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Evolution of Antiurolithiatic Activity of Gardenia Jasminoides

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ABSTRACT

Kidney stone disease (urolithiasis) is a common urinary tract disorder associated with significant pain, recurrence, and complications affecting renal function. The present study aims to evaluate the antiurolithiatic activity of leaf extract of Gardenia jasminoides using experimental models and to assess its potential as a natural therapeutic agent for kidney stone management. The extract was subjected to phytochemical investigation and evaluated through in vitro and/or in vivo experimental models of urolithiasis. Parameters such as crystal formation, crystal aggregation, urinary biochemical markers, renal function indicators, and histopathological changes were analyzed to determine the efficacy of the extract.1-2

Keywords: Inflammation, Rhizomes, Labdane Diterpenes, Coronarin D, Inflammatory Mediators, Neutrophil-Induced Inflammation, Anti-Inflammatory Mechanisms, Pharmacology.

1. Introduction -

Urolithiasis, commonly known as kidney stone disease, is one of the most prevalent disorders affecting the urinary system worldwide. It is characterized by the formation of hard crystalline deposits or calculi in the kidneys, ureters, urinary bladder, or urethra due to the supersaturation of urine with stone-forming substances. The disease affects people of all age groups and has become a major public health concern because of its increasing incidence, recurrence, and associated complications. Kidney stones are mainly composed of calcium oxalate, calcium phosphate, uric acid, struvite, or cystine, among which calcium oxalate stones account for nearly 70–80% of all urinary calculi. 1-2

The formation of kidney stones is a complex process involving supersaturation, nucleation, crystal growth, aggregation, and retention within the renal tubules.Several factors contribute to the development of urolithiasis, including dehydration, dietary habits, metabolic disorders, obesity, urinary tract infections, genetic predisposition, and environmental conditions. Insufficient water intake and excessive consumption of oxalate-rich foods, salt, and animal proteins can increase the concentration of stone-forming substances in urine, thereby promoting crystal formation. In addition, oxidative stress and inflammation are believed to play important roles in renal tissue injury and crystal retention during stone formation. Patients suffering from kidney stones commonly experience severe abdominal pain, hematuria, nausea, vomiting, urinary obstruction, recurrent infections, and impaired renal function.

Recurrence is a major problem in urolithiasis, as nearly 50% of patients may develop stones again within five to ten years after the first episode.Conventional management of kidney stones includes increased fluid intake, dietary modifications, pharmacological therapy, extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and surgical removal of stones. Drugs such as diuretics, citrate preparations, and alkalizing agents are commonly used to reduce stone formation and facilitate stone expulsion. Although these therapies are effective, they are often associated with several limitations including high treatment cost, side effects, recurrence of stones, and risk of renal injury. Surgical procedures and lithotripsy may also cause tissue damage, infection,

bleeding, and incomplete stone removal. Due to these limitations, there has been growing interest in the use of medicinal plants and natural products as safer, affordable, and effective alternatives for the prevention and treatment of urolithiasis.Medicinal plants have been used since ancient times in various traditional systems of medicine such as Ayurveda, Traditional Chinese Medicine, and Unani for the treatment of kidney stones and urinary disorders. Herbal remedies are considered beneficial because they possess multiple pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, diuretic, nephroprotective, and crystal inhibitory properties.4-5

Iridoid Glycosides

Iridoid glycosides are considered the major active constituents of Gardenia jasminoides. Important iridoids include:

Geniposide Genipin Gardenoside Shanzhiside Genipingentiobioside Deacetylasperulosidic acid methyl ester

- Pathophysiology of Kidney Stone Formation

Kidney stones mainly consist of calcium oxalate, calcium phosphate, uric acid, cystine, or struvite crystals. Among these, calcium oxalate stones are the most prevalent.
Major Steps in Stone Formation

1. **Supersaturation of Urine**

Excess concentrations of calcium, oxalate, phosphate, or uric acid in urine exceed their solubility limits, leading to crystal formation.

2. **Nucleation**

Initial crystal particles form from supersaturated urine.

3. **Crystal Growth** and Aggregation Small crystals combine to form larger aggregates.
4. **Crystal Retention**

Crystals adhere to renal epithelial cells, causing tubular obstruction and inflammation.
Oxidative Stress and Renal Injury Reactive oxygen species (ROS) damage renal cells and promote crystal attachment and inflammation. Experimental Models for Antiurolithiatic Evaluation
In Vitro Models

A. Calcium Oxalate Crystallization Assay
Evaluates inhibition of:

- Nucleation
- Aggregation
- Crystal growth

B. Turbidity Method Measures crystal formation spectrophotometrically.
C. Microscopic Crystal Analysis
Assesses crystal size and morphology.

- **Gardenia Jasminodes :-** Is an evergreen flowering shrub naxtive to parts of East Asia, especially China Japan, Taiwan, and Vietnam.
- **Synonyms :** Gardenia Florida L, Gardenia radicans thunb, Gardenia grandiflora Lour
- **Scientific name :-** Gardenia Jasminodes J. Ellis
- **Genus :-** Gardenia
- **Family :-** Rubiaceae
- **Common Name :-** Cape Jasmine
- **Kingdon :-** Plantae
- **Chemical constituents:** Iridoid glycosides, Carotenoids, flavonoids , volatile oil, triterpenoids, mannitol sucrose.
- **Geographical Distribution:** 1, Native to china, Japan and Taiwan

2. Cultivated in india, southeast Asia, southern Europe

Morphology of Gardenia jasminodes leaves :-

Leaf Type :-Simple leaf **Arrangement :-**
Opposite or sometimes whorled
Shape :-
Elliptic to belong or lanceolate

1. To evaluate the antiurolithiatic activity of leaf extract of Gardenia jasminoides using experimental models.
2. To study the effect of the leaf extract on inhibition of calcium oxalate crystal formation, growth, and aggregation.

