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Research

Antiurolithiatic Activity of Extract of *Moringa oleifera* Leaf Stalk

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Abstract

Urolithiasis, a prevalent and painful condition, involves the formation of stones in the kidneys or urinary tract, predominantly composed of calcium oxalate. Despite extensive research, effective non-invasive treatments remain limited. This study explores the antiurolithiatic potential of Moringa oleifera leaf stalk extract through in vitro models. The extract was evaluated for its ability to dissolve calcium oxalate and calcium phosphate stones, as well as its efficacy in inhibiting nucleation and aggregation of these crystals. In the dissolution study, Moringa oleifera extract showed a significant dissolution of both calcium oxalate (46.45%) and calcium phosphate (48.33%) stones, compared to the standard drug Cystone, which exhibited higher dissolution rates of 54.67% and 74.16%, respectively. Nucleation assays confirmed the extract's ability to prevent initial crystal formation, with higher concentrations showing increased efficacy. Aggregation assays demonstrated that the extract effectively inhibited the clustering of calcium oxalate crystals, reducing the potential for stone growth and renal injury. Phytochemical analysis identified alkaloids, flavonoids, terpenoids, glycosides, and saponins in the Moringa oleifera extract, which may contribute to its antiurolithiatic properties. The results indicate that Moringa oleifera leaf stalk extract can significantly inhibit stone formation and promote dissolution, offering a promising natural alternative for the prevention and treatment of urolithiasis. Further pharmacological and clinical studies are needed to fully understand the mechanisms and efficacy of this herbal remedy in managing kidney stones.

Keywords: Urolithiasis, Moringa oleifera, kidney stones, stone dissolution, herbal medicine.

INTRODUCTION

Urolithiasis is one such disease that after extensive research in the field of urology has remained incurable in Allopathy. It is a process of stone formation which occurs either in the kidney (commonly known as nephrolithiasis) and or in any part of the urinary tract, including the Ureters (known as ureteral stone) and bladder (bladder stone). Urolithiasis has an important effect on the health care system with a prevalence of >10% and an expected recurrence rate of ~50%.[1] The worldwide incidence of Urolithiasis is quite high, and more than 80% of

urinary calculi are calcium oxalate (CaOx) stones alone or CaOx mixed with calcium phosphate[2]. Epidemiological studies revealed that the Nephrolithiasis is more prevalent in men (12%) than in women (6%) and is more prevalent between the ages of 20–40 in both sexes[3].

Kidney stones, one of the most painful of the urologic disorders, have beset humans for centuries. Scientists have found evidence of kidney stones in a 7,000-year-old Egyptian mummy. Unfortunately, kidney stones are one of the most common disorders of the urinary tract. Each year, people make almost 3 million visits to health care providers and more than half a million people go to emergency rooms for kidney stone problems. (Ravindra Kumar, Tirath Kumar etal., 2012).

Most kidney stones pass out of the body without any intervention by a physician. Stones that cause lasting symptoms or other complications may be treated by various techniques, most of which do not involve major surgery. Also, Research advances have led to a better understanding of the many factors that promote stone formation and thus better treatments for preventing stones.

Today large number of population suffers from kidney stone, gall stone and urinary calculi. Stone disease has gained increasing significance due to changes in living conditions *i.e.* industrialization and malnutrition. Changes in prevalence and incidence, the occurrence of stone types and stone location, and the manner of stone removal are explained. The problem of urinary stones or calculi is a very ancient one and many remedies have been employed during the ages these stones are found in all parts of the urinary tract, the kidney, the ureters and the urinary bladder and may vary considerably in size.

Nature bestowed our country with an enormous wealth of medicinal plants. Plants have been used as traditional healthcare system from the centuries. The WHO has listed 20 000 medicinal plants globally in which contribution of India is 15–20%. The WHO reported that 80% of global countries depend on the medicinal plants. A large body of evidence has collected to show potential of medicinal plants used in various traditional systems. In the last few years more than 13 000 plants have been studied for the various diseases and ailments all over the world.

An attempt has been made during the last decade to study the identical, chemistry, pharmacology and clinical investigations of Pashanbheda plants used for dissolving kidney stones.

