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IN VITRO ANTI-UROLITHIATIC ACTIVITY OF MIXTURE OF TRIDAX PERCUMBENES & KALANCHOE PINNATA EXTRACT BY UV SPECTROSCOPY

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ABSTRACT

In medical terminology, kidney stone disease is referred to as renal calculus disease, nephrolithiasis, or urolithiasis. The Latin word "renal" means kidney, and the Greek word "nephro" means kidney. Both "calculus" (Lat.-pl. calculi) and "lithiasis" refer to stone. A crystallopathy known as kidney stone disease is brought on by an excess of minerals in the urine combined with dehydration. Small crystal fragments collect in the upper urinary system as calculi, or stones, as a result of this imbalance. If renal calculi are tiny enough, they can exit the urinary tract through the urine stream because they usually develop in the kidney. A little calculus could go away without producing any symptoms. But, if a stone becomes larger than 5 millimeters (0.2 inches), it may obstruct the ureter, which can lead to renal colic, a strong, excruciating pain in the lower back that frequently travels down to the groin.[1] The majority of calcium stone patients (75%–80%) have nephrolithiasis, which is mainly caused by calcium oxalate monohydrate or dehydration. Hypercalciuria, hyperoxaluria, hyperuricosuria, and hyppcitraturia are the four most prevalent chemical factors that contribute to the production of urinary stones.[2] The primary need and goals are: Preventing kidney stones is the main goal of anti-urolithiatic activity. Inhibiting crystal formation stops them from growing and aggregating. To avoid kidney stone-related urinary diseases. **Materials and Procedures:** Fresh Kalanchoe pinnata leaves were extracted in water and arranged in various concentrations. Artificial stones like calcium phosphate and calcium oxalate were made using the homogenous precipitation method, and semi-permeable egg membranes were utilized as dissolution bags. Following a 72-hour incubation period, spectrophotometric estimation was used to determine the total content of the dissolving bags. The weight variation test of calcium oxalate tablets was used to assess the inhibitory effect of a combination of Kalanchoe pinnata and tridax percubenes extract on the nucleation of calcium oxalate crystals and the rate of aggregation in calcium oxalate crystals. **Findings:** While the Cystone standard has demonstrated stronger demineralization for calcium phosphate than the extract of Kalanchoe pinnata, the extract of a combination of Kalanchoe pinnata and tridax percubenes had a greater capacity to dissolve calcium oxalate in dissolving models. In the nucleation assay as opposed to the aggregation assay, cysteine demonstrated a considerable inhibitory effect. Tridax percubenes extract and Kalanchoe pinnata extract together had a strong inhibitory effect in both the nucleation and aggregation tests. **Discussion:** The current study will contribute to the body of knowledge regarding in-vitro research. Understanding the molecular mechanism of the litholysis process and identifying the phytochemicals in the extract that dissolve or disintegrate renal calculi may be aided by correlation between in vitro investigations. **Conclusion:** Tridax percubenes extract and Kalanchoe pinnata extract together demonstrated strong in-vitro anti-urolithiatic activity.

KEYWORD: Kalanchoe Pinnata, Tridax Percumbenes, Spectrophotometer, Semipermeable membranes.

INTRODUCTION

In medical terminology, kidney stone disease is referred to as renal calculus disease, nephrolithiasis, or urolithiasis. The Latin word "renal" means kidney, and the Greek word "nephro" means kidney. Both "calculus" (Lat.-pl. calculi) and "lithiasis" (Greek) refer to stone or stones. A crystallopathy known as kidney stone disease is brought on by an excess of minerals in the urine combined with dehydration. Small crystal fragments collect in the upper urinary system as calculi, or stones, as a result of this imbalance. If renal calculi are tiny enough, they can exit the urinary tract through the urine stream because they usually develop in the kidney. A little calculus could go away without producing any symptoms. But, if a stone becomes larger than 5 millimeters (0.2 inches), it may obstruct the ureter, which can lead to renal colic, a strong, excruciating pain in the lower back that frequently travels down to the groin.

