride dihydrate
- sodium oxalate
- p-phenylene diamine
- Potassium permanganate
- sodium meta-bisulfite
- Tris buffer (Tris hydroxy methyl aminomethane)
- Cystone (Himalaya Herbal Tablet)

Preparation of the Hydroalcoholic Extract

***Dry powder extract*:**

- The *Corallocarpus epigaeus* leaves was collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction.
- 10g of Fresh leaf *Corallocarpus epigaeus* were put into a conical flask. 100ml of distilled water was

added to the conical flask and boiled for 30 min at 50°C a while to maximize the extraction.
- After cooling it was filtered through Whatmann filter paper and as aqueous extract stock solution transferred to a suitable container.[18]

Preliminary Phytochemical Screening

A preliminary phytochemical screening of PSE was conducted to determine the presence or absence of carbohydrates, steroids, anthocyanins, saponins, tannins, phenols, triterpenoids, flavonoids, glycerides, alkaloids, and furans according to the methods. [21]

GC-MS Analysis

- The prepared sample was analyzed using an Agilent GC–MS system operated with Mass Hunter software.
- The sample (Sample ID: T-26422424-17-R CC-T) was analyzed as a standard sample without dilution (dilution factor: 1).
- Data acquisition was performed using the specified acquisition method, and the chromatographic data were processed using the Mass Hunter data analysis software.
- The analysis was carried out on 6 July 2026, with sample acquisition beginning at 2:27 PM and data processing completed at 3:34 PM.
- The generated chromatograms and mass spectra were used for the identification of the chemical constituents present in the sample by comparing the obtained mass spectral data with the instrument's spectral library.[27]

Molecular Docking Analysis

- A computational technique used in drug discovery to predict the interaction between a ligand (small molecule) and a target protein.
- Helps in understanding binding affinity and molecular interactions.
- It is used to investigate how potential drug interact with proteins involved in kidney stone formation, such as those that influence crystal growth or matrix formation.[29]

Tools & Methods Used:

- Docking Software: AutoDock Vina, SwissDock
- Visualization: PyMOL
- Ligand & Protein Sources: PubChem and RCSB PDB
- Docking Strategy: Blind docking with defined grid box; exhaustiveness adjusted
- Validation: Binding site prediction and pharmacophore analysis.[30]

In Vitro Antiurolithiatic Activity Assay

UV Spectrophotometric Estimation of Calcium Oxalate By Using Dissolution Model:

Preparation of the semi-permeable membrane from eggs

- Apex of eggs was punctured by a glass rod to squeeze out the entire content.
- Empty eggs were washed thoroughly with distilled water and placed in a beaker consisting of 4ml concentrated HCl in 200ml distilled water.
- It was kept for overnight which led to the complete decalcification of semi permeable membrane.
- On the next day, semi permeable membranes were removed carefully from eggshells; washed thoroughly with distilled water and placed it in ammonia solution for neutralization of acid traces and then rinsed it with distilled water.
- It was stored in refrigerator at a pH of 7-7.4 in the moistened condition.[33]

Synthesis of calcium oxalate by homogenous precipitation

- 47gm of calcium chloride dihydrate was dissolved in 100ml distilled water and 1.34gm of sodium oxalate was dissolved in 100 ml of 2N H_2SO_4.
- Both were mixed equally in a beaker to precipitate out calcium oxalate with stirring.
- The resultant calcium oxalate was freed from traces of sulfuric acid by ammonia solution; washed with distilled water and dried at a temperature 60°C for 2hours. [15]

Preparation of 0.02M $KMnO_4$ solution

0.32gm of $KMnO_4$ was dissolved in 100ml of distilled water. It was boiled for 30min. After cooling, excess of $KMnO_4$ was removed by filtration.

Preparation of 0.1M Tris Buffer Solution

12.1gm of tris buffer was dissolved in 1000 ml water. Adjust solution pH 7.2 by using HCl.

Negative control

10mg of the calcium oxalate was suspended in 10ml of distilled water(1mg/ml).

Positive control (Standard Solution Preparation)

500mg tablet of Cystone® was placed in absolute ethanol for removing colour coating and 400mg was obtained. Cystone® tablet was crushed into powder form and 10gm cystone powder dispersed into 100ml of distilled water and filtered(100mg/ml).

Sample solution

10gm of Dry rhizome powder was extact with 100ml distilled water(100mg/ml).

Method:

Group 1: 1ml of calcium oxalate(1gm/ml) +1ml of distilled water (blank)

Group 2: Five series of standard solution is prepared.

1ml of calcium oxalate (1mg/ml) + 1ml of cystone solution (10mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of cystone solution (20mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of cystone solution (30mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of cystone solution (40mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of cystone solution (50mg/ml)

Group 3: five series of dry powder of rhizome extract sample solution is prepared.

