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# PHYTOCHEMICAL SCREENING AND DETERMINATION OF *IN-VITRO* ANTILITHIATIC ACTIVIY OF *SOLANUM ANGUIVI LAM.*

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## ABSTRACT

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system or the process of forming stones in the kidney, bladder or ureters. Herbal medicines have their own emphasis since they have very less side effects when compared to synthetic drugs. Recent studies have demonstrated an increasing overall prevalence, as well as an increase in the proportion of peoples with urinary stone disease over the last decade. By knowing the importance of herbal medicine, we selected *Solanum anguivi Lam.root* to evaluate antiurolithiatic activity. The present study was aimed to determine the antilithiatic activity of *Solanum anguivi Lam.root* belonging to the family Solanaceae. The results of the present study indicate that methanolic extract of *Solanum anguivi Lam.root* exhibit strong antilithiatic activities.The antilithiatic activities observed by complexometric titration and by semi permeable membrane against standard drug cystone, lead us to propose *Solanum anguivi Lam.root* as promising natural source of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention and treatment of lithiatic diseases.

## INTRODUCTION

Natural products are excellent sources of lead compounds in the search for new medication for many kinds of clinical disorders. Herbal medicines have their own emphasis since they have very less side effects when compared to synthetic drugs. Synthetic drugs have more side effects and also has to be used for long term[1].On the other hand, careful analysis has led to the conclusion that traditional medicine represents a more multifaceted approach to health care than conventional medicine, because it addresses a multi-factorial approach to restoring health, seeks equilibrium between mind, body and the environment, and places a greater emphasis on the multidimensional elements of health than on pathology alone.

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system or the process of forming stones in the kidney ,bladder or ureters. Kidney stones are a common cause of blood in the urine and pain in the abdomen ,flask or grain. Kidney stones occurs in one in 20 people at sometime in their lives. The stones form in the urine collecting area of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself. Urolithiasis is a problematic condition,especialy with regards to its treatment in all the systems of medical sciences.In the system of modern medicine which is supposed to be the most advanced and highly scientific system,the problem of urolithiasis has no satisfactory answer. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value.The rising prevalence of urinary stone disease has had a significant impact on the healthcare system due to the direct costs involved and the morbidity associated with complications such as infection and chronic renal failure. The most common symptom in patients with urolithiasis is acute flank pain, with dramatic relief upon passage of the stone . Stones that are impacted at the ureteropelvic junction produce flank pain, whereas stones lodged in the proximal ureter (between the ureteropelvic junction and the iliac vessels) cause flank pain radiating to the genitals . Stones lodged at the ureterovesical junction produce voiding urgency and suprapubic discomfort, and they cause pain that radiates into the groin and genitals . Associated symptoms include gross or microscopic hematuria, nausea, and vomiting[2]. Struvite stones often remain asymptomatic without causing obstruction.

***Solanum anguivi*** Lam. (SAG) is a rare ethnomedicinal herb belonging to the family Solanaceae. The plant can be found in many places throughout non arid part of Africa. It is highly polymorphic and variable in its plant structure, fruits and leaf characters. The

domesticated species are consumed as leafy and/or fruit vegetables that are rich in essential minerals and vitamins and are recommended as a dietary staple or supplements for nursing mothers, the young, the aged, and anaemic patients. The plant is used as therapeutic agent for various diseases. The roots are carminative and expectorant useful in coughs, cultarrhal affections, dysuria, colic, nasal ulcers, ingredient of dasamula, asthma, difficult parturition, tooth ache, cardiac disorder, worm complaints, spinal guard disorder, nervous disorder and fever. The leaves and fruits rubbed up with sugar are used as external application for itch. The fruit of SAG is a ready sources of vegetable commonly consumed in Nigeria and other African countries because of the traditional believe that it reduces the risk of diabetes and artherosclerosis.By knowing the importance of herbal use of medicine, we have choosen *Solanam anguivi Lam.root* to evaluate antiurolithiatic activity

**MATERIALS AND METHOD**

**1. PLANT SOURCE** :

Plant material consist of dried roots of *Solanum anguivi Lam.root* were collected from the Pathanamthitta district, Kerala during the month of June and authentified by the botanist as *Solanum anguivi Lam.root*.

