Assessment of In-Vitro Antiurolithiatic Potential of Methanolic Extract of Origanum Majorana by Titrimetric Method

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Abstract

Kidney stones, also called urolithiasis. These stones are usually formed from calcium oxalate crystals that build up in the urinary tract. This study focused on a plant called marjoram (Origanum majorana), which has long been recognized for its therapeutic properties like antioxidant, anti-inflammatory, and diuretic effects. The dried leaves of the plant were collected and processed using a method called Soxhlet extraction with methanol to obtain a concentrated extract. The obtained concentrate was subsequently evaluated to find out what types of natural compounds it contains. The results showed the presence of useful substances such as flavonoids, phenols, tannins, saponins, and terpenoids. To check its effectiveness, the extract acquired formulation was later examined.study how kidney stones form and grow. One test looked at how the extract prevents the initial formation of crystals, while another test checked whether it can stop already formed crystals from sticking together and becoming larger.

This suggests that the leaf has strong potential in preventing kidney stones. The beneficial effect is likely because of the synergistic effect of its natural compounds, especially flavonoids and phenols, which can bind to calcium, reduce crystal formation, and stop their growth. Its free radical scavenging abilities could additionally help by reducing stress in the body that contributes to stone formation

Key words: Antiurolithic activity, Origanum majorana,In-Vitro study, Kidney Stone,

Introduction

A summary of Kidney Stones:

Kidney stones are hard, small lumps that form inside the kidneys from minerals and salts. They may likewise occur in various regions of the urinary tract, including the ureters, urinary bladder, and urethral canal. These calculi develop when compounds like calcium and uric acid become excessively saturated in the urine and begin crystallizing. Over time, these crystals join together and grow into stones [1].

Kidney stones usually form due to reasons like drinking less water, unhealthy eating habits, certain health conditions, or family history. When the organism lacks sufficient hydration, urine becomes concentrated, which makes it easier for stones to form.Some stones stay in the kidneys without causing problems, while Others pass along the renal passageways and can cause pain. Common symptoms include strong Discomfort in the lower back or side, pain while passing urine, blood in urine, feeling of nausea, and frequent urge to urinate. Sometimes, stones can also lead to infections or other complications. Kidney stones are known to be very painful and can come back again if proper care is not taken [2].

In simple terms, kidney stones are a common health issue, but they can be prevented by drinking plenty of water, eating a healthy diet, and receiving appropriate healthcare treatment whenever required [3].

Epidemiology of kidney stone:

Renal calculi are increasingly prevalent and represent an escalating medical concern around the world. They arise more frequently in certain regions compared to others owing to differences in weather, food habits, lifestyle, and living conditions. People living in hot regions are at higher risk because they lose more water from the body, which can lead to dehydration [4].

About 1 in ten to one in 7 people may develop kidney stones at a particular stage of their lifetime. Once a person gets a stone, there is a significant possibility of recurrence within several years. Renal calculi are predominantly observed among grown individuals between 20 and 50 years of age [5]. Males are generally impacted more frequently than females, but nowadays, cases in women are also increasing due to changes in lifestyle and diet.

Food and drinking habits play a very important role.consuming excessive sodium-rich meals, animal protein, and foods high in oxalate, along with drinking less water, increases the risk of stone formation. Certain body conditions like high calcium, oxalate, or uric acid levels in the urine also contribute to stone formation.

Modern lifestyle factors such as lack of physical activity, obesity, diabetes, and hypertension further elevate the risk of getting kidney stones. In some cases, it can run in families, meaning genetics also play a role [6].

Kidney stones and urinary system:

The urinary system helps keep our body clean and balanced by removing waste and extra fluid as urinary excretion. It consists of four primary components: kidneys, ureters, urinary bladder, and urethra. All these structures function collectively smooth to perform this important function.

The kidneys are the main organs that clean the blood. They remove waste substances, toxins, and extra water from the body. Inside the kidneys, there are tiny units called nephrons where this cleaning process happens [7].Here, useful things like water, sugar, and some salts are taken back into the body, while harmful waste is removed.

