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Phytochemical and *In-vitro* Anti-urolithiasis Activity of Roots of *Epipremnum aureum* L.

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ABSTRACT:

Approximately 60% of the world's population uses traditional medicines made from medicinal plants. Numerous conventional medications are made from organic materials, minerals, and medicinal plants. The majority of practitioners in Indian medical systems create and administer their own medications. In the present study ornamental plant *Epipremnum aureum* L. roots are screened for anti-urolithiasis activity. The maceration extraction method performed by using ethanol (90%) solvent. Additionally, a phytochemical examination was conducted, which demonstrating the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, tannins & phenolics and carbohydrates. The *in-vitro* anti-urolithiasis activity performed by calcium oxalate dissolution method. The ethanolic roots extract shows calcium oxalate dissolved is 74%. In current research article we found that ethanolic roots extract of *Epipremnum aureum* L. shows significantly anti-urolithiasis activity.

Keywords: Roots, ethanolic (90%), phytochemical, *in-vitro*, anti-urolithiasis.

1. INTRODUCTION:

About 80% of people on the planet utilize herbal medicines for basic healthcare, mostly in developing nations. Their safety, effectiveness, cultural acceptance, and lack of adverse effects have allowed them to endure over time. [(1)] Herbal medications are in high demand right now, and their acceptance is growing daily. Approximately 800 plants have been employed in traditional medical systems, and 500 plants having therapeutic uses have been documented in ancient literature. [(2)] Plants have long been used for therapeutic purposes, and this practice is the basis for a large portion of modern medicine. [(3)]

Epipremnum aureum L. is a member of the *Araceae* family. [(4)] It is also referred to as the devil's lvy, money plant, taro vine, silver vine, golden pothos, and others. As a scrambler shrub, *Epipremnum aureum* L. may scale trees and other plants via its aerial roots. [(5)] This plant eliminates benzene, xylene, and formaldehyde, among other indoor contaminants. [(6)] It is found in all across the world, from China, Japan, India, and Indochina to Northern Australia. The plant has been shown to have anti-cancer, anti-bacterial, anti-helminthic, anti-fungal, analgesic and aphrodisiac properties. [(7)] The existence of strong phytoconstituents that are in accountable for the majority of the pharmacological activity of *Epipremnum aureum* L. is revealed by the phytochemical analysis. [(8)]

Ouron (urine) and lithos (stone) are Greek terms which means urolithiasis. Hard, solid, nonmetallic mineral accumulation in the bladder and kidneys is referred to as urolithiasis. Nephrolithiasis is more prevalent in males and females around the ages of 20 and 40, with a higher prevalence in men (12%) compared to women (6%), according to epidemiological study. [(9)] Among other areas of the urinary tract, stones can form in the ureters, kidneys, bladder, and urethra. Medical diseases such metabolic abnormalities or urinary tract infections, as well as lifestyle choices, food, and genetics, can all have an impact on the formation of stones.[(10)] The most frequent types of stones are calcium oxalate or magnesium ammonium phosphate.[(11)] The present investigation we examine phytochemical & pharmacological characteristics of ethanolic roots extract of *Epipremnum aureum* L.

2. Materials & Methods:

2.1. Collection of plant material:

The fresh roots of *Epipremnum aureum* L. were collected from local area of Pandharpur, Solapur (District), Maharashtra, India during October to December 2024. Herbarium was prepared and authentified by Dr. R. F. Suryvunashi, Head of Botany at Dr. Ganpatrao Deshmukh Mahavidyalaya in Solapur, India. The roots of *Epipremnum aureum* L. was shade dried at room temperature for 2 weeks. The dried parts were later coarsely powdered with help of electric grinder after passed through sieve obtain coarse powder. For additional research, this powder was kept in an airtight container to avoid moisture and contamination.