**2. APPARATUS AND CHEMICALS:**

Soxhlet apparatus, 0.4M trihydroxy methyl amine methane, 0.4M Hydrochloric acid, Sodium chloride, Sodium oxalate, Calcium chloride dihydrate, Disodium EDTA, Solochrome blackT, Disodium hydrogen phosphate, Methanol, Ethyl acetate.

**3. EXTRACTION:**

The powdered root was subjected to hot continuous soxhlet extraction with petroleum ether, ethylacetate, methanol after the residue extraction solvent was distilled off and excess solvent was evaporated to reddish brown semi solid extract .The obtained extract were then evaluated for antilithiatic activity. All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents. TLC studies were done for identifying the presence of constituents which are detected in chemical tests and to known how many extracts are present in each extracts

**Cystone :**

Aqueus extract was prepared by grinding a tablet to powder. This powder was mixed with 5 ml water and kept for 2-3 hrs and then centrifuged at 1000 rpm. The clear supernatant was used for the study.

## 4. PHYTOCHEMICAL SCREENING[11,12]:

- **TEST FOR PROTEINS-**

***Biuret test***-to 3ml test solution add 4% sodium hydroxide and few drops of1% copper sulphate solution violet or pink colour appears .

- **TEST FOR FLAVANOIDS-**

To 1ml of alcoholic extract 3-5 drops of 2% lead acetate solution was added.Development of orange or yellow colour indicates the presence of flavanoids.

- **TEST FOR AMINO ACID-**

***Ninhydrin test*** –heat 3ml of test solution ad 3 drops of ninhydrin solution in boiling water bath for 1 min purple or bluish colour appears.

- **TEST FOR TANNINS-**

***Millons test*** heat 3ml of test solution add 3ml of millons reagent shows dark red colour.

- **TEST FOR CARBOHYDRATE**

**1-*fehlings test***-mix 1ml of fehlings A and B solution,.Add equal volume of test solution. Heat on a boiling water bath 5-1 min. First a yellow, the brick red precipitate is obtaine

**2-*Seliwanoffs test*** –heat 3ml seliwanoffs reagent and 1ml test solute in a boiling water bath for 12 min red colour formed.

- **TEST FOR ALKALOID**

**1. *Hagers Test***-add one drop of hagers reagent into 1ml of extract, yellow precipitate is formed

**2.*Dragondorffs test***-to 1ml of extract add 2-3 drops of dragondorffs reagent, a reddish brown precipitate is obtained.

- **TEST FOR TRITERPINOID SAPONIN-**

***Froth formation test***-shake 1ml of extract with water in a test tube forth will develop.

## 2.THIN LAYER CHROMATOGRAPHY:

Silica gel (60F254)and distilled water were used to prepare a slurry coating materials and aluminium plates were coated by using spreading device with a layer about.30mm thick, coated plates were then dried and activated in oven for 30 min. A small drop of methanolic and ethyl acetate extract solution were placed separately on it, and the spot were placed to become dry.Plates were placed in chromatographic chamber containing the toluene:ethyl acetate:formic acid,5:3:0.5 and Rf value were recorded.

**3. MACROSCOPIC ANALYSIS:**

Root was studied macroscopically for important identification points,odour taste and texture and for microscopical studies a transverse section was prepared stained using concentrated hydrochloric acid and fluroglucinol microscopy of powder was investigated .

**4. DETERMINATION OF INVITRO ANTILITHIATIC ACTIVITY METHOD 1: BY COMPLEXOMETRIC TITRATION[3]**

0.1M Tris buffer:0.1M tris buffer was prepared by using solution A and B.

**SolutionA**: 0.4M tris 48.4g of tris (trihydroxymethyl amino methane) was accurately weighed and dissolved in few ml of water and finally made upto 1000ml with water.

**SolutionB** :0.4M hydrochloric acid(33.6ml of concentrated hydrochloric acid was measured and added in few ml of water upto 1000 ml with water).A working solution was made by adding 25 ml of solution A and 20.7ml of solution B made upto100 ml by using water and then pH was adjusted to 7.4.

**Sodium chloride preparation** : It was prepared by dissolving 5.85g of sodium chloride in 10 ml of water. **Sodium oxalate preparation**: It was prepared by dissolving 1.3 g of sodium oxalate in 10ml of water .