The urine formed in the kidneys then travels through two thin tubes called ureters and reaches the urinary bladder. The bladder stores urine for some time. When it becomes full, urine is passed out within the organism through the urethra. In this way, the renal system assists in regulating adequate liquid balance, salts, and body pH.

Renal stones develop when specific compounds present in the urine become excessively concentrated and begin producing tiny crystalline particles. These constituents comprise calcium, oxalate, and uric acid, and phosphate. Normally, urine has some natural chemicals that stop crystals from forming, but when these are low, stones can develop.

These stones may remain within the renal organs or migrate into the urinary passage. As they travel through the ureters, they may induce intense agony and uneasiness. If a calculus obstructs the passage of urine, it may result in complications such as infection or renal impairment.

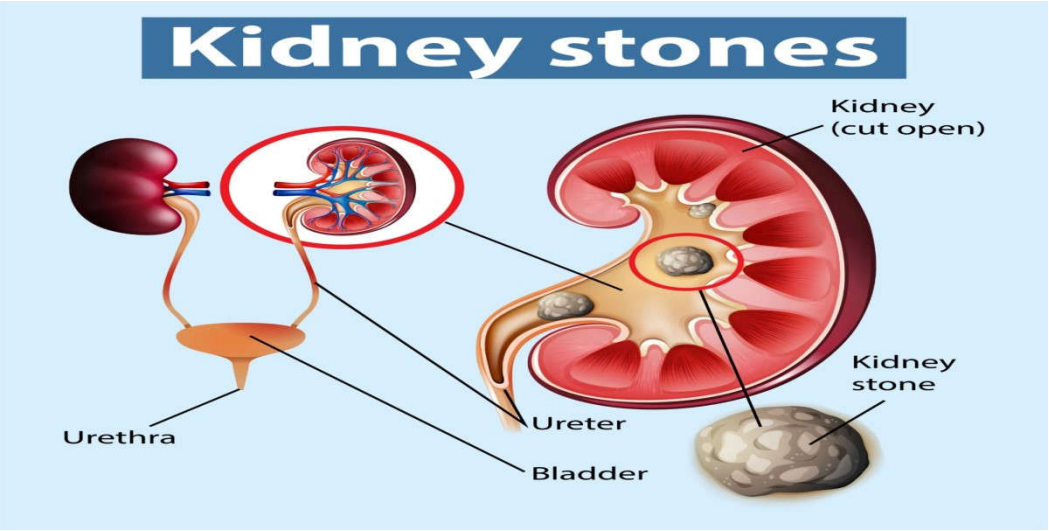

Figure 1: *kidney stones*

Kidney stones and Type:

Kidney stones are hard, stone-like substances that form in the kidneys when minerals and salts build up in the urine. These stones are not all the same; they are classified into various categories based on what they are made of a) Calcium stone

b) Urate calculus

c) Struvite stones

d) Cystine stone [8].

Nephrolithiasis represent the most prevalent form of renal stones. They are generally composed of calcium mixed with substances like oxalate or phosphate. These stones develop when excessive amounts of calcium or oxalate are present in the urinary fluid. Inadequate water consumption, excessive intake of sodium-rich foods, and certain medical conditions may elevate the likelihood of developing these calculi.

Uric acid stones develop when the urinary fluid becomes too acidic. This can happen in the event that an individual eats a lot of protein-rich foods like meat and fish or has conditions like gout.
Struvite stones develop as a result of infections within the urinary system caused by certain bacteria. These stones can grow very quickly and become quite large. They can cause serious problems if not treated on time [9].

Cystine stones are rare and occur due to a genetic problem where too much cystine is passed into the urine. This leads to stone formation, and these stones can come back again and again in affected people.

