

Gas Chromatographic and Mass Spectroscopic (GC-MS) Analysis of Rhizome of *Acorus Calamus* Linn. for Identification of Potent Antimicrobial Bio-active Compounds

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Abstract

Acorus calamus Linn. has wide applications as a herbal medicine since its roots, leaves and rhizome exhibit antimicrobial and insecticidal activities. The objective of this study was to identify some compounds in *Acorus calamus* Linn., which may be responsible for its antimicrobial activity. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis, for investigation of the compounds present, of the petroleum ether extract of *Acorus calamus* Linn. rhizome suggested presence of five compounds (which showed close match with those of the GC-MS database main library of *Novocatalyz*) viz., spiro [2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane); 9-octadecenoic acid (Z)-, phenylmethyl ester; shyobunone; asarone [syn. α -asarone]; benzene, 1,2,3-trimethoxy-5-(2-propenyl)-. α -asarone and benzene, 1,2,3-trimethoxy-5-(2-propenyl)- have been reported to be sensitive against plant pathogen and may be responsible for its anti-fungal activity. As per the compound contents of this plant, studies suggest that *Acorus calamus* Linn. could be a potential herbal medicine against certain microbial diseases.

Keywords: *Acorus calamus* Linn.; Bioactive compound; GC-MS analysis; α -Asarone.

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1. Introduction

Acorus calamus has been used widely as a herbal medicine as its roots, leaves and rhizomes (Figs. 1 and 2) have been reported to exhibit anti-microbial and insecticidal activities and as such it can be used for prevention and treatment of a wide number of diseases such as rheumatoid arthritis, gastrointestinal disorders, and various other mental ailments including epilepsy [1-3]. *A. calamus* is a traditional medicine [4]. In Ayurvedic medicine, *A. calamus* is an important herb, and is valued as a 'rejuvenator' for the brain and nervous system, and as a remedy for digestive disorders. *Acorus calamus* is also used as a herbal medicine for treating inflammation of eyes [5].

Research works have evaluated antimicrobial activity of *Acorus calamus* rhizome and leaf extracts obtained with different solvents viz., petroleum ether, chloroform, hexane and

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ethyl acetate. Petroleum ether extract of *Acorus calamus* has been found to be very effective against pathogenic fungi. Petroleum ether extract of *Acorus calamus* has been reported to exhibit 100 % inhibition of *Sclerotium rolfsii* with minimum inhibitory concentration (MIC) of 10.4 mg/mL [6].



Fig. 1. *Acorus calamus* plant.



Fig. 2. Rhizome of *Acorus calamus*.

Researchers have also identified some of the active compounds, which impart therapeutic potential to *A. calamus*. Antioxidant activity of ethanolic extract of *Acorus calamus* has been reported to be linked to its phenolics and flavonoid contents. A positive correlation of its anti-oxidant capacity with its phenolic and flavonoid contents has been reported for *Acorus calamus* Linn. rhizome [7]. As per some of the preliminary phytochemical studies, *A. calamus* is found to contain some secondary metabolites such as flavonoids, glycosides, tannins, saponins, volatile oil polyphenolic compounds, mucilage, etc. [8]. Several volatile bioactive organic compounds such as β -asarone, linalool, methyl eugenol, etc. have also been reported in Nepalese *Acorus calamus* rhizomes [9]. α - and β -asarones, caryophyllene, isoasarone, methyl isoeugenol, and safrol have been identified as the major chemical constituents in *A. calamus*, of which α - and β -asarones have been identified as responsible for imparting anti-microbial potential to *A. calamus* [10-14].

Antimicrobial activity of β -asarone derived from *A. calamus* against bacteria, yeasts and filamentous fungi has been evaluated and it was found that β -asarone exhibited high activity against filamentous fungi: *Trichophyton rubrum*, *Microsporium gypseum* and *Penicillium marneffeii* [15]. It has also been observed that the antifungal activity of *A. calamus* is because of haem peroxidase, which is synthesized and accumulated in the plant during pathogenesis [16].