1ml of calcium oxalate (1mg/ml) + 1ml of hydro alcoholic extract of *Corallocarpus epigaeus* (10mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of hydro alcoholic extract of *Corallocarpus epigaeus* (20mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of hydro alcoholic extract of *Corallocarpus epigaeus* (30mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of hydro alcoholic extract of *Corallocarpus epigaeus* (40mg/ml)

1ml of calcium oxalate (1mg/ml) + 1ml of hydro alcoholic extract of *Corallocarpus epigaeus* (50mg/ml).[34]

Procedure:

1. All groups were packed it together in egg semi permeable membrane tied with thread at one end and were suspended in a conical flask containing 150ml of 0.1M Tris buffer each.
2. At another end of thread tied by a stick placed on the mouth of conical flask and covered with aluminium foil.
3. All groups were kept in an incubator, pre heated to 37degree Celsius for 4 hours kept for three days.
4. The entire content of each group was removed from sutured semi permeable membrane and was transferred into test tube individually.
5. 4ml of 1N h2so4 and 60-80microlitre of 0.02M Kmno4 were added and kept aside for 2 hours. Colour change dark pink to colourless was observed after 2 hours.
6. Change of colour intensity was measured against 620nm UV spectrophotometry. Concentration of undissolved calcium was determined from standard calibration curve of calcium oxalate by using the measured absorbance readings.

Redox reaction:

$$\mathbf{2KMnO_4 + 5CaC_2O_4 + 8H_2SO_4 \rightarrow 2MnSO_4 + K_2SO_4 + 5CaSO_4 + 10CO_2 + 8H_2O}$$

Calcium oxalate (CaC_2O_4) reacts with potassium permanganate ($KMnO_4$) in an acidic medium, specifically in the presence of sulfuric acid (H_2SO_4), in a redox reaction. $KMnO_4$ oxidizes the oxalate ions ($C_2O_4^{2-}$) to carbon dioxide (CO_2), while itself being reduced to colourless Mn^{2+}. [34]

Results and Discussion

Preliminary Phytochemical Screening Results

The dust and debris on the collected rhizomes were washed using running tap water followed by distilled water. The rhizome skin was peeled off. The peeled rhizome was chopped into small pieces and then they were dried well in shade and converted into moderately coarse powder by mechanical grinder. Then the powder was sieved with a commercial sieve of mesh size approximately 60mm to make the particle size uniform. Finally, stored in airtight containers for further use.

Preparation of Hydroalcoholic Extract of Corallocarpus epigaeus (HAECER)

About 30g of air-dried coarse powder of rhizome of *Corallocarpus epigaeus* was macerated with 500ml of hydro alcoholic solvent (75:25) in the closed flask for 72hrs. The flask was shaken frequently. The extracts were then filtered through whatmann filter paper No.42 (125 mm) to remove all non- extractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness and stored in sterile bottles for further use.

Preliminary Phytochemical Screening of Hydroalcoholic Extract of Corallocarpus epigaeus Rhizome

Hydroalcoholic extract of *Corallocarpus epigaeus* was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract for the identification of phytochemicals and secondary metabolites.

Table 1 : Preliminary phytochemical screening of HAECER

S. No.	Test	Observation	Inference
1	Alkaloids – Mayer's test	Cream colour precipitate	Presence of Alkaloids
2	Alkaloids – Dragendroff's test	Orange brown precipitate	Presence of Alkaloids
3	Alkaloids – Wagner's test	Reddish brown precipitate	Presence of Alkaloids
4	Alkaloids – Hager's test	Yellow precipitate	Presence of Alkaloids
5	Flavonoids – Shinoda' test	Red colour is obtained	Presence of Flavonoids
6	Flavonoids – Alkali's test	White precipitate occurs	Presence of Flavonoids
7	Flavonoids – Lead acetate test	White precipitate occurs	Presence of Flavonoids
8	Glycosides – Borntrager's test	No pink colour in ammoniacal layer	Absence of glycosides
9	Glycosides – Modified Borntrager's test	No pink colour in ammoniacal layer	Absence of anthraquinone glycoside
10	Glycosides – Keller Killiani test	No reddish-brown layer was observed	Absence of sugars
11	Glycosides – Raymond's test	No violet colour appeared	Absence of Cardiac glycoside
12	Glycosides – Legal's test	No pink to red colour	Absence of Cardiac glycoside
13	Sterols – Salkowski's test	The lower chloroform layer of the solution turned red in colour.	Presence of Sterols
14	Sterols – Libbermann-Burchard's test	At the junction of two layer a brown ring was formed.	Presence of Sterols
15	Tannins – Ferric chloride test	No Bluish black colour is formed	Absence of Tannins
16	Tannins – Lead acetate test	No Precipitate is formed	Absence of Tannins
17	Carbohydrates – Molisch's test	Formation of purple colour	Presence of Carbohydrates
18	Carbohydrates – Fehling's test	Formation of reddish-brown precipitate	Presence of free reducing sugars
19	Carbohydrates – Benedict's test	Red precipitate is formed	Presence of Reducing sugar
20	Proteins – Millon's test	White precipitate turned red on heating	Presence of Proteins
21	Proteins – Biuret test	Violet colour is obtained	Presence of Proteins
22	Amino acid – Ninhydrin test	Appearance of purple colour	Presence of amino acid
23	Amino acid – Nitric acid test	Presence of yellow coloration	Presence of proteins and free amino acids
24	Gum	White precipitate is formed	Presence of gum
25	Terpenoids	Appearance of pink colour	Presence of Terpenoid
26	Triterpenoids	Reddish violet colour is formed	Presence of Triterpenoid
27	Mucilage	Red colour is formed	Presence of Mucilage
28	Saponins	Foam is formed	Presence of Saponins
29	Phenolic content	Appearance of deep blue colour	Presence of Phenolic content