Figure 2: *Category of renal stone*

Process kidney stones works:
Renal stones develop when minerals and crystalline salts present in the urinary fluid become excessively saturated. Whenever the body lacks an adequate quantity of water, these compounds begin generating minute crystals. Gradually, these crystalline particles combine with one another and progressively transform into solid masses within the kidneys.These stones may remain inside the renal organs or migrate into the urinary passageways. Once a stone reaches the ureter, it may obstruct the movement of urine and trigger intense discomfort. This

pain may appear intermittently as the stone advances toward the urinary bladder.Smaller stones can be eliminated from the body through urinary excretion without difficulty, whereas larger stones may become lodged and require medical intervention. In certain situations, renal stones may additionally produce discomfort during urination or even result in the presence of blood within the urine [10].

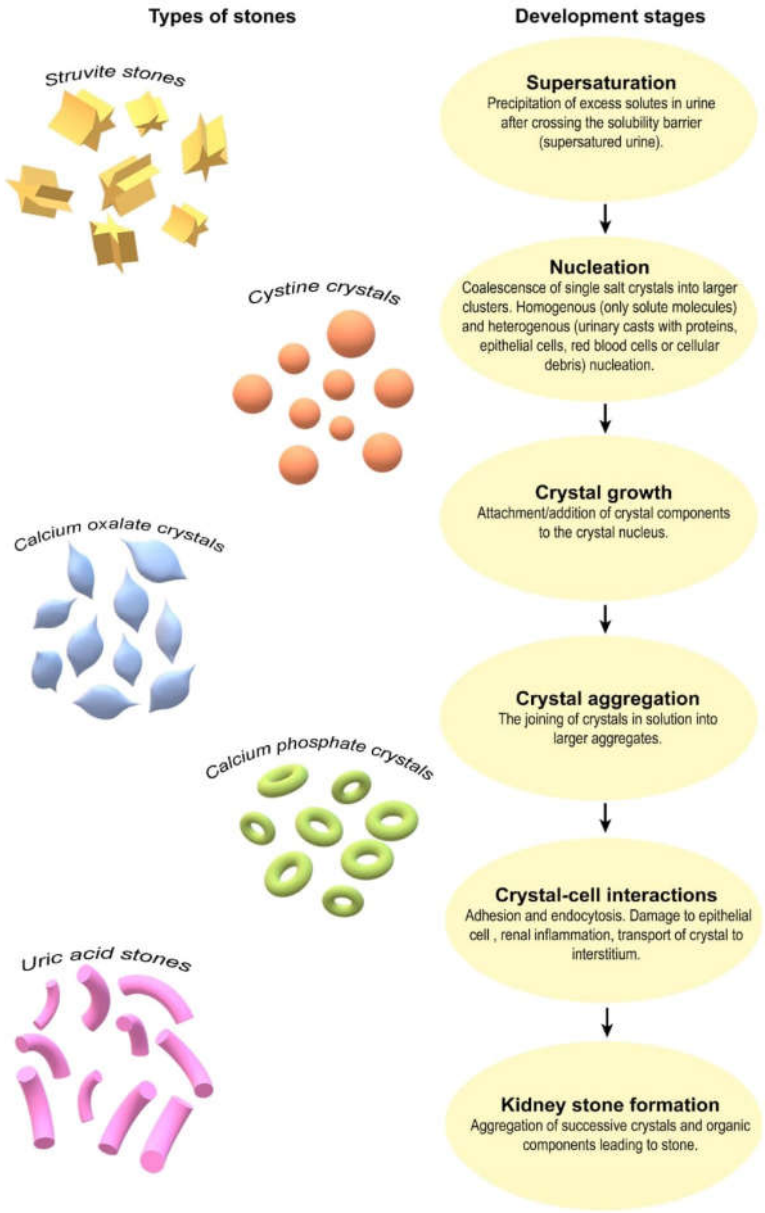

Figure 3: *way of kidney stones work*

MATERIAL AND METHODOLOGY

Plant Collection and Authentication:

Sangola is located in the Solapur District of the Indian State of Maharashtra, where Origanum Majorana leaves were gathered in March 2026.The division of botanical science and plant physiology's Dr. Pawar.R.G. Attested to the plant's validity. The collected crude drug was washed and then allowed to dry in the shade [11].