Pashanbheda is a drug mentioned in the Ayurvedic system of medicine for various ailments but mainly as a diuretic and lithotriptic. It is said to have properly of breaking and disintegrating the stones and is widely used drug. However, its identity is yet debatable. Many diuretic and other plants such as *Alternanthera sessalis*and *Aerva spp*. In South India. *Rotula aquatica* in Mysore, *Ammaunia baceifera* in Kerala,*Bauhinia racemosa, Coleus spp., Bryophyllum spp., Didymocarpus pedicellata, Ocimum basilicum* in Bengal and many other have been referred to as Pashanbheda from time to time(Shashi Alok, Sanjay Kumar Jain,et.al.,2013). Now *Bergenia ligulata syn. Saxifrega ligulata* is being widely accepted under this name. Chemical efficiency of *Bergenia ligulata* is dissolving the urinary stones fully justifies the use of various names attributed to it, *viz.*, Pashanbheda, Pashana, Asmaribheda, Ashmabhid, Ashmabhed, Nagabhid, Upalbhedak, Parwatbhed and Shilabhed (dissolving or piercing stones or slabs) etc.

Effective cure of urinary calculi have been prescribed by practitioners in unani system of medicine, while in Homoeopathic system of medicine, *Berberis vulgaris, cantharis spp*., and *Lycopodium spp*. are being use. To increase the acceptability and awareness among the people, there is an urgent need to develop trust and faith towards the safer indigenous system by establishing its validity in treatment for various diseases. Health care systems are going to become more and more expensive, therefore we have to introduce herbal medicine systems in our health care. The Literature revealed that there are no scientific studies carried out regarding antilithiatic activity in *Moringa oleifera*(*in vitro*). As the plant is easily available and economical, studies on the medicinal activity of the leaf stalk can be done with great interest. The present article deals with evaluating the potential of medicinal plant *Moringa oleifera Lam.* leaf stalk in stone dissolving activity.

PLANT PROFILE

Scientific Classsification

Kingdom	:	Plantae
Unranked	:	Angiosperms
Unranked	:	Eudicots
Unranked	:	Rosids
Order	:	Brassicales
Family	:	Moringaceae
Genus	:	Moringa
Specius	:	*Moringa oleifera*
Binomial name	:	*Moringa oleifera* lam.

Synonymn

Guilandina moringa l.

Hyperanthera moringa l.
Moringa pterygosperma gaertn.nom.illeg.

Description[19]

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. English common names include: moringa, drumstick tree (from the appearance of the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish), ben oil tree, or benzoil tree (from the oil which is derived from the seeds). It is a fast-growing, drought-resistant tree, native to the southern foothills of the Himalayas in north western India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. It can also be used for water purification and hand washing, and is sometimes used in herbal medicine.

Moringa oleifera is a fast-growing, deciduous tree. It can reach a height of 10–12 m (32–40 ft) and the trunk can reach a diameter of 45 cm (1.5 ft). The bark has a whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up feathery foliage of trip innate leaves.

The flowers are fragrant and bisexual, surrounded by five unequal, thinly veined, yellowish-white petals. The flowers are about 1.0-1.5 cm (1/2") long and 2.0 cm (3/4")broad. They grow on slender, hairy stalks in spreading or drooping later flower clusters which have a length of 10–25 cm. Flowering begins within the first six months after planting. In seasonally cool regions, flowering only occurs once a year between April and June. In more constant seasonal temperatures and with constant rainfall, flowering can happen twice or even all year-round.

The fruit is a hanging, three-sided brown capsule of 20–45 cm size which holds dark brown, globular seeds with a diameter around 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water. In cultivation, it is often cut back annually to 1–2 m (3–6 ft)and allowed to regrow so the pods and leaves remain within arm's reach.

Traditional Medicine[20]

All plant parts of *Moringa oleifera* are traditionally used for different purposes, but leaves are generally the most used. In particular, they are used in human and animal nutrition and in the traditional medicine. Leaves are rich in protein, mineral, beta-carotene and antioxidant compounds, which are often lacking among the populations of underdeveloped or developing countries. *Moringa* leaves are added to food preparations as integrators of the diet. In traditional medicine, these leaves are used to treat several ailments including malaria, typhoid fever, parasitic diseases, arthritis, swellings, cuts, diseases of the skin, genito-urinary ailments, hypertension and diabetes. They are also used to elicit lactation and boost the immune system (to treat HIV/AIDS related symptoms) , as well as cardiac stimulants and contraceptive remedy. One can directly consume either raw and dried leaves or the extract of an aqueous infusion.

Similarly, the use of seeds concerns both human nutrition and traditional medicine. Barks are boiled in water and soaked in alcohol to obtained drinks and infusions that can be used to treat stomach ailments (ease stomach pain, ulcer and aiding digestion), poor vision, joint pain, diabetes, anemia and hypertension, toothache, hemorrhoids, uterine disorder. In a well known practice, *Moringa* seeds are used to sediment impurities of water .Roots are soaked in water or alcohol and boiled with other herbs to obtained drinks and infusions as remedies for toothache, as anthelmintic and antiparalytic drugs and as sex enhancers.

