Project Report

Studies on purification and detoxification (sodhana prakriya) of toxic Ayurvedic medicinal plants.

Principal Investigator
K S Laddha

Institute
Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai

Supported by
Department of AYUSH, Ministry of Health & Family Welfare, Govt. of India, New Delhi
Project Title: Studies on purification and detoxification (sodhana prakriya) of toxic Ayurvedic medicinal plants.

1. Principle Investigator: K S Laddha  
   Address: Professor in Pharmacognosy and Phytochemistry,  
            Department of Pharmaceutical Sciences and Technology,  
            Institute of Chemical Technology, Nathalal Parikh Marg,  
            Matunga, Mumbai- 400019

2. Co-Investigator: Dr. P B Pimpalgaonkar  
   Address: Government Ayurvedic College,  
            Tuljapur Road, Madhuban, Osmanabad

3. Other Scientific Staff engaged in the Study: Manasi P Nabar (SRF)  
4. Non Scientific Staff engaged in the study: NA

5. Date of Start: 16th July 2009

6. Duration: 2 years

7. Objectives of Proposal: The objective of the proposed project is to study the chemical changes occurring during classical sodhana prakriya, which are used to make toxic medicinal plants into therapeutically active drugs in Ayurveda. As per the recommendation of the PEC Committee at CCRAS, work has been undertaken on five plants namely Langli, Vacha, Bhallatak, Gunja and Vatsnabha under the said project.

8. A] Methodology followed till end of period of reporting:

   1) Abrus precatorius seeds (Gunja)  

   Abrus precatorius Linn. (Leguminosae) seeds synonymously called Crab’s eye or Jequirity are also known by the common names gunja, rati and gunchi. Abrus precatorius is a plant used in the Ayurvedic system of medicine. In the indigenous system of medicine, seed extracts are used externally for the treatment of ulcers and skin affections. The seeds have also been used internally in the affections of the nervous system and their paste is applied locally in sciatica, stiffness of shoulder joints and paralysis. They are said to be useful in diarrhea, dysentery and possess anthelmintic activity. The seeds contain a toxic principle called abrin. Abrin is a toxalbumin similar to ricin found in castor seeds. A hemagglutinin and a glucoside abralin have also been reported in A. precatorius seeds. Hence the seeds have been subjected to the Ayurvedic sodhana prakriya for detoxification.

The study is related to the Abrus precatorius seeds having scarlet colour with a black spot round the hilum. The seeds of this plant are poisonous. Abrus seeds were collected from the local market of Mumbai. Seed sample was authenticated by Dr. H M Pandit, Botanist, Guru Nanak Khalsa College, Mumbai.

Sample preparation: The process of sodhana was carried out on 3 samples, namely the entire seed, separated seed coat and the embryo to ascertain the localization of the hemagglutinin in the seeds. Abrus precatorius seeds (Gunja), seed coats and embryos were then subjected to the subsequent procedures. 1g of the seed sample was macerated in 10ml of water for 1 day at room temperature. The sample was filtered and NaCl equivalent to 0.9%w/v was added to the filtrate and filtered through a 0.2 micron filter. The filtrate so obtained was used for the study. Pre as well as post-sodhit samples were subjected to the same procedure.
Ayurvedic sodhana prakriya for Gunja: The entire seeds (Gunja), seed coats and embryos were steamed using kanjika (rice gruel) in a dolayantra (round bottom vessel) for 1 prahar (3 hours) Fig.1. Subsequently the seed coats and embryos from the intact seeds were separated, washed with warm water and dried. The dried samples were then treated in a similar manner as that of the pre-sodhit samples and then subjected to hemagglutination assay.

With an aim to find an alternative to the traditional sodhana prakriya, studies were also carried out using water as the medium for the sodhana prakriya in place of kanjika. In both the media studies were carried out in two conditions, with the seeds immersed in the medium (boiling) and with the seeds exposed to the steam produced by the medium (steaming).

Fig. 1 Dolayantra used for the process of swedana (steaming of the drug material).

Haemagglutination assay:
Rat (Sprague Dawley) blood was used for the haemagglutination assay procedure. Procedure for obtaining Washed Red Blood Cells (RBC’s): 2 ml of rat blood was collected in Appendorff tubes containing 0.2 ml of 5% EDTA solution to prevent clotting of blood. It was centrifuged at 3000rpm for 15 min to remove soluble blood constituents. Plasma was separated and replaced with equal volume of normal saline. The re-suspended components were further washed (centrifuged and re-suspended alternately) four times using normal saline to obtain washed RBC’s. The washed RBC’s were finally suspended in normal saline to obtain a concentration of 4% v/v. A 24 well plate was used to carry out the assay.