**Preparation of extract**: The powdered root of *Solanum anguivi lam.root* was subjected to continuous hot perculation in soxhlet apparatus using methanol and ethyl acetate for 48hrs and the obtained extract was concentrated by heating.

**Estimation of calcium**: Two sets of test tube(A1,A2)where taken .To these test tube 2ml of tris-HCl buffer ,1ml of sodium chloride1ml of calcium chloride dihydrate and 1ml of sodium oxalate added now 2ml of extract (methanolic extract ,ethyl acetate extact) and vehicle under investigation was added, amount of calcium was estimated by titrimetry using disodium edetate as titrant and solochrome blackT as indicator.

**Formula**

*Amount=(titre value ×equivalent factor ×actual molarity)/(weight taken × expected molarity)*

**Estimation of phosphate**:Two sets of test tubes (B1 and B2) were taken .To these test tubes 2ml of tris – Hcl buffer 1ml disodium hydrogen phosphate were added .Now 2ml of extract(methanolic extract, ethyl acetate extract) and vehicle under investigation was added .Amount phosphate present was estimated by colorimetric analysis at 530 nm against blank.

**Formula**

*Amount = (sample absorbance /standard absorbance)×standard concentration*

*Percentage inhibition = {(control- test)/control} ×100*

**METHOD2:**

**BY USING SEMI PERMEABLE MEMBRANE[4]**

**Step1: Preparation of experimental kidney stones**

Calcium oxalate stones by homogenous equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10ml of 2N sulphuric acid were allowed to react in sufficient quantity of distilled water in a beaker .The resulting precipitate was calcium oxalate.Equimolar solution of calcium chloride dihydrate in distilled water and di sodium hydrogen phosphate in 10ml of(2N sulphuric acid )was allowed to react in sufficient quantity of distilled water in a beaker . The resulting precipitate was calcium phosphate .Both precipitate freed from traces of sulphuric acid by ammonia solution. Washed with distilled water and dried at 60°c for 4 hours.

**Step2: Preparation of egg membrane:**

Egg was washed and treated with concentrated hydrochloric acid to dissolve the outer shell. Then the inner contents were discarded by making a small incision in the egg membrane and it was washed thoroughly with distilled water.

**Step 3: Estimation of calcium oxalate and calcium phosphate by titrimetry**

Weighed separately 1mg of calcium oxalate and calcium phosphate and 10mg of extract /standard and packed it in together in semi permeable membrane by suturing. This was allowed to suspend in conical flask containing100ml 0.1M tris buffer one group served as negative control (containing only1mg of calcium oxalate and calcium phosphate separately).Placed the conical flask of all groups in an incubator preheated to 37°c for 2hours for about 7-8 hours. Removed the contents of semi permeable membrane from each group into a test tube. Added 2ml of 1N sulphuric acid and titrated with 0.0871N potassium permanganate till a light pink colour endpoint obtained. 0.1 ml of 0.0871N potassium permanganate equivalent to 0.01741of calcium. The amount of undissolved calcium oxalate was subtracted from the total quantity used in the experiment in the beginning to know how much quantity of calcium oxalate actually test substance could dissolve.

**Data analysis:**

Statistical calculations were carried out with the Graph pad Prism 5.00 for Windows. The data are presented as the mean + SEM of five different sets of experiments. The statistical analysis was performed using the Students t-test with $P < 0.05$ being considered significant.Comparisons were made between the test and control of each group and also between *Solanum anguivi Lam.root* extracts and cystone groups

**RESULTS**

**1. PHYTOCHEMICAL SCREENING**

Phytochemical analysis of various extract showed the presence of flavanoid, tannins, steroid, carbohydrates, alkaloid, glycoside, saponin, reducing sugar, amino acid phytochemical screening.

***Table No:1*** **Phytochemical Screening of various extracts**

| Phytochemical | Methanolic extract | Ethyl acetate extract |
|---|---|---|
| Proteins | + | + |
| Flavanoid | + | + |
| Tanins | + | + |
| Carbohydrate | + | + |
| Amino acid | + | + |
| Alkaloid | + | + |
| Triterpenoid saponin | + | + |
| Reducing sugar | + | + |

The yield of Methanolic and Ethyl Acetate extracts was 4.25% and 6.45% respectively. Phytochemical studies shows that both the extract contains flavanoids tannins, terpenoidal saponoins ,carbohydrate , protein, amino acid, reducing sugar, alkaloids.