Finally, flowers are used to produce aphrodisiac substances and to treat inflammations, muscle diseases, hysteria, tumors and enlargement of the spleen.

Malnutrition Relief

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Since moringa thrives in arid and semiarid environments, it may provide a versatile, nutritious food source throughout the year. *Moringa* leaves have been proposed as an iron-rich food source (31% Daily Value per 100 g consumed, table) to combat iron deficiency. However, further study is needed to test practical applications of using this dietary source and its iron bioavailability.

MATERIALS AND METHODS

Chemicals

Calcium chloride dihydrate, Sodium oxalate, p-Phenylenediamine were purchased from Sigma-Aldrich ltd,Potassium Permanganate, Sodium metabisulphite and Tris buffer were purchased from Loba chemicals. Cystone was purchased from Himalaya drug company.

Collection Of Plant

The *Moringa oleifera* leaf stalk were collected from parts of Chennai, in the month of April-May.The collected Plant material was identified by Prof.Sasikala Ethirajulu, Consultant(Pharmacognosy) and approved by Dr.P.Sathiyarajeswaran, Assistant Director Incharge, The Siddha Central Research Institute, Arignar Anna Govt. Hospital Campus, Arumbakkam, Chennai-600106.

Extraction[21]

Freshly collected leaf stalk of *Moringa oleifera* were cut into small pieces and shade dried. The dried leaf stalk were finely powdered using a domestic food processor. The powdered leaf stalk were extracted with 70% Alcohol and 30% Distilled water by a process Soxhlet Extraction. The yield of extract was found to be 5.07%.

Preparation Of Semipermiable Membrane From Eggs[22]

Apex of eggs was punctured by a glass rod in order to squeeze out the entire content. Empty eggs were washed thoroughly with Distilled water and placed in a beaker containing 4ml of Concentrated Hcl in 200ml Distilled water. It was kept for overnight which let to the complete decalcification of semipermiable membrane. On the next day, semipermiable membrane was removed carefully from egg shells, washed throughly with distilled water and placed it in ammonia solution for neutralisation of acid traces, and then rinsed it with distilled water. It was stored in Refrigrator at a pH of 7 -7.4 in the moistened condition. 10mg of the Calcium Oxalate suspended in 10ml of Distilled water was considered as Negative Control. 100mg of Extract of *Moringa oleifera* dissolved in 5ml of distilled water was taken. 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 dispersed into 100ml of distilled water and filtered. Filtrate of Cystone was used as Positive Control for *in vitro* Anti-urolithiatic activity.

Spectrophotometric Estimation Of Calcium Oxalate By Using Dissolution Model[23]

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 100ml of 2N Sulphuric acid. Both were mixed equally in a beaker to precipitate out Calcium Oxalate with stirring. The resultant Calcium Oxalate was freed from traces of Sulphuric acid by Ammonia Solution, washed with distilled water and dried at room temperature 60°C for two hours.

Preparation of 0.02M $KmnO_4$ Solution

0.32gm of Potassium Permanganate was dissolved in 100ml of Distilled Water. It was boiled for 30 mins. After cooling, excess of Mno4 was removed by Filtration.

Table 1: Spectrophotometric estimation of calcium oxalate by using dissolution model

Group I	Control	1ml of Calcium Oxalate (1mg/ml) + 1ml of Distilled Water.
Group II	Standard Cystone(400mg/ml)	1ml of Calcium Oxalate (1mg/ml) + 1ml of Cystone Solution.
Group III	Test Extract of *Moringa oleifera* leaf stalk(20mg/ml)	1ml of Calcium Oxalate (1mg/ml) + 1ml of Extract of *Moringa oleifera* Leaf Stalk.

Method

All Groups were packed together in Egg Semipermiable Membrane tied with thread at one end and were suspended in a Conical Flask containing 150ml of 0.1m Tris buffer each. At another end of the thread tied by a stick placed on a mouth of Conical Flask and covered with Aluminium Foil. All groups were kept in an Incubator, preheated to 37 °C for 4 hours, kept for three days. The entire content of each group was removed from sutured Semipermiable Membrane and was transfered into test tube individually. 4ml of 1N Sulphuric acid and 60-80microlitre of 0.02M Potassium Permanganate were added kept aside for 2 hours. Colour change from Dark Pink to Colourless was observed after 2 hours. Change of Colour Intensity was measured against 620nm Spectrophotometrically. Concentration of undissolved Calcium was determined from Standard Calibratiom Curve of Calcium Oxalate by using the measured Absorbance Readings.

