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Essential Oil Constituents and Pharmacognostic Evaluation of Java Citronella (*Cymbopogon winterianus*) stem from Bandung, West Java, Indonesia

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Abstract

BACKGROUND: *Cymbopogon winterianus* essential oil contains citral-derived chemicals with a variety of pharmacological effects, although there has been minimal research on pharmacognostic, phytochemical, and biological aspects.

AIM: This research aims to evaluate the pharmacognostic and chemical components of *C. winterianus* stem essential oil.

METHODS: The pharmacognostic studies were carried out in terms of macroscopic, microscopic, water content, water extractive values, ethanol extractive values, and essential oil yield. The oil was extracted by ethanol steam distillation method, the oil composition was analyzed by gas chromathography-mass spectroscopy.

RESULTS: *C. winterianus* stems are 15–35 cm long and 0.5–2 cm broad, with a rough, stiff, and thin texture, with prominent fibers on the top and bottom surfaces. It has a bitter and slightly spicy taste with a distinctive lemony aroma and was greenish yellow in hue with a blend of purplish-red colors. The upper and lower epidermis was examined under a microscope, which revealed sclerenchyma fibers, trichomes, parenchyma, calcium oxalate (rosette), cortex, stem pith, oil cells, stomata (Gramineae type), trachea (ladder and spiral thickening), and collenchyma. The water content was 7.16 ± 0.72%, the water-soluble extractive value was 12.152 ± 0.003%, the ethanol-soluble extractive value was 12.290 ± 0.76%, and the essential oil content was 6.46 ± 0.50%. Essential oil constituents were 6-octen-1-ol, 3,7-dimethyl-, formate; trifluoroacetyl-lavandulol; n-hexadecanoic acid; 9,12-octadecadienoic acid (Z,Z)-; oxacyclotetradecane-2,11-dione, 13-methyl-; palmitic acid vinyl ester; glycidyl palmitate; 9,17-Octadecadienal, (Z)-; glycidyl palmitoleate; 1,4-bis(trimethylsilyl)benzene; and cyclotrisiloxane, hexamethyl.

CONCLUSION: The essential oil constituents and preliminary pharmacognostic evaluation of *C. winterianus* stem can provide useful data for further phytochemical analysis, quality control, and standardization of *C. winterianus*. Citronellyl formate detected as citral derivatives as the main compound in the ethanol steam distillation method.

Introduction

Indonesia, with 30000–40000 medicinal herbal plant species [1], has become the second-largest number of indigenous medicinal plants [2]. About two-thirds of these medicinal plants are grown and produced in Java. According to the Ministry of Trade Republic of Indonesia (RI) (2019), Bandung, Tasik, Garut, and Sukabumi highlands were identified as appropriate growth locations in West Java. The *Cymbopogon* genus was one of the medicinal plants cultivated in Java as a source of essential oils [3]. *Cymbopogon* grows well in the highlands and it was reported that it had a higher volatile oil concentration than in the lowlands [4]. In addition to the growing area, the quality of the raw materials and the process of extracting these essential oils affect the essential oil content [5].

The *Cymbopogon* genus plant has a wide range of applications, including common tea, medicinal supplement, insect repellant, pesticide, flu control, anti-inflammatory, and analgesic properties [6], [7], [8]. Conventionally, *Cymbopogon winterianus* was used to make aromatic tea, as a vermifuge, diuretic, and antispasmodic [9], and to treat fever, intestinal parasites, digestive, and menstrual issues. It was used in Chinese medicine to treat rheumatic pain [10]. Several studies have found that *C. winterianus* possesses anticonvulsant, antibacterial, and antifungal properties [11] and can be used to treat epilepsy, sedatives, and anxiety [6], [12].

