

Fate of β -asarone in Ayurvedic *Sodhana* process of *Vacha*

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ABSTRACT

Background: *Calamus* (*Acorus calamus* Linn., Araceae) rhizome synonymously called sweet flag or *Vacha* is an aromatic herb indigenous to Central Asia and Eastern Europe. It has been used by the Ayurvedic practitioners since time immemorial for diseases ranging from weakness of memory to being used as an anthelmintic. Reports of its use have been found in books like *Charak Samhita*, *Sushruta Samhita*, etc., The major constituent of the oil of *Vacha* is a phenyl propanoid called β -asarone, which is reported to show carcinogenic properties. Due to the toxic effects of β -asarone, *sodhana prakriya* (detoxification process) has been prescribed for *Vacha* before its inclusion in the Ayurvedic medicines. *Shodhanaprakriya* (*S.prakriya*) of *Vacha* has been mentioned in the Ayurvedic texts. **Objectives:** This study was undertaken with an aim to find out the mechanism involved in the *S. prakriya* of *Vacha* and also to suggest an alternate method for the conventional one. **Materials and Methods:** The conventional method was studied in the laboratory and equivalent alternate methods were designed based on the mechanism involved. *Vacha* samples were subjected to the conventional method as well as the alternate methods and the content of β -asarone in the different samples was monitored using Gas Chromatography technique. **Results:** Various alternate methods have been devised based on the mechanism involved in the *S. prakriya* which have given results comparable with those of the conventional method. **Conclusion:** The scientific mechanism involved in the *S.prakriya* of *Vacha* has been established and alternate methods have been proposed.

Key words: *Acorus calamus*, β -asarone, *Sodhana*, *Vacha*

INTRODUCTION

Calamus (*Acorus calamus* Linn., Araceae) rhizomes synonymously called sweet flag, sweet roots are also known by the common names *Golomi*, *Uragandha*, *Vacha*, *Vekhandha* and *Bach*.^[1] It is an aromatic herb, indigenous to Central Asia and Eastern Europe. It has been used since time immemorial by the inhabitants of these continents. In India, reference of the use of *A. calamus* is available in books like *Charak Sambita*, *Sushruta Sambita*. In the Ayurvedic system

of medicine, *A. calamus* rhizomes are considered to possess antispasmodic, carminative, and anthelmintic properties.^[2] The dried rhizomes have been used in the treatment of epilepsy, schizophrenia, constipation, tympanitis, colic, otitis media, cough, and asthma and to treat weakness of memory.^[1] 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. The rhizomes are used in the treatment of kidney and liver troubles, rheumatism, and eczema. The skin of the rhizome is said to be hemostatic. 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 fibers. Internodes are ridged and furrowed. The undersurface shows zigzag 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 fragrant oil obtained by the alcoholic extraction of the rhizomes is used in the pharmaceutical industry and oenological industry. Rhizomes contain a component called β -asarone, which possesses toxic properties. β -asarone is a phenyl propanoid [1,2,4-trimethoxy-5-prop-1-enyl-benzene] possessing carcinogenic properties. It induces unscheduled

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Received: 13-Jun-2012

Revised: 04-Jul-2012

Accepted: 19-Sep-2012

Access this article online

Quick Response Code:



Website:

www.jaim.in

DOI:

10.4103/0975-9476.109545

deoxyribonucleic acid (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 *A. 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 *sodbhana* (detoxification/potentiation).^[5,6]

The aim of this study was to evaluate the chemical changes involved in the classical *sodbhana prakriya* (detoxification process). Also an attempt was made to put forth a modern alternate method for the *shodhanaprakriya* (*S.prakriya*) based on the fate of β -asarone in the conventional method.

MATERIALS AND METHODS

A.calamus or *Vacha* rhizomes were obtained from the market and were authenticated by Dr. H. M. Pandit, Botanist, Department of Botany, Guru Nanak Khalsa College, Matunga, Mumbai. Voucher sample was deposited at the Institute of Chemical Technology, Matunga, Mumbai.

