Research Article

In-vitro antiurolithiatic activity of Strychnos potatorum L.F.

Theradum Vareed Binu1,*, Bhavaniamma Vijayakumari1

1Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 641 043, Tamil Nadu, India

* Corresponding author: Theradum Vareed Binu; E-mail: binuabin2011@gmail.com; Tel.: +919496677218

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Abstract

With the passage of time, many problems associated with frequent use of synthetic drugs become prominent, like severe side effects and resistance of microbes against these drugs. On the other side, these drugs are expensive and a large population cannot afford these drugs. In recent times, research on medicinal plants has been intensified all over the world. Kidney stone formation (Urolithiasis) is a complex process that includes many chemical events such as super saturation, nucleation, growth, aggregation and retention of urinary stone constituents within the renal tubules. The currently employed medical management of urinary calculi includes lithotripsy and surgical procedures are expensive and complete recurrence is rare. In this respect, it was noted that there was little attempts reported on in vitro crystallization, growth and dissolution studies of Strychnos potatorum L.f. Strychnos potatorum L.f. is a dry deciduous tree and coming under the family Loganiaceae. Mainly four parts of the plant were taken for the study, leaf, stem, bark and seed. The four parts were extracted with petroleum ether, chloroform, methanol and water in the increasing order of polarity. The treatment of urolithiasis is mainly considered with the dissolution of existing stones and preventing recurrence of stones. The per cent inhibition of turbidity increased with concentration of extract and methanol extract of seed showed maximum dissolution of calcium oxalate stones in vitro.

Keywords: Urolithiasis, Loganiaceae, lithotripsy, Strychnos potatorum L.f.

1. Introduction

Human race is constantly being challenged by many dreadful diseases and it is an uphill task to combat them in the present scenario. Medicinal plants have a promising future because there are about half million plants around the world and most of their medical activities have not yet been investigated that could be decisive in the treatment of present or future studies (Hassan 2012). Kidney stone formation (Urolithiasis) is a complex process that includes many chemical events such as super saturation, nucleation, growth, aggregation and retention of urinary stone constituents within the renal tubules. Three distinct stages can be recognized in the process of stone formation. The first stage involves crystal
nucleation, growth and aggregation. If the size of the stone is small, it will flush out during urination. But, if it is bigger, it will lead to bleeding through urine. Small stones are more likely to pass spontaneously than large ones (Ibrahim et al., 1991). To cope up with alarming situation, the recent exciting development in the medicinal plant based drugs has come as a boon. One of them is the pharmacognosy technique. There are no satisfactory drugs in modern medicine which can dissolve the urinary stone and patients mostly rely on alternative systems of medicine for better relief (Miller et al., 2007). In this respect, it was noted that there was little attempts reported on in vitro crystallization, growth and dissolution studies of Strychnos potatorum L.f. and utilizing bioactive compound of indigenous medicinal plant. It is expected that traditional botanical knowledge on medicinal plants and screening their efficacy by developing novel experimental strategies, incorporating the emerging tools and techniques in phytochemistry, pharmacognosy would help us to generate additional knowledge, provide a better explanation, placement and direction for further research in the area of urolithiasis.

2. Materials and methods
2.1. Collection and identification of plant
The fresh plant materials viz., leaf, stem, bark and seed of Strychnos potatorum L.f. belonging to the family Loganiaceae were collected from Vattaparai, Palakkad , Kerala, India. The plant material was identified by Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, India. In vitro crystallization studies were carried out in the research lab of the Department of Botany, Avinashilingam University for Women, Coimbatore, Tamil Nadu, India.

2.2. Successive solvent extraction
The air dried powdered plant materials were extracted in soxlet extractor successively with petroleum ether, chloroform, methanol and water according to the increasing order of polarity. The different solvent extracts were concentrated by rotary evaporator and then air dried. The extracts were freeze dried and stored in desiccators until further analysis.