Additionally, a calculus may cause painful urination, vomiting (from extreme pain), or blood in the urine. Within ten years, around half of all kidney stone patients are at risk of getting another one. Nowadays, because of dietary and lifestyle changes, stone production is the most common and severe urologic illness, causing great pain. [1] The majority of patients with calcium stone-related nephrolithiasis (75%–80%) are mostly formed of calcium oxalate monohydrate or dehydrate. Hypercalciuria, hyperoxaluria, hyperuricosuria, and hypocitraturia are the four most prevalent chemical factors that contribute to the production of urinary stones.[2]

Types of kidney stone

Calcium stones: The most prevalent kinds of kidney stones are calcium stones, including calcium phosphate and calcium oxalate stones. They may develop as a result of excessive calcium in the urine, which is frequently brought on by nutritional or metabolic issues. **Uric acid stones:** These are a particular kind of kidney stone. Small stones grow when your body produces too much uric acid. It might result in blood in your urine and excruciating discomfort when you urinate. A heavy consumption of purin-rich foods may be linked to gout, and little uric acid stones may be passed on their own.[3] **Struvite stones:** One kind of hard mineral deposit that can develop in your kidneys is called a struvite stone. When minerals like calcium and phosphate crystallize and adhere to one another inside your kidneys, stones can result. Gram-negative bacteria in your urinary tract produce the mineral struvite, which is created when urea is broken down into ammonia.[4] Stones in the cyst: Kidney stones known as **cystine** stones are brought on by the hereditary condition cystinuria. They may need to be treated with more water, dietary adjustments, or surgery, and they can cause pain and blood in the urine.

Mechanism of action

1] Phenol Test

Phenolic compounds, particularly those found in plants, show promise in preventing and managing kidney stones (urolithiasis) due to their antioxidant and anti-inflammatory properties, which can inhibit crystal formation and aggregation.[5]

Mechanisms of Action of Phenolic Compounds

Kidney stones (urolithiasis): Are hard mineral deposits that form inside the kidneys. Oxidative stress and reactive oxygen species (ROS): are implicated in kidney stone formation.

Phenolic compounds: in plants may help prevent kidney stone formation by scavenging free radicals and ROS, modulating antioxidant and pro-oxidant enzymes, and regulating signalling pathways associated with oxidative stress.

2] Flavonoids Test

Flavonoids, plant-derived compounds, have shown promise in preventing kidney stone formation, particularly calcium oxalate stones, due to their antioxidant, anti-inflammatory, and diuretic properties.

Mechanisms of Action of Flavonoids

Kidney stones, also known as urolithiasis, are a common health problem characterized by the formation of hard mineral deposits inside the kidneys. Calcium oxalate stones are the most prevalent type. Flavonoids, a group of plant-derived compounds, have been studied for their potential to inhibit kidney stone formation. Research suggests that flavonoids can reduce the formation of calcium oxalate crystals in vitro and in vivo.

Diuretic Effects: Some flavonoids may increase urine production, which can help flush out minerals and prevent stone formation.

Inhibition of Crystal Aggregation: Certain flavonoids can inhibit the aggregation of calcium oxalate crystals, preventing them from growing into larger stones.[6]

3] Triterpenoid saponins test

Triterpenoid saponins, naturally occurring compounds found in various plants, show potential in preventing and treating kidney stones (urolithiasis) by inhibiting calcium oxalate crystal formation and affecting stone-forming constituents.

Mechanisms of Action of Triterpenoid saponins

Inhibiting Calcium Oxalate Crystal Formation: Studies have shown that saponin-rich extracts can inhibit the formation and growth of calcium oxalate crystals, which are the main type of kidney stones.

Affecting Stone-Forming Constituents: Some spooning may influence the urinary concentration of substances that contribute to stone formation.[7]

Kalanchoe pinnata

Fig. No. 01: Kalanchoe pinnata leaves.

