Evaluation of Antiurolithiatic Potential of Standardized *Asteracantha longifolia* Linn aerial Parts

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**Abstract**

Background

The formation of kidney stones is a very painful disease that follows a complex process which results from serious physiochemical events in the kidney. The treatments include surgical removal, extracorporeal shock wave lithotripsy and drug treatment in Western medicine. In Sri Lanka, decoction prepared from the roots of *Asteracantha longifolia* Linn is used as a remedy for urolithiasis. If we use the roots continuously, it may wipe off *A. longifolia* from the site. Therefore, an attempt was made to evaluate the antiurolithiatic potential of standardized *A longifolia* Linn aerial parts.

Methods

Aerial parts of *A. longifolia* were standardized according to WHO guidelines. The effects of aqueous extract (100, 500, 1000 and 2500 µg/ml) of *A. longifolia* aerial parts or the reference drug, stonil (100, 500, 1000 and 2500 mg/ml) was studied by the measurement of turbidity in presence or absence of extract at 620 nm using a spectrophotometer.

Results

The percentages of total ash, water soluble ash, acid insoluble ash, hot water extractable matter, hot ethanolic extractable matter, cold water extractable matter and cold ethanolic extractable matter were as follows for aerial parts: 18.1±0.3, 9.3±0.1, 0.02±0.00, 32.3±0.3, 27.8±0.3, 19.8±0.3, 3.4±0.0 respectively. Moreover, compared to the reference drug stonil, aqueous extract of *A. longifolia* aerial parts exhibited significantly higher antiurolithiatic activity at a dose of 2500 µg/ml.

Conclusion

Hot aqueous extract of *A. longifolia* aerial parts can be used to treat urolithiasis.

**Keywords**

*Asteracantha longifolia*; standardization; antiurolithiatic activity; alternative medicine

**Introduction**

Urolithiasis or kidney stone formation is a complex process including super saturation, nucleation, growth, aggregation, and retention within the kidneys. The formation of kidney stones is a very painful disease that has afflicted a wide sector of human population since ancient times [1]. The overall probability of forming stones differs in various parts of the world, and is estimated at 1-5% in Asia, 5-9% in Europe, and 13% in North America. The recurrence rate of renal stones is 75% in a 20 year span [2].
Though it is not a life threatening disease, most of the times, the discomfort is intolerable. It is more prevalent between the ages of 20 and 40 in both sexes [3].

Surgical removal and extracorporeal shock wave lithotripsy are the most common ways to treat urinary stones. However, stone recurrence is very common with these methods. Urolithiasis can also be treated with Western drugs and many of these drugs produce a number of metabolic adverse effects that limit their long term use [2]. Thus, herbal medicines are efficacious and have lesser side effects compared to modern medicines and also reduce the recurrence rate of renal stone. Therefore, it is valuable to search for alternative treatment strategies to treat urolithiasis.

*Asteracantha longifolia* Linn (Family: Acanthaceae) is a perennial herb with an ascending rhizome. Scientific investigations have been shown that anti-tumor [4], anti-inflammatory and analgesic [5], anti-diabetic [6, 7] and hepatoprotective [8] activities of *A. longifolia*. It is widely distributed in Asian countries and used by local population for different medicinal purposes. The burnt ash of the plant with cow urine is given for oedema and dropsy. The seeds are given for gonorrhoea, jaundice, anasarca and to serve as an aphrodisiac and used externally as a poultice or embrocation for rheumatism. In Sri Lanka, decoction prepared from the roots of *A. longifolia* is used as a remedy for urolithiasis. Therefore, if we use the roots continuously, it may wipe off *A. longifolia* from the site. Therefore, in the present study, an attempt was made to evaluate the antiurolithiatic activity of aerial parts of *A. longifolia* grown in Sri Lanka.

**Collection of Plant Material**

*A. longifolia* plants were collected from paddy fields of Western Province, Sri Lanka during the period of March to May 2014. The plant material was identified and authenticated by Senior Scientist, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka. The aerial parts were separated, cut into small pieces, air dried for 3-5 days and made into fine powder using a grinder. The powdered *A. longifolia* aerial parts were stored separately in air tight container until use.

**Standardization of Aerial parts of Asteracantha longifolia**

Total ash content, acid insoluble ash content, water soluble ash content and extractable matter (both hot and cold) were determined according to the WHO guideline [9] using *A. longifolia* aerial parts.