Spectrophotometric Estimation Of Calcium Phosphate By Using Dissolution Model[23]

Synthesis of Calcium Phosphate by Homogenous Precipitation

1.47gm of Calcium Chloride dihydrate was dissolved in 100ml of Distilled Water and 1.42gm of Disodium Hydrogen Phosphate was dissolved in 100ml of 0.1N Sulphuric acid. Both were mixed with equally in a beaker to precipitate out Calcium Phosphate with stirring. The Resultant Calcium Phosphate was freed from

traces of sulphuric acid by Ammonia Solution, washed with Distilled Water and dried at a temperature 60°C for 2hours.

Preparation of Molybdate-Sulphuric acid Reagent

Molybdate -Sulphuric acid Reagent was prepared by 5% w/v of Sodium Molybdate Solution, 13ml of Concentrated Sulphuric acid in 80ml of Distilled Water. Finally, Volume was adjusted to 100ml with distilled water.

Preparation of Reducing solution

1gm of p-Phenylenediamine was dissolved in 100ml of 3%w/v of Sodium metabisulfite Solution.

Table 2: Spectrophotometric estimation of calcium phosphate using dissolution model

Group I	Control	1ml of Calcium Phosphate(1mg/ml) + 1ml of Distilled Water
Group II	Standard Cystone(400mg/ml)	1ml of Calcium Phosphate(1mg/ml) + 1ml of Cystone Solution(400mg/ml)
Group III	Test Extract of *Moringa oleifera* leaf stalk(20mg/ml)	1ml of Calcium Phosphate(1mg/ml) +1ml of Extract of *Moringa oleifera* Leaf Stalk

Method

All Groups were packed together in Egg Semipermiable Membrane tied with thread at one end and were suspended in a Conical Flask containing 150ml 0.1M tris buffer each. At another end of the thread tied by a stick placed on a mouth of Conical Flask and covered with Aluminium Foil. All groups were kept in an Incubator, preheated to 37 °C for 4 hours, kept for three days. The entire content of each group was removed from sutured Semipermiable Membrane and was transfered into test tube individually. 4ml of 1N Sulphuric acid and 3ml of Molybdate-Sulphuric acid Reagent,1ml of Reducing Solution were added and kept aside for 2 hours. Colour change from dark pink to colourless was observed after 2 hours. Change of colour intensity was measured against 620nm Spectrophotometrically. Concentration of undissolved Calcium was determined from Standard Calibration Curve of Calcium Phosphate by using the measured Absorbance Readings.

In a pilot study, We found that the Inhibition of Nucleation and Aggregation by the Extract was not effective at below 5mg/ml. Extract exhibited good inhibition activity at above 10mg/ml, and thus Concentration 10-50mg/ml for both the Nucleation and Aggregation Assays.

Assay (Turbidity Method)[24]

The Inhibitory Activity of the Extracts on the Nucleation of Calcium Oxalate Crystals was determined by a Spectrophotometric Assay. Crystalisation was initiated by adding 100**µl** of 4mM Calcium Chloride and 100**µl** of 50mM Sodium Oxalate Solution to 0.5ml of Human Normal Urine, both prepared in a buffer containing 0.5ml of 0.05mM Tris buffer and 0.5ml of 0.15mM Nacl Solution at Ph 6.5 and 37°C and adjusted to volume by addding 1.5ml of Distilled Water. The Rate of Nucleation was determined by comparing the Induction Time of crystals(time of appearance of crystals that reached a critical size and thus became optically detectable) in the presence of the Extract and that of the Control with no Extract. The Inhibition of Nucleation was observed by plotting a graph between Absorbance values and Concentration in the presence of the Extract against Control.

Aggregation Assay[24]

The Rate of Aggregation of the Calcium Oxalate Crystals was determined by a Spectrophotometric Assay with slight modifications. The Calcium Oxalate Monohydrate (COM) crystals were prepared by mixing both the Solutions of Calcium Chloride and Sodium Oxalate of 50mM each.Both solution were then equilibrated. The Solutions were then cooled to 37°C and then evaporated. The COM crystals were then dissolved with 0.5ml of 0.05mM Tris buffer and 0.5ml of 0.15mM Nacl Solution at Ph 6.5 to a Final Concentration of 1mg/ml.Absorbance at 620nm was recorded. The Inhibition of Aggregation was estimated by plotting a graph between Absorbance and Concentration in the presence of the Extract against Control.

