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Purification of Beta Asarone from *Acorus Calamus.* L

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ABSTRACT

β-asarone (cis-isomer of 2, 4, 5-trimethoxy-l-propenylbenzene) is a constituent of oil derived from the dried rhizome of *A. calamus* which is used as herbal medicine. Ethyl acetate extract was prepared from fresh leaves of *acorus calamus*. The extract was separated in silica gel column chromatography until single spot of beta asarone was observed on TLC. The pure fraction analyzed in NMR spectroscopy. ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker 400 MHz NMR spectrometer at 298K. Spectral assignments of fraction were correlated with standard beta asarone. The comparison confirmed pure beta asarone in the fraction.

Keywords: *cis*-asarone, *trans*-asarone, *Acorus calamus,* chemical shift

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INTRODUCTION

Beta asarone an important chemical constituent of *Acorus calamus* has also been reported to possess most of the biological activities of the plant. The α- and β-asarones identified as the major constituents have often been attributed to antibacterial properties of the *A. calamus* whole plant, roots, rhizomes and essential oils [1]. Content of β-asarone depends on the ploidy condition of the plant. The volatile oil from the tetraploid form of the rhizome contains up to 95 to 96% β-asarone as major component on the other hand oil from the European or triploid form contains less than 11% of β-asarone. A diploid form of the plant contains virtually no asarone [2]. The root containing β-asarone is used in traditional medicine to treat diabetes [3]. A study by Geng *et al*. has shown β-asarone as a potential candidate for a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease [4]. In a number of *in vitro* experiments, β-asarone was found to have anticholinergic activity and was reported to stop frog hearts in diastole and to reduce the tone and longitude of intestinal movement [5]. *A. calamus* essential oil also possesses antigonadal activity in insects [6, [7]. Aromatic oils obtained by alcoholic extraction of the rhizome are used in the pharmaceutical and oenological industries [8]. These studies revealed the presence of α- and β-asarones as the major component in different plant parts.

MATERIALS AND METHODS

Plant Source

A. calamus (L.) plants were collected from the Horticultural Research Station, Yercaud in Tamil Nadu, India and grown in the Herbal Garden of Vellore Institute of Technology University, Vellore, Tamil Nadu, India. The plant was authenticated at the Horticulture Research Station, Yercaud.

Extract Preparation

A known amount of fresh leaves (100 g) of *A. calamus* was homoginised and extracted with ethyl acetate for 24 h as per the procedure published previously [9]. Extracts thus prepared were concentrated and stored at 4 ^{o}C.

Column Chromatography

Silica gel column chromatography is a common technique to purify a mixture of chemicals. A glass tube of about 45 cm length and 3 cm in diameter long was held vertically by two large clamps. At the base of the tube a plug of glass-wool was supported by short pieces of glass beads. Silica gel beads were packed 1/4 full. In brief, 2 g of extract of was mixed with 60-120 mesh silica gel and then the mixed sample was added onto the column and packed with silica gel and washed by different ratios of petroleum ether and ethyl acetate as gradient elution solvents. As the solvents moved through the column, the components of the extract mixture were carried along. The higher polarity part of the fractions eluted by petroleum

ether: ethyl acetate was collected and repeated the separation by silica gel column chromatography until the pure mixture of the asarone isomers (a single spot detected by TLC) was obtained. Then the target effluent was collected and condensed before analyzing in NMR spectroscopy.

TLC

About 5-10 µl of fraction separated by column chromatography were loaded on pre-coated silica gel-G plates along with standard β-asarone (Sigma, St. Louis, USA). The plates were developed in a chamber saturated with solvent system consisting of toluene: ethyl acetate (8:2 v/v) as described by Oprean *et al.* [10]. The plates were removed from the chamber, dried and observed under visible and UV light for visualization of the spots. Presence or absence of the β-asarone in the fraction was confirmed by comparing with standard β-asarone.

NMR Spectroscopy Analysis

The pure fraction obtained from column chromatography was analysed in NMR spectroscopy.^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker 400 MHz NMR spectrometer at 298K. The spectra were recorded in $CDCl_3$. Spectral assignments were confirmed with the help of Homonuclear Proton Correlation Spectroscopy as well as ^{13}C-^{1}H Heteronuclear Single Quantum Correlation Spectroscopy (HSQC) and correlated with standard 70% β-asarone (Sigma, St Louis, USA) spectrum.

Spectral Acquisition for ^{13}C and ^{1}H-NMR

The following parameters were used to record the one dimensional proton spectra – spectral width: 6410 Hz; number of scans: 16; Dwell time: 78 µs; Acquisition time: 1.99 s; Pulse width: 14.1 µs.

The following parameters were used to record the one dimensional ^{13}C spectra – spectral width: 22058.824 Hz; number of scans: 1024; Dwell time: 22.6778 µs; Acquisition time: 0.499 s; Pulse width: 9.62 µs.