2.2. Sample preparation for extraction:

The extraction of plant material was carried out by using maceration. Roots powder of *Epipremnum aureum* L. was take 15gm for extraction with solvent 100ml ethanol (90%). Solution sonicated for 20 min after it placed into mechanical shaker at 120rpm for 24hrs. Whatman filter paper no. 1 was used to filter the extract and excess ethanol was then allowed to evaporate by air drying it. (12, 13)

2.3. Qualitative phytochemical analysis:

To determine whether plant materials contain secondary metabolites or phytoconstituents, phytochemical studies were conducted using established method mentioned in "Practical Pharmacognosy, Techniques and Experiments" by Khandelwal K. R. .(14) The phytoconstituents such as alkaloids, flavonoids, glycosides, tannins & phenolics, and carbohydrates were identified. The results are shown in Table 1.

2.4. *In-vitro* anti- urolithiasis activity:

Calcium oxalate dissolution method

Principle:

There are several methods for studying *in-vitro* anti-urolithiasis action, such as titrimetric CaOx measurement, nucleation assay, aggregation assay, turbidimetric assay, ultracal 30 or begostone. (15) The exact principle may vary depending on how the chemical being studied acts. By preventing the formation of stones, several medications have an anti-urolithiasis effect and stop the development of kidney stones. Prevent crystal nucleation or development, for instance, to interfere with the crystallization process. The reduction of stone-forming elements may have an anti-urolithiasis impact by reducing the solubility of crystallizing salts, such as calcium phosphate and calcium oxalate. (16)

Calcium oxalate dissolution method by titrimetric method

Experimental procedure:

Step 1: Preparation of Experimental Kidney Stones (Calcium Oxalate Stones) by Homogenous Precipitation

In two separate beaker 1.34gm of sodium oxalate and 1.47gm of calcium chloride dehydrate were dissolved in 100ml of distilled water and 2N sulphuric acid, respectively. In a one beaker, both were combined in equal amounts to cause calcium oxalate to precipitate. By using an ammonia solution, the precipitate made free form trace of sulphuric acid. After that, the precipitates were cleaned with water from distillation and left to dry at 60^0C for four hours.

Step 2: Using Eggs to Prepare a Semi-Permeable Membrane

To prepare semi-permeable membrane of eggs, A glass rod was used to pierce the top of the eggs, remove calcified shell and its inside components, such as the yolk and albumin. The complete decalcification is achieved by properly washing the empty egg shell with distilled water and leaving it in a beaker with 2M hydrochloric acid for the whole night. After then cleaned with purified water and it was submerged in ammonia solution for a time to neutralize any remaining acid in a wet state. Then it was rinsed with distilled water and kept in the refrigerator with a pH of 7 to 7.4.

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Step 3: Calcium Oxalate Determination by Titrimetric:

To determine the calcium oxalate dissolving percentage, three distinct groups are created:

Group I: Blank, 10ml of calcium oxalate only (Blank)

Group II: Standard, 10ml of calcium oxalate of Calcium oxalate + 10ml of Neeri (Standard)

Group III: Test, 10ml of calcium oxalate + 10ml of *Epipremnum aureum* L. ethanolic roots extract. (Test)

Measure 10ml of calcium oxalate & 10ml of extract, standard and packed in a semi-permeable membrane using a suturing model. Then semi-permeable membrane placed in petri dish containing 100ml of 0.lM Tris HCl buffer. The first group, which contained 10mg of calcium oxalate, acted as a negative control. The second group containing 10mg of crystals of calcium oxalate & 10ml of a standard drug, which serves as a positive control. The third group is made by combining 10ml of crystals of calcium oxalate with 10ml of plant extract. All of the groups in petri dish were kept in an incubator that was warmed to 37 °C for 2hrs for around 7 to 8 hours. Squeezed the semi-permeable membrane contents from each group into the conical flask, 2ml of 1N sulphuric acid titrated with 0.9494N $KMnO_4$ was added until the end point was a light pink colour. 0.1898mg of calcium is equal to 1ml of 0.9494N $KMnO_4$. Calculating the quantity of dissolving calcium oxalate using extracts involves deducting the amount of calcium oxalate that remains undissolved with the total amount. The dissolution percentage was measured. [17]

3. Results & Discussion:

3.1. Qualitative phytochemical analysis:

Table 1: Phytochemical analysis of *Epipremnum aureum* L. ethanolic (90%) roots extract results

Sr.no.	Chemical constituent	Observation
1	Alkaloids	++
2	Flavonoids	++
3	Glycosides	+
4	Tannins & phenolics	+
5	Carbohydrates	++

(+ + strongly Present, + Present)

Alkaloids, Flavonoids, Carbohydrates are strongly present. Glycosides, Tannins and phenolics compounds are slightly present. A significant part of pharmacological action is played by secondary metabolites.