Assay: 0.2ml RBC suspension was added to each well along with 0.2ml of the sample. A negative control (blank) was maintained using 0.2ml of saline in place of the sample. The plate was incubated at 37± 2°C for 2 hours. Plates were read manually at the end of the incubation period. The assay was carried out in triplicates.

9.  A] Interim modification of objectives/methodology, if any: Not Applicable

10.  A] Summary on progress (during the period of report):

The presence of agglutination in the blood samples is an indication of the presence of haemagglutination factor in the sample. The results stated below are with reference to rat blood samples.

Column 1 and 2 represents post-sodhit samples treated using kanjika as the medium. Column 3 and 4 represents post-sodhit samples treated with water as the medium. Column 5 represents the
blank. Column 6 represents the pre-sodhit samples. Samples in column 1 and 3 were steamed with the medium whereas those in column 2 and 4 were boiled in the medium. (Table 1)

Row A: seed coats separated pre-treatment,
Row B: embryo separated pre-treatment,
Row C: seed coat separated post-sodhana prakriya,
Row D: embryo separated post-sodhana prakriya.
Well 6 C represents the pre-sodhit entire seed sample.
Wells 5B, 5C, 5D and 6D are empty wells without any samples.

Well 5A contains the blank with an equivalent amount of saline in place of the sample and this was used as the negative control for the experiment.

Pre-sodhit seed coat and entire seed samples did not cause agglutination. The pre-sodhit embryo sample caused high degree of agglutination. All the post-sodhit samples did not show agglutination. (Fig.2)

The results obtained with both the media, namely kanjika and water as well as in both the conditions; boiling and steaming were the same. All the post-sodhit samples did not show signs of agglutination.

Table 1 Table showing hemagglutination activity of different samples of Gunja (Abrus precatorius seeds)

<table>
<thead>
<tr>
<th></th>
<th>Post-sodhit samples</th>
<th>Blank</th>
<th>Pre-sodhit samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanjika</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 steamed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Boiled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 steamed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 boiled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig.2 Agglutination studies in the various pre and post-sodhit samples of Gunja (Abrus precatorius seeds) and arrow indicating the well in which agglutination has occurred.
Fig. 3A Photographs of samples showing the seeds (X), seed coat (Y) and embryo (Z) prior to the *sodhana prakriya*.

Fig. 3B Photographs of samples showing the embryo and seed coats post *sodhana prakriya*. The figure represents columns 1, 2, 3& 4 and rows A, B, C& D of table 1. Labeling as per Table 1.
Discussion

The principle toxic constituent of *Gunja* is abrin, a toxalbumin and hemagglutinin. Abrin is a highly toxic protein present in the seeds to an extent of about 0.15 per cent. Abrin consists of two polypeptide chains A and B linked by a disulphide bond. The toxic action of abrin is associated with the A-chain and that the B-chain functions as a “carrier” moiety necessary for binding of the toxin to the cell surface. Abrus agglutinin is a bivalent tetramer of 134,900 Daltons. The toxic effects seen after ingestion of *gunja* are hemorrhagic gastroenteritis with erosions, hemolysis, acute renal damage, hepatotoxicity with elevated liver enzymes and seizures.

*Gunja* contains an agglutinating factor that is responsible for the agglutination of the erythrocytes from the blood. The screening of the pre-sodhit samples of seed coat, embryo and the entire seed led to the conclusion that only the embryo contained the hemagglutinating factor. The hemagglutination assay carried out using the various post-sodhit samples indicated that the treatment, steaming or boiling with *kanjika* or with water led to the destruction of the hemagglutinating factor, the factor being protein in nature. The sodhana procedure prescribed for the detoxification of *Gunja* involves treatment at elevated temperature. Hence the reduction in the toxic properties of the samples can thus be correlated with the denaturation of this protein factor leading to destruction of the hemagglutinating activity. Water when used as an alternate medium in place of *kanjika* has also led to destruction of the hemagglutinating factor. So it has a potential to be used as an alternate medium in the sodhana procedure for *Gunja*. Since the study is related to the detoxification of the toxic Ayurvedic medicinal plants, water is suggested to be an alternate medium in the sodhana prakriya taking into consideration only the reduction in toxicity of *Gunja* when treated with water.