Figure 2. Phytochemical screening of HAECER

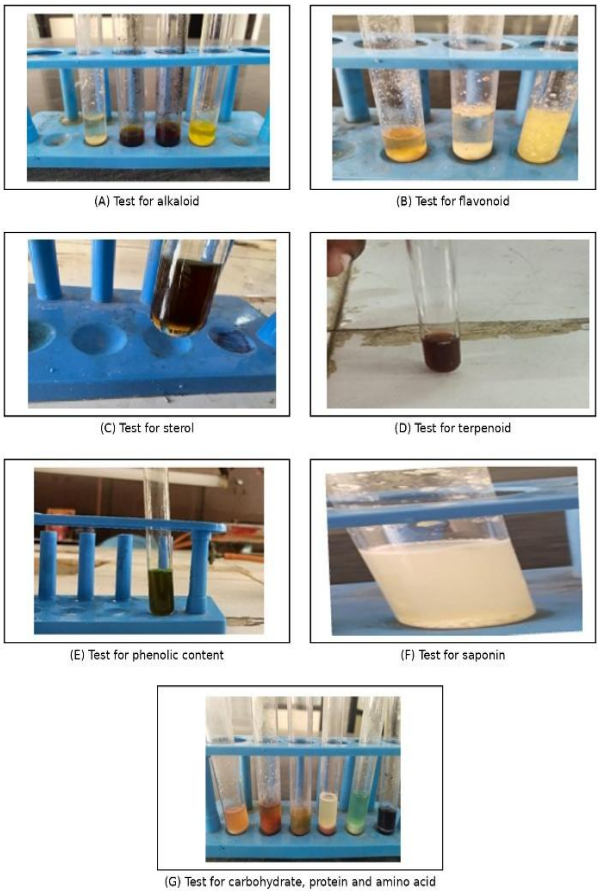

Table : 2 Result for phytochemical screening

S.NO	PHYTOCHEMICAL	RESULT
1	Alkaloid	+
2	Flavonoid	+
3	Glycoside	-
4	Sterol	+
5	Tannin	-
6	Carbohydrate	+
7	Protein	+
8	Amino acid	+
9	Gum & Mucilage	+
10	Terpenoid	+
11	Saponin	+
12	Phenolic content	+

(+) Presence (-) Absence

Discussion

Phytochemical screening of HAECER indicated the presence of alkaloids, flavonoids, sterols, carbohydrates, proteins, amino acids, gum and mucilage, terpenoids, triterpenoids and saponins. Such screening is a key step in identifying the bioactive constituents of medicinal plants and supports drug discovery efforts, since a plant's secondary metabolites are often closely linked to its therapeutic effects.

GC-MS Analysis of HAECER

GC-MS analysis was carried out to profile the phytochemical constituents of the extract. Among the compounds detected, γ-Sitosterol, α-Tocopherol (vitamin E), hexadecanoic acid methyl ester and 2,4-di-tert-butylphenol emerged as the major bioactive constituents. Each of these has documented antioxidant, anti-inflammatory, antimicrobial and anticancer properties, which may collectively underlie the biological activity of the extract.GC-MS analysis was carried out to profile the phytochemical constituents of the extract. Among the compounds detected, γ-Sitosterol, α-Tocopherol (vitamin E), hexadecanoic acid methyl ester and 2,4-di-tert-butylphenol emerged as the major bioactive constituents. Each of these has documented antioxidant, anti-inflammatory, antimicrobial and anticancer properties, which may collectively underlie the biological activity of the extract.

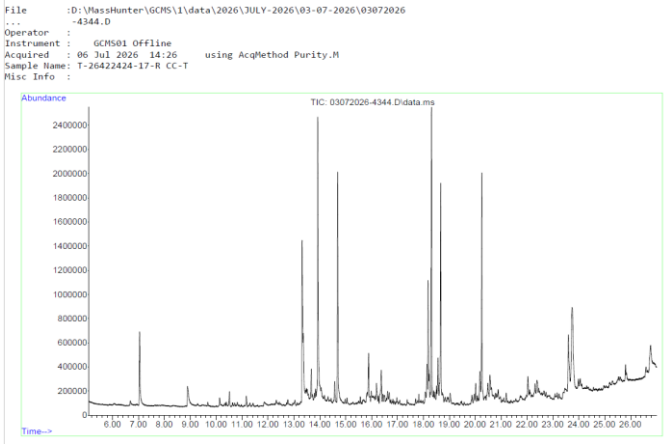

Figure 3. GC-MS total ion chromatogram of the hydroalcoholic extract of Corallocarpus epigaeus