Color :-
Dark green
Surface :- 3.
Smooth and glossy
Texture :-
Leathery
Margin :-
Entire (smooth edge)
Apex :-
Acute or pointed
Base :-
Rounded to tapering
Venation :-
Pinnate reticulate venation
Size :-
Usually 5-12 cm long
Petiole :-
Short petiole present **Persistence** :-Evergreen

Uses:-
The leaves of Gardenia jasminoides are glossy and dark green.
Gardenia leaves are mainly used for ornamental purposes. The leaves help the plant prepare food by photosynthesis.

Major Phytochemicals :-
Geniposide , Genipin, Crocin, Crocetin, Gardenoside, Chlorgenic acid, Flavonoids, Tannins, Linalool, Benzyl acetate6-7

Need of Work:-

1. To assess the antioxidant and nephroprotective properties of the leaf extract in kidney stone conditions.
2. To determine the ability of the extract to reduce urinary stone-forming constituents and renal damage.
3. To compare the activity of the leaf extract with standard antiurolithiatic drugs.

Aim:-
To the antiurolithiatic activity of leaf extract of gardenia jasminoides using experimental Models and to assess its potential as a natural therapeutic agent for kidney stone.

Objective:-
1. To evaluate the antiurolithiatic activity of leaf extract of Gardenia jasminoides using experimental models.
2. To study the effect of the extract on kidney stone formation and crystal deposition.
3. To assess biochemical parameters related to urolithiasis such as calcium, oxalate, and creatinine levels.
4. To evaluate the protective effect of the extract on kidney tissues.
5. To compare the activity of the extract with a standard antiurolithiatic drug.8-9

Plan of Work:-
1. Literature review.
2. Collection and authentication of plant.
3. Drying of plant material. 4.Extraction of plant material. 5.Phytochemical testing.
4. In vitro antiurolithiatic evaluation
5. Result and discussion.
6. Conclusion.

Literature review :-

1. Mr. Ravindra D. Hiremath
Urolithiasis is reported at a ratio of 1:20 persons at one or the other time throughout their life span. newlineAim and Objectives: The study undertaken was to develop the fingerprints of hydro-alcoholic extracts of Vernonia cinerea L. and Elephantopus scaber L. Further, an attempt to provide a scientific base to validate the claims made and use of Vernonia cinerea L. and Elephantopus scaber L. in management and treatment of kidney stones followed by evaluation of anti-oxidant and antimicrobial potential.

1. Material:-
Collect leaves and check the activity of antiurolitiatic. newlineMethodology: The plants namely Vernonia cinerea Less and Elephantopus scaber Linn were collected from surrounding areas of Belagavi and authenticated. 1-2

2. Binu T V
Isolation and Characterization of an Antiurolithiatic Compound from Strychnospotatorum L f Human race is constantly being challenged by many dreadful diseases and it is an uphill task to combat them in the present scenario. With the onset of the synthetic era, pharmaceutical industries are producing a lot of synthetic drugs, that help to alleviate the chronic diseases. With the passage of time, many problems associated with frequent use of synthetic drugs become prominent, like severe side effects and resistance of microbes against these drugs.

3. Y.Mohan Reddy
Studies In Vitro Propogation And Molecular Characterization of Gardenia Latifolia Ait

4. Ravi Kant
A Study to Evaluate the Efficacy Safety and Tolerability of New Formulation of Dolichos biflorus Seeds in Urolithiasis The aim of the present study was to assess the safety, tolerability and efficacy of dietary Dolichos biflorus seeds as a functional beverage and as natural dietary supplements for prevention and/or management of urolithiasis and explore the underlying pharmacological mechanisms responsible for this activity.

5. Ahire, Sunita D
Exploring Potential Of Selected Medicinal Plants For Treatment Of Urolithiasis Herbal drugs are used by peoples for its clinically proven effects like immunomodulation, adaptogenic and antimutagenic. newlineDiet with low amounts of proteins and high intake of animal proteins might cause the risk of stone formation. Urolithiasis is very common in Northern India compared to

- **Material And Methods:-**

southern India. 6-7 Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while.10-11

Fig No. 1

2. Dried Form:-

Fig No. 3

3. Powder Form:-

Fig No. 4

- **Methods:-**

1. Soxhlet extraction
2. Solvent extraction
3. Steam distillation
4. Supercritical cor extraction
5. Wtrasonic – assisted extraction
6. Aqueous (boiling) extraction
7. Gas ms
8. Macerution
9. Soxhlet extraction

- **Leaf extraction:-**
- **Material requirement:-**

1. Dried leaves of Gardenia jasminoides
2. Soxhlet apparatus
3. Round-bottom flask
4. Condenser
5. Heating mantle
6. Whatman filter paper or thimble
7. Solvent (ethanol, methanol, hydroalcohol, or petroleum ether)

8. Rotary evaporator or water bath
9. Analytical balance
10. Grinder12-13

- **Procedure:-**

Collection of Plant Material
↓
Washing with Distilled Water
↓
Shade Drying (5–10 days)
↓
Grinding into Coarse Powder
↓
Weighing of Powdered Sample
↓
Loading Powder into Thimble
↓
Placement of Thimble in Soxhlet Apparatus
↓
Addition of Solvent(Ethanol/Methanol/Hydroalcoholic Solvent)
↓
Heating and Reflux
↓
Continuous Soxhlet Extraction(6–24 hours)
↓
Solvent Siphoning Repeated Cycles
↓
Extraction Completion(Colorless Solvent in Siphon Tube)
↓
Collection of Extract Solution
↓
Filtration (if required)
↓
Solvent Evaporation(Rotary Evaporator/Water Bath)
↓
Obtaining Crude Extract
↓
Drying of Extract
↓
Storage at 4°C in Airtight Container
↓
Further Phytochemical Analysis