Synonym

Bryophyllum calycinum, Crassula pinnata, Cotyledon pi nnata Biological source:- It is a glabrous, ornamental, crassulenscent herb, cultivated in houses and gardens it

is green in color odour is sweet and sour tastes:- sour, bitter, and astringent Shape:- oval or elliptical leaves Size:- typically grows to a height of 1 to 1.5 meters (3-5 feet).Chemical constituents:-Kalanchoe pinnata is composed of several chemical components, including flavonoids (Quercetin, Kaempferol, Luteolin), phenolic acids, triterpenes, steroids, and bufadienolides, some of which are linked to its recognized toxic properties.[8] Conventional Applications and Characteristics:- Kidney Stones: In traditional medicine, the juice from the leaves is utilized for kidney stones Healing Wounds & Reducing Inflammation: Kalanchoe pinnata is recognized for its ability to heal wounds, with a paste made from its leaves commonly used on cuts, injuries, and burns to arrest bleeding and enhance the healing process. Relieving Pain: This plant exhibits analgesic (pain-relieving) and anti-inflammatory effects, making it effective for alleviating discomfort, such as headaches and muscle soreness. Anti-diabetic and Anti-cancer Properties: Several studies indicate that Kalanchoe pinnata might possess anti-diabetic and anti-cancer attributes, though further research is necessary to validate these claims. Other Traditional Applications like Jaundice: The juice extracted from the leaves combined with black pepper powder is consumed to help manage jaundice. Fever: A mixture of leaf juice and black pepper powder can be ingested to alleviate fever.

Tridax procumbenes

Fig. No. 02: Tridax Procumbenes Leaves.

Synonym:- Tridax daisy, Mexican daisy, and wild daisy Balbisia elongata Willd. Biological source:- Often referred to as coat buttons or tridax daisy, encompasses the whole plant, which is a herbaceous weed originally from tropical America but has since become established in tropical regions of Africa, Asia, Australia, and India. Family:- Asteraceae. The color of plant bears daisy-like yellow-centered white or yellow flowers with three-toothed ray florets. Odour is somewhat pungent, and slightly bitter odor taste is slightly bitter taste. Shape:- annual, sometimes perennial prostrate to ascending herb Size:- can grow up to 50 cm (20 inches) tall, with flowering axes reaching that height.[10] Chemical constituents:- including alkaloids, flavonoids, carotenoids, tannins, saponins, and fatty acids Alkaloids: Studies have detected various alkaloids, including akuammidine. Flavonoids: Tridax procumbens is rich in flavonoids, with some studies identifying kaempferol, epicatechin, and procumbenetin. Carotenoids: The plant contains carotenoids, including lutein. Tannins: Tannins, including tannic acid, have been identified. Saponins: Saponins are also present in the plant. Traditional Uses & Properties: Wound Healing: The juice from the leaves is traditionally applied directly to wounds to promote healing. Skin Diseases: Leaf extracts have been used in folk medicine to treat infectious skin diseases. Liver Disorders: It's used in Ayurvedic medicine for liver disorders, hepatoprotection, gastritis, and heartburn.[11]

MATERIAL AND METHOD

Chemical

The chemicals calcium chloride dihydrate, sodium oxalate, and p-phenylenediamine were sourced from Sigma-Aldrich Ltd. Potassium permanganate, sodium metabisulfite, and tris buffer were acquired from Loba Chemicals Ltd. Cystone was obtained from the Himalaya Drug Company. The plant materials were collected from Dighanchi village Tal –Atapadi, Dist-Sangali The plant specimens were recognized and verified by the Department of Botany at Ishwarrao More-Patil Arts, Commerce & Science Mahila Mahavidyalaya Ekata Nagar Dighanchi. Fresh leaves were gathered in FEB 2025.

Extraction of kalanchoe pinnata

The fresh leaves of Kalanchoe pinnata were diced into small bits by hand and placed into a conical flask. A volume of 100 ml of distilled water was then added to the flask and boiled for a period to enhance the extraction process. Once cooled, the mixture was filtered using Whatman filter paper, and the resulting aqueous extract stock solution was transferred to an appropriate container.[13]

Fig. No. 03: Extraction of Kalanchoe Pinnata.