Some studies, *in vitro* and *in vivo*, however, have raised concerns relating to side-effects of *A. calamus* as it was observed to induce malignant tumours due to β -asarone. Nevertheless, there are several polyploid varieties of *A. calamus*, which do not contain toxic materials. It has been argued that for toxicity, products should contain no or a negligible amount of β -asarone [17]. Ethanol extract of *A. calamus* (upto a dose of 600 mg/kg BW) has been reported to lack potential toxicity in studies relating to rats [18].

Given the presence of a number of compounds in *Acorus calamus*, this study seeks to identify few phytochemical compounds that may be responsible for its therapeutic properties using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

2. Materials and Methods

2.1. Collection, identification and authentication of plants material

Plant materials were collected in wild condition from various localities of Ranchi. The collected *A. calamus* plant material was preserved in the form of herbarium, which matched with the specimen No. 2574 of M. R. Almeida, preserved in the Blatter Herbarium, St. Xavier's College, Mumbai, India. The characteristics of the plant were identified using 'Flora of Bihar and Orissa' [19]. The material was then washed with distilled water 2-3 times and shade dried. The dried plant material was then ground using grinder. The powder, thus obtained, were relocated to air tight jars and stored in the dark for future use.

2.2. Sample preparation

10 g of powdered plant material was soaked overnight in 50 mL of petroleum ether (40-60 °C) separately with intermittent shaking. The extract was then filtered through Whatman no. 1 filter paper and the filtrates were collected separately. To the residue 50 mL of solvent (in which the powder was soaked) was added, stirred well and left for 4 h. at room temperature with intermittent shaking. The extracts were filtered again and the filtrates were collected. This procedure was repeated once again and the filtrates of the plant was pooled separately. The filtrate, thus obtained, was transferred separately to pre-weighed evaporating dishes and the solvent was evaporated. The sample so obtained was stored in an air tight poly-tube and sent to Novacatalyz Laboratory (based in Bangalore) for GC-MS analysis for authentic identification of constituent compounds present in the plant extracts.

2.3. Gas chromatography-mass spectroscopy (GC-MS) analysis

2.3.1. Preparation of extract

0.1080 g of sample was put into a 5 mL volumetric flask (VF) and then dissolved in 2 mL of solvent, which was then sonicated and made up to mark with solvent. 0.1 mL of solvent was pipetted out from the solution and it was mixed with 0.9 mL of methanol solvent in a separate flask to make the final volume 1 mL.

2.3.2. Method of analysis

Modified method was used for GC-MS analysis [20], which is as follows. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. An injection volume of 1 µL was

employed. The injector temperature was adjusted to 280 °C. The ion source temperature was adjusted to 230 °C. The oven temperature was programmed from 150 °C for 2 min, to 290 °C at 8 °C per min. and held for 10 min. Mass spectra were taken at eV; a scan interval of 0.5 second and 40 to 800 Da. Total GC-MS running time was 30 min.

3. Results and Discussion

3.1. Compound analysis

GC-MS analysis (for investigation of the compounds present) of the petroleum ether extract of *A. calamus* rhizome suggested presence of five compounds, viz., Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane); 9-Octadecenoic acid (Z)-, phenylmethyl ester (C₂₅H₄₀O₂); Shyobunone; α-Asarone; and Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(C₁₂H₁₆O₃) (Tables 1-3).

Table 1. Compounds and their synonyms.

