IJBPAS, January, 2026, 15(1): 396-405

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COMPREHENSIVE INSIGHTS FROM BIOSYNTHESIS TO TOXICOLOGY ON β-ASARONE

PARMAR U[1], DODIYA T[2*] AND PRAJAPATI D[2]

1: Department of Quality Assurance, Parul Institute of Pharmacy & Research, Parul University, Limda, Vadodara 391760, Gujarat, India

2: Department of Pharmacognosy, Parul Institute of Pharmacy & Research, Parul University, Limda, Vadodara 391760, Gujarat, India

***Corresponding Author: Dr. Tanvi Dodiya: E Mail: tanvi.dodiya@paruluniversity.ac.in**

Received 13th Sept. 2024; Revised 25th Nov. 2024; Accepted 25th Jan. 2025; Available online 1st Jan. 2026

https://doi.org/10.31032/IJBPAS/2026/15.1.9792

ABSTRACT

β-asarone is essentially isolated from the "*Acorus* species" and "*Guatteria gaumeri* Greenman". β-asarone is an extensively studied phytochemical. β-asarone has significant pharmacological action reported by preclinical studies. The goal of the thorough review is to examine every piece of knowledge about β-asarone's Isolation, biosynthesis, analytical techniques, and pharmacological activities. The review delves extensively into the stages that plants' enzymes take to synthesize β-asarone. It can be biosynthesised via the Shikimate Pathway. Ultrasonic-assisted extraction, Chromatotron method, Soxhlet extraction, and other methods can be employed to extract and isolate β-asarone from different plant parts and other medicinal products. Furthermore, the review addresses the identification and characterization of β-asarone through a range of analytical methods, including GC-MS, HPLC, HPTLC, and others. The β-asarone could be evaluated quantitatively and qualitatively using these methods. The compound has characteristics that include neuroprotective, anti-depressant, anti-Alzheimer, and genotoxic, which are useful in pharmacology and toxicology. To improve understanding of biological activities, β-asarone isolation, and other subjects, a wealth of new information is included. establishing the most of each aspect for upcoming investigations will be greatly aided by this.

Keywords: β-asarone, Sweet Flag, β-asarone extraction, Analytical characterization

1. INTRODUCTION

When extracting essential oil components from herbal compounds, β-asarone is the most bioactive compound found. It is primarily sourced from *Acorus calamus* and

is present in roots, leaves, rhizomes, and essential oils. This compound belongs to the phenylpropanoids class and has different ploidy levels. The tetraploid variety is widespread in countries like India, Singapore, Taiwan, Japan, Thailand, Indonesia, and Russia. *Acorus calamus* is a perennial semi-aquatic plant valued in traditional medicine. β-asarone has been studied for its potential medicinal properties, especially its effects on the central nervous system, despite concerns about its genotoxic and mutagenic effects **[1-4]**.

1.1 Organoleptic Properties of β- Asarone

Organoleptic characteristics are evaluated based on sensory impressions. In pharmaceutical assessment, this includes attributes like color, taste, size, form, and unique features such as texture and touch. The characteristics of β-asarone are detailed in **Table 1 [5]**.

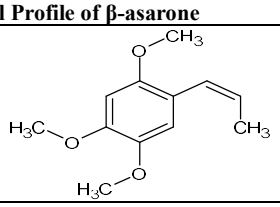
1.2 Physicochemical Profile of β- Asarone

Physicochemical characterization seeks to define the Chemical and Physical properties such as identification, purity, stability, and quality of the substance to determine its potential effects. Some of the Physicochemical profiles of β-asarone are shown in **Table 2 [6-8]**.

Table 1: Organoleptic Properties of β-asarone

Color	**Light Brownish Yellow (Buff) color**
Odour	**Aromatic**
Texture	**Fine having tiny fibers present**
Taste	**Sharp strong Pungent**

Table 2: Physicochemical Profile of β-asarone

Structure	
IUPAC Name	**Cis-2,4,5-trimethoxy-1-allyl phenyl**
Molecular Formula	**$C_{12}H_{16}O_3$**
Molecular Weight	**208 g/mol**
Melting Point	**60-65°C**
Boiling Point	**264-267°C**
logP	**3.406**
Solubility	**Soluble in n- hexane.**

2. BIOSYNTHESIS

β-asarone is a phenylpropene found in various medicinal plants through the shikimate pathway. Erythrose 4-phosphate (E4-P) and phosphoenolpyruvate (PEP) can both be converted into chorismate with the help of six enzymes that catalyze seven distinct reactions. Additionally, three more enzymes are involved in further reactions during the biosynthesis process **[9, 10]**.