Results of studies carried out on *Abrus precatorius* seeds have indicated that the proposed method is identical to the conventionally used sodhana prakriya and involves the denaturation of the toxic protein due to exposure to high temperature leading to the detoxification of the seeds.

References:

9. B) Methodology followed till end of period of reporting

2) Gloriosa superba roots (Langli)

Gloriosa superba L. is a plant belonging to the Liliaceae family. It is synonymously called Glory Lilies, Climbing Lilies or Malabar Glory Lily. In Hindi it is known by the names karihari, kalihar or languli and in Marathi indai, kariannag, nagkari or kallavi. The tubers of Gloriosa are used as drug. The drug is collected in Bengal, Hardwar and a few other parts of India.\(^1\)

The toxic component of Gloriosa tubers is the alkaloid colchicine (\(C_{22}H_{25}O_6N\)). The colchicine content of the tubers from Ceylon, quantified according to the USP method, is reported to be 0.3\% and the content of the tubers collected from the Amritsar market is 0.03\% as per the BP method. A new alkaloid allied to colchicine, provisionally named gloriosine (\(C_{22}H_{25}O_6N\)) has been isolated from fresh tubers. The tubers of Gloriosa are regarded as tonic, stomachic and anthelmintic when taken in doses of 5-10 grains; in larger doses they are intensely poisonous. It is a gastro-intestinal irritant and may cause vomiting and purging. It is sometimes used for promoting labour pains and also as abortifacient. It is considered useful in colic, chronic ulcers and piles.

Gloriosa superba roots were collected from Salem, India. Root sample was authenticated by Dr. H M Pandit, Botanist, Guru Nanak Khalsa College, Mumbai.

**Sample Preparation:** The process of sodhana was carried out on the roots of Gloriosa and then the sample was subjected to the following process. 5 g of the root sample was extracted with methanol in a Soxhlet assembly till exhaustion (24 hours). The extract obtained was evaporated till dryness and then stored in a cool dry place. The extract so obtained was further used for the quantification studies. Pre as well as post-sodhit samples were subjected to the same process.

**Ayurvedic sodhana prakriya for Gloriosa:** Gloriosa roots (10 g) were cut into small pieces and soaked in 100 ml Gomutra (Cow’s urine, pH between 7.8 to 8.2) for 1 day at room temperature (Fig. 1) The roots were then washed with warm water and air dried.\(^2\) The dried samples were then treated in a manner similar to the pre-sodhit samples and then subjected to the quantification process. The medium used for sodhana as well as the pooled washings were preserved for further analysis.

With an aim to find an alternative to the traditional sodhana prakriya, studies were also carried out using alkaline medium in place of gomutra which is used in the conventional technique. The alkaline medium was prepared by adjusting the pH of water to 8 using sodium hydrogen carbonate.

Fig. 1 Ayurvedic sodhana prakriya of Gloriosa superba roots
Quantification Studies: The High Performance Thin Layer Chromatography (HPTLC) technique was used for the quantification of the colchicine in the samples of *Gloriosa superba*. HPTLC was performed on Silica gel 60 F<sub>254</sub> plates (Merck, Germany) using chloroform: Acetone: diethylamine, 7:2:1 (v/v) as the mobile phase. Samples were applied to the plates as 8 mm bands by means of Camag Linomat V applicator. The plates were developed in Camag twin trough chamber previously equilibrated with mobile phase for 20 min along with the plate. The development distance was 8 cm. Plates were then removed from the chamber and dried in a current of air. Densitometric scanning was done at 357 nm using Camag TLC Scanner III with winCATS software. The wavelength was selected after acquiring spectra from the standard and the samples.

Samples were prepared by dissolving appropriate quantity of the extract in methanol (Table 1). A solution of standard colchicine (S. D. Fine-Chem. Ltd., India) in methanol of 0.03% w/v concentration was used as the standard. All reagents used were of Analytical Grade. A standard curve was developed between 2-12 µl of 0.003% w/v colchicine standard. Samples in duplicates were applied on the plates for quantification studies.