Figure 4: *Origanum Majorana* **Introduction:**

Origanum majorana, commonly called sweet marjoram, is a well-known medicinal and aromatic plant. It belongs to the mint family, Lamiaceae. This plant is mainly grown in Mediterranean countries, North Africa, and as well as a regionof asian .It is likewise grown in india because of its health benefits and use in cooking. Sweet marjoram has a pleasant smell and is known for its healing properties [12]. From a botanical point of view, it is a perennial herb, due to often grown as an annual plant. It usually grows about 30 to 60 cm tall. The stem is soft, covered with fine hairs, and has many branches. The leaves are small, oval in shape, and grayish-green in color. These leaves contain tiny oil glands that produce essential oil. The flowers are small and can be white or light pink, growing in clusters. The plant contains many important chemical compounds. These include essential oils, flavonoids, and phenolic compounds. It also has tannins and terpenoids. Important active components like thymol and carvacrol are present in it. These substances are responsible for its medicinal effects [13]. Traditionally,It is beneficial for digestion issues like indigestion and gas. It can relieve cough, asthma, and bronchitis. The plant also helps reduce inflammation and muscle spasms. It is sometimes used to treat headaches and reduce anxiety. It has strong antioxidant properties. It also works well against bacteria and fungi. The plant may help in managing diseases like diabetes and heart problems. Its essential oil is used in medicines, cosmetics, and perfumes.

Scientific Classification

Kingdom: Plantae
Division: Angiosperms
Subdivision: Eudicots
Class: Magnoliopsida
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae
Subfamily: Nepetoideae

Genus: Origanum
Species: Origanum majorana

Synonyms of plant:

English: Sweet Marjoram, Knotted Marjoram
Hindi: Marua, Marwa
Sanskrit: Marubaka, Maruvaka
Marathi: Marva, Marua
Bengali: Marua
Urdu: Marwa, Marzanjosh[14].

Morphological characteristic:

1. Root:
The roots are fibrous and spread widely in the soil. They are shallow and help the plant quickly absorb water and nutrients.
2. Stem:
The stem is soft, green, and has many branches. It is slightly square in structure and covered with fine hairs.
3. Leaves:
The leaves are small, simple, and grow opposite to each other. They are oval in shape with smooth edges and a grayish-green color. The surface has tiny oil glands that give the plant its smell. **4. Flowers:**
The flowers are small and grow in clusters. They are usually white or light pink in color and give the plant a compact look.
5. Calyx:
The calyx is tube-shaped and has five small teeth. It stays attached even after the fruit is formed.
6. Corolla:
The petals are joined together and form two lips. The upper part has two small lobes, and the lower part has three lobes.
7. Androecium:
There are four stamens in the flower. Two are longer and two are shorter.
8. Gynoecium:
The pistillate section of the blossom possesses an upper-positioned ovary. It is divided into four sectionand has a single style arising from the base.
9. Fruit:
The fruit splits into four small parts when it becomes mature [15].

Parts used:
Root, Leaves, Flower, seeds, Steam.

Propagation:

1. Propagation by Seeds:

Growing Origanum majorana from seeds is for large-scale farming.The kernel are small and light, to ensure better growth, they are cultivated in seedbed nursery rather than in the field.They are usually planted during a suitable season. For even spreading, the grains are blended with fine soil and then covered with soil. They start to germinate within 7 to 14 days if moisture and temperature are suitable [16].

2. Propagation by Stem Cuttings:

This method is used to get plants that are exactly like the parent plant. Healthy stems of about 8–10 cm are selected, and the lower leaves are removed. These cuttings are planted in moist soil or sand. Roots usually develop within 2 to 3 weeks. This method gives uniform plants with the same quality and oil content.

3. Propagation by Division:

This is a simple method used for small-scale cultivation. Fully grown plants are carefully uprooted. These parts are then replanted in soil, where they grow into new plants quickly [17].

4. Environmental Conditions:

The plant grows well in a warm and slightly humid climate. It prefers well-drained and fertile soil, especially sandy soil. Regular watering is needed, excessive fluid intake is avoided.
Good sunlight is important for proper growth and oil production.