The chemical shift assignments of the peaks for 13C and ^{1}H-NMR are as follows:

^{1}H spectra ($CDCl_3$): δ (ppm) 6.845 (1H, s, H6); 6.537 (1H, s, H3); 6.481 (1H, qd, H7); 5.77 (1H, dq, H8); 3.901 (3H, s, 2-OCH_3); 3.841 (3H, s, 5- OCH_3); 3.813 (3H, s, 4- OCH_3); 1.839 (3H, dd, H9).
^{13}C spectra ($CDCl_3$): δ (ppm) 151.61 (C-4); 148.64 (C-2); 142.52 (C-1); 125.88 (C-8); 124.86 (C-7); 118.21 (C-5); 114.28 (C-6); 97.75 (C-3); 56.72 (5-O CH_3); 56.54 (4-OCH_3); 56.17 (2-OCH_3); 14.70 (C-9).

Both the proton and carbon chemical shifts were in correlation with the values reported by Zuo *et al.* (2012). The spectral correlation of standard β-asarone and sample were given in figure 4.3- 4.8 and chemical shift value correlation in Table 1 and 2.

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RESULTS

In order to confirm the presence of β-asarone in the fractions separated in column chromatography, initially TLC analysis was carried out. The fraction on lane 4 (Figure 1) was identified as pure in comparison with standard β-asarone spot. For further confirmation ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker 400 MHz NMR spectrometer at 298K. The spectra were recorded in $CDCl_3$. Correlation of chemical shift of standard β-asarone with sample β-asarone (Figure 2) of ^{1}H-NMR Spectroscopy and ^{13}C-NMR was done (Table 1 and 2).

Table 1: Correlation of chemical shift of standard β-asarone with sample β-asarone of ^{1}H-NMR Spectroscopy

Position	Chemical shift value (ppm)	
	Standard	Sample
H3	6.534	6.537
H6	6.844	6.844
H7	6.462- 6.499	6.462 – 6.499
H8	5.724 – 5.806	5.729 - 5.811
H9	1.827 – 1.850	1.828 – 1.851
$2OCH_3$	3.896	3.901
$4OCH_3$	3.807	3.801
$5OCH_3$	3.838	3.827

Table 2: Correlation of chemical shift of standard β-asarone with sample β-asarone of ^{13}C-NMR

Position	Chemical shift value (ppm)	
	Standard	Sample
C1	142.41	142.52
C2	148.71	148.64
C3	97.64	97.75
C4	151.51	151.61
C5	118.10	118.21
C6	114.18	114.28
C7	124.77	124.86
C8	125.73	125.88
C9	14.61	14.70
$2OCH_3$	56.06	56.17
$4OCH_3$	56.42	56.54
$5OCH_3$	56.61	56.72

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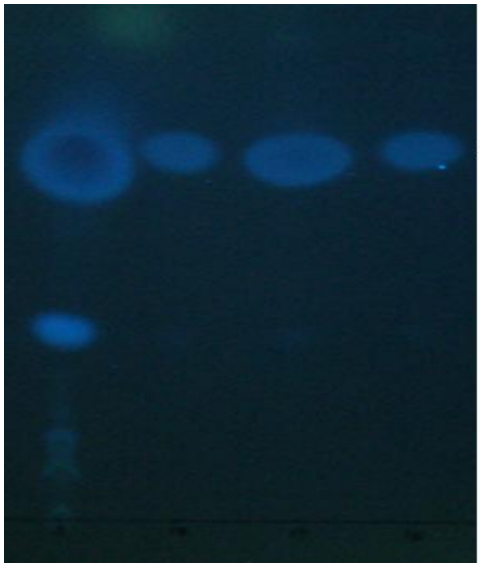

1 2 3 4

Figure 1: TLC plate of fractions separated in column chromatography

Lane 1 Standard β-asarone ; **Lanes 2, 3 and 4** - fractions of column chromatography of petroleum ether and ethyl acetate solvents (88:12 v/v).

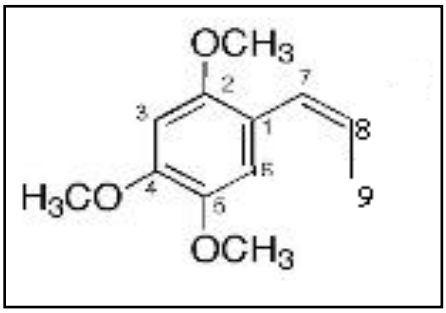

Figure 2: Chemical structure of β-asarone

DISCUSSION

Till date no report has been puplished for the purification of β-asarone from the fresh leaves or rhizomes of *A. calamus*. However, previously published report described the procedure for separation of *cis* and *trans* asarone from essential oil of *A. tatarinowii* by preparative gas chromatography [11]. So far, HPLC and GC-MS are two most frequently used techniques for the analysis of asarone. The fraction separated from column chromatography of leaf extract was confirmed for pure asarones on thin layer chromatography. The sample separated were directly analyzed in NMR and the spectra was compared with standard asarone. The standard analyzed was 70% asarone and has other analogs in it, so other spectral graph along with *cis* and *trans* asarone was observed. On the other hand, the sample analyzed

was pure but spectral graph of both *cis* and *trans* asarone was predicted. Conventional silica gel column chromatography is often insufficient to resolve closely related substances and isomers. But, it could be also inferred that the *cis*- form of the asarone was dominant when compared to the *trans*-isoform in the fractions. Furthermore, the fractions obtained from 88:12 ratio of petroleum ether and ethyl acetate were identified by TLC, ^{1}H, ^{13}C and HSQC NMR spectra as β-asarone.

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