Extraction of tridax procumbenes

The dried flowers & leaves were shade dried and powdered in a mixture grider and stored in an airtight jar for study. The extraction was performed using the soxhlet apparatus according to the standard procedure. Taken 50 gm powder and 100 ml of methanol for the extration and boiled for a period to enhance the extraction process. Once cooled, the mixture was filtered using Whatman filter paper, and the resulting aqueous extract stock solution was transferred to an appropriate container.[14]

Fig. No. 04: Extraction of Tridax Procumbenes.

Phyatochemical Test for Kalanchoe Pinnata

Standard techniques were used to test the extracts for the presence of active phytochemical components.

1. Phenols test

1 ml of extract was mixed with 1% ferric chloride solution. The green colour showed that phenols were present.

2. Flavonoids test

3-5 drops of a lead acetate solution with a concentration of 2 Percent were added to 1ml of alcoholic extract. The appearance of orange or yellow colour shows that flavonoid is there.

3. Alkaloid test

Alkaloids were detected by adding one drop of Drangendroff reagent to one millilitre of extract and looking for a yellow precipitate.

4. Triterpenoid Saponins Test

Test of Froth Formation In a test tube, when 1ml of extract was mixed with 1ml of water, foam formed.

5. Bendict's Test:-The extract mixed with Bendict's reagent and boiled until a red colour showed. This showed that the extract contained carbohydrate.

6. Biuret Test:-A few drops of a 5 percent Sodium Hydroxide solution and a 1 percent Copper Sulphate solution were added to 2-3 ml of the extraction. When there is protein, the colour will be violet or pink.

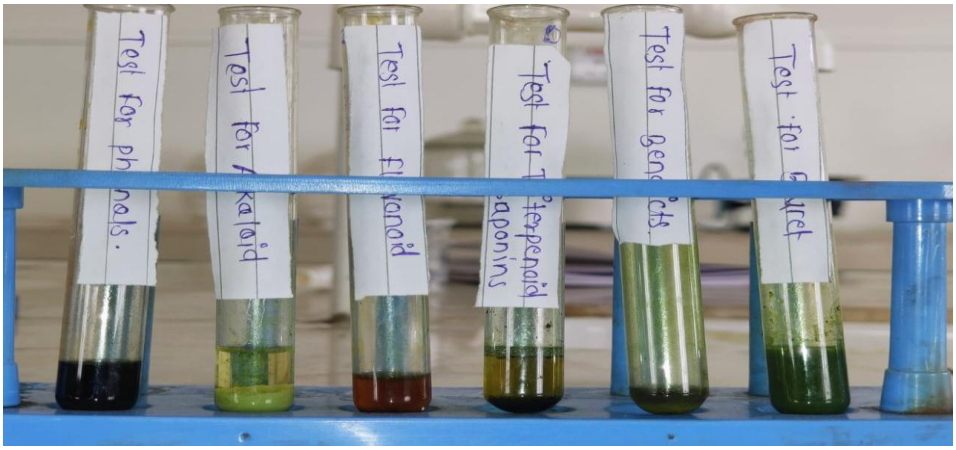

Fig. No. 05: Phytochemical Tests.

Phyatochemical Test for Tridax Procumbenes

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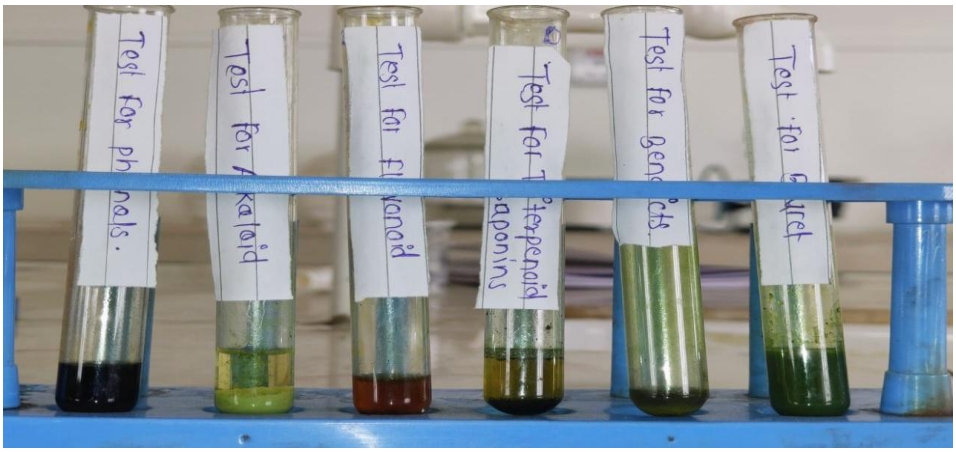


Fig. No. 05: Phytochemical Tests.