5. Care during Propagation:

Proper attention is essential during the early stages of growth. The ground must remain damp but not waterlogged. Seedlings should be protected from pests and diseases. Weed needs to be eliminated regularly, and light fertilizers may be utilized to aid and reinforce support healthy growth [18].

Uses:

Origanum majorana, also called sweet marjoram, is a useful plant with many benefits in health, food, and industry. It has been traditionally used to treat breathing problems like cough, asthma, and bronchitis. It also helps in digestion by reducing problems like indigestion, gas, and stomach pain. The plant possesses characteristics that assist in decreasing inflammation, fight germs, and protect the body from damage. In addition, it could assist in regulating glucose level in the bloodstream and support heart health. In cooking, it is widely used to add flavor to soups, sauces, and meat dishes due to its pleasant smell.The oil extracted from the herb is utilized in medicines, cosmetics, and perfumes. its healing and aromatic properties. Overall, sweet marjoram is a valuable plant with many practical uses [19].

PREPARATION OF PLANT EXTRACT ORIGANUM MAJORANA :

The newly harvested foliage was washed thoroughly beneath flowing water, ground into a coarse powder in a machine grinder.

Preparation of methanolic Extract:

Research on medicinal plants is being done by many colleges and institutes in India.

First, collect the plant material and dry it in the shade. Once it is completely dry, grind it into a coarse powder using a grinder. Take about 20 grams of this powder.

Next, choose a suitable solvent like ethanol or methanol, depending on what compounds you want to extract. Place the powder into a thimble made of filter paper or cellulose, and keep it inside the Soxhlet apparatus. Then add around 250 tmL of the solvent into a round-bottom flask attached to the setup.

Heat the flask using a heating mantle so the solvent starts to boil. The vapors rise, cool down in the condenser, and fall back onto the plant powder. This process helps pull out the phytochemicals from the plant. Once the chamber fills up, the liquid flows back into the flask. This cycle keeps repeating.Continue the process for about 6 to 8 hours, or until the solvent

becomes clear, which means most of the compounds have been extracted. After that, remove the solvent using a water bath or a rotary evaporator under reduced pressure. What remains is a thick, semi-solid plant extract [20].

Figure 5: *Extraction process*

PHYTOCHEMICAL INVESTIGATION:

Using the following chemicals analyses,Preliminary phyto-constituents where identified in the Origanum majorana of methanol extract.

Sr. No.	Name of The Test	Observation	Inference
1.	**Test for Saponins:** A test tube containing the extract 2 mg was filled with 10 ml water and shake vigorously	Persistent Foam	Saponins Present
2.	**Test for Phenols:** Mixture of extract and 2 ml of 2% $FeCl_3$	Deep blue Colour	Phenols Present

3.	**Test for Tannins:** Mixture of extract and 2 ml of 2% $Fecl_3$ solution.	Greenish-black colour + White precipitate	Tannins Present
4.	**Test for Terpenoids:** Chloroform (2ml) was combined with the extract. After that, 2 ml of conc. H_2SO_4 was add and gently shaken.	Reddish- brown ring at interface	Terpenoids Present
5.	**Test for Flavonoids:** A few drops of sodium hydroxide solution were added to the extract.	Turns colourless on adding acid	Flavoinoids Present
6.	**Test for Carbohydrates:** 2 ml glacial acetic acid with a few drops of $Fecl_3$ were combined with the extract and then transferred into a second tube that that had two milliliters of concentrated sulfuric acid.	Violet/ Purple ring at interface.	Carbohydrates present
7.	**Test for Proteins:** **A] Biuret Test:** Take Two mililitreof the sample solution in a test tube, add 1 ml of sodium hydroxide (NaOH) solution, and mix well. Then add 2–3 drops of dropwise and shake gently.$_4$ copper sulfate (CuSO) solution	Violet/ Purple colour	Proteins Present
8.	**Test for Alkaloids:** **A] Dragendroff's Test:** One or Two ml of Dragendroff's reactant $KBiI_4$ sol^n was added to a few ml of extract.	No Orange or reddish-brown precipitate.	Dragendroff's Test Absent
9.	**B] Mayer's Test:** Two drops of Mayer's reagent (potassium mercuric iodide solution) were added to a few milliliters of extract.	No Cream or pale yellow color precipitate.	Mayer's Test Absent