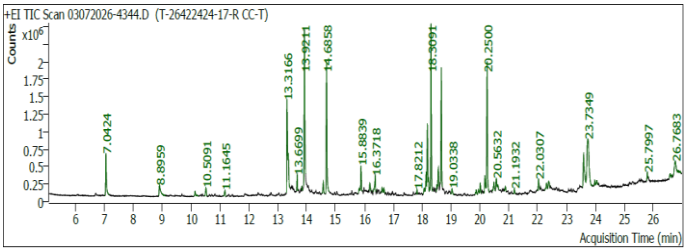

Figure 4. Annotated GC-MS chromatogram showing retention times of major peaks

Table 3 : Identified Bioactive Compounds and Molecular Docking Analysis

S. No	Compound	Chemical Class	RT (min)	Molecular Formula	Match Score	Activity
1	γ-Sitosterol	Phytosterol	26.7683	$C_{29}H_{50}O$	86.5	Antiurolithiatic, Antioxidant, Anticancer, Anti-inflammatory
2	α-Tocopherol (Vitamin E)	Vitamin E	23.7349	$C_{29}H_{50}O_2$	94.0	Antiurolithiatic, Powerful antioxidant, Skin protection
3	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	18.3091	$C_{17}H_{34}O_2$	97.4	Antiurolithiatic, Antioxidant, Antimicrobial
4	2,4-Di-tert-butylphenol	Phenolic compound	13.6699	$C_{14}H_{22}O$	96.5	Antiurolithiatic, Antioxidant, Antibacterial

Molecular Docking Analysis

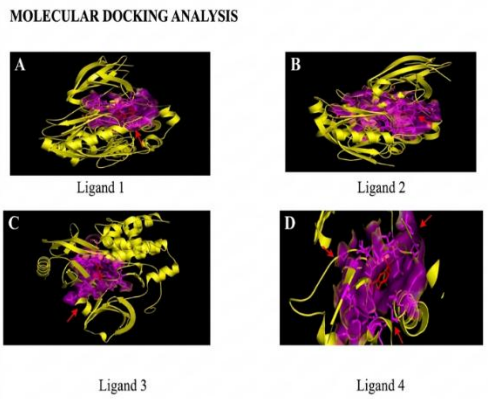

Figure 5. Molecular Docking Analysis

Table 4: Docking report for hydro-alcoholic extract of Corallocarpus epigaeus rhizome isolated compounds (Ligands) by using GC-MS studies

S.NO	*Compound Name*	Compound Code	Calculated Affinity (kcal/mol)
1	*Gamma Sitosterol*	Ligand 1	-10.991
2	*Alpha tocopherol*	Ligand 2	-9.529
3	*2,4-Di-tert-butylphenol*	Ligand 3	-7.135
4	*Hexadecenoic acid, methyl ester*	Ligand 4	-6.048

Molecular Interaction of Ligand 1 (Gamma sitosterol) with AKT1 Protein and its Anti-Urolithiatic Potential

γ-Sitosterol, one of the phytosterols identified in *Corallocarpus epigaeus*, was chosen for docking against AKT1 (protein kinase B) to explore a possible protein-binding interaction relevant to antiurolithiatic activity, given AKT1's central role in cell survival, proliferation, metabolism, and oxidative and inflammatory responses.

Docking was carried out to predict the orientation and strength with which γ-sitosterol binds AKT1; a more negative docking score points to a more favourable predicted affinity, with the interaction likely involving hydrophobic contacts, van der Waals forces and other non-covalent interactions within the binding pocket.

The predicted binding pattern suggests γ-sitosterol may engage AKT1-linked signalling pathways involved in cellular stress and inflammation, offering computational support for its possible antiurolithiatic role — though this would need to be confirmed through further *in vitro* and *in vivo* work.

Molecular Interaction of Ligand 2 (α-Tocopherol) with AKT1 Protein and its Anti-Urolithiatic Potential

α-Tocopherol, another constituent of *Corallocarpus epigaeus*, was likewise docked against AKT1 to examine a possible binding interaction relevant to antiurolithiatic activity, given the protein's involvement in cell survival, metabolism and broader cellular responses.

Docking predicted the binding orientation and affinity of α-tocopherol for AKT1, with a more negative score indicating a stronger predicted interaction, likely mediated by hydrophobic and van der Waals contacts and other non-covalent forces within the binding site.

Combined with its known antioxidant properties, α-tocopherol's predicted interaction with AKT1 offers a computational rationale for a possible role in limiting the oxidative stress and inflammation associated with stone formation, again warranting confirmation through *in vitro* and *in vivo* studies.

Molecular Interaction of Ligand 3 (2,4-Di-tert-butylphenol) with AKT1 Protein and its Anti-Urolithiatic Potential

2,4-Di-tert-butylphenol, a phenolic constituent of the plant, was similarly docked against AKT1 — a protein central to the PI3K/AKT pathway that governs metabolism, oxidative stress and inflammation — to assess its possible relevance to anti-urolithiatic activity.