Preparation of the semi-permeable membrane from eggs

The apex of the eggs was punctured using a glass rod to extract all the contents. The emptied eggs were thoroughly rinsed with distilled water and then placed in a beaker containing 4ml of concentrated HCl mixed with 200ml of distilled water. This mixture was allowed to sit overnight, resulting in the complete decalcification of the semi-permeable membrane. The next day, the semi-permeable membranes were carefully extracted from the egg shells, thoroughly washed with distilled water, and then immersed in ammonia solution to neutralize any remaining acid traces, followed by another rinse with distilled water. They were then stored in a moist condition in the refrigerator at a pH of 7 to 7.4.[12]

Fig. No. 06: Semi-Permeable Membrane.

Synthesis of calcium oxalate by homogenous precipitation

1.47gm of calcium chloride dihydrate was dissolved in 100ml distilled water and 1.34gm of sodium oxalate was dissolved in 100 ml of 2N H2SO4. 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 KMnO4 solution

1) 0.32gm of KMnO4 was dissolved in 100ml of distilled water.
2) It was boiled for 30min.
3) After cooling, excess of MnO4 was removed by filtration.[12]

Procedure

1) A suspension of 10mg calcium oxalate was created in 10ml of distilled water to serve as a negative control.
2) An aqueous extract of fresh Kalanchoe pinnata leaves measuring 5ml was utilized.
3) To eliminate the color coating, a 500mg Cystone tablet was immersed in absolute ethanol, resulting in a recovery of 400mg.
4) The Cystone tablet was then ground into a powder and dissolved in 100ml of distilled water, followed by filtration.
5) The filtrate obtained from the Cystone was utilized as a positive control for assessing in vitro anti-urolithiatic activity.

Spectrophotometric Estimation of Calcium Oxalate By Using Dissolution Model

Procedure

A) Group I: 1ml of calcium oxalate (1mg/ml) + 1ml of distilled water

B) Group II: 1ml of calcium oxalate (1mg/ml) + 1ml of Cystone solution (400mg/ml)

C) Group III: 1ml of calcium oxalate (1mg/ml) + 1ml of hot aqueous extract of Kalanchoe pinnata (20mg/ml)

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 150 ml 0.1 M Tris buffer each. At another end of thread tied by a stick placed on the mouth of conical flask and covered with aluminum foil.

2) All groups were kept in an incubator, pre C for 4 hours, kept for three days. The entire°heated to 37 content of each group was removed from sutured semi permeable membrane and was transferred into test tube l of 0.02Mμindividually. 4ml of 1N H2SO4 and 60-80 KMnO4 were added and kept aside for 2 hours. Colour change from dark pink to colorless was observed after 2 hours.

3) Change of color intensity was measured against 620nm spectrophotometric.[11]

4) Concentration of undissolved calcium was determined from standard calibration curve of calcium oxalate by using the measured absorbance readings as shown in table No: 03.

RESULT AND DISCUSSION

Phytochemical Tests for Kalanchoe Pinnata

Table No. 01: Phytochemical Test.

Test	Observation	Inference
Test For Phenols 1 ml of extract was mixed with 1% Ferric chloride solution.	Green color appears	Positive
Test For Flavonoids: 3-5 drops of lead acetate sol^{n} with a conc of 2% were added 1 ml of alcoholic extract.	Yellow color formed	Positive
Test For Alkaloids: Alkaloids were detected by adding 1 drop of drangendroff reagent to one milliliter of extract.	Yellow ppt not formed	Negative
Test For Triterpenoid Saponins: In a test tube 1 ml of extract was mixed with 1 ml of water.	Foam are formed	Positive
Test For Bendicts :	Carbohydrate	Negative

The extract mixed with bendicts reagent and then boiled.	Not present	
Test For Biuret: A few drops of a 5% sodium hydroxide solution and a 1% copper sulphate solution were added to 2-3 ml of the extraction.	Violet color are not appears	Negative

Phytochemical Tests for Tridax percubenes

Table No:-02 Phytochemical Test.