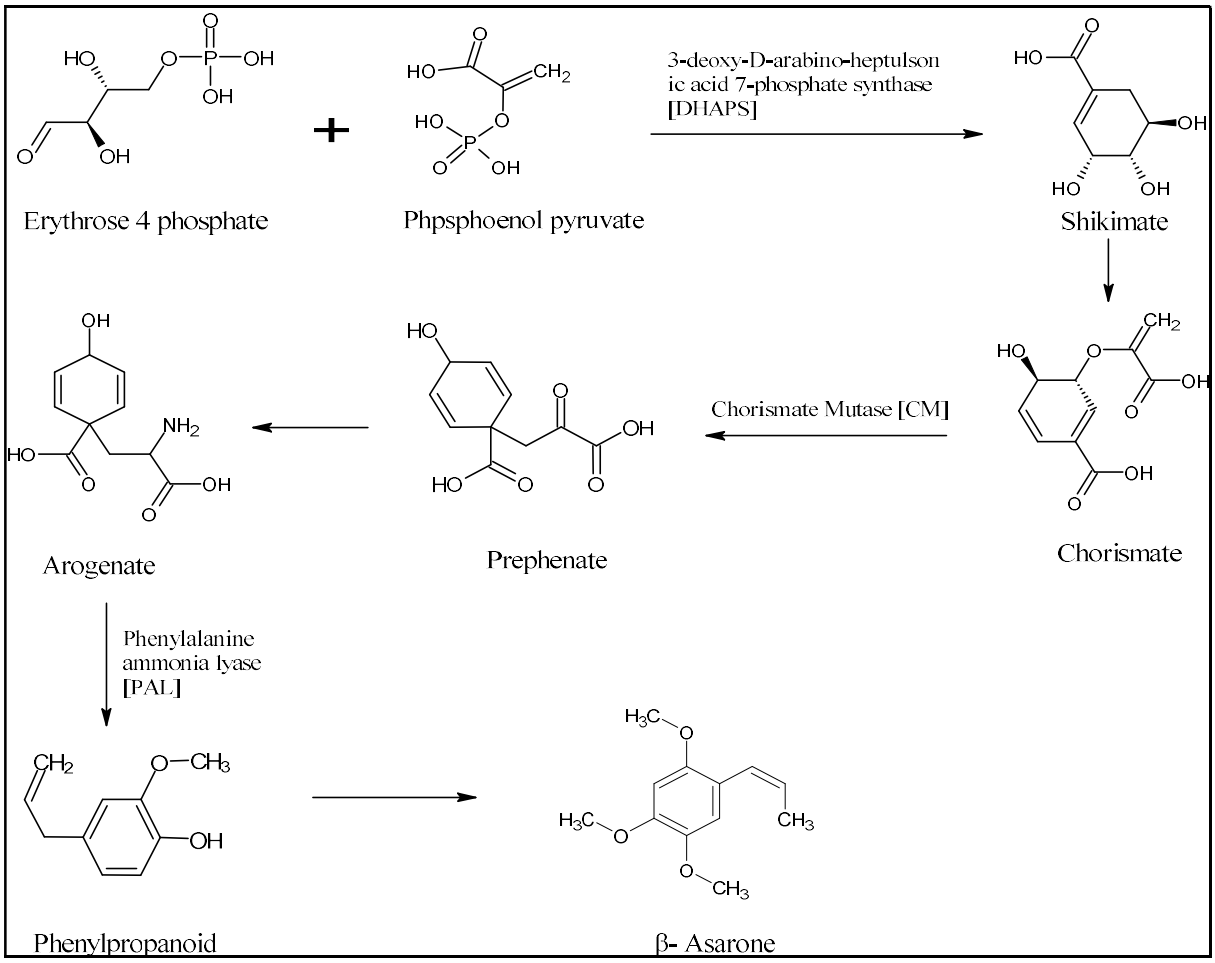


Figure 1: Biosynthetic Pathway of β-asarone [11]

3. EXTRACTION TECHNIQUES

3.1. Conventional Solvent Extraction of *Acorus calamus* [AC]

0.5 or 1 gram of ground AC was mixed with 50 milliliters of ethanol and kept at 40-45°C for 30 minutes. The extract was then filtered and purified using chromatography. NMR, IR, and UV spectroscopy were used to confirm the identity of the purified β-asarone **[12]**.