Fig No. 5

Fig No. 6

Fig No. 7

Fig No. 8

Fig No. 9

2. Distillation method

- Leaf extraction for oil:-
- Materials Required

1. Fresh or shade-dried leaves of Gardenia jasminoides
2. Distilled water
3. Clevenger apparatus
4. Round-bottom flask
5. Heating mantle
6. Condenser
7. Separating funnel
8. Anhydrous sodium sulfate
9. Beaker
10. Measuring cylinder

- **Procedure:-**

Collection of Fresh Leaves
↓
Washing
↓
Shade Drying
↓
Pulverization (Coarse Powder)
↓
Weighing Plant Material
↓
Addition of Distilled Water
↓
Loading into Round-Bottom Flask
↓
Assembly of Clevenger Apparatus
↓
Heating/Boiling
↓
Steam Carrying Volatile Oil
↓
Condensation
↓
Collection of Oil + Water Distillate
↓
Separation of Essential Oil
↓
Drying with Anhydrous Sodium Sulfate
↓
Filtration
↓
Measurement of Oil Yield
↓
Storage in Amber-Colored Bottles
↓
Essential Oil for Pharmacological Studies14-15

4. Phytochemical Test:-

- Detection of Glycosides :-Table No. 1

Test	Procedure	Observation
Keller Killani Test	Take plant extract in a test tube + add 2 ml glacial acetic acid containing a drop of ferric chloride	Formation of brown ring at the junction indicates presence of cardiac glycosides.
Bromine water Test	Plant Extract + few ml of bromin water	Yellow colour is form

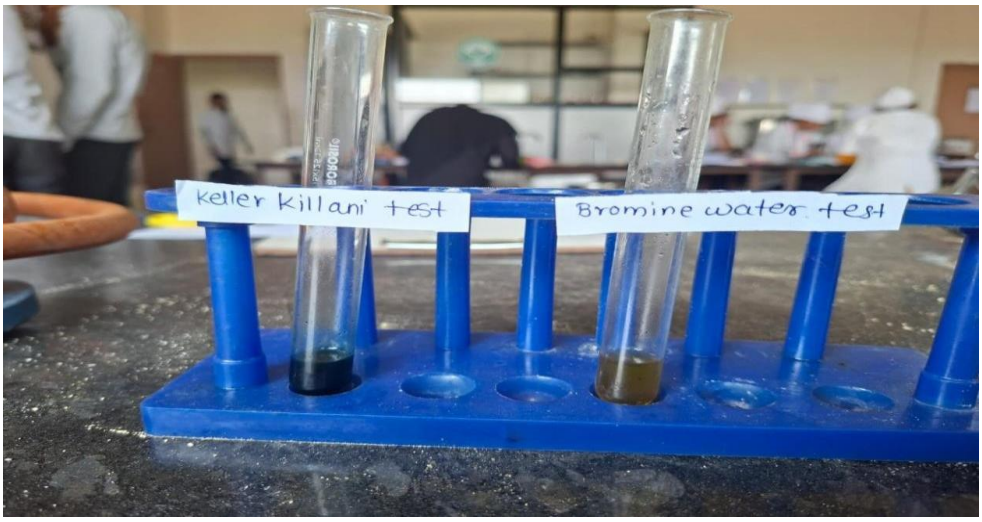


Fig No. 10

Test for reducing sugar:-Table No. 2

Test	Procedure	Observation (Indicating Positive Test)	Results
Benedict's test	0.5mL filtrated + 0.5mL Benedict's reagent + Boiled for 2 min.	Green/Yellow/red colour	Present
Fehling's test	1mL each of Fehling's solution A & B + 1mL filtrated + boiled in water bath	A red precipitate	Present