C. winterianus, popularly known as Java citronella (Indonesian synonym: Sereh Wangi) [13], is a perennial tropical grass plant of the Poaceae family that produces citronella oil. According to Verma *et al*. (2020), *C. winterianus* was the most productive type of citronella oil source [5]. Citronella

oils are frequently used in cosmetics, medicines, perfumery, and food and beverage flavoring [6], [9]. This secondary metabolic was high in monoterpene alcohols including citronellal, geraniol, and citronellol, which are known to have a variety of pharmacological properties [5], [9], [14]. Geraniol and citronellal are more abundant in stems than in leaves [13]. Plant essential oils are derived by distilling the roots, stems, leaves, flowers, and seeds [15], and their application for therapeutic purposes necessitates pharmacognostic investigations. However, there has been little research into its pharmacognostic, phytochemical, and biological properties [13]. The purpose of this study was to evaluate the pharmacognostic and chemical components of *C. winterianus* stem essential oil as preliminary studies for their biological properties.

Materials and Methods

Materials preparation

Fresh *C. winterianus* collected from Lembang, Bandung, West Java, Indonesia. The plant was identified by the Indonesian Institute of Sciences (LIPI). Separation of the materials was carried out, and only the stem section of the lemongrass (reddish white) was used, which was removed from the green part (leaf). The samples were washed and dried by the air-drying method. Essential oil extraction was carried out immediately to avoid loss in the storage process.

Physicochemical evaluation

Macroscopic and microscopic

Macroscopic identification was carried out by observing dried samples of *C. winterianus* stem shape and organoleptic characteristics (color, smell, and taste). Microscopic identification of dried sample *C. winterianus* stems was performed using a digital light microscope (Leica®) to examine the identification fragments. Observations were made on a powdered dry sample at magnifications of 100× and 400×. Lower magnification as medium lens power was used to localize the fragments for initial examination. A stronger lens was utilized to examine bigger identifying fragments, such as stomata. Typical anatomical fragments were observed in this study included stem epidermis, transport tissue, cortex tissue, and stem pith [16], [17]. The fragments were identified using phloroglucinol and chloral hydrate as reagents. Phloroglucinol consists of 1% solution with 96% ethanol, which was used to color the fragments containing wood substance (lignin) change to pink-red violet. Chloral hydrate, which was composed of 5 parts chloral hydrate dissolved in two parts water, was used to clean the slides sample. It dissolves starch, allowing the form of tissues or cells to be clearly observed [17].

Water content

The weighing cup was heated in an oven at 105ºC for 1 h. After that, the cup was chilled in a desiccator and then weighed as an empty weight. One g samples were weighed and then placed in the oven (Memmert®) at 105ºC for 5 h. After that, the cup containing the sample was then chilled in a desiccator and weighed. Repeat the test in 1-h intervals until a constant weight was obtained. The weighing was declared to have reached a constant weight if the difference in weighing 3 times in a row after being ignited was not more than 0.25% or the difference in weighing does not exceed 0.5 mg with an analytical balance (Mettler Toledo®) [17].

$$\%\,water\ content\,(w/w) = \frac{(W1-W0)-(W2-W0)}{W1-W0}$$

Description:
W0 = Weight of empty cup
W1 = Weight of the cup + sample used
W2 = Weight of cup + drying result

Water-soluble extractive values

Determination of water-soluble extract content was carried out by weighing 5.0 g of dried samples that had been air-dried. Dried samples were extracted with 100 mL of chloroform (0.25 ml of chloroform in 97.5 ml of aqua distillate) for 6 h using a stoppered erlenmeyer flask and the sample was shaken occasionally, then left for 18 h. The sample was filtered quickly using filter paper to obtain 20 ml of filtrate. The filtrate was evaporated over a water bath to dry and the remainder in the cup was heated using an oven at 105°C until a constant weight was obtained. The water-soluble extract content was determined by the formula [17]:

$$\%\ water-soluble\,extractive\,values\,(w/w)$$
$$\frac{W2-W0}{W1-W0}\times\frac{100}{20}\times 100\%$$

Description:
W0 = Weight of empty cup
W1 = Weight of the cup + sample used
W2 = Weight of cup + drying result