**Preparation of Aqueous Extracts using Aerial parts of Asteracantha longifolia**

Sample (~10 g) was taken into a conical flask and distilled water (100 mL) was added. The contents were shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask, boiled gently for 2 h and allowed to cool and filtered rapidly using a dry filter paper (Qualitative filter paper, 90 mm Diameter Whatman®). Then the filtrate was transferred to a round bottom flask and evaporated to dryness under the reduce pressure (at 700C) using a rotor vapor and stored at 40C until use.

**Evaluation of Antiurolithiatic Activity for Aqueous extract of Asteracantha longifolia Aerial parts**

**Preparation of Artificial urine**

The artificial urine (AU) was prepared according to the method described by Srinivasa and co-workers [10] with slight modification and the following chemicals were added to prepare 200 ml of AU. Sodium chloride 105.5 mM, Sodium phosphate 32.3 mM, Sodium citrate 3.21 mM, Magnesium sulfate 3.85 mM, Sodium sulfate 16.95 mM, Potassium chloride 63.7 mM, Calcium chloride 4.5 mM, Sodium oxalate 0.32 mM, Ammonium hydroxide 17.9 mM, and Ammonium chloride 0.0028 mM. The AU was prepared freshly each time and pH was adjusted to 6.0.

**Study without Inhibitor (control test)**

A volume of 1.0 ml of AU was transferred into the cell containing 0.5 ml of distilled water and blank reading was taken. Then 0.5 ml of 0.01M sodium oxalate was added to the previous cell and the absorbance was measured for a period of five minutes at 620 nm.

**Study with Inhibitor**

The aqueous extract of aerial parts of *A. longifolia* was dissolved in distilled water, filtered through membrane filter and the concentrations of 100, 500, 1000 and 2500 mg/ml were obtained separately. A mixture of 1 ml of AU and 0.5 ml of plant extract was added to the cell and reading was taken. Then 0.5 ml of 0.01M sodium oxalate solution was added to the same cell and the absorbance was measured for a period of five minutes at 620 nm. The percentage of inhibition of calcium oxalate crystal formation was calculated using the following formula:
Estimation of extractive values determine the material. ISSN:XXXX-XXXX SFJHM, an open access journal was very low which indicate the purity of the A. longifolia material [11]. Acid insoluble ash content of aerial parts of mainly silica and indicate contamination with earthy A. longifolia. In the present study, water soluble ash of aerial parts of amount of inorganic compounds present in the drugs [11]. Physico-chemical parameters of A.longifolia the powder of aerial parts. Physico-chemical extractable matter (both hot and cold) were investigated for such as total ash, acid insoluble ash, water soluble ash and in the present study, we investigated the drug. Physico-chemical parameters of A. longifolia aerial parts were illustrated in Table 1. The water soluble ash is used to estimate the amount of inorganic compounds present in the drugs [11]. In the present study, water soluble ash of aerial parts of A. longifolia was 9.3% + 0.1. Acid insoluble ash consist mainly silica and indicate contamination with earthy material [11]. Acid insoluble ash content of aerial parts of A. longifolia was very low which indicate the purity of the material.

Estimation of extractive values determine the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. Thus, these values are representative of the polar or non-polar extractable compounds in a plant material [13]. According to the results, aerial parts of A. longifolia contain high content of cold water extractable matter and low content of cold ethanolic extractable matter. This indicates the presence of more polar compounds in aerial parts of A. longifolia. In the present study, we used a classical model of synthetic urine supersaturated with calcium chloride and sodium oxalate to determine the growth and aggregation of CaOx crystals. The normal urine in the human is not a static solution, as new solutes are constantly being added and subtracted from the solution. However, it is difficult to mimic the urinary tract in vitro, but the growth of crystals in synthetic urine in a static environment can be useful to some extent for explaining the growth of urinary calculi in humans [2]. Roots of A.longifolia are used as a remedy for urolithiasis in traditional medicinal systems of Sri Lanka. Continuous usage of the roots and growing demand for A. longifolia may cause threat for the plant. Therefore, it is a necessity to find possibilities to use leaves or stems or any other part as alternatives to tubers and roots for medicinal purposes.

In the present study, we investigated the antiurolithiatic activity of A. longifolia aerial parts (Table 2) and compared the efficacy with a reference drug, stonil (Table 2). Aqueous extracts of A. longifolia aerial parts showed significant (P≤0.05) antiurolithiatic activity and the effect was significantly (P≤0.05) higher than that of the positive control, stonil. Stonil consist of number of plant extracts and herbal formula; Gokshur extract, Punarnava extract, Kulathi extract, Varun extract, Mooli extract, Palashpuspa extract, Sunthi, Pashanabhed, Shuddha Shilajit, Shuddha Guggulu, Yavakshar, Hajarul Yahud Pishhi and Shwet Parpati. However, antiurolithiatic activity of aqueous extracts of A. longifolia aerial parts (r²=0.342) were not dose dependent. In this assay, absorbance of tested concentrations of A. longifolia extracts and stonil were measured to detect whether there was any interference due to the color at measured wave length. However, there was no marked interference for the absorbance due to the color of the extract or stonil.