10.	**C]Hager's Test:** One or two milliliters of Hager's reagent (a saturated solution of picric acid) were added to a few milliliters of extract.	No yellow Precipitate	Hager's Test Absent
11.	**D] Wagner's Test:** To a few ml of the extract, few drops of Wagner's reagent (iodine in potassium iodide) were added.	No reddish Precipitate	Wagner's Test Absent

Table 1: *Phytochemical components of the Extract Origanum Majorana*[21].

Figure 6: *Chemical Test for Origanum Majorana*

EXPERIMENTAL WORK:

Apparatus:

Beaker, Burette, conical flask, burette stand, volumetric flask, measuring cylinder.

Chemicals Used:

Calcium chloride, Sodium oxalate, Sulfuric acid, Potassium permanganate, Ammonia solution, Distilled water, Test sample / Plant extract, Egg membrane, Hydrochlorothiazide.

Preparation of semi-permeable membrane using eggs:

First, a small hole was made at the top of the egg. The outer shell is carefully removed without damaging the inner membrane. A small opening is made to remove the egg contents, and the membrane is washed thoroughly with distilled water.

After cleaning, the empty membrane is filled with the test sample or plant extract. The opening is then tied properly, and the membrane is placed in distilled water or a buffer solution for incubation.

Once the incubation period is complete, the contents are taken out, and the remaining calcium oxalate is measured by titration. This helps in determining the antiurolithiatic activity of the sample [22].

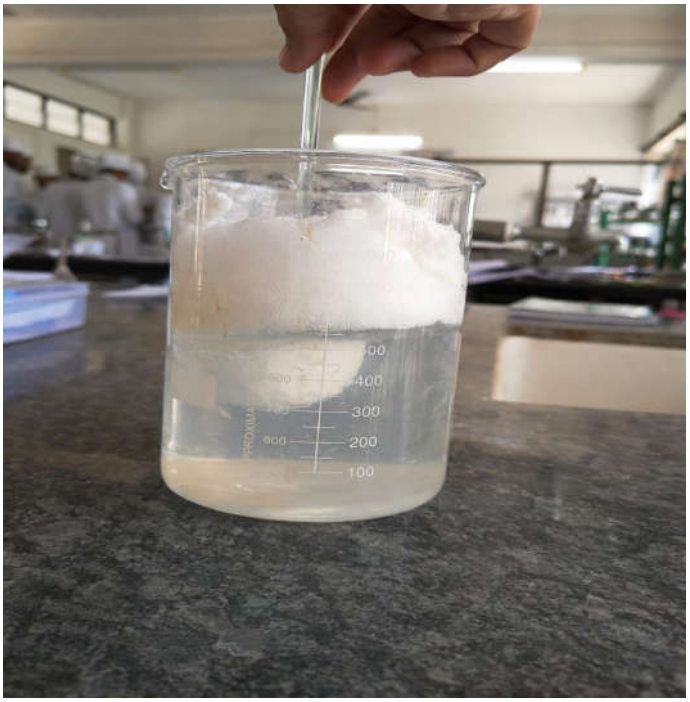

Figure 7: *Decalcification of egg shell*

EXAMINING THE IN VITRO ANTI-UROLITHIC ACTIVITY TEST USING TITRATION:

In vitro antiurolithiatic study using the titrimetric method, $CaC_2O_4.2H_2O$ are first prepared by mixing equal volumes (50 mL each) of 0.1 M calcium chloride and 0.1 M sodium oxalate solutions. The precipitate formed is collected and washed well with distilled water.A fresh hen's egg is then used to obtain the inner membrane by carefully removing the hard outer shell without damaging it. This membrane is filled with about 10 mg of $CaC_2O_4.2H_2O$ with one hundred mg of the test sample or plant extract, and then tied securely with thread.The prepared membrane is positioned in a beaker containing One hundred mL a suitable buffer solution and Conditioned at 37°C for about 2 hours. After incubation, the contents are transferred into a

conical flask, and 10 mL of 1 N sulfuric acid is added to dissolve the remaining calcium oxalate.The solution afterward,it was titrated using $KMnO_4$ until a light pink colour appears and persists for a few seconds. The quantity of calcium oxalate dissolved is used to analyse the antiurolithiatic activity of sample [23].