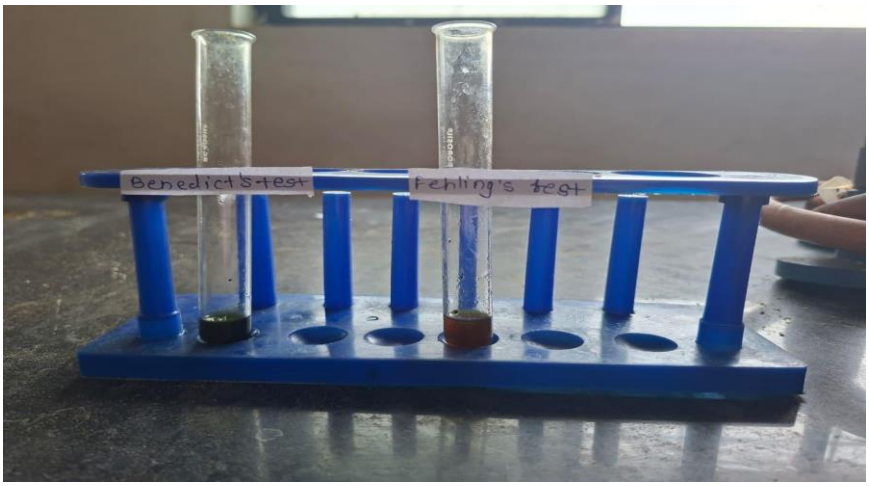
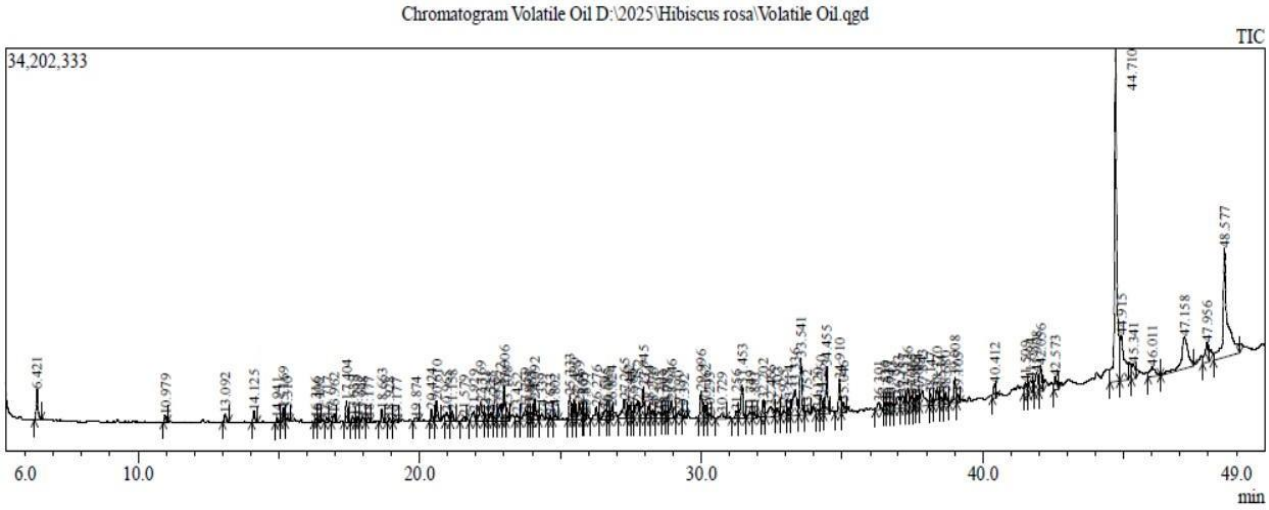
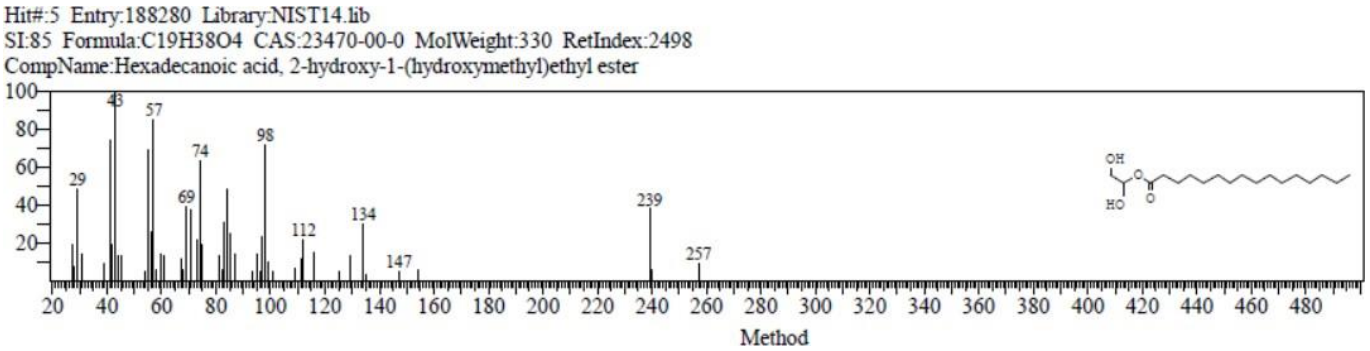


Fig No. 11

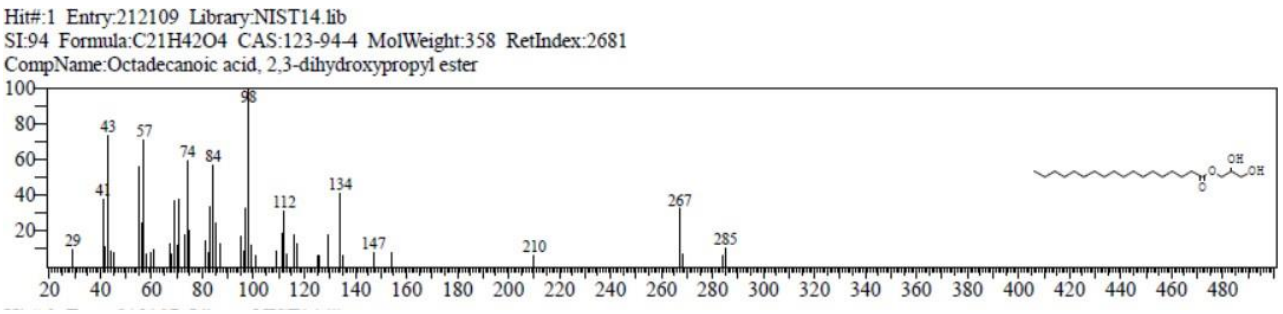
5. Qualitative Analysis:-
GC/MS(Gas Chromatography & Mass Spectrometry