Test	Observation	Inference
Test For Phenols : 1 ml of extract was mixed with 1% Ferric chloride solution.	Green color appears	Positive
Test For Flavonoids: 3-5 drops of lead acetate sol^{n} with a conc of 2% were added 1 ml of alcoholic extract.	Yellow color formed	Positive
Test For Alkaloids: Alkaloids were detected by adding 1 drop of drangendroff reagent to one milliliter of extract.	Yellow ppt not formed	Negative
Test For Triterpenoid Saponins: In a test tube 1 ml of extract was mixed with 1 ml of water.	Foam are formed	Positive
Test For Bendicts : The extract mixed with bendicts reagent and then boiled.	Carbohydrate Not present	Negative
Test For Biuret: A few drops of a 5% sodium hydroxide solution and a 1% copper sulphate solution were added to 2-3 ml of the extraction.	Violet color are not appears	Negative

Absorbance

Calcium oxalate, and other chemicals in the urine crystallize on the inside of the kidneys when kidney stones form. The early mineral phase formation is the term used to describe this stage. When crystals come together over time to form tiny, hard masses called stones, this process is called crystal growth. Calcium oxalate monohydrate stones (COM) and calcium oxalate dihydrate stones (COD) are the two types of calcium oxalate stones.

Calcium oxalate estimation using UV spectrophotometry

The primary component for stones, calcium oxalate, can be more readily dissolved by combination of extract of Kalanchoe pinnata and Tridax procumbenes. Developing in the urinary system. A lower proportion denotes greater calcium oxalate crystal dissolving potency shown in table No: 3.

Table No. 03: Absorbance.

Sample	Absorbance
Positive	4.242
Negative	1.196
Dil 1 ml	1.706
Dil 2 ml	1.602
Dil 3 ml	1.196

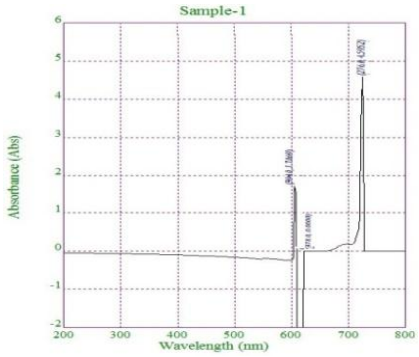


Fig. No. 07: Dil of 1ml.

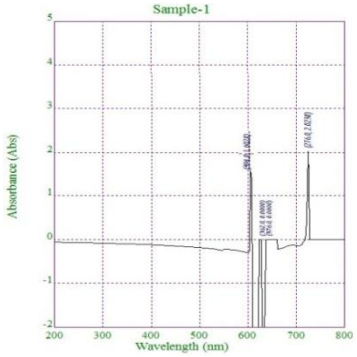


Fig. No:-08 Dil of 2ml

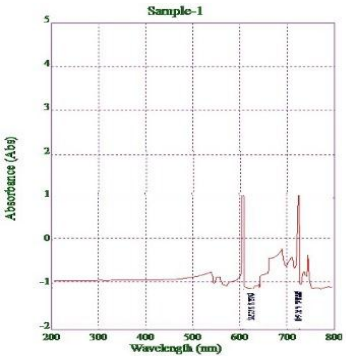


Fig. No:-09 Dil of 3ml

Assay for Nucleation

The crystallisation of calcium oxalate particles in the urinary system is the cause of urine supersaturation. This is Stone-forming salts start to join into clusters during the nucleation phase when fresh constituents are added. When it came to the nucleation of calcium oxalate ions, the Cystone standard solution shown greater inhibitory action. Since nucleation is a crucial initial step for the initiation of crystals, which subsequently grow and form aggregates, an in vitro crystallisation study was conducted. The combination of extract of kalanchoe pinnata and tridax percubenes show inhibition of nucleation as well as aggregation of calcium oxalate stone.