The docking results indicated favourable binding energy for 2,4-di-tert-butylphenol at the AKT1 site, suggesting interactions mediated largely through hydrophobic and van der Waals forces, with the possibility of hydrogen bonding as well.

Taken together with its antioxidant character, this compound's predicted AKT1 interaction may bear on the oxidative and inflammatory mechanisms involved in stone formation, lending computational support to its anti-urolithiatic potential and pointing to a need for further *in vitro* and *in vivo* validation.

Molecular Interaction of Ligand 4 (Hexadecenoic acid, methyl ester) with AKT1 Protein and its Anti-Urolithiatic Potential

Hexadecanoic acid methyl ester (methyl palmitate) was also selected as a docking ligand against AKT1, a serine/threonine kinase central to the PI3K/AKT pathway that regulates cell survival, metabolism, oxidative stress and inflammation, in order to probe its possible contribution to anti-urolithiatic activity.

Docking analysis predicted the binding affinity of methyl palmitate for the AKT1 site, with hydrophobic contacts, van der Waals forces and other non-covalent interactions likely involved; the favourable score obtained points to a potentially stable ligand–protein complex.

This predicted AKT1 interaction may be relevant to the cellular, oxidative-stress and inflammatory pathways underlying stone formation, offering computational support for methyl palmitate's possible contribution to the plant's anti-urolithiatic potential and warranting further experimental confirmation.

In-Vitro Antiurolithiatic Activity: Results

UV Spectrophotometric Estimation Of Calcium Oxalate By Using Dissolution Model

1. Preparation of the semi-permeable membrane from eggs

2. Synthesis of calcium oxalate by homogenous precipitation

3. Preparation of Standard solution:

4. Preparation of Sample solution:

Method

- **Group I:** 1ml of calcium oxalate (1mg/ml) + 1ml of distilled water
- **Group II:** Five series of positive control solutions were prepared.
- **Group III:** Five series of dry powder extract sample solutions were prepared.

Observation

Change of colour intensity was measured against 620nm UV spectrophotometrically

Standard solution absorbance at 620nm:

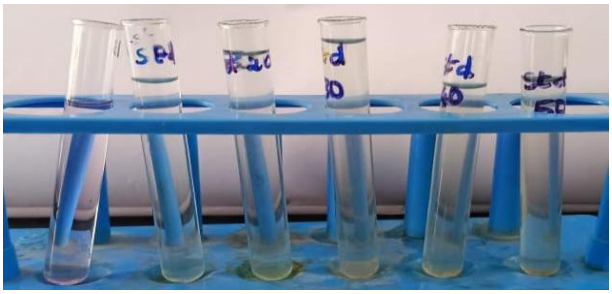

Figure 6. Standard solution

Table 5: Standard (Cystone) Absorbance

Concentration (mg/mL)	Control absorbance(620nm)	Extract absorbance
10	0.850	0.710
20	0.850	0.650
30	0.850	0.560
40	0.850	0.470
50	0.850	0.360

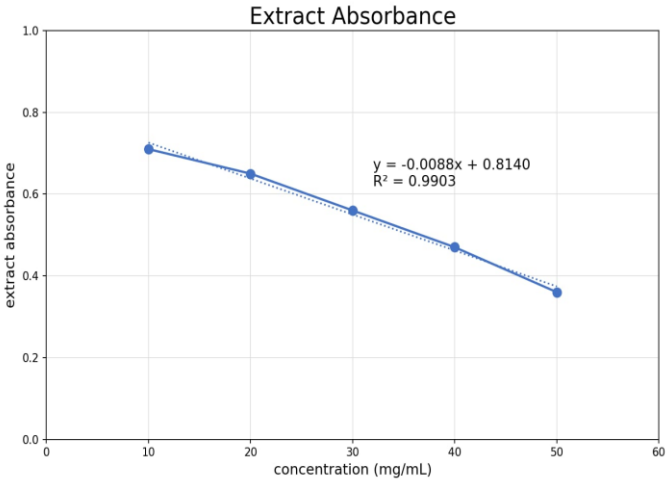

Figure 7. Standard curve for Standard solution

Dry powder extract solution absorbance at 287nm:

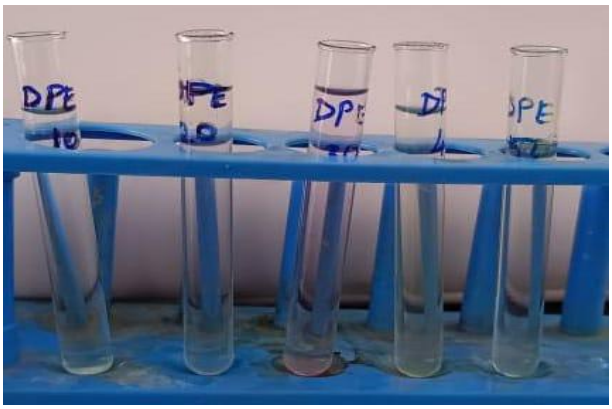

Figure 8. Dry powder extract solution

Table 6 : Dry Powder Extract Solution Absorbance

Concentration (mg/mL)	Control absorbance(620nm)	Standard (cystone) absorbance
10	0.850	0.620
20	0.850	0.540
30	0.850	0.460
40	0.850	0.380
50	0.850	0.290