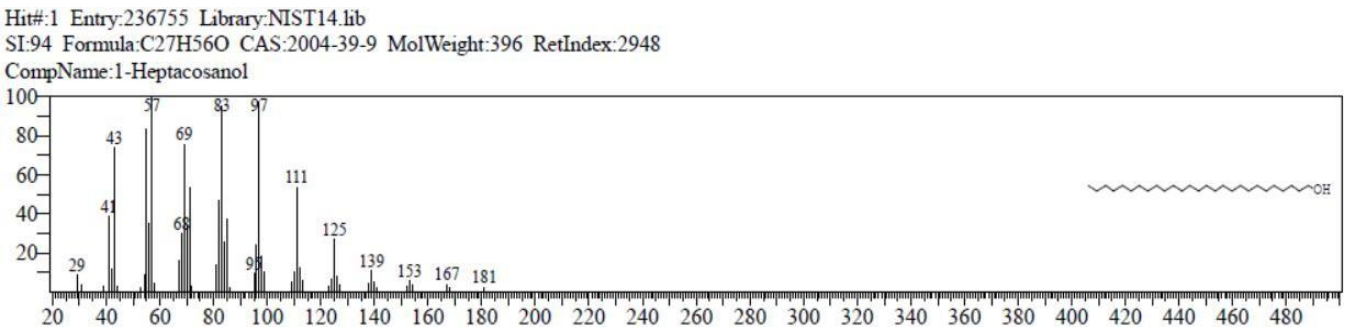
1. Compound Name:Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester



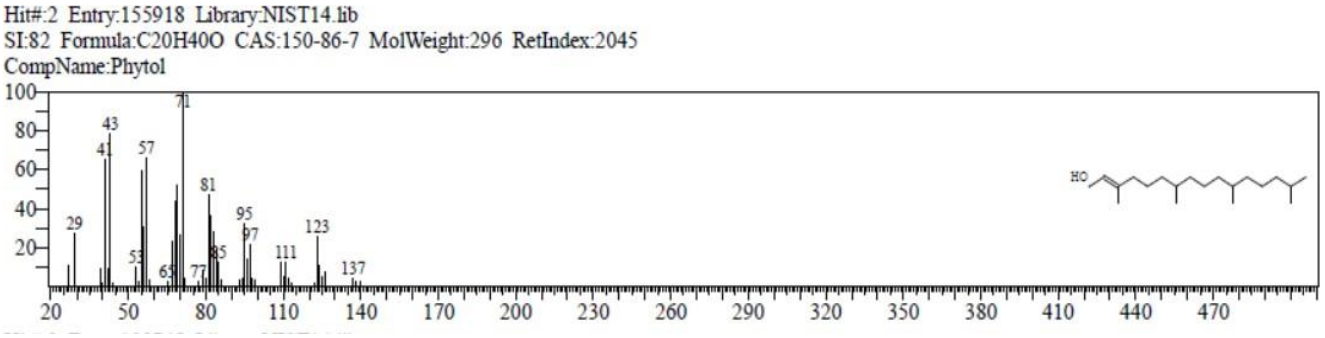
2. Compound Name:Octadecanoic acid,2,3-dithydroxypropyl ester



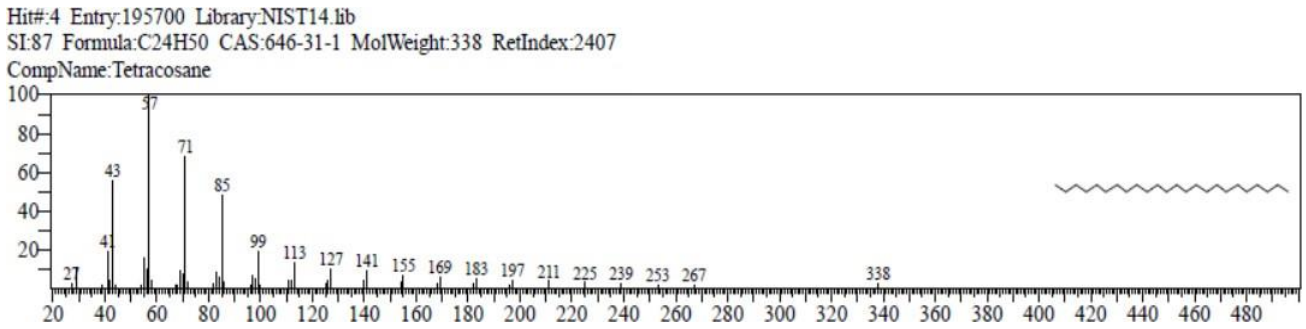
3. Heptacosanol



4. Phytol



5. Tetracosane



Activity Check By In Vitro Study:-

Anti-urolithiatic activity:-
Sample Description:- extraction of Gardenia jasminoides.

sss
Activity:- Anti-urolithiatic activity by nucleation assay and crystal aggregation assay

1. Nucleation Assay

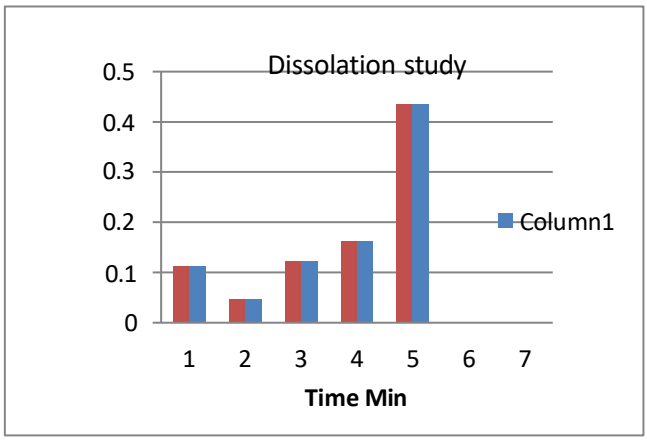
Mix 1.0 ml of calcium chloride (cacl2) and 2.0 ml Tris buffer (PH 7.4) in test tube
↓
Add 1.0 ml of sodium oxalate(Na2C2O4)
↓
Immediately followed by the essential oil at varying concentration (ex-100-1000 micro gram per ml)
↓
measurement
↓
Uv visible spectrophotometer at 620 nm over 10 min