3.2. *In-vitro* anti- urolithiasis activity:

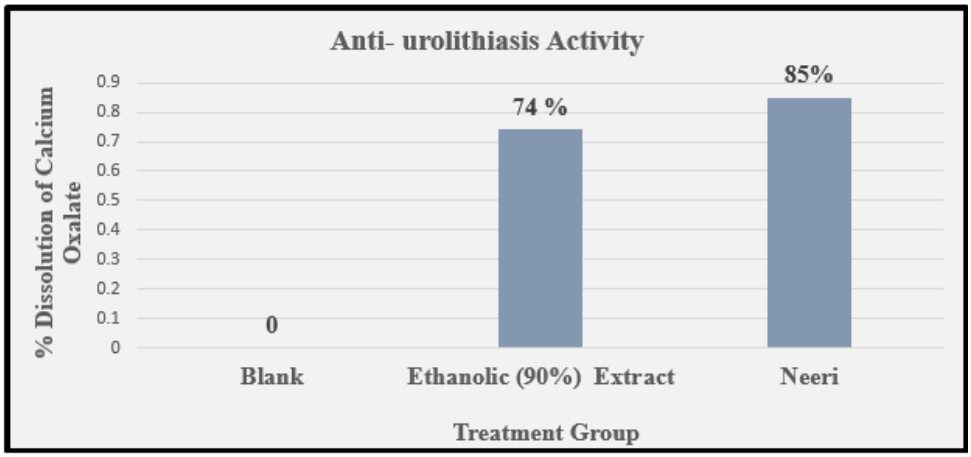
Calcium oxalate dissolution method

Fig. no.1: A. Decalcified of egg shell in 10% acetic acid, B. Egg membrane along with the content suspended into 0.1M Tris buffer.

The percentage of dissolution of the ethanol extract were evaluated based on the amounts of dissolved and undissolved calcium oxalate. The results are recorded in following table.

Table 2: Result of *in-vitro* anti-urolithiasis activity of *Epipremnum aureum* L. ethanolic roots extract

Drug	Undissolved Calcium Oxalate (mg)	% Dissolution
Blank	**0**	**0**
Neeri (Standard)	**1.5**	**85%**
Ethanol roots extract	**2.4**	**74%**



Graph No. 1: Percentage of calcium oxalate dissolution of *Epipremnum aureum* L. ethanolic roots extract

As per the present in-vitro study, the calcium oxalate dissolution by the ethanolic (90%) roots extract was shown to be 74%, whereas the standard medication (Neeri) showed 85% dissolution. In the semi-permeable membrane of eggs, the quantity of undissolved calcium oxalate was found to be 2.6mg for the test sample and 1.5mg for the standard. These results demonstrate that the plant extract exhibits anti-urolithiasis activity nearly comparable to that of the standard medication, Neeri. The percentage of calcium oxalate dissolution of the roots extract, when compared with Neeri, shows that the extract's efficacy is slightly lower than that of the standard drug Neeri.

4. Conclusion:

The study's findings revealed presence different phytoconstituents like alkaloids, glycosides, flavonoids, tannins & phenolics and carbohydrate in ethanolic roots extract *Epipremnum aureum* L. It gives significant anti- urolithiasis activity. The calcium oxalate percentage dissolution found to be 74%. Here we conclude that ethanolic roots extract *Epipremnum aureum* L. can be used to treat urolithiasis.

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Conflict of Interest Statement: All authors have nothing else to disclose.

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