3.2. *Acorus calamus* extraction by Ultrasonic-Assisted Extraction Technique

The ultrasonic-assisted extraction used the same solvent volume, duration, and solid-to-solvent ratio as conventional extraction. A 20 kHz ultrasonic probe with a 1.0 cm tip diameter was used, positioned 1.0 cm below the solvent surface. Sonication power was set at 30%, 50%, and 70%, resulting in power densities of 471.2, 981, and 1571.6 Wdm^{-3}, respectively. The extraction procedures were the same as the conventional approach **[12]**.

3.3. Extraction of *P. sarmentosum Roxburgh*

After splitting the *P. sarmentosum* plant specimens into their leafy parts, stems, roots, and fruits, an electric grinder (SM-100) was used to pulverize them. The powdered material was macerated with 99% ethanol, 50% ethanol, and water solvents at 60°C for 48 hours as part of the extraction process. After filtering, each of the resultant extracts was dried by solvent evaporation in a rotary evaporator called R-100 set at 40°C. PS stood for *P. sarmentosum*, L for Leaves, S for Stems, R for Roots, F for Fruits, and EW for Ethanol: Water (50:50), with E standing for Ethanol **[13]**.

3.4. Simple Distillation Method (Aqueous extract)

A 500 mL round-bottom flask was filled with 150g of shade-dried and coarsely powdered *Acorus calamus* rhizome. Approximately 400 mL of water was added to the flask, and the resulting mixture was extracted over 24 hours in a water bath kept at a temperature lower than 80°C. After filtering the mixture, a dark brown residue was obtained. The filtrate was then concentrated and evaporated until completely dry. The resulting residue was kept for further examination, and the percentage recovery was between 99.63-100.64% **[14]**.

3.5. Soxhlet Extraction (ethanolic extract and ethyl acetate)

A 150g of dried and roughly ground *Acorus calamus* rhizome was placed in a Soxhlet extractor with 600 ml of ethanol/ethyl acetate. A 1-liter round-bottom flask in a water bath was connected to the extractor's base via a condenser and water bath. To enable the directed flow of the condensed alcohol/ethyl acetate onto the stuffed material, the dried powder was first compressed using filter paper and placed inside a thimble. The extraction process was conducted with great care to keep the water bath temperature below 100°C. This process involved continuous boiling for 48 hours. Afterward, a rotary evaporator was utilized to evaporate the extract at a lower pressure. After being dried in a desiccator, the finished product was put in a freezer and sealed in an airtight jar for later use **[14]**.

3.6. Supercritical fluid / Supercritical carbon dioxide (SC-CO_2) extraction

The supercritical fluid extraction method was implemented for obtaining the oil from the rhizomes of *Sweet Flag* (AC L.) under various pressures (100, 150, and 200 bar) and temperatures (45, 55, and 65°C) for an uninterrupted dynamic extraction time of 120 minutes **[15]**.

3.8. Isolation of β-asarone

Thin Layer Chromatography (TLC) to analyze Phytoconstituents from ethanol extracts of *Acorus*. The rhizome extract underwent vacuum liquid chromatography (VLC) over silica gel, using a gradient solvent system of hexane and ethyl acetate. The most active fraction was further

separated using TLC, yielding 15.6 mg of a yellow oil compound with high purity **[16]**.

4. ANALYTICAL TECHNIQUES

Pharmaceutical analysis is vital for maintaining high standards in drug formulations. As the pharmaceutical sector expands globally, there is a growing need for advanced analytical techniques. Technological developments have led to faster analysis, improved precision, and lower costs. These methods analyze various constituents, including bioactive compounds in plant extracts. Plants are valued for their pharmacological activities, and their bioactive compounds are identified in different plant parts for medicinal use. Detection techniques such as FTIR, NMR, and MS are used alongside extraction processes like HPLC, TLC, HPTLC, and OPLC **[17, 18]**.