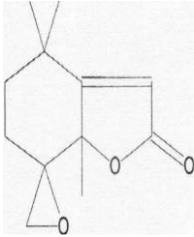
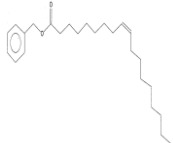
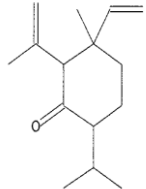
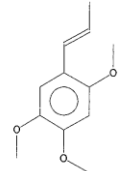
Sl. No.	Compound name	Synonyms
1	Spiro [2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)	No synonym.
2	9-Octadecenoic acid (Z)-, phenylmethyl ester	1. Benzyl oleate. 2. Benzyl (9Z)-9-octadecenoate.
3	Shyobunone	No synonym.
4	Asarone	1. (E)- 2,4,5-Trimethoxypropenylbenzene. 2. trans-2,4,5-Trimethoxypropenylbenzene 3. Benzene, 1,2,4-trimethoxy-5-(1-propenyl)-, (E)- 4. Benzene, 1,2,4-trimethoxy-5-propenyl)-, (E)- 5. Asarone. 6. Asarone, trans- 7. α-Asarone. 8. trans-Asarone.
5	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	1. Benzene, 5-allyl-1,2,3-yrimethoxy. 2. Elemicin. 3. 3,4,5-Trimethoxyallylbenzene. 4. 5-Allyl-1,2,3-trimethoxybenzene. 5. 1,2,3-Trimethoxy-5-(2-propenyl)-benzene. 6. 1,2,3-Trimethoxy-5allylbenzene. 7. 4-Allyl-1,2,6-trimethoxybenzene. 8. Benzene, 5-(2-propenyl)-1,2,3-trimethoxy.

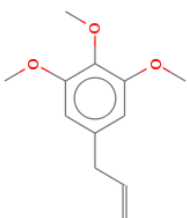
Table 2. Compounds and their sources.

Sl. No.	Compound	Chemical Formula	Class	Molecular weight	Source
1.	Spiro [2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane)	C ₁₂ H ₁₆ O ₃	-	208	1. <i>Isodon excise</i> , 2. <i>Loranthus micranthus</i>
2	9-Octadecenoic acid (Z)-, phenylmethyl ester	C ₂₅ H ₄₀ O ₂	Oleic acid	372	1. <i>Cleidion nitidum</i> (Muell. – Arg.) Thw.

			ester		ex Kurz., 2. <i>Sarcostemma secamone</i> (L) Bennet, 3. <i>Jatropha tanjorensis</i> *J.L.Ellis &Saroja <i>Acorus calamus</i>
3.	Shyobunone	$C_{15}H_{24}O$	Ketone compound	220	
4.	Asarone [syn. A++asarone]	$C_{12}H_{16}O_3$	Aromatic compound	208	1. <i>Acorus calamus</i> 2. <i>Acorus gramineus</i>
5.	Benzene, 1,2,3- trimethoxy-5-(2- propenyl)-	$C_{12}H_{16}O_3$	Phenyl- propenes	208	1. <i>Gloriosa superb</i> 2. <i>Dalbergia barbidensis</i>

Table 3. Phytochemical compounds (with their biological activity) found in *Acorus calamus*.

Sl. No.	Compound	Structure of Compound	Biological activity	References
1.	Spiro [2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethyl-benzofuran]-7,2'-(oxirane) ($C_{12}H_{16}O_3$)		No activity reported	Channabasava, <i>et al.</i> [21]
2.	9-Octadecenoic acid (Z)-, phenylmethyl ester ($C_{25}H_{40}O_2$)		1. Insectifuge, Flavor 2. Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor 3. Anti-inflammatory	Kumari, <i>et al.</i> [22] Bharathy, <i>et al.</i> [23] Das, <i>et al.</i> [24]
3.	Shyobunone ($C_{15}H_{24}O$)		1. No activity reported 2. Insecticidal activity 3. Insecticidal and repellent activity against LS and TC.	Chen, <i>et al.</i> [25] Kumar, <i>et al.</i> [26]
4.	Asarone[syn. A++asarone] ($C_{12}H_{16}O_3$)		1. Antipyretic, Antispasmodic, Emetic Fungicide, Mutagenic, Sedative Myorelaxant, Tranquilizer, Pesticide, Cardio depressant, Psychoactive,	Liu, <i>et al.</i> [27] Kumar, <i>et al.</i> [28] Manikandan and Devi [29] Devi <i>et al.</i> [30] Devi, <i>et al.</i> [31] Ganjewala <i>et al</i> [14] Lee [32]

		Anticonvulsant	
		2. Fungicidal action against Phytopathogenic fungus <i>R.solani</i> and <i>P.infestans</i>	
5.	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- ($C_{12}H_{16}O_3$)		1. Anti-microbial 2. Hypnotic and Antiseptic effect.
			Nikhila <i>et al.</i> [33] Yang <i>et al.</i> [34]

3.2. Spiro [2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane) ($C_{12}H_{16}O_3$)

GC-MS analysis suggested the presence of Spiro in *Acorus calamus* (Fig. 3). This compound is found to be present in various other plants, including *Loranthus micranthus*. Channabasava *et al.* [21] through GC-MS analysis of methanol extracts of *Loranthus micranthus* reported presence of spiro, but it was not found to exhibit any biological activity.