**2.THIN LAYER CHROMATOGRAPHY**

***Table No:2*** **Thin layer chromatography of various extracts**

| ***Solvent system*** | ***sample*** | ***Number of spot*** | ***Colour with sulphuric acid*** | ***$R_f$ value*** |
|---|---|---|---|---|
| Toluene:ethyl acetate:formic acid(5:3:0.5) | Methanolic extract | 4 | Purple colour | 0.12,0.24,0.52,0.80 |
| Toluene:ethyl acetate:formic acid(5:3:0.5) | Ethyl acetate extract | 4 | Purple colour | 0.17,0.34,0.48,0.75 |

Both the extracts showed four bands of separation . Corresponding Rf values of the bands is presented in table2. Purple color with vanilline sulphuric acid confirmed the presence of steroid.

**3.MACROSCOPIC ANALYSIS**

**Root:**

Yellowish brown colour roots in which striations and root scars are present. It is 1-2.5cm in diameter with a number of secondary roots. Outer surface of root is rough due to presence of longitudinal striations and root scar fracture short splintery and no distinct odour and taste. Root of *Solanum anguivi Lam.* shows thin cork composed of 5-15 layers of thin walled tangentially elongated rectangular cells filled with yellowish brown content cork cambium single layered secondary layered secondary cortex composed of 5-9 layers of thin –walled oval and tangentially elongated cells. Stone cells present in single or in groups of 2-5 or more in this region secondary phloem composed of sieve elements parenchyma much abundant thin walled stone cells present in outer phloem region in singles or in groups of 2-5 varying greatly in shape and size phloem rays1-3 cells wide iso- diametric to slightly radially elongated in inner phloem region and radially elongated in outer phloem region ,occasionally stone cells also found in medullary rays wood occupies stone cells found in medullary rays wood occupies bulk of root and composed of vessels tracheids fibres and xylem parenchyma traversed by xylem rays all elements being lignified vessels occur singly or in groups of 2-5

with simple pits xylem fibres moderately thick walled with simple pits, xylem fibres moderately thick walled with simple pits and pointed ends found in abundance xylem parenchyma have simple pits or reticulate thickening xylem rays uniseriate to biseriate thick walled cells radially elongated and pitted microsphenoidal crystals of calcium oxalate as oxalate as sandy masses and simple starch grains present in some cells of secondary cortex phloem and medullary rays ,simple and rounded to oval starch grains.

**3.*IN-VITRO* ANTILITHIATIC ACTIVITY**

The result of present study clearly indicate the cystone which is a prescribed treatment for urinary for renal calculi which showed a good inhibitory effect on the formation of the precipitate of calcium oxalate and phosphate (Table 3 and4 ).

Methanol extract of *Solanum anguivi Lam.root* was more active than ethyl acetate extract of same plant. Methanolic extract of *Solanum anguivi Lam. root* showed comparable activity to the marketed formulation in terms of inhibiting the formation of phosphate precipitate but showed a significantly better potential in preventing the formation of the calcium oxalate precipitate. (Table 3)

Cystone (as positive control) which is a prescribed for treatment for urinary and renal calculi, showed a good inhibitory effect on the formation of the precipitates of calcium and phosphate (Table 3and 4 and Figure1and 2) *Solanum anguivi Lam.root* extract in Methanol inhibited the precipitation of calcium oxalate and phosphate whiles the same in ethyl acetate caused very little inhibition.