Table 3: Different Analytical Methods for β-asarone

Sr. No.	Methods	Description			Results	Ref.
		Stationary Phase	Mobile Phase	Wavelength		
1.	HPLC	Agilent 5 µm T-C-18(2) 250 × 4.4 mm	Methanol: water (80:20 v/v)	251 nm	R_t: 5.43 min.	19
		C-18 Gemini, 250 × 4.6 nm, 5µm	Acetic acid: alcohol (1:3 v/v)	210 nm	R_t: 7.14 min.	20
		C-18 column (250 × 4.6 mm, 5µm)	0.1 % Phosphoric acid: Acetonitrile: Methanol (50:40:10 v/v/v)	210nm	R_t: 11.89 min.	21
2.	HPTLC	HPTLC plate 5 × 10 cm, precoated with silica gel 60 F_{254}	Toluene: Ethyl acetate: Acetic acid (90:10:2 v/v/v)	254nm, 366nm	R_f: 0.38	22
		Silica gel 60 F_{254} 20 × 10 cm	Toluene: Ethyl acetate (93:7 v/v)	313nm	R_f: 0.55	23
		HPTLC plate 20 × 10 cm, precoated with silica gel 60 F_{254} TLC Plate	Toluene: Methanol: Tri-ethyl amine (9.2:0.5:0.3 v/v/v)	282nm	R_f: 0.81	24
3.	TLC	Precoated Silica gel 60 F254	n-hexane: ethyl acetate (8:2 v/v)	UV chamber 254nm	R_f: 0.66	25
			Chloroform: Methanol (9:1v/v)	UV chamber 254nm	R_f: 0.42	26
4	GC-MS	Rtx-5 capillary column (5% biphenyl)	Nitrogen: 1.18mL/min for 10 min.	Flame ionization	R_t: 6.8 min.	14
		Column: HP-5 MS, 30×25mm ID. 0.25µm thick film	Helium: 1.3mL/min	Mass Selective Detector	R_t: 6.7min.	27

5. PHARMACOLOGICAL AND TOXICOLOGICAL ACTIVITIES

5.1. Pharmacological Effects

5.1.1. Neuroprotective

When rats were given a 50% ethanolic rhizome extract at a dose of 25 mg/kg for 10 days while additionally receiving acrylamide, the amount of acrylamide-induced neurotoxicity in the rats was minimized. This reduction was measured by figuring out the degree of limb paralysis. Additionally, the elevated receptor for dopamine content and lowered glutathione levels returned to normal as a result of this treatment **[28]**.

5.1.2. Anti-depressant

After being injected into laboratory rats for seven days, an extract made with methanol

of *Acorus calamus* containing 50–100 mg/kg demonstrated dose-dependent antidepressant effects with a potency equivalent to 5 mg/kg imipramine **[28]**.

5.1.3. Anti-Alzheimer

Acetylcholinesterase (AChE) was found to be inhibited by both β-asarone. On the other hand, rats that suffered from long-term exogenous corticosterone-induced impairment of memory, after receiving β-asarone therapy demonstrated improved geographical learning and memory. β-asarone was found to reduce an overexpression of Bax (apoptotic protein) in the hippocampus **[29]**.

5.1.4. Anti-Parkinson

The treatment with β-asarone improved Parkinsonism in rats exposed to 6-hydroxydopamine (6-OHDA), a neurotoxin affecting dopamine-producing cells. Parkinson's disease is mainly caused by the breakdown of dopamine receptor cells in the substantia nigra, leading to increased Alpha-synuclein expression and persistent neuroinflammation. β-asarone reversed the 6-OHDA-induced changes in cell markers and dopamine metabolites **[29]**.

5.1.6. Anti-hyperlipidemic

A significant hypolipidemic effect was seen with oral administration of the *Acorus calamus* rhizome 50% ethanol extract at amounts of 100 and 200 mg/kg as well as the extract's isolated saponins at a dose of 10 mg/kg. On the other hand, the aqueous extract didn't show hypolipidemic effects until 200 mg/kg. Furthermore, α-Asarone, which is derived from the rhizomes of *Acorus*, demonstrated hypolipidemic effects in mice **[30]**.

5.1.8. Antioxidant

The treatment of β-asarone (20 & 30 mg/kg via the oral route for 30 days) reversed decreased antioxidant levels and increased lipid peroxidation caused by a high-fat diet in rats. However, it may not change elevated indicators of oxidative stress in the hippocampal regions of SAMP-8 mice, which mimic Alzheimer's disease **[29]**.

5.2. Toxicological Effects

5.2.1. Chronic toxicity

In this study, 12-day-old male B6C3F1 pre-weanling mice were given β-asarone at a single dose of 52 mg/kg or multiple doses of roughly 1 mg on days 1, 8, 15, and 22. Mice developed hepatomas at 10 months for a single shot and 13 months for subsequent doses. Autopsies at 10 months after a single dose of β-asarone (156 mg/kg) revealed no hepatoma development, even after pretreatment with the sulfotransferase inhibitor pentachlorophenol. These results suggest that β-asarone may be involved in the development of hepatocellular cancer **[29]**.