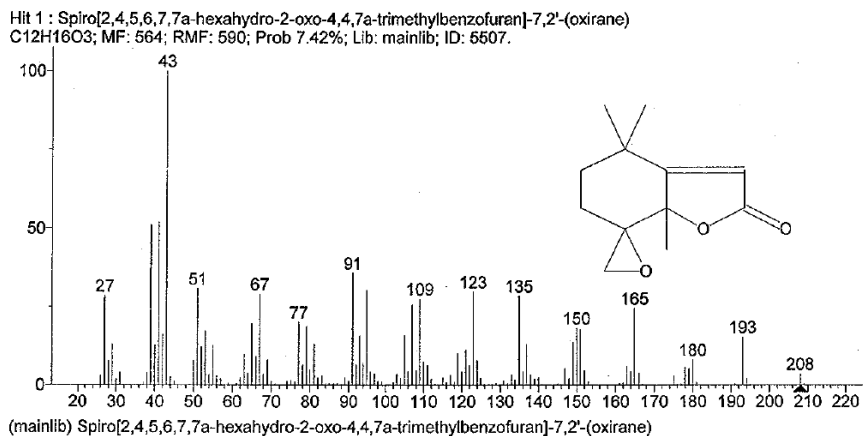


Fig. 3. Chromatogram of compound Spiro [2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane).

3.3. 9-Octadecenoic acid (Z)-, phenylmethyl ester

GC-MS analysis suggested the presence of this compound in *Acorus calamus* (Fig. 4). This compound present in several plants, including *Sarcostemma secamone*, *Jatropha tanjorensis*, and *C. Nitidum* has been reported to exhibit anti-inflammatory activity and

some biological activity. Kumari *et al.* [22] investigated possible bioactive components of whole plant of *Sarcostemma secamone* using GC-MS analysis and found 14 compounds in the ethanolic extract in which 9-octadecenoic acid (Z)-, phenyl methyl ester (comprising 6.96 % of all the found compounds) was found to show some biological activity. Bharathy *et al.* [23] carried out GC-MS chromatogram of ethanolic extract of *Jatropha tanjorensis* leaves and identified 16 phyto-constituents and reported that 9-Octadecenoic acid (Z)-, phenyl methyl ester (belonging to ester class) exhibited anti-inflammatory activity. Das *et al.* [24] reported presence of 9-octadecenoic acid (Z)-, phenyl methyl ester (1.47 %) (belonging to oleic acid ester class) as a major compound in the ethanolic extracts of leaves of *C. Nitidum* and found that it exhibited anti-inflammatory, antiandrogenic cancer preventive, dermatitogenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic insectifuge, and flavor.

3.4. Shyobunone ($C_{15}H_{24}O$)

GC-MS analysis suggested the presence of shyobunone in *Acorus calamus* (Fig. 5). Shyobunone, found in *Acorus calamus*, has been reported to exhibit biological activity. Ganjewala *et al.* [3] reported presence of Shyobunone (besides other compounds) in the rhizome extract of *A. calamus*, which are reported for anti-oxidant, anti-microbial, anti-inflammatory, nematicide and hypocholesterolemic activities.

Chen *et al.* [25] found that shyobunone (9.60 %) was one of the main components of essential oil derived from *A. calamus* rhizomes and it displayed insecticidal and repellent activity against *Lasioderma serricorne* (LS) and *Tribolium castaneum* (TC). On the contrary, Akram *et al.* [26] reported that Shyobunone, a keto compound found in the ethanolic extract of rhizome of *A. calamus*, did not exhibit any biological activity.