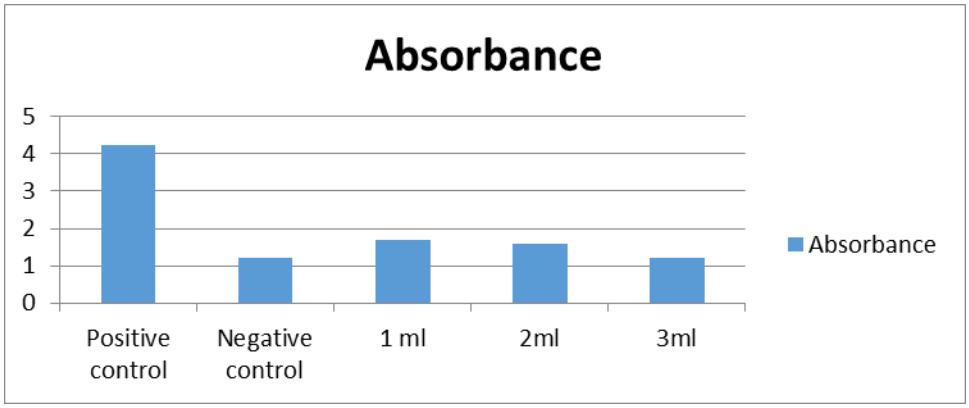
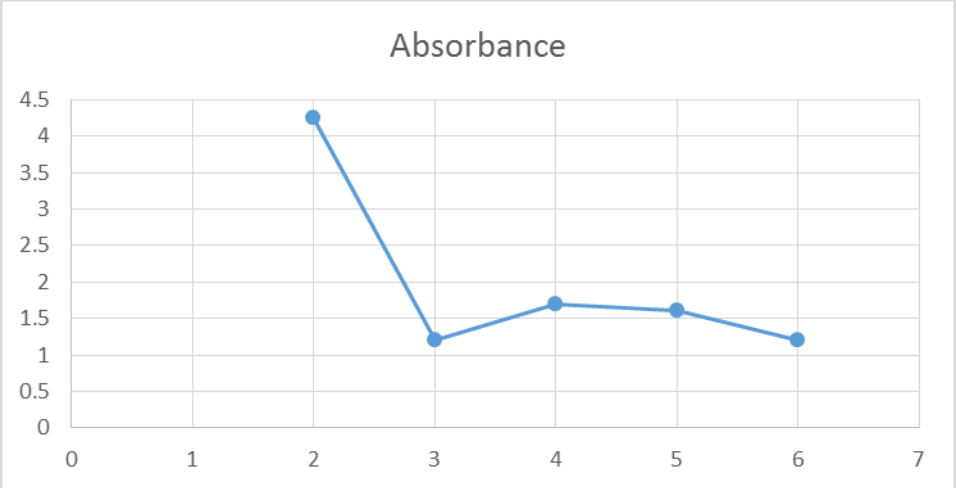


Fig. No:-10 Absorbance.

The 3ml extract mixture of kalanchoe pinnata and tridax procumbenes shows the lower absorbance as composed to control. This show that extraction of kalanchoe pinnata and tridax procumbenes prevented crystallization as well as nucleation of calcium oxalate crystal.



CONCLUSION

Kalanchoe pinnata and tridax procumbenes is one of the most widely used herbal medicine in India and has stood the test of centuries for the treatment of various aliments This research put the light on the importance and need to continuously carry out research. In preventing and treating issue such as kidney stones, which is becoming more common in the peoples as a result of sedentaey lifestyles and poor eating habits. In vitro studies, extract of combination was discovered to have strong anti urolithiatic efficieny in the current investigation. Calcium oxalate stone dissolve well in 3ml extract mixture of kalanchoe pinnata and tridax procumbenes. Result shows lower absorbance as composed to control. This show that extraction of kalanchoe pinnata and tridax procumbenes prevented crystallization as well as nucleation of calcium oxalate crystal.

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