Standardization of *etti kottai* (*Strynous nux-vomica*) *suthimurai* a Siddha detoxification method by estimating the total alkaloid content at different stages

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Abstract

Potentially toxic medicinal plants and mineral drugs have an important role in Siddha medicine preparation. Since these drugs produce side effects to humans their toxic principles have to be reduced for using in medicines. To overcome this, siddhars found a specific *Suthimurai* called detoxification procedures which are practiced right from ancient period. These procedures are to be scientifically standardized. These detoxifications procedures have made the acceptability of drugs for many internal medicine preparations. *Strychnous nux-vomica* seeds are widely used in neurological disorders. In Siddha medicine, it is an important ingredient of medicines used in treating *vatha* disease. The *Strychnous nux-vomica* seeds were collected and detoxified according to Siddha literature. Total alkaloid content of the detoxified seeds was quantified at every stage of the process for developing and understanding the scientific rationale behind the detoxification process. Compared to unprocessed seeds the total alkaloid content of detoxified seeds was reduced from 3.28mg to 0.90mg which indicates decrease in toxic alkaloidal content of the seeds with increased therapeutic potency.

Keywords: Siddha medicine, medicinal plants, *Strychnous nux-vomica*, alkaloidal content.

Introduction

The Siddha medicine is an ancient traditional medicine followed in India. It works with the three humors *Vatha, Pitha* and *Kapha* which are the root cause of the disease. Usually medicines are formulated in the view of eradicating the root cause to get rid of particular disease. In Siddha literature, sage *Yugimunni* classified *vatha* diseases into 84 types mostly associated with muscular and neurological problems. *Strychnous nux-vomica* seeds used in internal medicine preparations like *rasagandhi melugu*, *ramapana melugu* commonly used for treating cancer and external medicine preparations like *Vida Mutti Thilam* and *Ilaguvudumutti Thilam* useful in pain management. *Strychnos nux-vomica* is an evergreen tree native to South East Asia and India belonging to family *Loganiaceae*. It is a medium size tree found mostly in hills. The seeds have to be processed in order to reduce or eliminate the toxic effects of alkaloids existing in the seeds. It is possible that the toxic components are chemically changed after the *suthimurai* process such as heating and soaking while the therapeutic activities of the seeds remain same(1). At low dose level it is used as stimulant, laxative and for the treatment of other stomach ailments but in higher doses it is toxic. Pharmacologically *Strychnos nux-vomica* showed anticancer, antimicrobial, anti-inflammatory, antioxidant...
(2). The seeds are mainly used as aphrodisiac, appetizer, anti-periodic, digestive, purgative, and stimulant. Further the seeds are also used in the treatment of anemia, asthma, bronchitis, intermittent & malarial fever and in weakness of extremities (3). It is also reported that nux-vomica seeds in large doses, producing tetanic convulsions and eventually death and in lesser doses it may manifest mental derangement (4). Strychnine (C21H22O2N2; m.p. 286 to 2880 C) and brucine (C23H26O4N2; m.p. 1780 C) have been reported as the most important and strongly toxic alkaloids present in this, besides other minor alkaloidal constituents (5). So it is mandatory to purify or properly process Nux-vomica seeds prior to its administration in therapeutics. The purpose of this study was to evaluate the role of detoxification on the quantitative reduction of total alkaloids of Strychnos nuxvomica

Materials and Methods

Collection of the raw drug

Matured and ripened etti kottai (Strychnos nuxvomica) fruits were collected from the Kalvarayan hills, in Tamilnadu, India during the month of November and were botanically authenticated by botanist at Sivaraj Siddha medical college, Salem and sample specimen were kept in the museum for future reference. Seeds were removed from the fruit pulp, thoroughly washed with water and dried in shade.

Selection of seeds

The dried seeds were first dropped in a vessel containing water and the seeds which floated on the surface of water, found broken, difference in color were rejected and the seeds which were settled at the bottom of the beaker were selected for detoxification process.