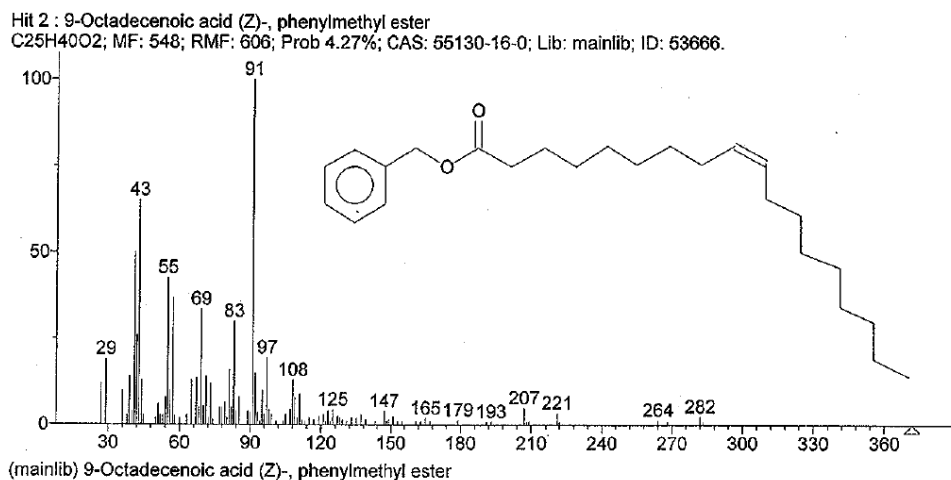


Fig. 4. Chromatogram of compound 9-Octadecenoic acid (Z)-, phenylmethyl ester.

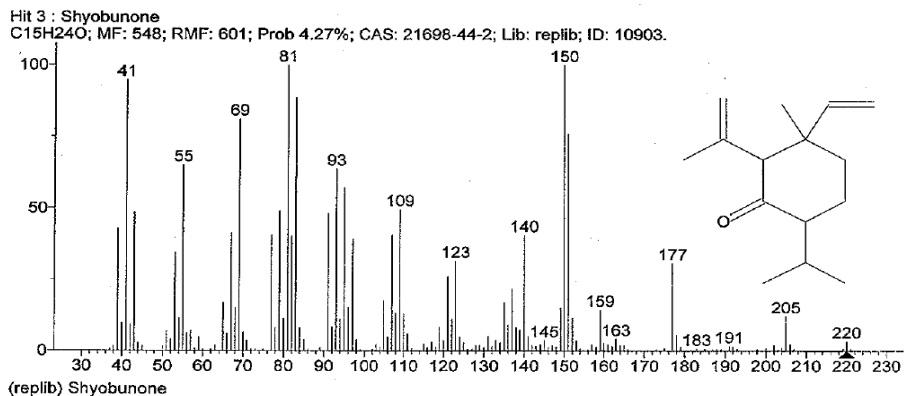


Fig. 5. Chromatogram of compound Shyobunone.

3.5. Asarone

GC-MS analysis suggested the presence of asarone in *Acorus calamus* (Fig. 6). Asarone found in *Acorus calamus* has been reported to exhibit anti-microbial activity. Liu *et al.* [27] determined the chemical composition of the essential oil of *Acorus calamus* rhizomes in China and identified 32 components of the essential oil of *A. calamus* rhizomes, in which α -asarone (comprising 50.1 % of total compounds present) was the principal compound. They reported the insecticidal activity of *A. calamus* against the booklouse (*Liposcelis bostrychophila*). Kumar *et al.* [28], Manikandan and Devi [29]; Devi and Ganjewala [30]; Devi *et al.* [31] have reported presence of α -asarone in the rhizome extract of *A. calamus*. Ganjewala *et al.* [3] evaluated anti-microbial activity of *Acorus calamus* rhizome and leaf extracts obtained with different solvents *viz.*, petroleum ether, chloroform, hexane and ethyl acetate and reported that α and β -asarones were primarily responsible for anti-microbial activities of *A. calamus*. The anti-fungal activity of petroleum ether and ethanolic extracts of the rhizome of *Acorus calamus* could, *inter alia*, probably be due to the presence of compounds (*viz.* Shyobunone and α -Asarone). Kumar *et al.* [28] had also reported presence of Shyobunone, and α -Asarone, (besides other compounds) in the rhizome extract of *A. calamus*, which are reported for anti-oxidant, anti-microbial, anti-inflammatory, nematicide and hypo-cholesterolemic activities. Manikandan and Devi [29]; Devi and Ganjewala [30]; Devi *et al.* [31] have attributed α -Asarone, which is one of major phyto-chemical compounds found in methanolic extract of *A. calamus*, for anti-oxidant activity. Lee [32] reported fungicidal activity of alpha-asarone and asaron aldehyde derived from *Acorus gramineus* rhizomes against *Rhizoctonia solani* and *Phytophthora infestans*.