**10 B] Interim modification of objectives/methodology, if any:** NA

**11. B] Summary on progress:**

The roots of *Gloriosa superba* were subjected to the Ayurvedic sodhana prakriya. *Gloriosa superba* samples were subjected to HPTLC analysis to determine the content of colchicine in these samples. In addition to the *Gloriosa* samples the media used for sodhana prakriya and the subsequent water washings were also analyzed for their content of colchicine. The various extracts obtained and their concentrations analyzed are summarized in Table 1.

Table 1. Weight of extract obtained from 5 g of sample and the concentrations of the test solutions prepared.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Weight of Sample (g)</th>
<th>Weight of Methanol extract obtained (mg)</th>
<th>Concentration of test solution (µg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-sodhit</td>
<td>5</td>
<td>500</td>
<td>1.84</td>
</tr>
<tr>
<td><em>Gomutra</em> treated</td>
<td>5</td>
<td>160</td>
<td>1.08</td>
</tr>
<tr>
<td>Aqueous alkaline medium treated</td>
<td>5</td>
<td>120</td>
<td>1.19</td>
</tr>
</tbody>
</table>

A linear curve was set up in the range of 60-360ng of colchicine standard with a correlation coefficient of 0.9994 and sdv of 2.37. The R<sub>f</sub> value for standard colchicine was found to 0.39. The amount of colchicine present in the sample was calculated from the colchicine standard curve. Quantity of colchicine in 10 g of the *Gloriosa* roots pre-treatment, post-treatment and in the medium used as well as the washings is as stated in Table 2.

Table 2. Quantity of colchicine (mg per 10 g) of the *Gloriosa* roots in the samples, media and the pooled washings, as quantified using HPTLC system for.

<table>
<thead>
<tr>
<th>Quantity of colchicine (mg) in</th>
<th>Medium of treatment</th>
<th>Quantity of colchicine (mg) in</th>
<th>Medium</th>
<th>Washings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treated drug</td>
<td></td>
<td>Post-treated drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.20</td>
<td><em>Gomutra</em></td>
<td>2.36</td>
<td>10.51</td>
<td>3.85</td>
</tr>
<tr>
<td>18.20</td>
<td>Aqueous alkaline medium</td>
<td>1.21</td>
<td>12.39</td>
<td>4.53</td>
</tr>
</tbody>
</table>
Plate 1: Standard curve of colchicine and samples obtained by using gomutra for sodhana prakriya of Gloriosa superba roots.

Track 1-8: Standard colchicine in decreasing order of volume (12 µl-2 µl) with a duplicate for 12 µl and 2µl.

All the other samples were spotted in duplicates.
Track 9, 10: Pre-sodhit sample 5µl
Track 11, 12: Post-sodhit sample 5µl
Track 13, 14: Medium (gomutra) 2µl
Track 15, 16: Pooled washings 5µl
Photo documentation of the plates at 254nm as well as at 366nm was done.

Plate 1. 254 nm

Plate 1. 366 nm

Plate 2 : Samples obtained by using aqueous alkaline medium for the sodhana prakriya of Gloriosa superba roots.

Track 1, 2: Pre-sodhit sample, 5µl
Track 3, 4: Post-sodhit sample, 5µl
Track 5, 6: Medium (alkaline), 2µl
Track 7, 8: Pooled Washings, 5µl
Discussion

The roots of *Gloriosa superba* have been used in Ayurveda since many years. They have been used as an emetic. But the roots contain a toxic principle, colchicine which is alkaloidal in nature and in high doses is not recommended for internal use. Hence the roots have been subjected to the Ayurvedic *sodhana prakriya* so as to either chemically modify the component to some less toxic derivative or to reduce the concentration of that component to tolerable limits.