Conventional *S.prakriya*

A. calamus rhizomes (100 g) were boiled in *gomutra* (cow's urine) (2 L) for one *prabar* (3 h) then in *Gorakhmundi* (*Sphaeranthus indicus*) *kwath* (2 L) for one *prabar* and subsequently in *Panchapallav kwath* (2 L) for one *prabar*. The rhizomes were dried, washed with *gandhodak*, and dried again. *Svedana prakriya* was carried on the rhizomes using *gandhodak* as the medium for 1 h. *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 1 h.^[7]

Alternate modified method I

A. calamus rhizomes (100 g) were boiled in aqueous medium (2 L) (to maintain similar pH environment as in the conventional method using *gomutra* as medium, 0.84% w/v sodium hydrogen carbonates solution showing pH 8 was used) for one *prabar* (3 h), then in *Gorakhmundi kwath* (2 L) for one *prabar*, and subsequently in *Panchapallav kwath* (2 L) for one *prabar*. The rhizomes were dried, washed with *gandhodak*, and dried again. *S. prakriya* was carried on the rhizomes using *gandhodak* as the medium for 1 h.

Alternate modified method II

A. calamus rhizomes (100 g) were boiled in water (2 L) for one *prabar* (3 h). The sample was further boiled in water (2 L) for one more *prabar*. The sample was finally again boiled in water (2 L) for one *prabar*. The conventional method involves treatment of the roots in three different media totally for 9 h hence to resemble the time period for treatment, the roots were boiled in water for a period of 9 h in divided intervals of 3 h each. The rhizomes were dried, washed with water, and subsequently dried for use in further processing. *S. prakriya* was carried on the rhizomes using water as the medium for 1 h. Samples were collected after 3, 6, and 9 h of boiling with water and after the entire *S. prakriya* was completed for subsequent quantitative studies.

Quantitative studies

The samples of *Vacha* (5 g) before the *S. prakriya* and the samples (5 g) post treatment were soxhlet extracted with *n*-hexane and volume made up to 50 ml. Only the alternate method I sample post aqueous medium treatment. 3.04 g of this sample was extracted with *n*-hexane and volume made up to 50 ml. 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.

GC Parameters: The analysis was done on Agilent System (7820A). The column used was HP-5 (5% Phenyl methylsiloxane), capillary column (30 m \times 320 μ m \times 0.25 μ m), 325°C attached to a Flame Ionization Detector (FID). One

Table 1: Quantification of β -asarone in the various samples of *Vacha*

Sample name	Dilutions of samples used for GC analysis	Quantity of β -asarone (μ l) in 5 g sample
Pre- <i>shodhit</i>	1:2500	117.94
Post- <i>shodhit</i> conventional post <i>gomutra</i> treatment	1:200	47.72
Post- <i>shodhit</i> conventional post <i>gorakhmundi</i> treatment	1:50	17.21
Post- <i>shodhit</i> conventional post <i>panchapallav</i> treatment	1:50	9.97
Post- <i>shodhit</i> modified I post pH 8 medium treatment	1:100	39.29
Post- <i>shodhit</i> modified I post <i>gorakhmundi</i> treatment	1:200	35.41
Post- <i>shodhit</i> modified I post <i>panchapallav</i> treatment	1:50	14.17
Post- <i>shodhit</i> modified II (3 h)	1:500	66.21
Post- <i>shodhit</i> modified II (6 h)	1:500	38
Post- <i>shodhit</i> modified II (9 h)	1:500	20.50
Post- <i>shodhit</i> modified II	1:500	15.20

GC=Gas chromatographic

microliter of the sample was injected in the splitless mode (10 ml/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 1 μ l/ml was prepared. Solutions of 0.1 μ l/ml-0.5 μ l/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 μ l/ml of β -asarone.

To ascertain the identity of the marker, gas-chromatography mass spectroscopic (GC-MS) analysis of the isolated asarone was carried out.

RESULTS

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 Figure 1.^[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 Figure 2.

Quantification of the β -asarone in the various samples was done based on the standard curve set between 0.04660 and 0.2330 μ l/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.