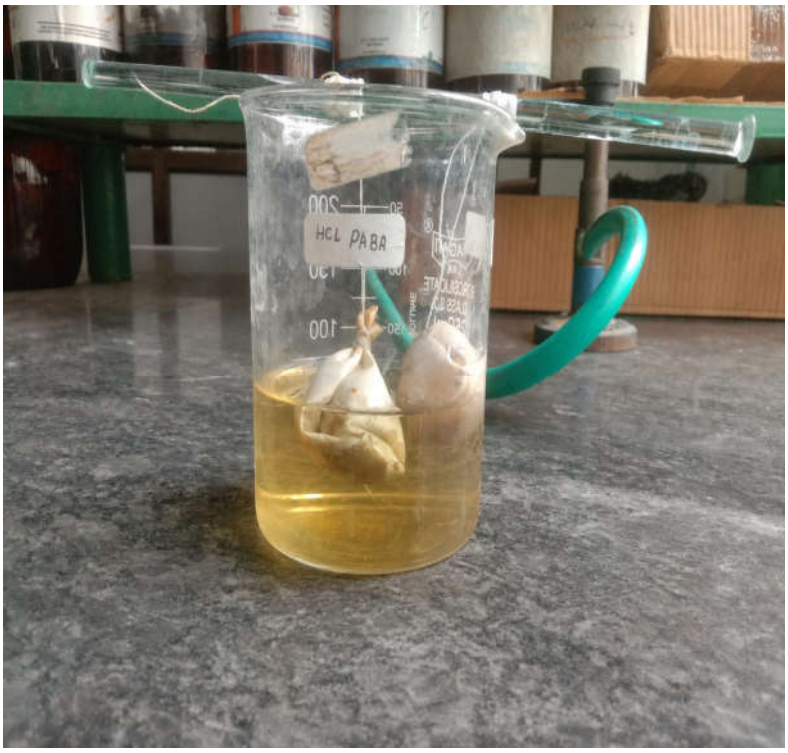

Figure 8: *Incubation of semi- permeable membrane*

Titration:

Figure 9: *Titration Process* **Burette**

Reading:

Sample	**Burette Reading**			**Mean**
	First	**Second**	**Third**	
Control [blank]	10.5	9.5	10	10
Hydrochloro -thiazide	4	3.5	3.2	3.5
Methanolic Extract	4.2	4.1	4	4.1

Table 2: *Burette Reading*

Calculation:

% Inhibition= $\overline{ControlControl}-Test \times 100$

Where,
Control= Vc
Test = Vt

%Inhibition of Hydrochlorothiazide

% Inhibition = $\overline{Vc\frac{10}{}vt-10\underline{-}vt\underline{3.5}}\times\times100100$

$=$

$= 610.5 \times 100$

% Inhibition = 65%

%Inhibition of Methanolic Extract of Origanum Majorana

%Inhibition = $\underline{\underline{5Vc}}10\underline{.9}vt-\underline{-}4\underline{vt}\times.1\times100100\times100$

$= 1010$

$=$

% Inhibition = 59%

RESULT:

Sr.No.	Constituents in methanolic Extract	Observation
1.	Saponins	+
2.	Phenols	+
3.	Tannins	+
4.	Terpenoids	+
5.	Flavonoids	+
6.	Carbohydrates	+
7.	Proteins	+
8.	Alkaloids Detection Test A] Dragendroff's Test	-
9.	B] Mayer's Test	-
10.	C] Hager's Test	-
11.	D]Wagner's Test	-