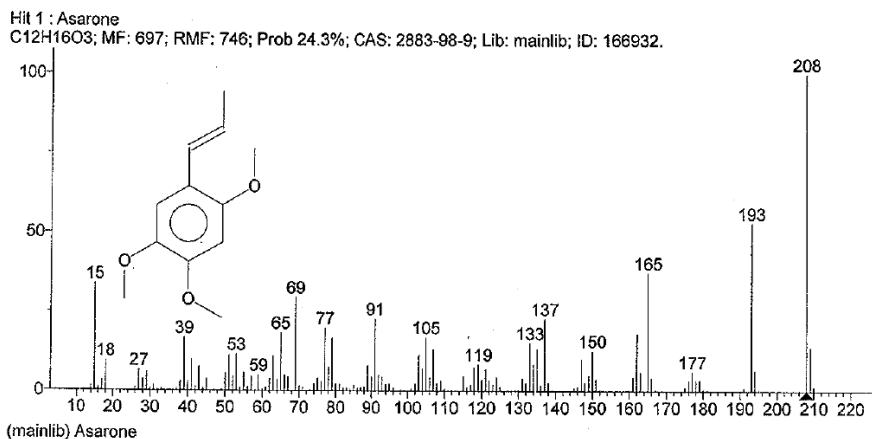


Fig. 6. Chromatogram of compound Asarone.

3.6. Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(C₁₂H₁₆O₃)

GC-MS analysis suggested the presence of this compound in *Acorus calamus* (Fig. 7). Nikhila *et al.* [33] found benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(C₁₂H₁₆O₃) as one of the medicinally active compounds from tuber of *Gloriosa superba* by GC-MS, which possessed anti-microbial activity. Yang *et al.* [34] reported that 'Benzene, 1,2,3-trimethoxy-5-(2-propenyl) (Elemicin)' is one of the organic compounds in *Dalbergia bariensis* wood and it has hypnotic and ant-septic effects. Atalar and Turkan [35] reported the presence of this compound in rhizome of *Acorus calamus*, besides asarone.

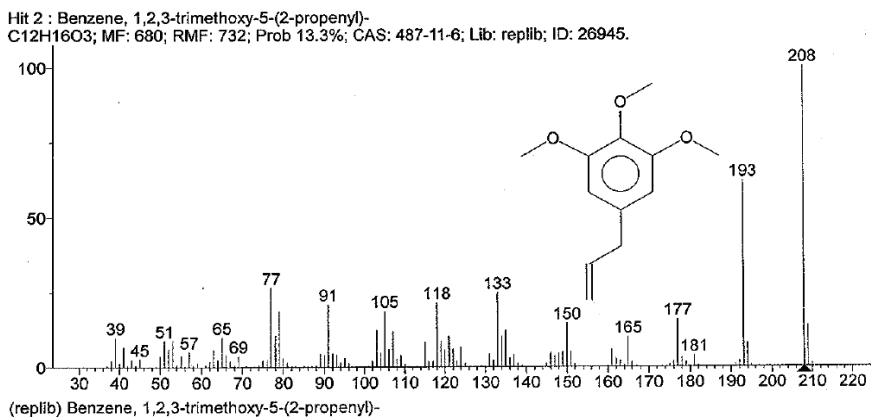


Fig. 7. Chromatogram of compound Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(C₁₂H₁₆O₃).