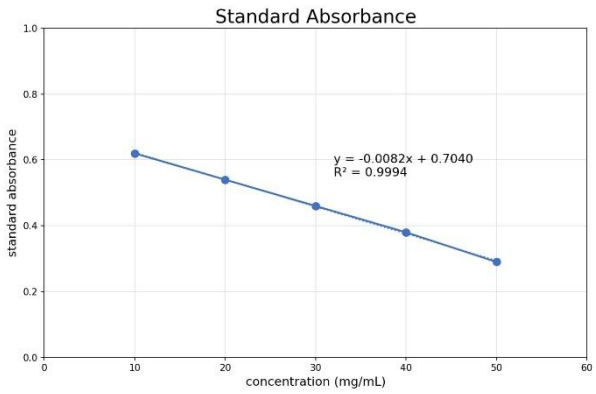

Figure 9. Standard curve for Dry powder extract solution

PERCENTAGE INHIBITION IC50 VALUE OF THE EXTRACT

The IC_{50} is the concentration that produces 50% inhibition. Since 50% lies between 40 mg/mL (44.71%) and 50 mg/mL (57.65%), we calculate it by linear interpolation:

IC50 = X1+(50-Y1) (X2-X1)/Y2-Y1

Result

IC_{50} of the hydroalcoholic extract of *Corallocarpus epigaeus* = 44.09 mg/mL (approximately 44.1 mg/mL)

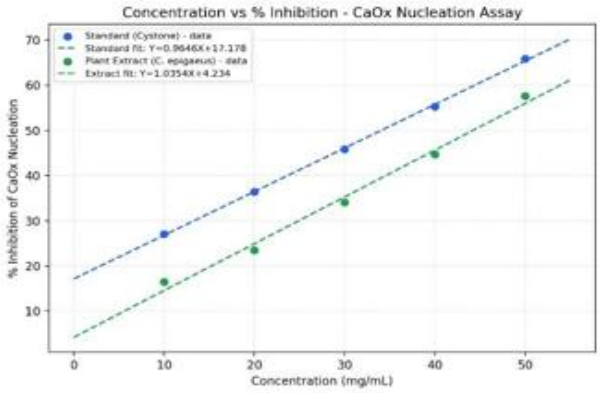

Figure 10. Concentration vs. Percentage Inhibition of Calcium Oxalate Nucleation by HAECER

Conclusion

This study, titled 'In Silico Docking and *In Vitro* Antiurolithiatic Activity of Hydroalcoholic Extract of *Corallocarpus epigaeus*', set out to examine the phytochemical composition of the plant's rhizome extract and evaluate its potential antiurolithiatic activity.

The hydroalcoholic extract, prepared by maceration, was subjected to preliminary phytochemical screening, GC-MS profiling, molecular docking analysis and in-vitro evaluation via a calcium oxalate dissolution assay.

Preliminary screening confirmed the presence of a range of bioactive constituents — alkaloids, flavonoids, sterols, carbohydrates, proteins, amino acids, gum and mucilage, terpenoids, saponins and phenolic compounds — reflecting a diverse secondary-metabolite profile that may underlie the extract's biological activity.

GC-MS analysis reinforced this phytochemical picture and identified γ-sitosterol, α-tocopherol, hexadecanoic acid methyl ester (methyl palmitate) and 2,4-di-tert-butylphenol as the principal bioactive constituents based on their reported biological effects; together, the phytosterols, phenolics, antioxidants and fatty acid esters present point to promising pharmacological potential.

Docking against AKT1 produced favourable predicted binding affinities for all four compounds, with γ-sitosterol scoring highest (−10.991 kcal/mol), followed by α-tocopherol (−9.529 kcal/mol), 2,4-di-tert-butylphenol (−7.135 kcal/mol) and hexadecanoic acid methyl ester (−6.048 kcal/mol) — results that offer preliminary computational support for interactions between these phytoconstituents and AKT1-linked pathways governing cellular, oxidative-stress and inflammatory processes.

The in-vitro dissolution assay showed a clear concentration-dependent effect, with percentage inhibition rising from 16.47% at 10 mg/mL to 57.65% at 50 mg/mL and an IC_{50} of approximately 44.09 mg/mL — indicating measurable calcium oxalate dissolution activity under the tested conditions.

Taken together, these findings offer preliminary experimental and computational evidence in support of the antiurolithiatic potential of *Corallocarpus epigaeus* rhizome extract.

The convergence of phytochemical, GC-MS, docking and in-vitro results points to a likely synergistic contribution from multiple constituents — particularly γ-sitosterol, α-tocopherol, hexadecanoic acid methyl ester and 2,4-di-tert-butylphenol — though it should be noted that the docking data are predictive rather than confirmatory, and the in-vitro results will need further validation.