2.3. In vitro antiurolithiatic studies
2.3.1. Nucleation assay
Nucleation assay was done by following the method of Hennequin et al. (1993). Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 μL of calcium chloride solution was mixed with 100 μL of herb extracts at different concentrations (100 μg/mL to 1000 μg/mL). Crystallization was started by adding 950 μL of sodium oxalate solution. The temperature was maintained at 37°C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control (Atmani and Khan 2000; Masao et al., 2000). The growth of crystals was expected due to the following reaction:

\[ \text{CaCl}_2 + \text{Na}_2\text{C}_2\text{O}_4 \rightarrow \text{CaC}_2\text{O}_4 + 2\text{NaCl} \]

2.3.2. Aggregation assay
Aggregation assay done by following the method of Atmani et al. (2004). “Seed” CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. The solution was equilibrated to 60°C in a water bath for 1 h and then cooled to 37°C overnight. The crystals

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were harvested by centrifugation and then evaporated at 37°C. CaOx crystals were used at a final concentration of 0.8 mg/mL, buffered with tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37°C in the absence or presence of the plant extract after stopping the stirring. The percentage aggregation inhibition rate (Ir) was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using the following formula

\[
Ir = (1 - \frac{\text{Turbidity}_{\text{sample}}}{\text{Turbidity}_{\text{control}}}) \times 100
\]

3. Results and discussion

3.1. CaOx crystal nucleation

CaOx crystallizations without (control) and with extract in different concentrations were recorded (Fig.1). The percentage inhibition of turbidity (aggregation) in the presence of herb extract was lower than the control, showing fewer crystals with less aggregation. The inhibition aggregation increased with increasing concentration of extract and it was greatest in methanol extract of seed compared to other extracts (Fig.2).

![Micrograph of CaOx crystal nucleation of methanol seed extract of Strychnos potatorum L.f.](image)

1; in the absence of plant extract (control); 2; 100μg/mL concentration; 3; 200μg/mL concentration; 4; 300μg/mL concentration; 5; 400μg/mL concentration; 5; 500μg/mL concentration

Herbal extracts may contain substances that inhibit the growth of CaOx crystals, the property of plants that may be important in preventing kidney stone formation. CaOx crystals induced by urinary macromolecules are less tightly bound to epithelial cell surfaces, which are then excreted with urine. Barros et al. (2003) reported the inhibitory effect of Phyllanthus niruri, on calcium oxalate crystal
growth and suggested that extract may interfere with early stages of stone formation. Farooq (2004) reported that the seed extract of Dolichos biflorus showed more in vitro antilithiatic activity, while comparing with Berginia ligulata. The in vitro assays performed in the different extracts of Crateva magna (petroleum ether, chloroform, methanol and aqueous) at various concentrations possess inhibitory effects against crystal nucleation and aggregation (Radha 2015).

3.2. Nucleation assay

The findings of the present study indicated that extracts from the plant parts inhibited the crystallization of CaOx in solution. There were less and smaller particles with increasing concentrations of extract as shown in various micrographs. The increasing concentration of plant extracts (100, 200, 300, 400 and 500 µg/mL) had inhibited the CaOX crystal growth. The inhibition aggregation increased with concentration of extract and it was greatest with methanol extract of seed as it showed maximum dissolution of crystals. It may be due to the bioactive component present in the methanol extract of seed.

3.3. Aggregation assay

Comparing the activities of the four different extracts namely, leaf, stem, bark and seed, the greater capacity to reduce all the crystallization process was shown by methanol seed extract with a concentration of 500 µg/mL. The higher concentrations of herb extracts were associated with fewer crystals and the size decreased proportionally. The present findings coincide with Barros et al. (2003) who reported that in the in vitro studies the plant extract of Phyllanthus niruri reduced CaOx crystal aggregation.

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**Fig. 2.** Effect of different concentrations of extracts of Strychnos potatorum L.f. on CaOx crystal nucleation.

1; leaf extract, 2; stem extract; 3; bark extract; 4; seed extract; 5
The results of the study were on par with the inhibitory effect of the root of *Rotula aquatica* as investigated against urinary crystal and reported the *in vitro* efficiency of the extract (Chauhan et al., 2011). Radha (2015) had also observed the *in vitro* inhibitory potency of different extracts of *Crataeva magna* aqueous bark extract on various stages of formation and growth of calcium oxalate crystals and reported the efficacy of the aqueous bark extract. The inhibitory activity of aqueous leaf extract of *Kalanchoe pinnata* on the nucleation of calcium oxalate crystals and the rate of aggregation in calcium oxalate crystals was determined by Phatak and Hendre (2015).

4. Conclusions

The treatment of urolithiasis is mainly considered with the dissolution of existing stones and preventing recurrence of stones. The pathogenesis of calcium oxalate stone formation is a multistep process and includes nucleation, crystal growth and crystal aggregation. The per cent inhibition of turbidity increased with concentration of extract and methanol extract of seed showed maximum dissolution of calcium oxalate stones *in vitro*. The result of the present study proved that methanol extract of seed of *S. potatorum* L.f. can be used for the further pharmacopeial preparations.

Conflict of interest statement

We declare that we have no conflicts of interest.

References