4. Conclusion

Acorus calamus finds wider application as a herbal medicine owing to its anti-microbial activity [36]. The therapeutic potential of *A. calamus* derives from the presence of some

compounds, which have been reported to retard the growth of various pathogens, as reported in the preceding sections. Given the incidence of such compounds (with therapeutic properties) in *Acorus calamus* rhizome, this study seeks to identify them using GC-MS analysis.

GC-MS analysis (for investigation of the compounds present) of the petroleum ether extract of *A. calamus* rhizome suggested the presence of five compounds (which showed close match with those of the GC-MS database main library of Novocatalyz) viz., Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2'-(oxirane); 9-Octadecenoic acid (Z)-, phenylmethyl ester (C₂₅H₄₀O₂); Shyobunone; α -Asarone; and benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(C₁₂H₁₆O₃). Presence of some of these phyto-chemicals may be responsible for the anti-microbial activity of *Acorus calamus*. Findings of various studies [3,27-31] in respect of rhizome of *Acorus calamus* suggest that presence of shynobunone and asarone may have been responsible for its anti-oxidant and anti-microbial activities.

However, in-depth research is needed on the bio-active constituent compounds of essential oils of *Acorus calamus*. This is because of the fact that there may be significant differences in biosynthesis, composition, structure, etc. of bio-active constituent compounds across various varieties, species (and even within the same species) owing to different environmental and various other abiotic constraints such as water stress, level of pollution, etc. Liu *et al.* [27] attributed that differences of chemical composition of the essential oils of *A. calamus* could be due to harvest time and local, climatic and seasonal factors as well as storage duration of medicinal herbs. Also, different species may have distinct characteristics. Future works on this area may, *inter alia*, attempt to correlate these isolated compounds with their anti-microbial activity and anti-oxidant capacity.

Acknowledgments

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References

1. M. H. Abass and N. H. Mohammed, Basra J. Date Palm Res. **13**, 26 (2014).
2. S. S. Kumar, A. A. Soban, A. T. S. Fareed, and J. M. S Mohamed, Orient. J. Chem. **26**, 223 (2010).
3. D. Ganjewala and S. A. Devi, Acta Biol. Szeged. **53**, 45 (2009).
4. R. Balakumbahan, K. Rajamani, and K. Kumanan, J. Med. Plant Res. **4**, 2740 (2010).
5. S. T. Runkel, A. F. Bull, L. A. Stone, T. Rosburg, C. Kurtz, L. Scarth, R. Scarth, Wildflowers of Iowa Woodlands (University of Iowa Press, 2009). <https://doi.org/10.2307/j.ctt20mverz>
6. U. C. Bapat, S. Prabha, and J. Kumar, Int. J. Bioassays **5**, 4714 (2016). <https://doi.org/10.21746/ijbio.2016.07.0012>