Table 3: Dissolution study of Calcium Oxalate by the Standard and Test groups

Group	Weight of Calcium estimated	Weight of calcium reduced	Percentage dissolution
GROUP I	0.62	-	-
GROUP II	0.281	0.339	54.67%
GROUP III	0.332	0.288	46.45%

Table 4 : Dissolution study of Calcium Phosphate

Group	Weight of calcium estimated	Weight of calcium reduced	Percentage dissolution
GROUP I	0.48	-	-
GROUP II	0.124	0.356	74.16%
GROUP III	0.248	0.232	48.33%

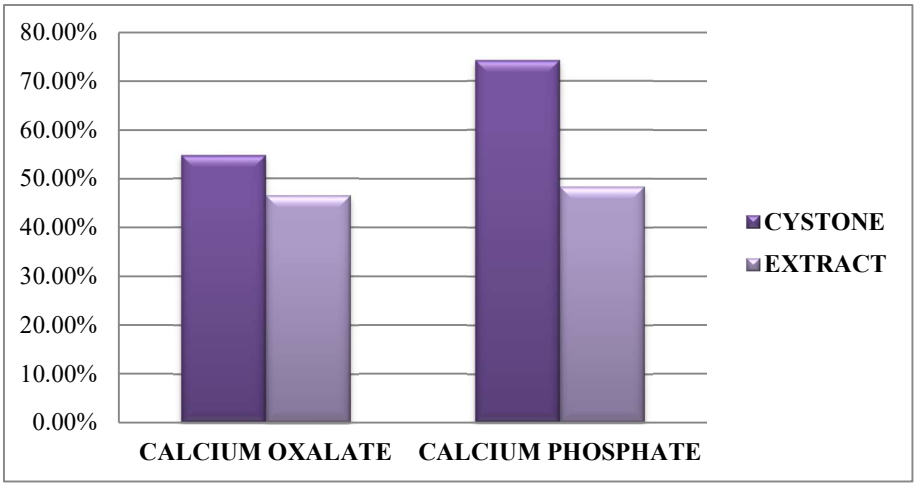

Fig 1: Percentage dissolution of calcium oxalate and calcium phosphate

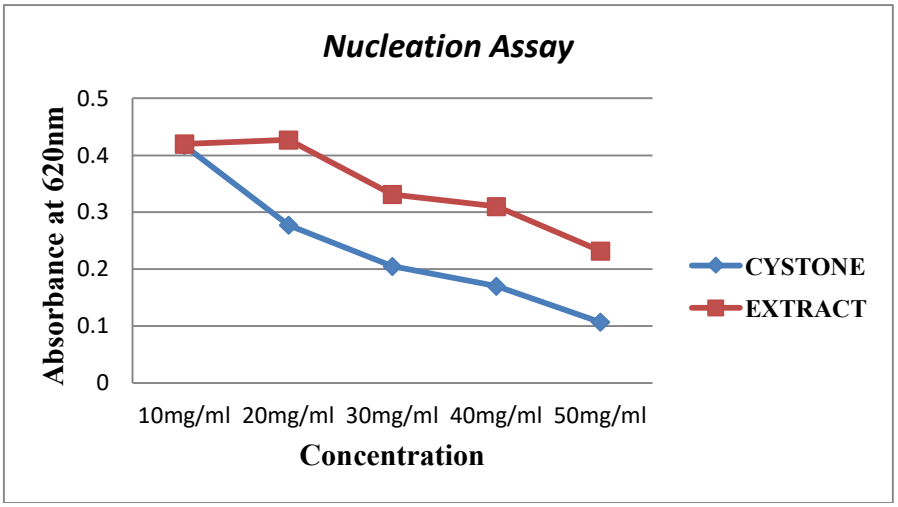

Fig 2: Nucleation assay showing the various concentration of *Moringa oleifera* extract and cystone