Observation table:-

Table No. 3

Concentration (micro gram per ml)	Wavelengt h(620 nm)
0.1	0.113
0.2	0.047
0.3	0.122
0.4	0.163
0.5	0.436

2. Crystal Aggregation assay:-
The rate of aggregation of the CaOx crystals was determined by the method of Atmani and Khan [20] with slight modifications. The COM crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate at 50 mmol/L. The solutions were equilibrated to 60° C in water bath, cooled to 37ºC and kept overnight. Percentage inhibition of aggregation rate was then calculated by comparing the turbidity slope of different concentrations of cystone / Extract with the turbidity slope of the control by the following formula.



[1-(Tsi/ Tsc)] × 100

Where Tsi was the turbidity slope of aggregation in the presence of inhibitor sample i.e, cystone/ plant extract and **Tsc**

Table no. 4: In Vitro Anti-urolithiatic Activity of Standard Drug Cystone at Different Concentrations and Time Intervals

Time	Concentration (µg/mL)	O.D	% Inhibition
Control	—	0.22	—
5 min	250	0.11	50.00
	500	0.11	50.00
	1000	0.12	45.45
10 min	250	0.10	54.54
	500	0.09	59.09
	1000	0.07	68.18
15 min	250	0.07	68.18
	500	0.08	63.63
	1000	0.07	68.18
20 min	250	0.07	68.18
	500	0.07	68.18
	1000	0.06	72.72
25 min	250	0.06	72.72
	500	0.05	77.27
	1000	0.04	81.81

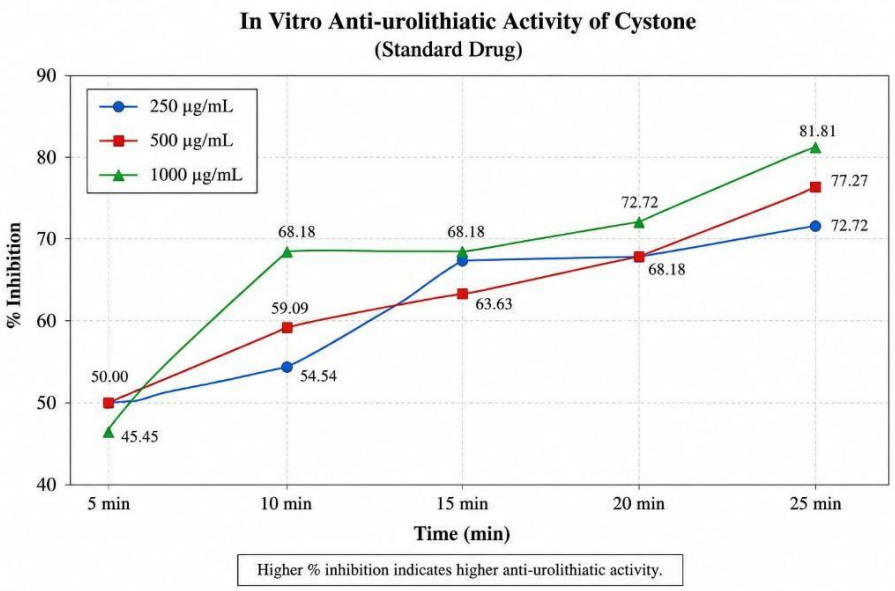
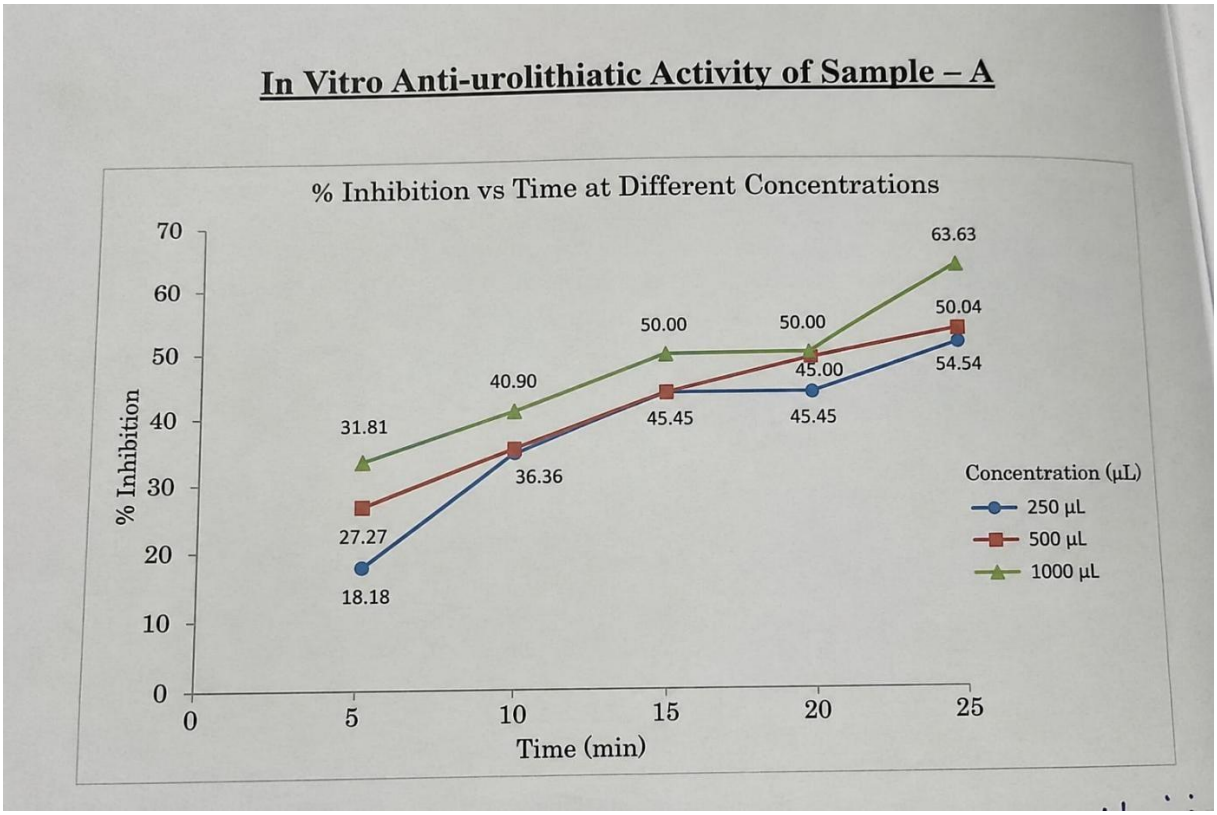