7. S. Prabha, U. C. Bapat, and J. Kumar. *Eur. J. Pharm. Med. Res.* **3**, 583 (2016).
8. W. Dong, D. Yang, and R. Lu, *Planta Med.* **76**, 454 (2010). <https://doi.org/10.1055/s-0029-1186217>
9. R. Gyawali and K. S. Kim, *Kathmandu Univ. J. Sci. Eng. Technol.* **5**, 51 (2009).
10. A. Muthuraman and S. Nirmal, *Asian Pac. J. Trop. Biomed.* **2**, S1017 (2012).
[https://doi.org/10.1016/S2221-1691\(12\)60354-2](https://doi.org/10.1016/S2221-1691(12)60354-2)
11. N. Mittal, H. S. Ginwal, and V. K. Varshney, *Pharmacog. Rev.* **3**, 83 (2009).
12. D. Wang, Y. Geng, L. Fang, X. Shu, J. Liu, X. Wang, and H. Luqi, *J. Sep. Sci.* **34**, 3339 (2011). <https://doi.org/10.1002/jssc.201100573>
13. D. Sandeep and C. K. Nair, *Mutat. Res.* **722**, 62 (2011).
<https://doi.org/10.1016/j.mrgentox.2011.03.005>
14. D. Ganjewala and A. Devi, *Acta Biol. Szeged.* **53**, 45 (2009).
15. S. Phongpaichit, N. Pujenjob, V. Rukachaisirikul, and M. M. Ongsakul, *J. Sci. Technol.* **27**, 517 (2005).
16. M. Ghosh, *Ann. Bot.* **98**, 1145 (2006). <https://doi.org/10.1093/aob/mcl205>
17. C. Singh, U. Jamwal, and P. Singh, *JMAPS* **23**, 687 (2001).
18. P. D. Shah, M. Ghag, P. B. Deshmukh, Y. Kulkarni, S. V. Joshi, B. A. Vyas, and S. R. Dinesh, *Int. J. Green Pharm.* **6**, 29 (2012). <https://doi.org/10.4103/0973-8258.97119>
19. H. H. Haines, *The Botany of Bihar & Orissa*, 1-3: 1-1372 (1924). [Reprint (1961)].
20. E. M. Kuskoski, J. R. José, M. B. Julia, F. Roseane, M. T. Ana, and, G. A. Agustín, *Open Analyt. Chem. J.* **6**, 1 (2012).
21. G. M. Channabasava, C. P. Chandrappa, and T. S. Sadananda, *Asian J. Pharm.* **4**, 34 (2014).
22. T. K. S. Kumari, S. Muthukumarasamy, and V. Mohan, *Sci. Res. Reporter* **2**, 187 (2012).
23. V. Bharathy and F. Uthayakumari, *Int. J. Pharm. Tech. Res. Coden (USA)*, **5**, 1839 (2013).
24. M. N. Das, S. Sivakama, K. S. Sundari. V. Mohan, and B. Parthipan, *Asian J. Pharm. Clin. Res.* **7**, 2 (2014).
25. H. P. Chen, K. Yang, L. S. Zheng, C. X. You, Q. Cai, and C. F. Wang, *Pharmacogn Mag.* **11**, 675 (2015). <https://doi.org/10.4103/0973-1296.165543>
26. S. Akram, S. S. Kumar, A. Fareed, T. S. Ahmed, and J. M. S. Mohamed, *Orient. J. Chem.* **26**, 223 (2010).
27. X. C. Liu, L. G. Zhou; Z. L. Liu, and S. S. Du. *Molecules* **18**, 5684 (2013).
<https://doi.org/10.3390/molecules18055684>
28. S. S. Kumar, A. S. Akram; T. S. F. Ahmed, and M. S. M. Jaabir, *Orient. J. Chem.* **26**, 223 (2010).
29. S. Manikandan and R. S. Devi, *Pharmacol. Res.* **52**, 467 (2005).
<https://doi.org/10.1016/j.phrs.2005.07.007>
30. S. A. Devi and D. J. Ganjewala, *Herbs Spiced Med. Plants* **17**, 1 (2011).
<https://doi.org/10.1080/10496475.2010.509659>
31. A. S. Devi, A. L. Mali, M. A. Rahee, and E. D. S. Belinda, *Asian J. Chem.* **9**, 107 (2014).
<https://doi.org/10.3923/ajb.2014.107.113>
32. H. S. Lee, *Bioresour. Technol.* **98**, 1324 (2007). <https://doi.org/10.1016/j.biortech.2006.05.018>
33. G. S. Nikhila, G. Sangeetha, T. S. Preetha, and T. S. Swapna, *J. Pharmacogn. Phytochem.* **5**, 17 (2016).
34. L. Yang, T. Jiang, H. Liu, and K. Li, *BioResources* **10**, 7092 (2015).
<https://doi.org/10.15376/biores.10.4.7092-7104>
35. A. M. Nuri and F. Türkan, *J. Inst. Sci. Technol.* **8**, 181 (2018).
<https://doi.org/10.21597/jist.433743>
36. S. Prabha, S. H. Gaurea, U. C. Bapat, and K. Jyoti, *Eur. J. Pharm. Med. Res.* **6**, 357 (2019).