In conclusion, aqueous extract of A. longifolia aerial parts exhibited potent antiurolithiatic activity, for the

\[
\text{% inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100\%
\]

Study with Positive Control (stonil)

Stonil tablets were dissolved in distilled water and kept for 24 h, filtered through membrane filter and the concentrations of 100, 500, 1000 and 2500 mg/ml were obtained separately. A mixture of 1 ml of AU and 0.5 ml of stonil was added to the cell and reading was taken. Then 0.5 ml of 0.01M sodium oxalate solution was added to the same cell and the absorbance was measured for a period of five minutes at 620 nm. The percentage of inhibition of calcium oxalate crystal formation was calculated by using above formula.

Statistical Analysis

All experiments were done in triplicate. Results were reported as Mean values ± SEM. Statistical analysis of the differences between mean values obtained for the experimental groups were done by Non parametric test of Mann- Whitney test. P values of ≤ 0.05 were considered as significant.

Results and Discussion

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and always suppose to be safe [11]. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. Medicinal plants being natural, non-narcotic, having less side effects, preventive and curative therapies which could be useful in achieving the goal of “Health for all” in a cost effective manner [12].

In the present study, physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash and extractable matter (both hot and cold) were investigated for the powder of A.longifolia aerial parts. Physico-chemical parameters of A. longifolia aerial parts were illustrated in Table 1. The water soluble ash is used to estimate the amount of inorganic compounds present in the drugs [11]. The study was carried out in a static environment so that new solutes are constantly being added and subtracted from the solution. Calcium oxalate crystal formation was calculated by using above formula.

\[
\text{Absorbance of control% inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100\%
\]
Table 1: Physico-Chemical Parameters of Asteracantha longifolia Aerial parts

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amount in aerial parts (%, dry weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>18.1 ± 0.3</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>9.3 ± 0.1</td>
</tr>
<tr>
<td>Hot Water Extractable Matter</td>
<td>32.3 ± 0.3</td>
</tr>
<tr>
<td>Hot Ethanol Extractable Matter</td>
<td>27.8 ± 0.3</td>
</tr>
<tr>
<td>Cold Water Extractable Matter</td>
<td>19.8 ± 0.3</td>
</tr>
<tr>
<td>Cold Ethanol Extractable Matter</td>
<td>3.4 ± 0.0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM., n=6

Table 2: Effect of Aqueous Aerial parts Extract of Asteracantha longifolia or Reference Drug, Stonil on Calcium Oxalate Crystallization

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Inhibition (%) of calcium oxalate crystallization</th>
<th>Parameters</th>
<th>Amount in aerial parts (%, dry weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min</td>
<td>2 min</td>
<td>3 min</td>
</tr>
<tr>
<td>2500 µg/ml of A. longifolia</td>
<td>73.53</td>
<td>45.09</td>
<td>28.61</td>
</tr>
<tr>
<td></td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.01*</td>
</tr>
<tr>
<td>2500 µg/ml of stonil</td>
<td>22.08</td>
<td>18.35</td>
<td>10.04</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1000 µg/ml of A. longifolia</td>
<td>50.98</td>
<td>45.57</td>
<td>35.87</td>
</tr>
<tr>
<td></td>
<td>0.00*</td>
<td>0.00</td>
<td>0.00*</td>
</tr>
<tr>
<td>1000 µg/ml of stonil</td>
<td>18.33</td>
<td>10.08</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>500 µg/ml of A. longifolia</td>
<td>50.98</td>
<td>36.42</td>
<td>31.34</td>
</tr>
<tr>
<td></td>
<td>0.00*</td>
<td>0.01*</td>
<td>0.01*</td>
</tr>
<tr>
<td>500 µg/ml of stonil</td>
<td>13.33</td>
<td>12.50</td>
<td>11.69</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>100 µg/ml of A. longifolia</td>
<td>53.92</td>
<td>48.94</td>
<td>41.62</td>
</tr>
<tr>
<td></td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.01*</td>
</tr>
<tr>
<td>100 µg/ml of stonil</td>
<td>0.42</td>
<td>0.81</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM., n=6
*Significant when compared to the respective dosage of reference drug, stonil; P ≤0.05
first time and A. longifolia would be a good candidate for future drug development. In addition, established physico-chemical parameters can be used as a tool to standardize A. longifolia aerial parts.

References