5.2.2. Mutagenicity

The term "mutagenicity" refers to the process that leads to mutations within an individual gene or an entire set of genes. In

the Ames test, the mutagenic effects of β-asarone were observed in the S. Typhimurium strain TA-100 only when S9 metabolic activation was present. Additionally, the β-asarone side-chain epoxide metabolites demonstrated mutagenicity **[29]**.

5.2.4. Teratogenicity

The outcomes of the trials showed that, in the chicken embryo test, eggs treated with β-asarone showed 100% mortality, suggesting the existence of teratogenicity. On the other hand, α-asarone caused embryotoxicity at doses of 15 to 60 mg/kg and maternal toxicity at 60 mg/kg when it was given p.o. to pregnant mice at doses of 5, 15, 30, and 60 (mg/kg) during days 6 to 15 of gestation. This led to fetal malformations such as hydrocephaly, extra ribs, club feet, and cleft lips. These results point to the possible teratogenic impact of β-asarone; however, additional *in-vivo* investigations are necessary **[30]**.

6. CONCLUSION

There is an intriguing paradox with β-asarone. Even with well-established methods for isolation and analysis, as well as fascinating preclinical evidence pointing to a wide range of pharmacological properties, its proven carcinogenicity still stands in the way. The suggested biosynthesis pathways provide a direction for further research, which might result in the creation of safer derivatives. To entirely figure out β-asarone pharmacological characteristics and reduce any potential toxicity, more research is necessary. To verify its safety and efficacy profile, future research should give priority to in vivo studies at well-defined dosages. At that point, it will be secure to assess β-asarone's actual therapeutic potential.

7. REFERENCES

[1] Yong-Qi F, Chen S, Lin L, Ruo-Ming F. Pharmacokinetics of β-asarone in rabbit blood, hippocampus, cortex, brain stem, thalamus and cerebellum. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2012; 67(2): p. 120- 123.

[2] Shailajan S, Menon S, Swar G, Singh D, Nair S. Estimation and quantitation of β-asarone from *Acorus calamus* rhizome and its formulations using validated RP-HPLC method. Pharmaceutical Methods. 2015; 6(2): p. 94-99.

[3] Nandakumar S, Menon S, Shailajan S. A rapid HPLC-ESI-MS/MS method for determination of β-asarone, a potential anti-epileptic agent, in plasma after oral administration of *Acorus calamus* extract to rats. Biomedical Chromatography. 2013; 27(3): p. 318-326.

[4] Qadry J. S., Textbook of Pharmacognosy, 16th edition 2010, CBS Publication Pvt Ltd; p. 216-218.

[5] Ashokan A, Shincymol VV, Ansary PY, Ommen SM. Pharmacognostical evaluation of Vacha (*Acorus calamus* Linn.) rhizome. Kerala Journal of Ayurveda. 2024; 3(1): p. 34-37.

[6] Quality standards of Indian medicinal plants, Volume 10; p. 25-37.

[7] National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 5281758, beta-Asarone. Retrieved September 26, 2024.

[8] Shenvi S, Vinod HR, Kush A, Reddy GC. A unique water-soluble formulation of β-asarone from sweet flag (*Acorus calamus* L.) and its *in-vitro* activity against some fungal plant pathogens. Journal of Medicinal Plants Research. 2011; 5(20): p. 5132-5137.

[9] Uebel T, Hermes L, Haupenthal S, Müller L, Esselen M. α-Asarone, β-asarone, and γ-asarone: Current status of toxicological evaluation. Journal of Applied Toxicology. 2021; 41(8): p. 1166-1179.

[10] Tzin V, Galili G. The biosynthetic pathways for shikimate and aromatic amino acids in Arabidopsis thaliana. The Arabidopsis book/American Society of Plant Biologists. 2010; 8, p. 114-137.

[11] Guillet G, Poupart J, Basurco J, De Luca V. Expression of tryptophan decarboxylase and tyrosine decarboxylase genes in tobacco results in altered biochemical and physiological phenotypes. Plant Physiology. 2000; 122(3): p. 933-44.

[12] Omer N, Choo YM, Ashokkumar M, Yusof NS. Ultrasound-Assisted Extraction of β-Asarone from Sweet Flag (*Acorus calamus*) Rhizome. Applied Sciences. 2022; 12(21):11007.

[13] Hamil MS, Memon AH, Majid AM, Ismail Z. Simultaneous determination of two isomers of asarone in *Piper sarmentosum* Roxburgh (Piperaceae) extracts using different chromatographic columns. Tropical Journal of Pharmaceutical Research. 2016; 15(1): p. 157-165.

[14] Vijayakumar KB, Bannimath G, Koganti VS, Iyer VB. Gas chromatographic method for analysis βAsarone in rhizome extracts of *Acorus calamus* and their microbiological evaluation.

Pharmaceutical Methods. 2016; 7(2): p. 121-126.

[15] Shreelaxmi MP, Sharanagouda H, Ramachandra CT, Roopa RS, Hanchinal SG. Supercritical fluid extraction of oil from sweet flag rhizome (*Acorus calamus* L.) and its insecticidal activity on pulse beetles (*Callosobruchus maculatus*). Int J Curr Microbiol Appl Sci, 2017; 6: p. 3608-3615.

[16] McGaw LJ, Jäger AK, Van Staden J, Eloff JN. Isolation of β-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* in South Africa. South African Journal of Botany, 2002; 68(1): p. 31-35.

[17] Payal Badhe, Vijay Barge, A review on High-Performance Thin Layer Chromatography, International Journal of Scientific Research in Science and Technology,2024; 11(1), p. 445-455.

[18] Ravisankar P, Gowthami S, Rao GD. A review on analytical method development. Indian journal of research in pharmacy and biotechnology. 2014 May 1;2(3):1183.

[19] Dhivya V, Nelson SJ, Paramasivam M. Comparing extraction efficacy of different solvents to extract *Acorus calamus* by using HPLC. Journal of Pharmacognosy and Phytochemistry. 2019; 8(3): p. 3074-3078.

[20] Mythili MN, Immanuel SC, Rajasekharan PE, Rajasekaran C, Tharachand C, Rao VK. HPLC profiling of B-Asarone content and cytogenetic studies of medicinally important Indian *Acorus calamus* L. Accessions. Int J Pharm Sci Rev Res. 2013; 22: p.73-78.

[21] Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry. 2017; 6(1): p. 32-36.

[22] Ashokan A, Shincymol VV, Ansary PY, Ommen SM. HPTLC Fingerprinting Profile of Vacha (*Acorus calamus* Linn.). International Research Journal of Ayurveda and Yoga. 2023; 6(12): p. 45-49.

[23] Widmer V, Schibli A, Reich E. Quantitative determination of β-asarone in calamus by high-performance thin-layer chromatography. Journal of AOAC

International. 2005; 88(5): p. 1562-1567.

[24] Shelar M, Lohidasan S, Gadgil S, Mahadik KR. Marker Based Standardisation of Sarasvata Ghrita, An Ayurvedic Polyherbal Formulation using Rapid Validated High-Performance Thin Layer Chromatography Method. Journal of Chemical and Pharmaceutical Research. 2017; 9(10): p. 10.

[25] Syarif RA, Rasyid R, Najib A. Dringo rhizome (*Acorus calamus* L.): A potential source high βasarone. Journal of Chemical and Pharmaceutical Research. 2015; 7(3): p. 258-61.

[26] Nath P, Yadav AK. Anthelmintic activity of a standardized extract from the rhizomes of *Acorus calamus* Linn. (Acoraceae) against experimentally induced cestodiasis in rats. Journal of Intercultural Ethnopharmacology. 2016; 5(4): p. 390.

[27] Mungkornasawakul P, Supyen D, Jatisatienr C, Jatisatienr A. Inhibitory effect of *Acorus calamus* L. extract on some plant pathogenic molds. International Conference on Medicinal and Aromatic Plants. Possibilities and Limitations of Medicinal and Aromatic Plant 576 2001, p. 341-345.

[28] Yadav D, Srivastava S, Tripathi YB. *Acorus calamus*: A review. Int. J. Sci. Res. in Biological Sciences 2019, 6(4); p. 62-67.

[29] Chellian R, Pandy V, Mohamed Z. Pharmacology and toxicology of α- and β-Asarone: A review of preclinical evidence. Phytomedicine. 2017, 32: p. 41-58.

[30] Mukherjee PK, Kumar V, Mal M, Houghton PJ. *Acorus calamus*: scientific validation of ayurvedic tradition from natural resources. Pharmaceutical biology. 2007; 45(8): p. 651-66.