Further work — including *in-vivo* studies, isolation and characterisation of the active constituents, mechanistic investigations and toxicity assessment — is therefore needed to firmly establish the therapeutic potential and safety of *Corallocarpus epigaeus* as an antiurolithiatic agent.

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References

1. Indian Medicinal Plants, 2nd Ed., vol.J.Jayyed Press, New Delhi, p.326
2. World Health Organization WHO Traditional Medicine Strategy 2014 2023 Geneva WHO 2013.
3. Petrovska BB Historical review of Medicinal plant usage pharmacognosy Rev.2012; 6(11): 1-5.
4. Kirtikar KR and Basu BD (1935) Kirtikar and Basu described *Corallocarpus epigaeus* in their classical book Indian Medicinal Plants.
5. Kokate CK, Purohit AP and Gokhale SB (2010) The authors explained pharmacognostic evaluation methods for medicinal plants, including macroscopic, microscopic, physicochemical, and phytochemical analyses.
6. Scales CD Jr. Smith Acetal prevalence of kidney stones in the United States.Eururol2012:62(1):160-165.
7. Ferraro PM. Curchan Gc et al kidney stones and risk of ESRD.J.AM Soc Nephrol 2017-28(10):2729-2735.
8. O'Flym KJ Urolithiasis in ancient Mesopotamia J R Soc Med 1993:86(10):609.
9. Tcfckil a, Cezayirli F The history of Urinary stones, in parallel with civilization scientific World Journal 2013:423964.
10. Kaushik H Nakum, Vipul B Vaja and Kalpesh B Ishnava, Ethnobotany and phytochemistry of *Corallocarpus epigaeus* CL-A candidate of potentials Medicinal plant, Journal of Medicinal plants studies 2025;13(3):382-385.
11. Sivkumar Y, Kannan K, Manavalan R pharmacognostical investigations of *Corallocarpus epigaeus* (Rotter) CB clark Lateral 2009;2(1):159-66.
12. Sethi P Micromorphological studies on Rhizome of *Corallocarpus epigaeus* Benth ex Hook F Researcher 2017:9(12).
13. Kirubha TS, Senthamarai R, Vasuki K, Rao AV,Selvadurai S.Anthelmintic activity of roots and rhizomes of *corollocarpus epigeaus*. J.Nat,Prod,Plant Resour.2011 Aug 24;1(1):81-4.
14. C.N "shri pharmacognostical screening of the preliminary phytochemical screening of the root and rhizome of *corollocarpus epigaeus* "International Journal of pharmaceutical and biomedical research, Vol (1) PP24-27 Feb 20100.
15. Saso L, Valentini G, Leone MG, Grippa E, Development of an *in vitro* assay for the screening of substances capable of dissolving calcium oxalate crystals, Urologia Internationalis, 1998;61(4):210-214.
16. Surendra KP, Karthik CP, Ranjit H. *In vitro* calcium oxalate crystallization inhibition of *Achyranthes indica* Linn. Hydroalcoholic