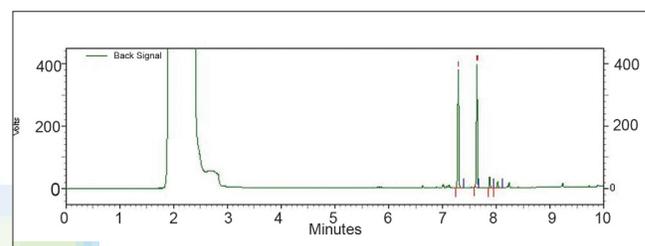


Figure 1: Gas chromatogram of isolated asarone from the oil of *calamus* using column chromatography

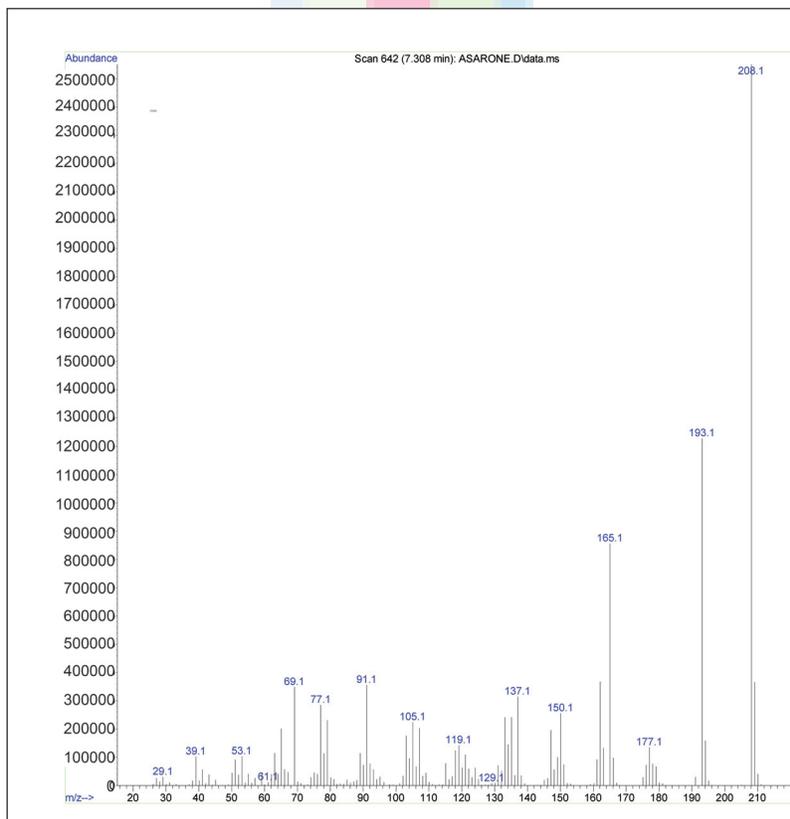


Figure 2: Mass spectroscopy data of the isolated β -asarone

DISCUSSION

A. 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 *A. 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 *shodhit* (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 kwath*, *panchapallava kwath*, and fomentation over *gandbodak* for specified 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 *S. 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 *S. 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, and results comparable with that of the conventional method were obtained. Whereas the alternate modified method II used water in every step, and with this method the decrease in the β -asarone content was not as much as that observed 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. Furthermore, 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 with that of the conventional method, may be achieved by increasing the treatment time in case of the modified alternate method II where water is used as the medium.

CONCLUSION

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 *S. prakriya* of *A. calamus (Vacha)*.

ACKNOWLEDGMENTS

The authors are thankful to AICTE for funding the necessary instrumentation for the research work undertaken.

We are also thankful to Dr. (Mrs.) Suchita Sheth (K.G.Mittal, Punarvasu Ayurvedic College, Mumbai) for helping us with the necessary equipment required, interpreting and carrying out the procedure as per the Ayurvedic system.

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How to cite this article: Gholkar MS, Mulik MB, Laddha KS. Fate of β -asarone in Ayurvedic *Sodhana* process of *Vacha*. *J Ayurveda Integr Med* 2013;4:19-22.

Source of Support: The research work published in the paper is supported by Central Council for Research in Ayurveda and Siddha (CCRAS), Department of AYUSH, Govt. of India. **Conflict of Interest:** None declared.