The screening of the pre-*sodhit* samples of *Gloriosa superba* has indicated the presence of colchicine in the samples to an extent of about 0.18% w/w on dry weight basis. The post-*sodhit* samples have shown to contain significantly reduced amounts of colchicine. The percentage of colchicine in the *gomutra* treated sample was found to be 0.024% and that in the sample treated with alkaline medium was found to be 0.012%. The results obtained from the HPTLC quantification studies have indicated that both the processes using the different media for the *sodhana prakriya* have led to a significant decrease in the content of colchicine in the sample. The *sodhana prakriya* prescribed for Gloriosa involves soaking of the sample in *gomutra* for 24 hours and then washing with warm water and subsequent drying. Since colchicine is an alkaloid, at alkaline pH it remains in its free base form. Colchicine has high solubility in water (1g dissolves in 22ml of water). The use of *gomutra* in *sodhana prakriya* exposes the sample to an alkaline pH in which colchicine readily dissolves. The analysis of the medium used for *sodhana prakriya* as well as the subsequent water washings show presence of colchicine. Also the post-*sodhit* samples of *Gloriosa* show small quantities of the colchicine. Hence it can be concluded...
that the decrease in the toxicity of the Gloriosa roots post- sodhana treatment is due to the decrease in its concentration. The mechanism involved in the above process is the solubilization of the colchicine in the gomutra. With this possible mechanism involved an attempt was made to put forth an alternate medium for gomutra and hence the sample was treated with aqueous alkaline medium. The results obtained from the alternate medium are comparable to the conventional method used and hence it could be used as an alternate medium for the sodhana prakriya in place of gomutra.

The experimental results suggest that the sodhana prakriya for Gloriosa superba roots leads to the decrease in the concentration of the toxic component, colchicine due to solubilization in the treatment medium. Further the results also indicate that the proposed method with a change in the treatment medium is at par with the conventionally used Ayurvedic sodhana prakriya with respect to the detoxification process.

References:

9. C] Methodology followed till end of period of reporting:

3) Acorus calamus rhizomes (Vacha)

Acorus calamus Linn. (Araceae) rhizomes synonymously called Calamus, Sweet flag, Sweet roots are also known by the common names vacha, vekhand and bach.1 In the Ayurvedic system of medicine, Acorus calamus rhizomes are considered to possess anti-spasmodic, carminative and anthelmintic properties. The dried rhizomes have been used in the treatment of epilepsy, schizophrenia, constipation, tympanitis, colic, otitis media, cough, asthma and to treat weakness of memory. They are also used in the treatment of diseases like chronic diarrhea and dysentery, bronchial catarrh, intermittent fevers, snake bite and glandular and abdominal tumors.2 The rhizomes are employed in the treatment of kidney and liver troubles, rheumatism and eczema. The skin of the rhizome is said to be haemostatic.1

The rhizomes are woody, branched, light brown, cylindrical to flattened with distinct nodes and internodes. Nodal regions are broad and bear leaf scars and also hair like fibres. Internodes are ridged and furrowed. The undersurface shows zig-zag line of circular root scars. Freshly exposed surface is granular and porous with a soothing aromatic odor.3,4

The rhizomes yield light brown to brownish yellow volatile oil called Calamus oil. The rhizomes contain a toxic component called β-asarone. β-asarone is a phenyl propanoid [1,2,4-trimethoxy-5-prop-1-enyl-benzene] and has been reported to possess carcinogenic properties. Reports also suggest, it induces unscheduled DNA synthesis in hepatocytes and possesses immunosuppressive, central nervous system inhibitory, sedative and hypothermic properties. Hence the use of this oil is restricted. The content of β-asarone in Acorus calamus depends on the ploidy level of the plant. The content increases as the ploidy level of the plant increases. The diploid variety found in North America is free from β-asarone. The triploid variety found in...
Europe contains 9-13% of β-asarone. The tetraploid variety of *A calamus* found in India contains around 75% of β-asarone. Due to this the Ayurvedic system uses *Acorus* which has undergone the process of *sodhana* (detoxification/potentiation).5,6

Acorus rhizomes were collected from Yamuna Nagar, Haryana. Microscopic evaluation was carried out to ascertain the identity of the sample. Rhizome sample was further authenticated by Dr. H M Pandit, Botanist, Guru Nanak Khalsa College, Mumbai.

**Sample preparation:** The samples of *Vacha* (five grams) before the *sodhana prakriya* as well as the samples (five grams) post treatment were soxhlet extracted with *n*-hexane and volume made up to 50ml except the alternate method I sample post aqueous medium treatment. 3.04g of this sample was extracted with *n*-hexane and volume made up to 50ml. The final dilutions of the samples used for the quantitative estimation of β-asarone are as per given in table 1. Quantification was carried out using the Gas Chromatographic (GC) technique.