[-] Indicates the Absence of compound

[+] Indicates the Presence of Compound

Table 3: *Results of Preliminary Phytochemicals Screening of Origanum Majorana*

%Inhibition

Sr.No.	Sample	%Inhibition
1.	Blank	00
2.	Hydrochlorothiazide	65%
3.	Methanolic Extract of Origanum Majorana	59%

Table 4: *Result of Percentage inhibition* **Graphical Presentation of % of Inhibition:**

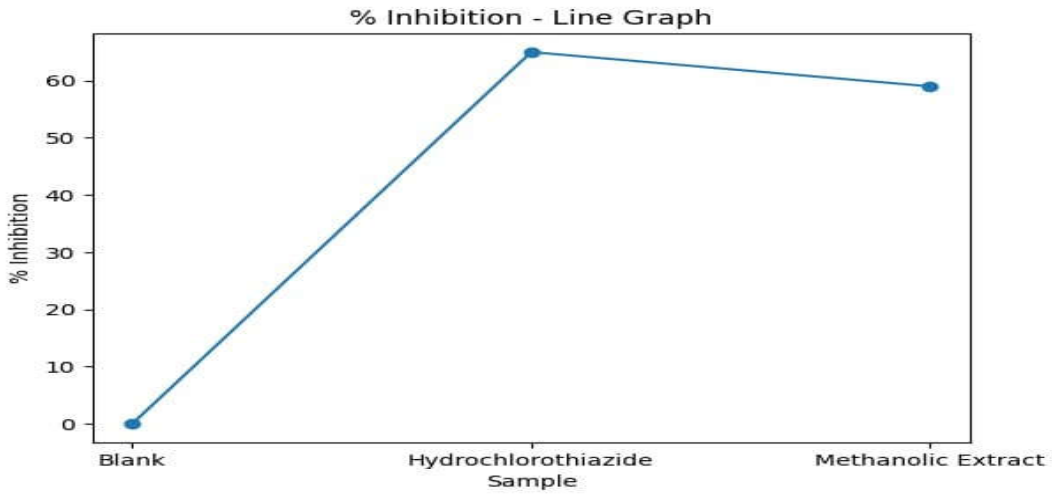

Figure 10: *Graphical presentation of Percentage Inhibition*

Discussion:

In this study, we tested the anti-kidney stone activity of the methanolic extract of Origanum majorana using in vitro methods. Calcium and oxalate become too concentrated in urine and start forming crystals. These crystals grow and stick together, leading to stone formation. So, any substance that can stop these steps may help in preventing or treating kidney stones. Flavonoids and phenols, in particular, can bind with calcium and reduce its availability, which helps prevent crystal formation. They also stop the crystals from sticking together and growing bigger.The in vitro results showed that the extract was effective in reducing both the formation and grouping of calcium oxalate crystals. This effect increased as the dose of the extract increased. This means the extract can interfere with the early stages of kidney stone formation and may help prevent stones from developing.The egg membrane method also supported these results. In this method, the membrane acts like a natural barrier. The extract was able to reduce or dissolve the calcium oxalate crystals, suggest that it may help in breaking down already formed stones. This is important because such damage can promote stone formation. By protecting kidney cells, the extract may help reduce the chances of stones forming again.Overall, the study shows that Origanum majorana has good potential as a natural remedy for preventing and managing kidney stones. However, more studies on animals and humans are needed to confirm its safety and effectiveness.

Conclusions:

This investigation demonstrated that the methanolic extract of Origanum majorana is effective in preventing kidney stones in laboratory conditions. It helped reduce the formation, growth, which are the main cause of kidney stones.

The extract contains important natural substance such as flavonoids, polyphenols, tannins, saponins, and terpenoids. These substances may help by reducing calcium levels, stopping crystal formation, and preventing crystals from becoming larger.

The egg membrane test also showed that the extract can reduce or break down already formed crystals. This means it may help both in preventing and treating kidney stones. The effect was stronger at higher doses.

Overall, Origanum majorana looks like a encouraging herbal alternative for controlling and treating kidney stones. However, more studies on animals and humans are needed to confirm its safety and effectiveness.

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