Table no. 5: In Vitro Anti-urolithiatic Activity of Sample–A at Different Concentrations and Time Intervals

Time	Concentration (µL)	O.D	% Inhibition
Control	—	0.22	—
5 min	250	0.18	18.18
	500	0.16	27.27
	1000	0.15	31.81
10 min	250	0.14	36.36
	500	0.14	36.36
	1000	0.13	40.90
15 min	250	0.12	45.45
	500	0.12	45.45
	1000	0.11	50.00
20 min	250	0.12	45.45
	500	0.11	50.00
	1000	0.11	50.00
25 min	250	0.10	54.54
	500	0.10	54.54
	1000	0.08	63.63



Observation:-
Standard:- 100 micro gram per unit

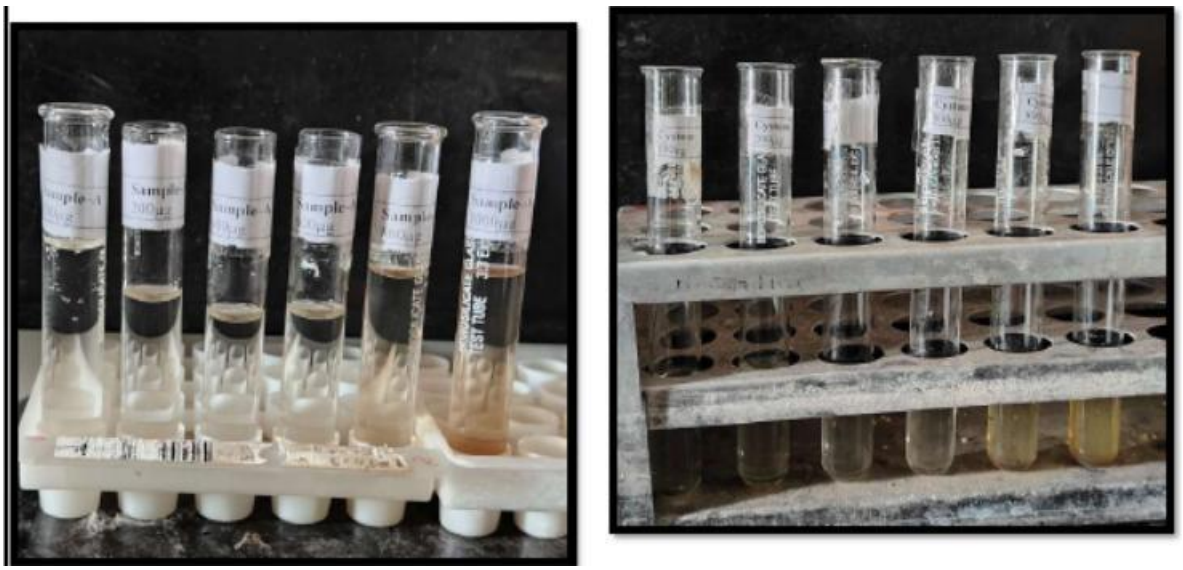


Fig No. 12

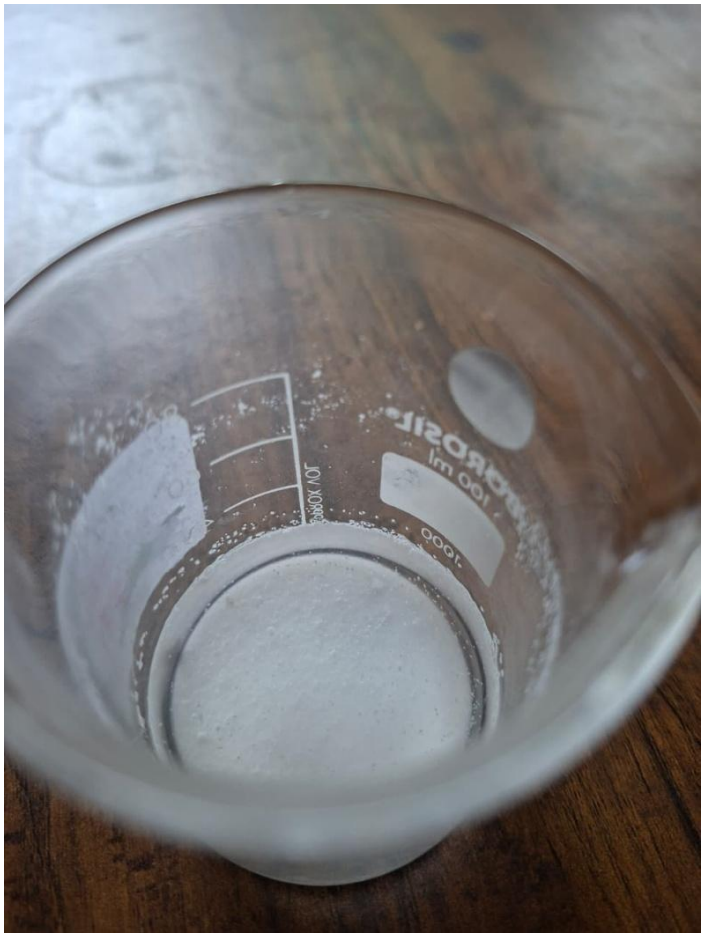


Fig No. 13

Result:-

1) Collection:-

The Whole plant Gardenia jasminoideas was collected in month of March from 2026 from sangli, India.

Crystal forms

2) Authentication:-

After collection the plant was identified, conformed and authenticated by Mr. S. M. Sabale Head of department of botany PVP college Kavthemahankal, sangli.

PADMABHUSHAN VASANTRAODADA PATIL MAHAVIDYALAYA
KAVATHE MAHANKAL, Dist. Sangli (Maharashtra) Pin- 416 405
Acting Principal (Dr.) G. D. Kore M.Sc., Ph.D. Mob.8668449680
NAAC 4thCycle accredited "B++" with CGPA 2.81
Jr. College Index No. J22.04.002 AISHE CODE: C-11026
Phone-02341-295220 Email: kmpvp@rediffmail.com Website:www.pvpkm.ac.in
kvt95.cl@unishivaji.ac.in

Date: 23/04/2026

Authentication Certificate

This is to certify that, the plant specimen submitted by **Dnyaneshwari Rajaram Manave** of Nootan Pharmacy College, Landagewadi has been authenticated and identified as follows:

Specimen:

- Scientific/ Botanical Name: ***Gardenia jasminoides*** J.Ellis
- Family: Rubiaceae
- Common Name: Gandhraj
- Collection Location: Kavathe Mahankal

The authentication has been carried out based on morphological characteristics of specimen and comparison with existing literature and Botanical Flora's.

Authenticated by,
Mr. S. M. Sabale

Acting Principal
Padmabhushan Vasantraodada Patil Mahavidyalaya,K.Mahankal,Dist-Sangli.

3) Extraction of Plant Material:-

Ethanol was selected because of its ability to extract a wide range of phytoconstituents including flavonoids, alkaloids, tannins, and phenolic compounds . The extraction process yielded a dark green semi-solid crude