**Conventional sodhana prakriya:** *Acorus calamus* rhizomes (hundred grams) were boiled in *gomutra* (cow’s urine) (two liters) for one *prahar* (three hours) then in *gorakhmundi* (*Sphaeranthus indicus*) kwath (two liters) for one *prahar* and subsequently in *panchapallav kwath* (two liters) for one *prahar*. The rhizomes were dried, washed with *gandhodak* and dried again. *Svedana prakriya* was carried on the rhizomes using *gandhodak* as the medium for one hour. *Gandhodak* was prepared as prescribed in the concerned reference. The *gandhodak* was filled in an earthen pot on which was placed another pot with many holes in its bottom and the two pots were sealed one over another. The earlier treated *A calamus* roots were placed in the upper pot with many holes and a plate was placed on top of this vessel. *Svedana* was carried out in this assembly for one hour.7

**Alternate modified method I:** *Acorus calamus* rhizomes (hundred grams) were boiled in aqueous medium (two liters) (To maintain similar pH environment as in the conventional method using *gomutra* as medium, 0.84% w/v sodium hydrogen carbonate solution showing pH 8 was used.) for one *prahar* (three hours) then in *gorakhmundi kwath* (two liters) for one *prahar* and subsequently in *panchapallav kwath* (two liters) for one *prahar*. The rhizomes were dried, washed with *gandhodak* and dried again. *Svedana prakriya* was carried on the rhizomes using *gandhodak* as the medium for one hour.

**Alternate modified method II:** *Acorus calamus* rhizomes (hundred grams) were boiled in water (two liters) for one *prahar* (three hours). The sample was further boiled in water (two liters) for one more *prahar*. The sample was finally again boiled in water (two liters) for one *prahar*. The conventional method involves treatment of the roots in three different media totally for 9 hours hence to resemble the time period for treatment; the roots were boiled in water for a period of 9 hours in divided intervals of three hours each. The rhizomes were dried, washed with water and subsequently dried for use in further processing. *Svedana prakriya* was carried on the rhizomes using water as the medium for one hour. Samples were collected after three, six and nine hours of boiling with water and after the entire sodhana prakriya was completed for subsequent quantitative studies.
GC Parameters: The analysis was done on Agilent System (7820A). The column used was HP-5 (5% Phenyl methylsiloxane), capillary column (30m x 320 µm x 0.25µm), 325°C attached to a Flame Ionization Detector (FID). One µl of the sample was injected in the splitless mode (10ml/min at 0.5 min). Carrier gas was nitrogen with a flow rate of 1.4 ml/min. Injector temperature was maintained at 250°C and the detector temperature at 300°C. The column temperature was held at 110°C for 2 min then increased at the rate of 20°C/min from 110-280°C and then held at 280°C for 1.5 min.

Asarone (mixture of α-asarone and β-asarone isomers), isolated in the laboratory using the column chromatographic method, was used to set up the standard curve. Stock solution of one µl/ml was prepared. Solutions of 0.1µl/ml to 0.5µl/ml were prepared from the stock solution for the standard curve. The percentage of β-asarone in these was 46.6 % v/v the rest being α-asarone. Hence the standard curve for β-asarone was set up between 0.04660 and 0.2330 µl/ml of β-asarone.

To ascertain the identity of the marker, Gas-Chromatography Mass Spectorscopic (GC-MS) analysis of the isolated asarone was carried out.

10. C] Interim modification of objectives/methodology, if any: Not Applicable

11. C] Summary on progress ( during the period of report):
α-asarone and β-asarone are trans and cis isomers of each other and hence could not be separated by common chromatographic methods. Hence the asarone mixture that was isolated using the column chromatography method was analyzed for its individual components using the GC method. The percentage of β-asarone in this was found to be 46.6 % v/v. β-asarone being cis isomer shows lower retention time and is eluted first as shown in fig 2.8,9 The GC-MS analysis of the isolated asarone gave M⁺ value of 208.1, hence ascertaining the identity of β-asarone whose molecular weight is 208.25. Fragmentation pattern for the GC-MS analysis is as given in
Quantification of the β-asarone in the various samples was done based on the standard curve set between 0.04660 and 0.2330 µl/ml of β-asarone and having a $R^2$ value of 0.9999. The results obtained for the various samples are shown in table 1.