***Table No:3 Comparison of percentage dissolution of calcium oxalate of various extracts of Solanum anguivi lam root with standard drug cystone by Complexometric Titration***

| | **Methanolic Extract** | **Ethylacetate Extract** | **Standard Drug(Cystone)** |
|---|---|---|---|
| **Calcium Oxalate Crystals (Dissolved) (% inhibition)** | 95± 0.01** | 50± 0.016 | 50± 0.025 |
| **Phosphate Crystals (%Inhibition )** | 95±0.053** | 70± 0.014 | 50± 0.01 |

***** Significant difference when cystone is compared with methanolic extract of Solanum anguivi Lam.root. P< 0.01***

***Table No:4 Comparison of percentage dissolution of calcium oxalateof various extracts of Solanum anguivi lam root with standard drug cystone by Using Semipermeable Membrane***

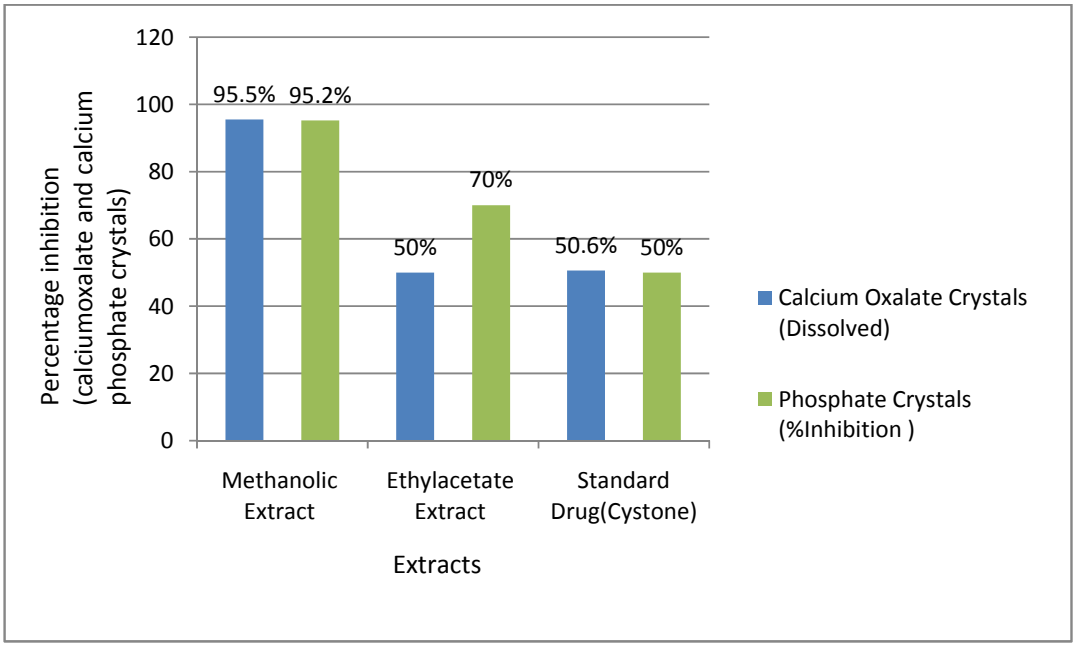
| | Methanolic Extract | Ethyl Acetate Extract | Standard Drug (Cystone) |
|---|---|---|---|
| **Calcium Oxalate Crystals (Dissolved)** | 0.61± 0.01** | 0.50± 0.61 | 0.50 |
| **Calcium Phosphate Crystals (Dissolved)** | 0.93± 0.63*** | 0.70 | 0.92 |

****** Significant difference when cystone is compared with methanolic extract of Solanum anguivi Lam.root. f P< 0.001.***

***** Significant difference when cystone is compared with methanolic extract of Solanum anguivi Lam.root. P< 0.01.***

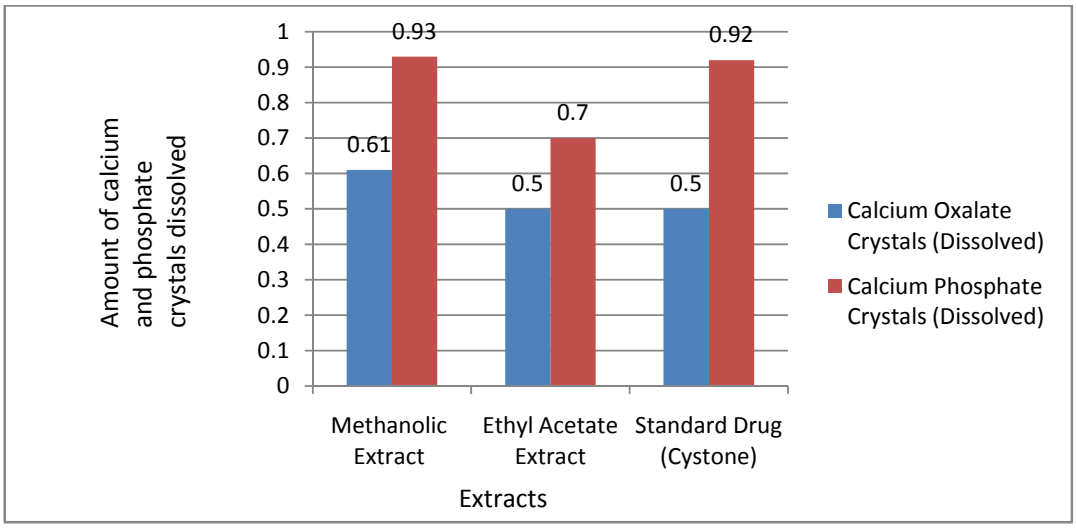
These results*(table no:3)& (table no:4)*shows dissolution of calcium oxalate and calcium phosphate by *in vitro* anti-urolithiatic activity of extracted fraction *Solanum anguivi Lam.root*. Methanolic extract at 1mg concentration produced higher dissolution of calcium oxalate and calcium phosphate as compared to other fraction.

***fig no:1 Comparison of percentage graphical representation of dissolution of calcium oxalate and calcium phosphate crystals vs various extracts of Solanum anguivi with standard drug cystone by Using complexometric titration***



From *the* complexometric titration *(fig no:1)* study results it is observed that methanolic fraction show highest dissolution of calcium oxalate and calcium phosphate in comparison to other fractions.

***Fig no:2 Comparison of percentage graphical representation of dissolution of calcium oxalate and calcium phosphate crystals vs various extracts of Solanum anguivi with standard drug cystone by Using Semipermeable Membrane***




From the Using Semipermeable Membrane*(fig no:2)*study results it is observed that methanolic fraction show highest dissolution of calcium oxalate and calcium phosphate when compared to other fractions.

**DISCUSSION**

The plant possesses numerous biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine. It was reported that most of the plants of *Solanaceae* contain alkaloids, tannins, steroids, saponins, as well as reducing sugars which can be confirmed from qualitative phytochemical tests. Different phytochemicals have been various protective and therapeutic effects which are essential to prevent disease and maintain a state of well being. Extract of root of *Solanum anguivi Lam.root* were analysed for its phytoconstituents. It contains carbohydrates proteins tannins,terpenoidal saponin, amino acid, alkaloid, flavanoids. Flavanoids shows anti oxidant property which place an important role as it is a part of mechanism of dissolving kidney stone[5].

The results of the present study indicate that methanolic extract of *Solanum anguivi Lam.root* exhibit strong antilithiatic activities. The antilithiatic activities observed by complexometric titration and by semipermeable membrane against standard drug cystone, lead us to propose

*Solanum anguivi Lam.root* as promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention of lithiatic diseases. Further studies are needed to explore the potential phenolics compound(s) from *Solanum anguivi Lam.root* and *in vivo* studies are needed for better understanding their mechanism of action.

**CONCLUSION**

Urolithiasis is a most common cause of blood in urine and pain in the abdomen flask or grain. Kidney stone occurs in one in 20 people at some time in their lives for this therapies developed along the principles of western medicine(allopathic )are often limited in efficacy, carry the risk of adverse effects and are often too costly, especially for the developing world. Therfore treating urolithiasis with plant derived compounds which are accessable and do not require laborious pharmaceutical synthesis seems highly attractive .In this research work ,an attempt has been made to compile the reported antilithiatic plants and may be useful to the health professionals scientist and scolars working in the field of pharmacology and therapeutics to develop evidence based alternative medicine to cure different kinds of urolithiasis in man. Isolation and identification of active constituents from these plants ,preparation of standardised dose and dosage regimen can play a significant role in improving the condition of patient. This study suggest that the extract of *Solanum anguivi Lam.root* posses significant urolithiasis activity which might be helpful in preventing or slowing the progress of diseases. Further investigation on isolation and identification of active components in this plant will lead to chemical entities with the potential for clinical research.

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