Suthimurai [Detoxification] of Strychnous nux-vomica seeds

Suthimurai (detoxification) process was carried out in Gunapadam Lab, Sivaraj Siddha medical college. Detoxification was made by boiling the seeds with nel (Oryza sativa) and soaked in Serukrai juice (Amaranthus polygonoides), then outer seed coat was peeled off and boiled in cow milk. The process was repeated three times with fresh cow milk and the seeds were dried. All the stages of samples were separated and subjected to chemical evaluation for total alkaloids and moisture content (6).

Estimation of moisture content

Strychnous nux-vomica seeds and petri-plates weights were taken separately. The Petri-plates were incubated in the oven for 24 hours at 105°C. The samples were removed from the oven and cooled to room temperature. Again the weights of the raw material along with the Petri-plates were measured. Moisture content was calculated by using the standard formula (7).

Preparation of extracts

The 100 gm of nux-vomica seeds was ground and then extracted with methanol for 24 hours in a continuous extraction using Soxhlet apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C to dryness (7).

Qualitative estimation (Test for alkaloids)

Presence of alkaloid was confirmed by Dragendorff’s method. A part of extract was dissolved in dilute HCL and 2 drops of Dragon drops was added. The formation of crystalline precipitate indicates presence of alkaloid. The sample which showed positive alkaloid was then subjected to further quantitative evaluation (7).

Determination of Total Alkaloid

To this extraction, 6 ml dilute ammonia is added, and the flask is shaken for 1 h and allowed to stand for 8 h with occasional shaking. To this, 10ml water is added and the mixture is shaken vigorously. When the drug has settled, 100 ml of the solution is drawn out and filtered into a separator and washed with a few milliliters of a mixture of solvent ether and chloroform, and shaken with 0.5N sulfuric acid for complete extraction of alkaloids. The combined acid extract is filtered and made alkaline with dilute ammonia solution. The alkaloids are liberated with chloroform and washed with chloroform. The chloroform washes and the extract are poured in a conical flask. The chloroform is distilled off and the solvent is removed completely in a vacuum desiccator. 5 ml of alcohol is added to the residue, and the solvent is again removed. The evaporation with alcohol is repeated and the residue is dried to constant weight in vacuum desiccator and weighed as total alkaloids (7).

Results and Discussion

The total alkaloidal content drastically reduced from 3.26 to 0.92 by the purification and variations in different stages were enumerated. Boiling in Oryza sativa reduced it from 3.26 to 2.74 mgs whereas Amaranthus polygonoides reduced it from 2.76 to 1.79mgs. Cow milk initially reduced it to 1.25, followed by 1.02 and finally to 0.92. The peeled out seed coat had alkaloid content of 0.27 mgs. The moisture content was improved from 13.18 to 21.28 initially followed by 20.05 and finally it was 26.15. The pH of the liquids used was acidic in nature and was altered by the process, which was further justified by dissolved solvent and its concentration. The study clearly demonstrated the reduction in total alkaloid content of the detoxified seeds to be minimal compared to the unprocessed seeds with...
an increase in moisture content and the process was stable at alkaline pH. The results were obtained based on three times repeated analysis of the processed seeds.

Table 1 Total alkaloids analysis

<table>
<thead>
<tr>
<th></th>
<th>Unprocessed seeds</th>
<th>Nel(oryza sativa)</th>
<th>Serukeerai(Amaranthus polygoniodes)juice</th>
<th>Cow Milk</th>
<th>Cow Milk</th>
<th>Cow Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST ANALYSIS</td>
<td>3.28</td>
<td>2.74</td>
<td>1.79</td>
<td>1.25</td>
<td>1.02</td>
<td>0.90</td>
</tr>
<tr>
<td>SECOND ANALYSIS</td>
<td>3.26</td>
<td>2.84</td>
<td>1.72</td>
<td>1.04</td>
<td>1.04</td>
<td>0.92</td>
</tr>
<tr>
<td>THIRD ANALYSIS</td>
<td>3.28</td>
<td>2.76</td>
<td>1.70</td>
<td>1.15</td>
<td>1.15</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Values in mg%

Conclusion

This study clearly confirms that this particular detoxification process is an effective method for reducing the toxicity of etti kottai (Strychnous nux-vomica). The Suthimurai detoxification method made the seeds suitable for medicine preparations providing increased therapeutic value and it was found to be easy and cost effective method. Further studies on individual alkaloids are hopeful to determine.

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