Figure 2: Gas chromatogram of isolated asarone from the oil of calamus using column chromatography

Figure 3: Mass spectroscopy data of the isolated β-asarone

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Dilutions of samples used for GC Analysis</th>
<th>Quantity of β-asarone (µl) in five g sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-sodhit</td>
<td>1:2500</td>
<td>117.94</td>
</tr>
<tr>
<td>Post-sodhit conventional post gomutra treatment</td>
<td>1:200</td>
<td>47.72</td>
</tr>
<tr>
<td>Post-sodhit conventional post gorakhmundi treatment</td>
<td>1:50</td>
<td>17.21</td>
</tr>
<tr>
<td>Method</td>
<td>Ratio</td>
<td>Value</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Post-sodhit conventional post panchapallav treatment</td>
<td>1:50</td>
<td>9.97</td>
</tr>
<tr>
<td>Post-sodhit conventional</td>
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</tr>
<tr>
<td>Post-sodhit modified I post pH8 medium treatment</td>
<td>1:100</td>
<td>39.29</td>
</tr>
<tr>
<td>Post-sodhit modified I post gorakhmundi treatment</td>
<td>1:200</td>
<td>35.41</td>
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<tr>
<td>Post-sodhit modified I post panchapallav treatment</td>
<td>1:50</td>
<td>14.17</td>
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<tr>
<td>Post-sodhit modified I</td>
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<td>4.81</td>
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<tr>
<td>Post-sodhit modified II (3 hours)</td>
<td>1:500</td>
<td>66.21</td>
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<tr>
<td>Post-sodhit modified II (6 hours)</td>
<td>1:500</td>
<td>38</td>
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<tr>
<td>Post-sodhit modified II (9 hours)</td>
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<td>20.50</td>
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<tr>
<td>Post-sodhit modified II</td>
<td>1:500</td>
<td>15.20</td>
</tr>
</tbody>
</table>

Fig. 4A Photographs of samples showing the rhizomes (a) and rhizomes pieces (b) of *Acorus calamus* prior to the *sodhana prakriya*.

![Photographs of samples showing the rhizomes (a) and rhizomes pieces (b) of *Acorus calamus* prior to the *sodhana prakriya*.](image)

Fig. 4B Photographs of samples showing the rhizomes pieces post *sodhana prakriya*.

![Photographs of samples showing the rhizomes pieces post *sodhana prakriya*.](image)

**Discussion**

*Acorus calamus* (*Vacha*) is a drug of importance in the Ayurvedic system of medicine which finds its use in diseases like epilepsy, schizophrenia, cough, asthma, to treat weakness of memory, etc. β-asarone is an important constituent of *Acorus calamus* (*Vacha*) with the Indian variety constituting large amounts of the same. β-asarone has been reported to possess carcinogenic properties and hence the Ayurvedic system uses *sodhit* (detoxified) *Vacha* in their medicines.

The conventional method of *sodhana* was studied for the mechanism involved in the detoxification process and also an alternate method was devised for the same. The conventional method involved the process of boiling in various media like *gomutra*, *gorakhmundi* (*Sphaeranthus indicus*) kwath, *panchapallava* kwath and fomentation over *gandhodak* for
specifies time intervals. β-asarone is a component of calamus oil which in turn is obtained from Vacha by the steam distillation process. Hence it can be said that the property of volatilization is used in the extraction process of β-asarone. Similarly the process of boiling during the sodhana prakriya of Vacha leads to the volatilization of the β-asarone from the Vacha samples. Hence it could be concluded that the mechanism involved in the process is the volatilization due to heating. The same principle was used in devising the alternate methods for the conventional sodhana prakriya. In the alternate modified method I, only the gomutra medium was replaced and the remaining media kept same as those of the conventional method, when results comparable to that of the conventional method were obtained. Whereas the alternate modified method II used water in every step when the decrease in the β-asarone content was not as much as in the conventional method. Thus it can be concluded that apart from the volatilization aspect, the media used in the conventional process also play some role in reducing the β-asarone content of the samples. Further it is observed that there is a constant decrease in the β-asarone content with time, hence it can also be stated that the decrease in content of β-asarone in the samples, comparable to that of the conventional method, can be achieved by increasing the treatment time in case of the modified alternate method II where water is used as the medium.

The study on the conventional method of sodhana as per the Ayurvedic text has revealed that the multiple processes of heating with different media have led to the decrease in the content of β-asarone. Hence it can be stated that the mechanism involved is primarily the volatilization of β-asarone during the heating processes along with some role played by the media involved. Alternate processes developed, simulating the conventional process, with respect to the mechanism involved, have also led to the decrease in the content of β-asarone. Hence the process of volatilization is primarily the principle underlying the sodhana prakriya of Acorus calamus (Vacha).

References: