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Volatile Components, Antibacterial and Antioxidant Activities of Komburongoh (*Acorus calamus* L.) Essential Oils as Potential Medicinal Herbs from Sabah, Malaysia

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ABSTRACT

Acorus calamus L., known for diverse therapeutic applications, was studied for its volatile components, antibacterial and antioxidant potential in essential oils from Sabah, Malaysia. Employing hydrodistillation with a Clevenger apparatus, the oils were analysed through gas chromatography-mass spectrometry. Antibacterial activity was assessed via disc diffusion against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. Antioxidant properties were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Both leaf and rhizome oils were rich in phenylpropanoids, oxygenated sesquiterpenes, and sesquiterpenes including α-asarone, γ-asarone, methyl isoeugenol, 6-epi-shyobunone, and (E)-β-farnesene. They demonstrated significant antibacterial activity at 400 µg/mL, while displaying lower DPPH (IC_{50} = 28.20 ± 4.99 µg/mL) and excelling in the FRAP (150.12 ± 0.10 mg TE/g). This ongoing phytochemical analysis of *A. calamus* holds promise for enhancing quality control, ensuring safety, and validating its traditional applications.

Keywords: *Acorus calamus*; antibacterial; antioxidant; essential oil; volatile components

INTRODUCTION

In recent years, there has been a notable increase in research focused on medicinal plants such as *Coleus forskohlii*, *Ranunculus isthmicus* subsp. *tenuifolius*, and *Ranunculus rumelicus*, mostly driven by their wide-ranging pharmacological potential (Khatun *et al.*, 2011; Fafal *et al.*, 2022). The increasing popularity of *Acorus* proliferates due to their medicinally and pharmacologically vital active components (Mukherjee *et al.*, 2007; Mittal *et al.*, 2009). These plants yield numerous natural compounds with therapeutic and pharmacological characteristics such as terpenoids, flavonoids, phenolics, and alkaloids (Sharma *et al.*, 2014; Sharma *et al.*, 2020). Thus, the various beneficial biological activities of natural plant products were extensively investigated. These well-documented advantages involved antibacterial, antioxidant, anti-inflammatory, antifungal, cytotoxicity, herbicide, and insecticide activities (Liu *et al.*, 2013; Ganesan and Gurumallesh Prabu, 2019; Loying *et al.*, 2019; Dinev *et al.*, 2021; Vakayil *et al.*, 2021).

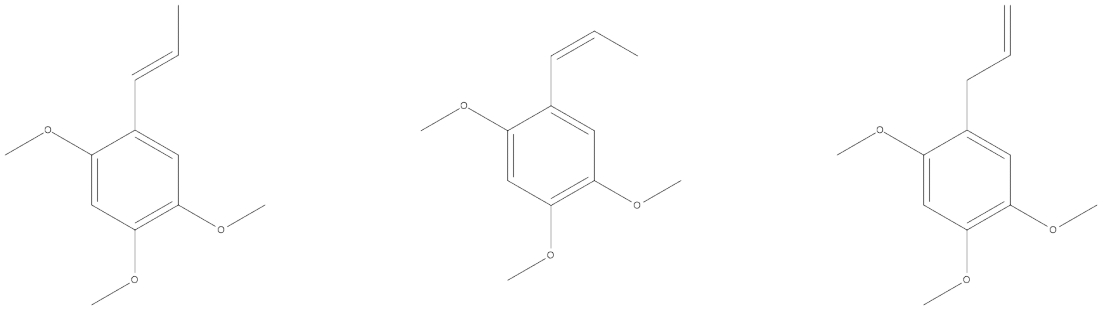
According to Rajput *et al.* (2014), the Acoraceae family contained approximately 110 genera and 1,800 species. Moreover, the morphological and anatomical distinctions of this family indicated that it was originally a member of the Araceae family (Grayum, 1987). Nonetheless, this family was viewed as a relatively primitive group within the family before its reclassification as Acoraceae (Heng *et al.*, 2010). Although the genus *Acorus* comprised 40 species, only several were identified based on their volatile components and bioactivities. These species included *A. calamus* L., *A. tatarinowii* Schott, and *A. gramineus* Solandin

Ait. (Ganjewala and Srivastava, 2011). These plants have been used in traditional medicine systems for centuries.

The *A. calamus* monocot plant is observed as grass-like with long and perennial characteristics. This plant typically thrives in moist and watery semi-aquatic habitats in tropical and subtropical climates (Sharma *et al.*, 2014). According to Ahmad and Holdsworth (2003), the Kadazan-Dusun communities in Sabah, Malaysia, employed around 50 plants (including *A. calamus*) as traditional herbal medicines daily. Furthermore, this plant is also known as 'sweet flag' or 'komburongoh' in the Dusun language, offering advantageous traditional medicine properties in healing various ailments including diarrhoea, insect repellent, poison antidote, and relieving gastritis (Kulip, 1997; Ahmad and Holdsworth, 2003). The leaves and rhizomes produce essential oils in the ethnobotany, phytochemistry, and biological fields, which were also widely investigated (Rajput *et al.*, 2014; Sharma *et al.*, 2014).

Both the leaves and the rhizomes of *A. calamus* contain various bioactive compounds that are believed to offer potential benefits in the pharmaceutical and nutraceutical industries. The antibacterial, antioxidant, antifungal, cardiovascular, and immunosuppressive effects of *A. calamus* essential oil were demonstrated in previous studies (Rajput *et al.*, 2014; Sharma *et al.*, 2014). Numerous researchers have investigated the volatile components of *A. calamus* essential oil due to its medicinal properties, leading to the identification of β-asarone as the primary constituent. However, the concentration of β-asarone in the essential oil varies depending on the geographical area and ploidy level of the plant (Raina *et al.*, 2003; Raal *et al.*, 2016).

α-Asarone (**1**), β-asarone (**2**), and γ-asarone (**3**) (Figure 1) are phenylpropenes that occur naturally in various plant groups, primarily in Acoraceae, Aristolochiaceae, and Lauraceae (Ganjewala and Srivastava, 2011; Uebel *et al.*, 2021). Plants containing asarone are commonly used for flavouring alcoholic beverages such as bitters. Research on the mutagenic effects of propenylic α-asarone and β-asarone has produced inconsistent findings, and there is a lack of available data regarding the potential carcinogenicity or genotoxicity of allylic γ-asarone (Varma *et al.*, 2002; Berg *et al.*, 2016; Atalar and Türkan, 2018).



(1) **(2)** **(3)**

Figure 1. Chemical structures of α-asarone (**1**), β-asarone (**2**), and γ-asarone (**3**)

There are no published studies on the antibacterial and antioxidant properties of essential oils derived from various parts of *A. calamus* plants. Furthermore, no prior findings on the chemical composition, antibacterial and antioxidant properties of essential oils produced from these plants have been reported in Sabah, Malaysia. Different studies may employ varied extraction methods or analytical techniques, making it challenging to directly compare results. Despite the extensive research conducted on *A. calamus*, there may still be undiscovered phytochemicals or components with potential therapeutic benefits. Hence, this research was undertaken to investigate the antibacterial activity using the disc diffusion method, as well as the antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays of essential oils isolated from various components of *A. calamus*. Additionally, the essential oils from different parts of *A. calamus* were evaluated using gas chromatography-mass spectrometry (GC-MS) analysis to identify their volatile components and determine which factors contribute to their

antibacterial and antioxidant properties. Overall, studying the phytochemical analysis of *A. calamus* is an ongoing process that can improve quality control and safety considerations, and further validate the traditional uses of this plant.

MATERIALS AND METHODS

Chemicals and Reagents

The ferric chloride ($FeCl_3$) heptahydrate and pentane were procured from Merck (Darmstadt, Germany). Subsequently, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) reagent, kanamycin, and glycerol were obtained from Sigma-Aldrich (Burlington, MA, USA). Meanwhile, acetic acid, anhydrous sodium acetate, hydrochloric acid, and methanol were sourced from Chemiz (Selangor, Malaysia). This study also utilised Trolox (Targetmol, Boston, MA, USA), dimethyl sulfoxide (DMSO) (Systerm, Selangor, Malaysia), and DPPH reagent (Tokyo Chemical Industry, Tokyo, Japan). Finally, all chemicals and reagents acquired from Bio3 Scientific Sdn. Bhd. and Apical Scientific Sdn. Bhd., Malaysia, were of analytical grade.

Plant Material

Whole plants of *A. calamus* were purchased from local farms in Ranau, Sabah, Malaysia (Coordinate: 5°57'26.4" N 116°40'24.0" E). The samples were not subjected to herbarium identification, as their identity was already well-established. *A. calamus* leaves and rhizomes were rinsed with distilled water to eliminate extraneous contaminants from the sample. After drying the samples in an oven at 50 °C (ED 23, Binder, Neckarsulm, Germany) until reaching a constant weight, the weights of each sample were measured. After collection, 250 to 300 g of *A. calamus* leaves and rhizomes were ground and pulverised using a grinder (EBM-9182, Elba, Borso Del Grappa, Italy). This increased the surface area, facilitating the extraction process and making it easier to fill the round flask.

Essential Oil Extraction

Following the method described by Cheng *et al.* (2005) with minor modifications, the dried *A. calamus* leaves and rhizomes were individually obtained using the hydrodistillation process. The ground samples were added to a 500 mL round flask with a capacity of 1000–2000 mL of distilled water. Subsequently, the substance was subjected to a six-hour hydrodistillation technique using a glass Clevenger-type apparatus. After the pentane dehydration process, the essential oil was transferred into dark-brown glass vials, sealed with aluminium foil, and stored at 4 °C until further analysis. The quantities of essential oil obtained were measured and expressed as a percentage (%) of the weight of the initial samples, following the established protocol described by Park *et al.* (2021). The oil yield was determined using the following equation, Eq.(1):

$$\text{Oil yield (\%, w/w)} = \frac{\text{Mass of oil extracted (g)}}{\text{Mass of sample (g)}} \times 100 \qquad \text{Eq.(1)}$$

Volatile Component Analysis

A fused silica capillary column (SH-Stabilwax-DA, Shimadzu, Kyoto, Japan) with dimensions of 30 m × 0.25 mm × 0.25 µm was connected to a GC-MS (GCMS-QP2010 SE, Shimadzu, Kyoto, Japan) for the analysis of the volatile components in the extracted oil. The injector was programmed to reach an injection volume of 1 µL, a split ratio of 1:40, and a target temperature of 250 °C. Initially, the GC oven temperature was set to 50 °C for 20 min, then ramped up to 250 °C at a rate of 3 °C per min, followed by a slower ramp to 300 °C at a rate of 1 °C per min. Helium was used as the carrier gas at a steady flow rate of 0.8 mL/min. Other analytical parameters included an ionisation potential of 70 eV and mass scanning from 35 to 450 m/z. In full scan mode, the MS detector revealed unique peak fragmentation patterns for several metabolites. The identification of volatile components from each peak was matched with reference mass spectra obtained from the National Institute of Standards and Technology (NIST) (MS Search Program Version 2.0) databases. The individual component concentrations were determined using a percentage of peak area (Adams, 2000).

Antibacterial Analysis

Bacterial strain growth

The American Type Culture Collection (ATCC) reference strains were received from the Faculty of Science and Technology, Universiti Malaysia Sabah. Two human pathogenic bacterial strains

were used: methicillin-resistant *Staphylococcus aureus* (MRSA) (Gram-positive, ATCC 43300) and *Escherichia coli* (Gram-negative, ATCC 25922).

Media preparation

The powdered 38 g Mueller-Hinton Agar (MHA) (Becton Dickinson, Franklin Lakes, NJ, USA) was added to 1 L distilled water. Subsequently, the media was carefully dissolved by shaking the conical flask. The media were then sterilised for 20 min at 121 °C in an autoclave (HMC Hiclave HV-25L, Gemini BV, Apeldoorn, Netherlands). Finally, the media were cooled before being transferred to separate 25 mL-capacity Petri dishes (nutrient agar).

Disc diffusion method

Using a modified version of the disc diffusion method outlined by Hong *et al.* (2021), the extracted oils were tested against two human pathogens. The 90 mm Petri dishes were prepared with 20 mL MHA and a 100 µL bacterial suspension, distributing them evenly. Sterilised filter paper discs with a 6 mm diameter were impregnated with 20 µL of essential oil. The oil was serially diluted with DMSO to achieve varying concentrations (100, 200, 300, and 400 µg/mL), following the protocol of Pintatum *et al.* (2020). Subsequently, the discs were left to dry for 15 min before being transferred using sterile forceps and then injected with two controls. These controls included a kanamycin susceptibility disc (100 µg/mL) for the positive control, and a DMSO susceptibility disc (100 µg/mL) for the negative control. The Petri dishes were then incubated at 37 °C for approximately 24 h. Finally, the diameters of the zone of inhibition (ZOI) were measured to determine the growth inhibition zones in millimetres (mm).

Antioxidant Analysis

DPPH assay

Based on the study by Benjamin *et al.* (2022), the DPPH free radical scavenging activity of the essential oil was assessed with minor adjustments. In a 96-well culture plate, 50 µL of dissolved essential oil was reacted at respective doses with 0.1 mM DPPH-methanolic solution (195 µL). The mixture was then gently stirred for 1 min before resting for 1 h. Finally, the absorbance of the final product was measured against a blank (methanol) using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, MA, USA) at 540 nm absorbance units. The positive control used was Trolox within a range of 6.25 to 100 µg/mL. Regression analysis was employed to determine the IC_{50} values (the concentration of a sample necessary to neutralise half of the DPPH radicals).

FRAP assay

The FRAP assay used in this investigation was modified based on the method outlined by Jinoni *et al.* (2024). The FRAP reagent was prepared by combining 300 mM acetate buffer (pH 3.6), TPTZ, and 20 mM $FeCl_3$ in a 10:1:1 ratio. In a 96-well culture plate placed in a water bath (WB-11, Daihan Scientific, Wonju, South Korea), 20 µL of dissolved essential oil and 180 µL of FRAP reagent were mixed and subjected to incubation at 37 °C for 40 min in the dark. The resulting absorbance readings of the combination were measured at 593 nm using a microplate reader, with a blank reference of methanol. The antioxidant positive control Trolox was used within a range of 0 to 100 µg/mL. The value was expressed as mg of Trolox per g (mg TE/g) of extract.

Statistical Analysis

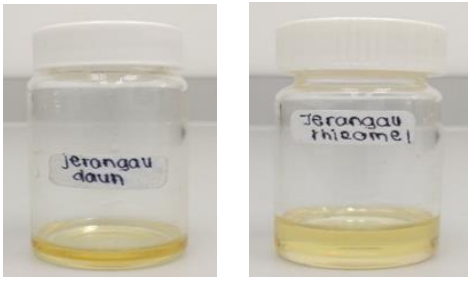
This study performed each procedure in triplicate. The findings were then denoted as mean ± standard deviation. Subsequently, independent samples t-tests (with a significance level of $p<0.05$) were applied in the antibacterial analysis to compare the statistical data. Statistical analysis was performed using IBM SPSS Statistics version 19.0 for Windows.

RESULTS AND DISCUSSION

Extraction Yield

When subject to hydrodistillation, both the *A. calamus* leaves and rhizomes yielded yellow-coloured essential oils with strong aromatic fragrances (Figure 2). On a fresh weight basis, the essential oil yield was 0.043%, w/w, for leaves and 0.370%, w/w, for rhizomes (Table 1). Notably, factors such as genotype and agronomic variables (including harvesting

period, plant age, soil fertility, and crop density) can significantly impact production (Marotti *et al.*, 1994). Additionally, Parki *et al.* (2017) pointed out that the oil output from *A. calamus* leaves and rhizomes varies based on climate, season, and geographic location.



(a) (b)

Figure 2. Essential oil extract from *A. calamus* leaves (a) and rhizomes (b)

Table 1. Essential oil yields from different tissues of *A. calamus*

Tissue	Fresh weight (g)	Yields (%, w/w)	Physical properties
Leaf	3293.79	0.043	Yellow
Rhizome	2229.08	0.370	Yellow

The *A. calamus* leaves and rhizomes exhibit a decreased oil yield compared to the findings published in prior research studies. For example, from a 500 g sample of fresh rhizomes, they managed to produce 1.30% and 1.23% to 4.80% oil yield (Raina *et al.*, 2003; Parki *et al.*, 2017).

Volatile Components of Essential Oils

A total of 78 volatile components were detected in both *A. calamus* leaf and rhizome essential oils. The identified components in *A. calamus* essential oils obtained separately from leaves and rhizomes, along with their relative percentages, retention times, and compound classes, are presented in Table 2. Moreover, the chromatographic oil profiles of *A. calamus* leaves and rhizomes are depicted in Figure 3 and Figure 4, respectively.

Fifty-nine (59) components were identified in the *A. calamus* leaf essential oil, comprising two monoterpenes (0.17%), 14 sesquiterpenes (8.09%), seven oxygenated monoterpenes (2.26%), one oxygenated diterpene (0.02%), 23 oxygenated sesquiterpenes (7.42%), five phenylpropanoids (80.07%), and seven miscellaneous (1.81%), accounting for 99.84% of the volatile components. Among the 41 components identified in the *A. calamus* rhizome essential oil, four were monoterpenes (2.90%), 11 were sesquiterpenes (2.97%), five were oxygenated monoterpenes (1.38%), 12 were oxygenated sesquiterpenes (6.78%), five were phenylpropanoids (84.66%), and four were miscellaneous (0.98%), representing 99.67% of the volatile components. The leaf essential oil was characterised by the presence of α-asarone (73.39%), (E)-β-farnesene (5.12%), methyl isoeugenol (4.63%), 6-epi-shyobunone (2.63%), γ-asarone (1.60%), linalool (1.43%), and Z-3-hexadecen-7-yne (1.21%) as the major components. Similarly, the rhizome essential oil was rich α-asarone (76.70%), methyl isoeugenol (6.38%), 6-epi-shyobunone (3.20%), trans-β-ocimene (2.02%), 4,6,6-trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.02,4]octane (1.96%), and γ-asarone (1.55%).

Previous research has extensively documented the diverse array of volatile components found in the leaves and rhizomes of *A. calamus* essential oils from various regions across the globe. For instance, Chaubey *et al.* (2018) reported the presence of 37 volatile components in the *A. calamus* leaf essential oil from the Himalayan region of Uttarakhand, India, with β-asarone constituting the highest proportion (77.7%), followed by α-asarone (6.8%). Raal *et al.* (2016) conducted research specifically on the essential oil extracted from *A. calamus* rhizomes and found it to be primarily composed of oxygenated sesquiterpenes (81.7 – 97.0%). The most abundant component in this oil was β-asarone, accounting for a substantial proportion ranging from 9.3% to 85.3%.

Table 2. Volatile components identified in the *A. calamus* leaf and rhizome essential oils

No.	Rt (min)[a]	Component[b]	Molecular formula	Percentage (%)[c]	
				Leaves	Rhizomes
1	8.206	α-Pinene	$C_{10}H_{16}$	–	0.13
2	8.918	Camphene	$C_{10}H_{16}$	–	0.53
3	9.059	1-Methylpentyl hydroperoxide	$C_6H_{14}O$	0.38	0.40
4	9.816	Sabinene	$C_{10}H_{16}$	–	0.22
5	10.06	β-Pinene oxide	$C_{10}H_{16}O$	–	0.05
6	12.34	Limonene	$C_{10}H_{16}$	0.06	–
7	12.562	trans-β-Ocimene	$C_{10}H_{16}$	0.11	2.02
8	15.659	Linalool	$C_{10}H_{18}O$	1.43	0.39
9	18.285	Camphor	$C_{10}H_{16}O$	0.27	0.78
10	19.753	Terpinen-4-ol	$C_{10}H_{18}O$	0.07	0.08
11	20.233	(E)-1,4-Undecadiene	$C_{11}H_2O$	0.06	–
12	20.526	α-Terpineol	$C_{10}H_{18}O$	0.06	–
13	24.241	Isopiperitenone	$C_{10}H_{14}O$	0.07	–
14	24.600	Bornyl acetate	$C_{12}H_{20}O_2$	0.34	0.08
15	26.761	δ-Elemene	$C_{15}H_{24}$	0.01	–
16	29.302	β-Elemene	$C_{15}H_{24}$	0.12	0.08
17	30.155	Methyl eugenol	$C_{11}H_{14}O_2$	0.03	0.01
18	30.677	Aristolene	$C_{15}H_{24}$	–	0.68
19	30.682	cis-β-Guaiene	$C_{15}H_{24}$	–	0.17
20	30.754	Isocaryophyllene	$C_{15}H_{24}$	0.96	–
21	31.200	β-Copaene	$C_{15}H_{24}$	–	0.62
22	31.207	β-Gurjunene	$C_{15}H_{24}$	–	0.29
23	32.009	(E)-β-Farnesene	$C_{15}H_{24}$	5.12	–
24	32.380	α-Humulene	$C_{15}H_{24}$	0.92	–
25	32.555	Methyl isoeugenol	$C_{11}H_{14}O_2$	4.63	6.38
26	33.490	Germacrene D	$C_{15}H_{24}$	0.12	0.03
27	33.833	6-epi-Shyobunone	$C_{15}H_{24}O$	2.63	3.20
28	34.113	Bicyclogermacrene	$C_{15}H_{24}$	0.04	0.06
29	34.114	γ-Elemene	$C_{15}H_{24}$	0.09	0.02
30	34.663	Dihydro-β-agarofuran	$C_{15}H_{26}O$	0.01	–
31	34.811	Shyobunone	$C_{15}H_{24}O$	0.49	–
32	35.004	δ-Cadinene	$C_{15}H_{24}$	0.27	0.51
33	35.634	Kessane	$C_{15}H_{26}O$	0.17	0.12
34	36.157	α-Calacorene	$C_{15}H_{20}$	0.02	0.39
35	36.381	α-Elemol	$C_{15}H_{26}O$	0.22	–
36	36.404	Elimicin	$C_{12}H_{16}O_3$	–	0.02
37	36.405	Cyclopentanecarboxylic acid, 3-methylene-2,2-dimethyl-5-[(E)-1-propenyl]-, methyl ester	$C_{13}H_{20}O_2$	–	0.03
38	36.733	Nerolidol	$C_{15}H_{26}O$	0.35	–
39	36.721	(E)-Farnesene epoxide	$C_{15}H_{24}O$	0.50	–
40	37.126	γ-Asarone	$C_{12}H_{16}O_3$	1.60	1.55
41	37.604	epi-Cubebol	$C_{15}H_{26}O$	0.12	–
42	37.667	Spathulenol	$C_{15}H_{24}O$	–	0.04
43	37.596	Germacra-diene-4-ol	$C_{15}H_{24}O$	0.22	–
44	37.897	Germacra-4(15),5,10(14)-trien-1-α-ol	$C_{15}H_{24}O$	0.04	–
45	37.907	Caryophyllene oxide	$C_{15}H_{24}O$	0.05	–
46	38.911	Z-3-Hexadecen-7-yne	$C_{16}H_{28}$	1.21	0.44
47	39.313	α-Asarone	$C_{12}H_{16}O_3$	73.39	76.70
48	39.907	Tetradecahydrophenanthrene	$C_{14}H_{24}$	0.05	–
49	39.928	Shyobunone @(2S,3S,6S)	$C_{15}H_{24}O$	0.05	–
50	39.922	Selina-3,11-dien-6-α-ol	$C_{15}H_{24}O$	0.14	–
51	40.177	Ledane	$C_{15}H_{26}$	0.03	–
52	40.307	Cadina-1(6),4-diene	$C_{15}H_{24}$	0.02	–
53	40.337	τ-Cadinol	$C_{15}H_{26}O$	0.30	0.21

Table 2. (continued)

No.	Rt (min)[a]	Component[b]	Molecular formula	Percentage (%)[c] Leaf	Rhizome
54	40.877	τ-Muurolol	$C_{15}H_{26}O$	0.11	0.07
55	40.879	α-Cadinol	$C_{15}H_{26}O$	0.63	–
56	41.21	Khusinol acetate	$C_{17}H_{26}O_2$	–	0.24
57	41.402	(Z)-Isogeraniol	$C_{10}H_{18}O$	0.02	–
58	41.441	Isolongifolol acetate	$C_{17}H_{28}O_2$	0.05	–
59	41.567	β-Asarone	$C_{12}H_{16}O_3$	0.42	–
60	41.572	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.02,4]octane	$C_{15}H_{22}O$	–	1.96
61	41.620	Aromadendrene	$C_{15}H_{24}$	0.23	–
62	41.668	Cyclocolorenone	$C_{15}H_{22}O$	0.63	–
63	42.035	Bulnesol	$C_{15}H_{26}O$	0.03	–
64	42.210	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	$C_{15}H_{24}O$	–	0.05
65	42.418	Isogermacrene D	$C_{16}H_{26}$	0.14	–
66	42.421	β-Vatirenene	$C_{15}H_{22}$	–	0.12
67	42.457	6-epi-Shyobunol	$C_{15}H_{26}O$	0.15	–
68	42.460	Shyobunol	$C_{15}H_{26}O$	0.45	–
69	42.896	N-Dodecanal	$C_{12}H_{24}O$	0.01	–
70	42.905	Octadecanal	$C_{18}H_{36}O$	0.04	–
71	43.202	Asaronaldehyde	$C_{10}H_{12}O_4$	0.06	0.11
72	43.703	Lippifoli-1(6)-en-5-one	$C_{15}H_{22}O$	–	0.33
73	44.513	Isocalamendiol	$C_{15}H_{26}O_2$	–	0.38
74	44.520	α-Bisabolol	$C_{15}H_{26}O$	0.02	–
75	44.530	Isocalamenediol	$C_{15}H_{26}O_2$	0.06	–
76	46.459	Cyperotundone	$C_{15}H_{22}O$	–	0.05
77	46.473	8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one	$C_{15}H_{22}O$	–	0.13
78	56.346	Phytol	$C_{20}H_{40}O$	0.02	–
		Monoterpenes		0.17	2.90
		Sesquiterpenes		8.09	2.97
		Oxygenated monoterpenes		2.26	1.38
		Oxygenated diterpenes		0.02	–
		Oxygenated sesquiterpenes		7.42	6.78
		Phenylpropanoids		80.07	84.66
		Miscellaneous		1.81	0.98
		Total		99.84	99.67

[a]Retention time (RT) identified using the NIST mass spectral library. [b]Compound are listed in order of elution from the SH-Stabilwax-DA column. [c]Percentage of components was calculated as the peak area of each component divided by the peak area of the total ion chromatogram × 100. (–) = Not detected.

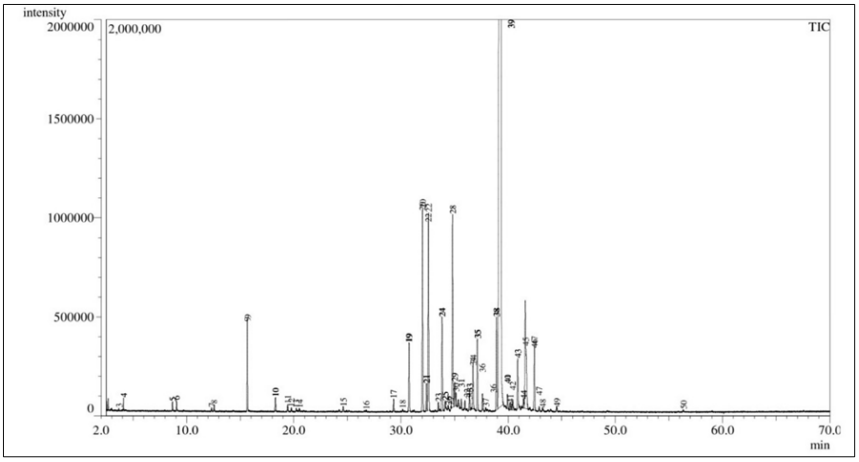


Figure 3. The chromatographic profiles of *A. calamus* leaf essential oil

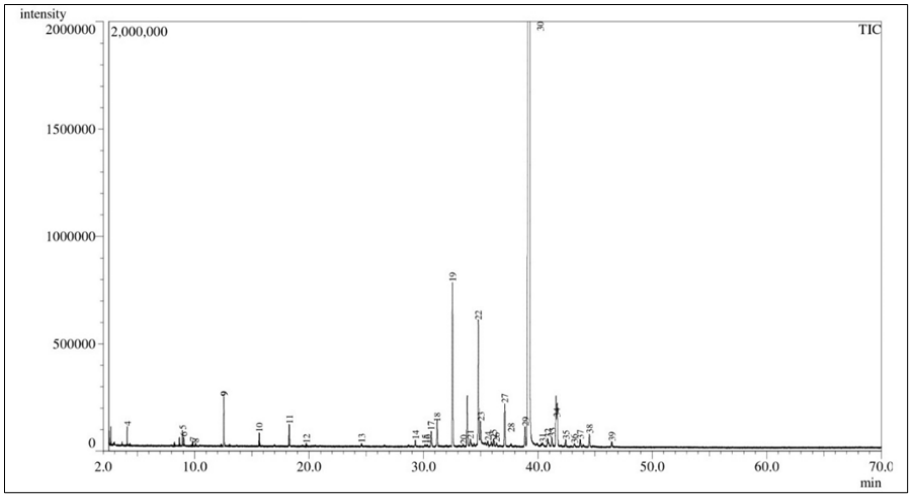


Figure 4. The chromatographic profiles of *A. calamus* rhizome essential oil

Additionally, α-acorenone, shyobunone, 6-epi-shyobunone, dehydroisocalamendiol, preisocalamendiol, and isoacorone were identified as notable constituents in the oil across four different accessions. Similarly, Venskutonis and Dagilyte (2003) reported that β-asarone was the predominant compound in *A. calamus* leaf essential oil, making up 27.0–45.5% of the total composition. Other studies have reported varying dominant compounds in *A. calamus* essential oil. For example, in a collection of triploid European calamus (*A. calamus*) oil, Mazza (1985) identified β-asarone (77.68%) and α-asarone (6.80%) as the primary constituents. Raina *et al.* (2003) found the tetraploid characteristic of *A. calamus* leaf and rhizome essential oils from Utaranchal, India, to contain β-asarone (83.2–85.6%), α-asarone (9.7%), and linalool (4.7%). Moreover, compounds like β-asarone, (Z)-methyl isoeugenol, (E)-caryophyllene, α-humulene, germacrene, linalool, camphor, and isoborneol have also been reported in the *A. calamus* leaf essential oil from Lithuania (Radušiene *et al.*, 2007). Furthermore, studies by Satyal *et al.* (2013), Parki *et al.* (2017), and Loying *et al.* (2019) consistently noted the presence of β-asarone as principal components in *A. calamus* leaf and rhizome essential oils.

The variability of volatile components within different plant parts is influenced by various factors such as geographical conditions, plant age, climate, and plant ploidy (Venskutonis and Dagilyte, 2003; Parki *et al.*, 2017). Various species of *A. calamus* demonstrate a global distribution pattern that correlates with their respective ploidy levels (Ogra *et al.*, 2009). Among the various research findings, Liu *et al.* (2013) reported the presence of α-asarone (50.09%) as the predominant constituent in *A. calamus* rhizome essential oil found in Hebei Province, China. This discovery aligns with the results obtained in this study, where it was observed that the *A. calamus* leaf and rhizome oils from Sabah, Malaysia, are predominantly composed of α-asarone. Furthermore, the identification of allylic γ-asarone in the essential oils derived from the leaves and rhizomes of *A. calamus* in Sabah, Malaysia, at concentrations of 1.60% and 1.55% respectively, provides compelling evidence for the exceptional distinctiveness of these oils compared to those obtained from *A. calamus* in different regions. Notably, β-asarone has consistently been associated with potential toxicity and carcinogenicity. However, as of now, there are no existing restrictions in place to prohibit the utilisation of α-asarone and γ-asarone (Uebel *et al.*, 2021).

The use of retention index (RI) in this analysis was limited by several factors. The main limitation was the analytical setup, which lacked the required standard compounds for RI calibration, making RI measurement impractical. Therefore, RT was used, along with mass spectral matching and similarity measures, which provided sufficient reliability for compound identification, as demonstrated in previous studies (Mus *et al.*, 2021; Manjarrez-Quintero *et al.*, 2024). Additionally, the consistent and well-documented conditions of the GC column ensured that RT remained a reliable metric across similar analyses, supporting its use in this study.

Antibacterial Activity

The ZOI against the Gram-positive bacterial strain (MRSA) and Gram-negative bacterial strain (*E. coli*) are presented in Table 3 and Table 4, respectively. The antibacterial activity of *A. calamus* leaf and rhizome essential oils was successfully assessed. The ZOI against MRSA ranged from 8.67 ± 0.58 mm to 11.00 ± 0.00 mm for leaves, and from 9.33 ± 0.58 mm to 11.33 ± 0.58 mm for rhizomes, respectively. Similarly, both *A. calamus* leaf and rhizome essential oils exhibited inhibitory effects against *E. coli*, with ZOI ranging from 8.67 ± 0.58 mm to 9.67 ± 0.58 mm, and from 8.67 ± 0.58 mm to 10.00 ± 0.00 mm, respectively. These values demonstrated a steady increase with increasing oil concentration, from 100 µg/mL to 400 µg/mL.

Table 3. ZOI of *A. calamus* leaf and rhizome essential oils against MRSA

Plant part	ZOI (mm)					
	Concentration of essential oil (µg/mL)				Control (µg/mL)	
	100	200	300	400	Kanamycin	DMSO
Leaves	8.67 ± 0.58	9.33 ± 0.58	10.00 ± 0.00	11.00 ± 0.00	10.33 ± 0.58	0.00
Rhizomes	9.33 ± 0.58	9.67 ± 0.58	10.33 ± 0.58	11.33 ± 0.58	10.67 ± 0.58	0.00

Values are expressed as means ± standard deviations based on three replicates. Significant differences were observed among concentrations within each plant part (independent samples t-test, $p<0.05$).

Table 4. ZOI of *A. calamus* leaf and rhizome essential oils against *E. coli*

Plant part	ZOI (mm)					
	Concentration of essential oil (µg/mL)				Control (µg/mL)	
	100	200	300	400	Kanamycin	DMSO
Leaves	8.67 ± 0.58	9.00 ± 1.00	9.33 ± 0.58	9.67 ± 0.58	8.67 ± 0.58	0.00
Rhizomes	8.67 ± 0.58	9.33 ± 0.58	9.67 ± 0.58	10.00 ± 0.00	8.67 ± 0.58	0.00

Values are expressed as means ± standard deviations based on three replicates. Significant differences were observed among concentrations within each plant part (independent samples t-test, $p<0.05$).

From the leaves and rhizomes at 400 µg/mL, the *E. coli* (9.67 ± 0.58 mm and 10.00 ± 0.00 mm) produced a significantly ($p<0.05$) lower ZOI than MRSA (11.00 ± 0.00 mm and 11.33 ± 0.58 mm), respectively. In addition, the kanamycin susceptibility disc (100 µg/mL) was a positive control against MRSA with ZOI values for the leaves (10.33 ± 0.58 mm) and rhizomes (10.67 ± 0.58 mm), respectively. Likewise, the ZOI values for the leaves (8.67 ± 0.58 mm) and rhizomes (8.67 ± 0.58 mm) were obtained based on *E. coli*. Consequently, the leaves and rhizomes acquired oil at the largest required concentration (400 µg/mL). Hence, this concentration was necessary to create a ZOI for both antibacterial strains greater than the positive control.

In the current investigation, *A. calamus* leaf and rhizome essential oils exhibited significantly ($p<0.05$) high susceptibility against MRSA and *E. coli* from the lowest to the highest, ranging from 100–400 µg/mL concentration. Vakayil *et al.* (2021) reported that the *A. calamus* rhizome essential oil was effective against *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *K. oxytoca*, and *Acinetobacter baumanii*, ranging from 20–60 µg to produce a ZOI. Loying *et al.* (2019) also mentioned that the *A. calamus* rhizome essential oil succeeded against *S. aureus*, *Salmonella typhimurium*, *Bacillus subtilis*, and *B. cereus*, at 500 µg/mL required to yield a ZOI. However, little is known about *A. calamus* leaf essential oil used in ZOI applications with different concentrations against bacterial pathogens.

Based on the current research findings, it has been observed that a significant proportion of the volatile components under investigation exhibit notable biological activity. Notably, asaronaldehyde, detected in both *A. calamus* leaf (0.06%) and rhizome (0.11%) essential oils, demonstrates particularly high levels of biological activity. Its bactericidal efficacy has been observed against several soil bacteria elucidated from *Piper cubeba* berries (Alqadeeri *et al.*, 2019). Furthermore, the *in vitro* antibacterial effects of the essential oil could also be attributed to a high concentration of α-asarone, which is known to occur at higher concentrations in *A. calamus* leaves and rhizomes as reported by Kumar *et al.* (2015).

Antioxidant Activity

The antioxidant activity of whole *A. calamus* (leaf and rhizome) essential oil was assessed using two methodologies: DPPH and FRAP assays. Employing multiple methods provides a comprehensive evaluation of the antioxidant properties in the sample, as a single method offers only basic insights (Číž *et al.*, 2010). Therefore, the results are summarised in Table 5. In the DPPH assay, the essential oil demonstrated strong radical scavenging effects with an IC_{50} value of 28.20 ± 4.99 µg/mL when compared to Trolox (IC_{50} = 62.40 ± 0.29 µg/mL). The essential oil also demonstrated a potent FRAP value, registering at 150.12 ± 0.10 mg TE/g.

Table 5. Antioxidant activity of *A. calamus* leaf and rhizome essential oils

Plant part	DPPH (µg/mL)	FRAP (mg TE/g)
Leaves Rhizomes	IC_{50} = 28.20 ± 4.99	150.12 ± 0.10
Trolox	IC_{50} = 62.40 ± 0.29	NA

Values are expressed as means ± standard deviations based on three replicates. NA = Not applicable.

Various studies have reported on the antioxidant activities of essential oils derived from *A. calamus* plants. Loying *et al.* (2019) found a DPPH radical scavenging activity of 1.68 µg/mL for *A. calamus* rhizomes, compared to the standard, ascorbic acid (IC_{50} = 1.48 µg/mL). Parki *et al.* (2017) described varying DPPH radical scavenging activity in *A. calamus* leaf and rhizome essential oils, with IC_{50} values ranging from 37.31 ± 0.19 µg/mL to 198.06 ± 0.07 µg/mL based on different seasons. In contrast, Devi and Ganjewala (2011) demonstrated that the methanolic extract derived from *A. calamus* leaves and rhizomes exhibited significant DPPH radical and superoxide anion-scavenging activities, as well as displayed the capacity to chelate ferrous ions.

Evidently, the significant antioxidant activity shown in *A. calamus* leaf and rhizome essential oils could be attributed to the presence of two primary volatile components, namely α-asarone and methyl isoeugenol. Both chemical substances are phenylpropanoids that have been found to exhibit diverse biological functions (Ilijeva and Buchbauer, 2016; Sharma *et al.*, 2020). This component possesses numerous biological activities with potential neurological and metabolic disorders for antimicrobial (Asha and Ganjewala, 2009; Kumar *et al.*, 2015), antioxidant (Manikandan and Devi, 2005), anti-inflammatory (Manikandan and Devi, 2005; Sundaramahalingam *et al.*, 2013; Jo *et al.*, 2018), anti-depression (Chellian *et al.*, 2016), anti-epilepsy (Wang *et al.*, 2014), antidiabetic (Das *et al.*, 2019a), and anticancer (Das *et al.*, 2019b) properties. Synthetic methyl eugenol has been extensively employed as an adjunctive flavouring agent in a diverse range of processed food products, beverages, sauces, fragrances, and aromatherapy oils, as evidenced by previous studies (Vargas *et al.*, 2010; Tan and Nishida, 2012).

The presence of epi-shyobunone had recently been identified in the essential oil of *Siparuna guianensis* (Siparunaceae). The essential oil derived from this species has demonstrated significant efficacy in safeguarding the brain and combatting Alzheimer's disease. This mechanism was achieved through the inhibition of cholinesterase, an enzyme that is closely linked to the pathogenesis of Alzheimer's disease, facilitated by the presence of certain shyobunone derivatives (Martins *et al.*, 2021). In this investigation, it was shown that the 6-epi-shyobunone was present in significant quantities (2.63% and 3.20%) in the respective leaves and rhizomes of *A. calamus*, which are part of the massive composites. Furthermore, it can be inferred that the *A. calamus* in Sabah, Malaysia, exhibits significant promise for use in the field of medicine. Due to the richness and active phytoconstituents of the volatile components, particularly α-asarone, γ-asarone, methyl isoeugenol, 6-epi-shyobunone, and (E)-β-farnesene, *A. calamus* can serve as benchmark research for modifying metabolic and neurological illnesses (based on compelling *in vitro*, *in vivo*, and clinical evidence).

CONCLUSION

This study successfully identified the essential oil extracted from *A. calamus* leaves and rhizomes. This study included 78 volatile

components, with α-asarone predominating the outcome. Based on prior studies, the volatile components possessed potent antibacterial and antioxidant effects. The identification of other unique components, namely γ-asarone, methyl isoeugenol, 6-epi-shyobunone, and (E)-β-farnesene, in significant quantities within the *A. calamus* leaves and rhizomes from Sabah, Malaysia, represents a novel finding not previously reported in samples obtained from other regions. Hence, undertaking comprehensive investigations into the molecular mechanisms underlying the distinct volatile components mentioned remains imperative, as these compounds have not yet been thoroughly studied. These endeavours aim to elucidate the untapped potential of these components in several domains, including pharmaceuticals, cosmeceuticals, and other related fields.

The extracted oil exhibited excellent antibacterial activity on MRSA and *E. coli*, with a maximum concentration necessary to demonstrate a ZOI of 400 µg/mL. Therefore, *A. calamus* can be a viable alternative for treating certain infectious disorders caused by human pathogenic bacteria. Additionally, the essential oil displayed significant antioxidant activity in radical scavenging, making it a potential anti-aging component. For future studies, the antioxidant activities of *A. calamus* leaf and rhizome should be analysed individually to provide more detailed insights and to assess mechanisms *in vitro* and *in vivo*. The toxicity of *A. calamus* leaves and rhizomes also needs to be emphasised using acute and subacute toxicity studies. These crucial steps will underscore the considerable potential for commercialisation of *A. calamus* from Sabah, Malaysia.

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REFERENCES

Adams, R.P. (2000). Systematics of *Juniperus* section *Juniperus* based on leaf essential oils and random amplified polymorphic DNAs (RAPDs). *Biochemical Systematics and Ecology*. 28(6): 515–528. DOI: 10.1016/S0305-1978(99)00089-7

Ahmad, F.B. & Holdsworth, D.K. (2003). Medicinal plants of Sabah, East Malaysia – Part I. *Pharmaceutical Biology*. 41(5): 340–346. DOI: 10.1076/phbi.41.5.340.15940

Alqadeeri, F., Rukayadi, Y., Abbas, F. & Shaari, K. (2019). Antibacterial and antispore activities of isolated compounds from *Piper cubeba* L. *Molecules*. 24(17): 3095. DOI: 10.3390/molecules24173095

Asha, D.S. & Ganjewala, D. (2009). Antimicrobial activity of *Acorus calamus* (L.) rhizome and leaf extract. *Acta Biologica Szegediensis*. 53(1): 45–49

Atalar, M.N. & Türkan, F. (2018). Identification of chemical components from the rhizomes of *Acorus calamus* L. with gas chromatography-tandem mass spectrometry (GC-MS\MS). *Journal of the Institute of Science and Technology*. 8(4): 181–187. DOI: 10.21597/jist.433743

Benjamin, M.A.Z., Ng, S.Y., Saikim, F.H. & Rusdi, N.A. (2022). The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. *Molecules*. 27(19): 6458. DOI: 10.3390/molecules27196458

Berg, K., Bischoff, R., Stegmüller, S., Cartus, A. & Schrenk, D. (2016). Comparative investigation of the mutagenicity of propenylic and allylic asarone isomers in the Ames fluctuation assay. *Mutagenesis*. 31(4): 443–451. DOI: 10.1093/mutage/gew007

Chaubey, P., Parki, A., Prakash, O., Kumar, R. & Pant, A.K. (2018). Comparative study of chemical composition and antioxidant activity of essential oil extracted from *Acorus calamus* L. leaves. *Journal of Herbal Drugs*. 8(4): 203–211. DOI: 10.14196/jhd.2018.203

Chellian, R., Pandy, V. & Mohamed, Z. (2016). Biphasic effects of α-asarone on immobility in the tail suspension test: Evidence for the involvement of the noradrenergic and serotonergic systems in its antidepressant-like activity. *Frontiers in Pharmacology*. 7: 72. DOI: 10.3389/fphar.2016.00072

Cheng, S.S., Lin, H.Y. & Chang, S.T. (2005). Chemical composition and antifungal activity of essential oils from different tissues of Japanese cedar (*Cryptomeria japonica*). *Journal of Agricultural and Food Chemistry*. 53(3): 614–619. DOI: 10.1021/jf0484529

Číž, M., Čížová, H., Denev, P., Kratchanova, M., Slavov, A. & Lojek, A. (2010). Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control*. 21(4): 518–523. DOI: 10.1016/j.foodcont.2009.07.017

Das, B.K., Choukimath, S.M. & Gadad, P.C. (2019a). Asarone and metformin delays experimentally induced hepatocellular carcinoma in diabetic milieu. *Life Sciences*. 230: 10–18. DOI: 10.1016/j.lfs.2019.05.046

Das, B.K., Swamy, A.V., Koti, B.C. & Gadad, P.C. (2019b). Experimental evidence for use of *Acorus calamus* (asarone) for cancer chemoprevention. *Heliyon*. 5(5): e01585. DOI: 10.1016/j.heliyon.2019.e01585

Devi, S.A. & Ganjewala, D. (2011). Antioxidant activities of methanolic extracts of sweet-flag (*Acorus calamus*) leaves and rhizomes. *Journal of Herbs, Spices and Medicinal Plants*. 17(1): 1–11. DOI: 10.1080/10496475.2010.509659.

Dinev, T., Tzanova, M., Velichkova, K., Dermendzhieva, D. & Beev, G. (2021). Antifungal and antioxidant potential of methanolic extracts from *Acorus calamus* L., *Chlorella vulgaris* Beijerinck, *Lemna minuta* Kunth and *Scenedesmus dimorphus* (Turpin) Kützing. *Applied Sciences*. 11(11): 4745. DOI: 10.3390/app11114745

Fafal, T., Sümer Tüzün, B. & Kıvçak, B. (2022). Fatty acid compositions and antioxidant activities of *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumelicus*. *International Journal of Nature and Life Sciences*. 6(2): 151–159. DOI: 10.47947/ijnls.1173088

Ganesan, R.M. & Gurumallesh Prabu, H. (2019). Synthesis of gold nanoparticles using herbal *Acorus calamus* rhizome extract and coating on cotton fabric for antibacterial and UV blocking applications. *Arabian Journal of Chemistry*. 12(8): 2166–2174. DOI: 10.1016/j.arabjc.2014.12.017

Ganjewala, D. & Srivastava, A.K. (2011). An update on chemical composition and bioactivities of *Acorus* species. *Asian Journal of Plant Sciences*. 10(3): 182–189. DOI: 10.3923/ajps.2011.182.189

Grayum, M.H. (1987). A summary of evidence and arguments supporting the removal of *Acorus* from the Araceae. *Taxon*. 36(4): 723–729. DOI: 10.2307/1221123

Heng, L., Guanghua, Z. & Bogner, J. (2010). Acoraceae. In Wu, Z.Y., Raven, P.H. and Hong, D.Y. (eds.). *Flora of China*, vol. 23. St. Louis, MO, USA, Missouri Botanical Garden Press. pp. 1–2

Hong, Y., Liu, X., Wang, H., Zhang, M. & Tian, M. (2021). Chemical composition, antibacterial, enzyme-inhibitory, and anti-inflammatory activities of essential oil from *Hedychium puerense* rhizome. *Agronomy*. 11(12): 2506. DOI: 10.3390/agronomy11122506

Ilijeva, R. & Buchbauer, G. (2016). Biological properties of some volatile phenylpropanoids. *Natural Product Communications*. 11(10): 1619–1629. DOI: 10.1177/1934578x1601101041

Jinoni, D.A., Benjamin, M.A.Z., Mus, A.A., Goh, L.P.W., Rusdi, N.A. & Awang, M.A. (2024). *Phaleria macrocarpa* (Scheff.) Boerl. (mahkota dewa) seed essential oils: Extraction yield, volatile components, antibacterial, and antioxidant activities based on different solvents using Soxhlet extraction. *Kuwait Journal of Science*. 51: 100173. DOI: 10.1016/j.kjs.2023.100173

Jo, M.-J., Kumar, H., Joshi, H.P., Choi, H., Ko, W.-K., Kim, J.M., Hwang, S.S.S., Park, S.Y., Sohn, S., Bello, A.B., Kim, K.-T., Lee, S.-H., Zeng, X. & Han, I. (2018). Oral administration of α-asarone promotes functional recovery in rats with spinal cord injury. *Frontiers in Pharmacology*. 9: 445. DOI: 10.3389/fphar.2018.00445.

Khatun, S., Chatterjee, N.C. & Cakilcioglu, U. (2011). Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq. *African Journal of Biotechnology*. 10(13): 2530–2535. DOI: 10.5897/AJB10.2526

Kulip, J. (1997). A preliminary survey of traditional medicinal plants in the West Coast and Interior of Sabah. *Journal of Tropical Forest Science*. 10(2): 271–274.

Kumar, S.N., Aravind, S.R., Sreelekha, T.T., Jacob, J. & Kumar, B.S.D. (2015). Asarones from *Acorus calamus* in combination with azoles and amphotericin B: A novel synergistic combination to compete against human pathogenic *Candida* species *in vitro*. *Applied Biochemistry and Biotechnology*. 175(8): 3683–3695. DOI: 10.1007/s12010-015-1537-y

Liu, X.C., Zhou, L.G., Liu, Z.L. & Du, S.S. (2013). Identification of insecticidal constituents of the essential oil of *Acorus calamus* rhizomes against *Liposcelis bostrychophila* Badonnel. *Molecules*. 18(5): 5684–5696. DOI: 10.3390/molecules18055684

Loying, R., Gogoi, R., Sarma, N., Borah, A., Munda, S., Pandey, S.K. & Lal, M. (2019). Chemical compositions, in-vitro antioxidant, anti-microbial, anti-inflammatory and cytotoxic activities of essential oil of *Acorus calamus* L. rhizome from North-East India. *Journal of Essential Oil-Bearing Plants*. 22(5): 1299–1312. DOI: 10.1080/0972060X.2019.1696236

Manikandan, S. & Devi, R.S. (2005). Antioxidant property of α-asarone against noise-stress-induced changes in different regions of rat brain. *Pharmacological Research*. 52(6): 467–474. DOI: 10.1016/j.phrs.2005.07.007

Manjarrez-Quintero, J.P., Valdez-Baro, O., García-Estrada, R.S., Contreras-Angulo, L.A., Bastidas-Bastidas, P.de.J., Heredia, J.B., Cabanillas-Bojórquez, L.A. & Gutiérrez-Grijalva, E.P. (2024) Optimized ultrasonic extraction of essential oil from the biomass of *Lippia graveolens* Kunth using deep eutectic solvents and their effect on *Colletotrichum asianum*. *Processes*. 12(7): 1525, DOI: 10.3390/pr12071525

Marotti, M., Piccaglia, R., Giovanelli, E., Deans, S.G. & Eaglesham, E. (1994). Effects of planting time and mineral fertilization on peppermint (*Mentha* x *piperita* L.) essential oil composition and its biological activity. *Flavour and Fragrance Journal*. 9(3): 125–129. DOI: 10.1002/ffj.2730090307

Martins, R.M.G., Xavier-Júnior, F.H., Barros, M.R., Menezes, T.M., de Assis, C.R.D., de Melo, A.C.G.R., Veras, B.O., Ferraz, V.P., Filho, A.A.M., Yogui, G.T., Bezerra, R.S., Seabra, G.M., Neves, J.L. & Tadei, W.P. (2021). Impact on cholinesterase-inhibition and in silico investigations of sesquiterpenoids from Amazonian *Siparuna guianensis* Aubl. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*. 252: 119511. DOI: 10.1016/j.saa.2021.119511

Mazza, G. (1985). Gas chromatographic and mass spectrometric studies of the constituents of the rhizome of calamus. I. The volatile constituents of the essential oil. *Journal of Chromatography A*. 328: 179–194. DOI: 10.1016/S0021-9673(01)87390-8

Mittal, N., Ginwal, H.S. & Varshney, V.K. (2009). Pharmaceutical and biotechnological potential of *Acorus calamus* Linn.: An indigenous highly valued medicinal plant species. *Pharmacognosy Reviews*. 3(5): 83–93

Mukherjee, P.K., Kumar, V., Mal, M. & Houghton, P.J. (2007). *Acorus calamus*: Scientific validation of Ayurvedic tradition from natural resources. *Pharmaceutical Biology*. 45(8): 651–666. DOI: 10.1080/13880200701538724

Mus, A.A., Gansau, J.A., Kumar, V.S. & Rusdi N.A. (2020) The variation of volatile compounds emitted from aromatic orchid (*Phalaenopsis bellina*) at different timing and flowering stages. *Plant OMICS*. 13(2): 78–85, DOI: 10.21475/POJ.13.02.20.2271

Ogra, R.K., Mohanpuria, P., Sharma, U.K., Sharma, M., Sinha, A.K. & Ahuja, P.S. (2009). Indian calamus (*Acorus calamus* L.): Not a tetraploid. *Current Science*. 97(11): 1644–1647

Park, Y.-S., Kim, I., Dhungana, S.K., Park, E.-J., Park, J.-J., Kim, J.-H. & Shin, D.-H. (2021). Quality characteristics and antioxidant potential of lemon (*Citrus limon* Burm. f.) seed oil extracted by different methods. *Frontiers in Nutrition*. 8: 644406. DOI: 10.3389/fnut.2021.644406

Parki, A., Chaubey, P., Prakash, O., Kumar, R. & Pant, A.K. (2017). Seasonal variation in essential oil compositions and antioxidant properties of *Acorus calamus* L. accessions. *Medicines*. 4(4): 81. DOI: 10.3390/medicines4040081

Pintatum, A., Laphookhieo, S., Logie, E., Berghe, W. Vanden & Maneerat, W. (2020). Chemical composition of essential oils from different parts of *Zingiber kerrii* Craib and their antibacterial, antioxidant, and tyrosinase inhibitory activities. *Biomolecules*. 10(2): 228. DOI: 10.3390/biom10020228

Raal, A., Orav, A. & Gretchushnikova, T. (2016). β-Asarone content and essential oil composition of *Acorus calamus* L. rhizomes from Estonia. *Journal of Essential Oil Research*. 28(4): 299–304. DOI: 10.1080/10412905.2016.1147391

Radušiene, J., Judžentiene, A., Pečiulyte, D. & Janulis, V. (2007). Essential oil composition and antimicrobial assay of *Acorus calamus* leaves from different wild populations. *Plant Genetic Resources: Characterisation and Utilisation*. 5(1): 37–44. DOI: 10.1017/S1479262107390928

Raina, V.K., Srivastava, S.K. & Syamasunder, K. V. (2003). Essential oil composition of *Acorus calamus* L. from the lower region of the Himalayas. *Flavour and Fragrance Journal*. 18(1): 18–20. DOI: 10.1002/ffj.1136

Rajput, S.B., Tonge, M.B. & Karuppayil, S.M. (2014). An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn. (sweet flag) and other *Acorus* species. *Phytomedicine*. 21(3): 268–276. DOI: 10.1016/j.phymed.2013.09.020

Satyal, P., Paudel, P., Poudel, A., Dosoky, N.S., Moriarity, D.M., Vogler, B. & Setzer, W.N. (2013). Chemical compositions, phytotoxicity, and biological activities of *Acorus calamus* essential oils from Nepal. *Natural Product Communications*. 8(8): 1179–1181. DOI: 10.1177/1934578x1300800839

Sharma, V., Singh, I. & Chaudhary, P. (2014). *Acorus calamus* (the healing plant): A review on its medicinal potential, micropropagation and conservation. *Natural Product Research*. 28(18): 1454–1466. DOI: 10.1080/14786419.2014.915827

Sharma, V., Sharma, R., Gautam, D.N.S., Kuca, K., Nepovimova, E. & Martins, N. (2020). Role of Vacha (*Acorus calamus* Linn.) in neurological and metabolic disorders: Evidence from ethnopharmacology, phytochemistry, pharmacology and clinical study. *Journal of Clinical Medicine*. 9(4): 1176. DOI: 10.3390/jcm9041176

Sundaramahalingam, M., Ramasundaram, S., Rathinasamy, S.D., Natarajan, R.P. & Somasundaram, T. (2013). Role of *Acorus calamus* and α-asarone on hippocampal dependent memory in noise stress exposed rats. *Pakistan Journal of Biological Sciences*. 16(16): 770–778. DOI: 10.3923/pjbs.2013.770.778

Tan, K.H. & Nishida, R. (2012). Methyl eugenol: Its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. *Journal of Insect Science*. 12: 56. DOI: 10.1673/031.012.5601

Uebel, T., Hermes, L., Haupenthal, S., Müller, L. & Esselen, M. (2021). α-Asarone, β-asarone, and γ-asarone: Current status of toxicological evaluation. *Journal of Applied Toxicology*. 41(8): 1166–1179. DOI: 10.1002/jat.4112

Vakayil, R., Nazeer, T.A. & Mathanmohun, M. (2021). Evaluation of the antimicrobial activity of extracts from *Acorus calamus* rhizome against multidrug-resistant nosocomial pathogens. *Research Journal of Agricultural Sciences*. 12(5): 1613–1617.

Vargas, R.I., Shelly, T.E., Leblanc, L. & Piñero, J.C. (2010). Recent advances in methyl eugenol and cue-lure technologies for fruit fly detection, monitoring, and control in Hawaii. In Litwack, G. (ed.). *Vitamins and Hormones*, vol. 83, Cambridge, MA, USA, Academic Press. pp. 575–595. DOI: 10.1016/S0083-6729(10)83023-7

Varma, J., Tripathi, M., Ram, V.J., Pandey, V.B. & Dubey, N.K. (2002). γ-Asarone – The fungitoxic principle of the essential oil of *Caesulia axillaris*. *World Journal of Microbiology and Biotechnology*. 18(3): 277–279. DOI: 10.1023/A:1014905111973

Venskutonis, P.R. & Dagilyte, A. (2003). Composition of essential oil of sweet flag (*Acorus calamus* L.) leaves at different growing phases. *Journal of Essential Oil Research*. 15(5): 313–318. DOI: 10.1080/10412905.2003.9698598

Wang, Z.-J., Levinson, S.R., Sun, L. & Heinbockel, T. (2014). Identification of both GABAA receptors and voltage-activated Na^+ channels as molecular targets of anticonvulsant α-asarone. *Frontiers in Pharmacology*. 5: 40. DOI: 10.3389/fphar.2014.00040