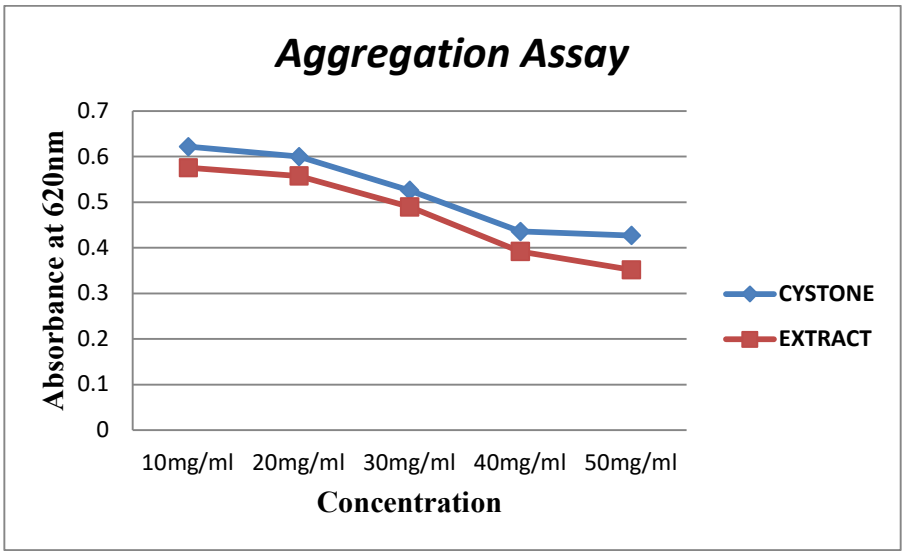

Fig 3: Aggregation assay shown the various concentration of *Moringa oleifera* extract and cystone

DISCUSSION

Urinary Lithiasis is generally the result of an imbalance between Inhibitors and Promoters in the Kidneys. Human Kidney stone are usually composed of Calcium Oxalate Crystals. The Urinary Supersaturation with Stone forming constituents is generally considered to be one of the causative factors in Calculogenesis.

In kidney stones formation, Calcium Oxalate and Calcium Phosphate or other chemicals in the Urine form Crystals on the inner surface of Kidney. This Stage is called as Initial Mineral Phase formation. Over the period of time, Crystals may combine to form a small hard mass called as stones and the stage is refered as Crystals Growth. Calcium Oxalate Stones have classified into two types i.e., Calcium Oxalate Monohydrate stones (COM) and Calcium Oxalate Dihydrate (COD).

The Hydroalcoholic Extract of *Moringa oleifera* Leaf Stalk was confimed by the Identification of the chemical constituents (Alkaloids, Flavonoids, Terpenoids, Glycosides and Saponins) present were carried out using various General Detection Reagents.

Estimation Of Calcium Oxalate And Calcium Phosphate

In the present study, Cystone Standard has shown more effective Demineralisation for Calcium Phosphate stones and a relatively lesser demineralisation for calcium Oxalate stones. It Causes dissolution of 74.16% of Calcium Phosphate stone and 54.67% of calcium Oxalate from the semipermeable membrane into the buffer solution. While The Extract of *Moringa oleifera* leaf stalk produces a dissolution of 48.53% of Calcium Phosphate and 46.45% of Calcium Oxalate from the semipermeable membrane. This shows that The Extract produces relatively similar dissolution of both Calcium Oxalate and calcium Phosphate somewhat lesser than that produced by Standard Cystone.

Nucleation Assay

Since Nucleation is an important first step for the Initiation of Crystals, Cystone Standard Solution exhibited stronger inhibitory activity than the Extract of *Moringa oleifera* in the Nucleation of Calcium Oxalate salts. Extract of *Moringa oleifera* inhibited the Crystallisation by inhibiting Nucleation of Calcium Oxalate through disintegrating into smaller particles with increasing concentration of the fraction. Nucleation assay confirms that the Extract contains Nucleation preventing agents.

Aggregation Assay

Calcium Oxalate crystals aggregate with other crystals and retains in the Kidney. This Aggregation process causes Renal injury. Calcium Oxalate Monohydrate crystals has stronger affinity with cell membranes. It may lead to higher potential risk for Renal Calculi formation. The extract of *Moringa oleifera* demonstrated better Inhibition of Aggregation slightly less than the inhibitory activity of Standard Cystone.

CONCLUSION

The Present Investigation provides useful information on Antiurolithiatic Activity of Extract of *Moringa oleifera* Leaf Stalk. The Extract shows considerable Dissolution with Standard in both type of stones Calcium Oxalate and Calcium Phosphate). The Nucleation and Aggregation assays have also shown better Inhibitory Activity of Crystal growth. The mechanism underlying this action is unknown, but it is related to lowering of Urinary Concentration of stone forming constituents. Further Pharmacological and Clinical studies are required to understand the Mechanism and Actual Efficacy of *Moringa oleifera* Leaf Stalk in treating kidney stones.

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