Extract: An approach to anti lithiasis. International Journal of Pharma and Bio Sciences,2011;2(8):432-437.
17. R. Acharya "phytochemical Screening of hydroalcoholic extract and antimicrobial activity of active principle of *eclipa alba* Asian Journal of emerging research vol 2, no 3 2020.
18. R.Sarojini, S. Selvakumar, and N.Chidambaranathan (2024). Preclinical Evaluation of Hydro alcoholic extract of *Corallocarpus epigaeus* for anti-inflammatory activity. International Journal of Research in pharmacology and Pharmacotherapeutics,13(2).176202.
19. Narendra ND, Suresh BV, Sandeep Veda NM, Rangu M. *In vitro* screening of *Corallocarpus epigaeus* tuber for its analgesic, anti-pyretic and anti-inflammatory activities int. J Ph. Pharm 2013;3(3)24-4.
20. Bhavani MB Shareef M, Sampath kumara KK, Nethra NS, Leelavathi S, Evaluation of *in vitro* antioxidant activity of a few wild Cucurbitaceae plant against cancer.
21. Priyavathi S, Vasantha K, TresinaSoris P, Mohan VR. Efficacy of phytochemical and antibacterial activity of *corollocarpus epigaeus* Hook. F. Int.J pharm Tech Res.2012 Apr 4;4:35-43.
22. Jeyaseelan M, Arumugam T, Thangaraj N. Evaluation of antioxidant and anti-inflammatory activities of *corollocarpus epigaeus* (Hook. F) rhizomes. Int J pharm Biomed res.2014; 591:18-24.
23. J.R.S.A.M. Patil, 'Qualitative test for preliminary phytochemical screening an overview "International Journal of chemical studies PP 603-608 192 2020.
24. Damodar K, Bhogineni S, Ramanjaneyulur B, phytochemical screening, quantitative estimation of total phenolic, flavonoids and anti-microbial evaluation of *Trachyspermum ammi* Journal of Atoms and Molecules 2011 Nov1:1(1):1.
25. Kandasamy S, Chinnappan S, Thangaaswamy S, Balakrishnan S. Facile approach for phytosynthesis of gold particles from *Corallocarpus epigaeus* rhizome extract and their biological assessment. Material Research Express. 2020 Jan 8;6(12):1250cl.
26. Muzahid AA Sharmin S. Hossain MS, Ahamed KU, Ahmed N, Yeasmin MS, Ahmed Nu, Saha B.K.,Rana GM, Maitra B, Bhuiyan MN, analysis of bio active compounds presents in different crude extracts of *Benincasa nisipida* and Curcurbitamoschata seeds by Gas Chromatography-Mass spectroscopy Heliyon 2023 Jan 1:9(1).
27. Arirudran B. Gas Chromatography-Mass Spectrometry (GC-MS) Determination pf Bioactive components from *corollocarpus epigaeus* L Rhizome. Asian Journal of Medicine and Biomedicine.2023 Oct 20;7(2):96-108.
28. Anwar Zeb AZ, Farhat Ullah FU, Muhammad Ayaz MA, Sajjad Ahamad SA, Abdul Sadiq AS. Demonstration of biological activities of extracts from Isodon rugosus wall. Ex Benth: separation and identification of bioactive phytoconstituents by GC-MS analysis in the ethyl acetate extract.
29. Islam S, Hosen MA, Ahmad S, ul QamarMT, Dey S, Hasan I, Fujii Y, Ozeki Y, Kawsar SM. Synthesis, antimicrobial, anticancer activities, PASS prediction, molecular docking, molecular dynamics and pharmacokinetics studies of designed methyl alpha D- glycopyranoside esters. Journal of Molecular structure.2022 Jul 15;12160:132761.
30. Torunoglu EL, Aytar EC, Aydin B,Durmaz A. Antioxidant and anti-urease activities of Cardamine bulbifera:Insights from molecular docking and density functional theory studies. European Journal of Integrative Medicine. 2024 Oct 1; 71:102410.
31. Ramalingam A, Sambandam S, Medimagh M, AI-Dossary O, Issaoui N, Wojcik MJ. Study of a new piperidone as an anti-Alzheimer agent: Molecular docking, electronic and intermolecular interaction investigations by DFT method. Journal of Kind Saud University-Sciene.2021 Dec1;33101632.
32. Nivatya HK, Singh A, Kumar N, Sonnam, Sharma L,Singh V, Mishra R, Gaur N, Mishra AK.bAssessing molecular docking tools: understanding drug discovery and design. Future Journal of Pharmaceutical Sciences. 2025 Aug 18'11 (1):111.
33. Christynal Oliviya R. and Dayana Devi A. Defence potential of Solanum Surattense and senna alexandrina against urolithiasis an *in vitro* approach.
34. Netra pal Sharma, Ajay Kumar, Sanjay Gupta "Investation the anti urolithiatic potential of horse gram (Macrotyloma Uniflorum) seeds extracts on experimental kidney stones from high altitude of Uttarakhand (India)".
35. Burns JR, Finlayson B. A proposal for a standard reference artificial urine *in vitro* urolithiasis experiment. J Urol 1980; 18:167-169.
36. Unnati A, Roshni B, Siddhi U, Umesh U. Anti-Urolithiatic Activity of Dolichos Biflorus seeds. Journal of pharmacognosy and phytochemistry,2013;2(2):209-213.
37. Martin IR, Carl WG, Michael BS and William HB:Urinary Calcium Binding Proteins and Renal calculi.Urolithiasis, 1981;675-684.
38. Agarval, V and Bajpai, M. Pharmacognostical and biological studies on senna and its products:an overview. International Journal of pharma and Bio Science 2010;1 (2):1-10.
39. Aggarwal A, Tandon S, Singala SK, Tandon C. Diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization in vitro by aqueous extract of Tribulus terrestris. Int Braz J Urol,2010;36:480-9.
40. Hennequin C, Lalanne V, Daudon M, Lacour Bm Drueke T. A new approach to studying inhibitors of calcium oxalate crystals growth.Urol Res,1993;21:101-8.
41. Hess B, Nakagawa Y, Coe Fl. Inhibition of calcium oxalate monohydrate crystal aggregation by urine protein. Am J Physiol, 1989;257: F99-F106.
42. Paarmatr, Sachin, Gangwal,Amit,Sheth,Nsvin. Solanum xanthocarpum (yellow) Berried Night Shade): A review. Scholars Research Library, 2010;2 (4):373-383.
43. Unnati A, Roshini B Siddhi U, Umesh U. Anti Urolithiatic activity of Dolichps Biflorus seeds. Journal of pharmacognosy and phytochemistry, 2013;2(2):209-213.
44. enkataswamy M Diffusion studies of drug through semi permeable membrane, 2018.DOI:10.13140/RG.2.2.16084.24966.
45. Khan SR. Interactions between stone forming calcific crystals and macromolecules. Urol Int, 1997;59(2);59-71.