extract with characteristic odor.The percentage yield of efficient extraction of bioactive constituents from the plant material.

4) Phytochemical Screening:-

Preliminary phytochemical analysis of ethanolic leaf extract showed the presence of various ethanolic extract indicated secondary metabolites such as alkaloids,Cardiac glycoside, reducing sugar and terpenoids this compounds are known to possess Anti-urolithiatic activity.

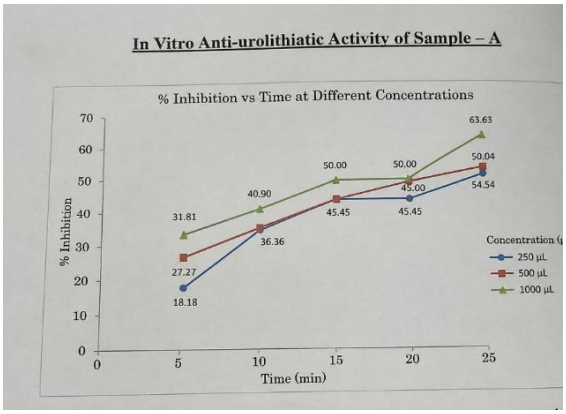
Table: Preliminary Phytochemical Screening

Table no. 6

Phytochemical	Result
Alkaloids	Trace amount
Cardiac glycoside	Present
Reducing sugar	Present

5) Anti-urolithiatic activity.

The anti-urolithiatic activity of ethanolic leaf extract of Gardenia jasminoides was evaluated by nucleation assay and crystal aggregation assay



Discussion:-

The present study evaluated the in vitro anti-urolithiatic activity of Sample–A in comparison with the standard drug Cystone at different concentrations and incubation time intervals.

Sample–A also demonstrated promising anti-urolithiatic activity, although the activity was lower than that of the standard drug. At 5 minutes, Sample–A showed 18.18–31.81% inhibition, which progressively increased with increasing incubation time. The highest inhibition observed for Sample–A was 63.63% at 1000 µL after 25 minutes. The gradual increase in inhibition suggests that the sample possesses active phytoconstituents capable of interfering with crystal nucleation, aggregation, or growth.

Phytochemicals such as flavonoids, phenolics, saponins, and terpenoids are known to contribute to anti-urolithiatic activity through antioxidant and crystal inhibitory mechanisms.

Conclusion :-

The present investigation demonstrated that Sample–A possesses significant in vitro anti-urolithiatic activity. However, the standard drug Cystone exhibited superior inhibitory activity compared to Sample–A.

Among all tested concentrations, Sample–A showed maximum inhibition of 63.63% at 1000 µL after 25 minutes, while Cystone showed 81.81% inhibition under similar conditions. These findings suggest that Sample–A contains bioactive constituents capable of preventing crystal formation and aggregation, thereby exhibiting potential anti-urolithiatic properties.

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