Ethanol-soluble extractive values

Determination of ethanol-soluble extract content was carried out by weighing 5.0 grams of dried samples that had been dried in the open air. Dried

samples were extracted with 100 ml of 96% ethanol for 6 h using a stoppered erlenmeyer flask and occasionally the sample was shaken, then left for 18 h. The sample was filtered quickly using filter paper to obtain 20 ml of filtrate. The filtrate was evaporated over a water bath to dry and the remainder in the cup was heated using an oven at 105°C until a constant weight was obtained. Ethanol soluble extract content was determined by the formula [17]:

$$\%\,ethanol-soluble\,extractive\,values\,(w/w) = \frac{W2-W0}{W1-W0}\times\frac{100}{20}\times 100\%$$

Description:
W0 = Weight of empty cup
W1 = Weight of the cup + sample used
W2 = Weight of cup + drying result

Essential oil steam distillation

Determination of essential oil content was done by the steam distillation method. Weighed 500 grams of dried samples that had been air-dried. Dried samples were extracted with 96% ethanol solvent using a one-liter round bottom flask. The flask was connected to a condenser and a scale burette. The sample was heated using an air bath with a distillation time of 6 hours. After the distillation was completed, the distillate was separated using a rotary vacuum evaporator (Buchi) at a temperature of 45°C to obtain the essential oil. Then it was allowed to stand for 15 minutes and the volume of essential oil was recorded. The essential oil content is determined by the formula [17]:

$$\%\,yield\,(w/w) = \frac{essential\,oil\,weight\,(g)}{sample\,weight\,(g)}\times 100\%$$

Gas chromathography-mass spectroscopy (GC-MS) analysis

The chemical components of *C. winterianus* essential oil were analyzed using GC-MS with an Agilent 122–5532 column (30 m × 250 μm with 0.25 μm thickness). The carrier gas was helium with the conditions of oven setpoint at the temperature of 50°C and slowly raised to 350°C. Total program time: 31 min with injection temperature at 200°C, injection mode: Split, pressure: 7,6522 psi, total flow: 54 mL/min, septum purge flow: 3 ml/min, split ratio 50:1, and column flow: 1 ml/min. The MS settings were source temperature: 250°C and quad temperature 200°C. Mass spectra fragmentation patterns identified the compounds. Identities of compounds were approved by comparing spectral data with library PubChem NCBI library and free-published literature.

Results and Discussion

Macroscopic and microscopic evaluation

Fresh *C. winterianus* (Figure 1a) changes shape and color as it dries, and the associated odor and taste become stronger. The dried stems of *C. winterianus* as shown in Figure 1b had an aromatic lemon-like scent, bitter and slightly spicy taste, and 15–35 cm thin shape (Table 1). These macroscopic identification results were not much different from *C. nardus* which was long and thin with rough and sharp edges, has a distinctive aroma, slightly spicy taste, and hairs on the top and bottom surfaces [18]. However, *C. nardus* was green in color, in contrast to *C. winterianus* which was greenish-yellow with a purplish red mixture (Figure 1).

Figure 1. Cymbopogon winterianus stem: (a) fresh chopped, (b) air-dried, and (c) distillate oil

Observations of *C. winterianus* powdered dry stems showed in Figure 2 at 100× magnification using phloroglucinol obtained the outer protective tissue modified from epidermal cells, hair-shaped trichomes, and help protect against damage [19]. There was parenchyma inside that had an elongated appearance as a thin-walled ground tissue with scattered starch grains. Long sclerenchyma fibers with pointy ends support the plant in the deeper portion.

Table 1: Morphological/organoleptic characteristic of *C. winterianus* stem

Parameters	Results
Shape	Thin pieces with a length of 15–35 cm and a width of 0.5–2 cm, has a rough and stiff texture and has prominent fibers on the surface
Odor	Aromatic lemon-like scent
Color	Greenish yellow with a mixture of purplish-red color
Taste	Bitter and slightly spicy

The observations of a sample utilizing chloral hydrate at a magnification of 100× revealed a cortex surrounded by sclerenchyma fibers. Observations with a magnification of 400× using phloroglucinol obtained the outermost layer of the sample, the upper epidermis which was in the form of long cells and tightly arranged with each other. Calcium oxalate, a metabolic product, spreads in an irregular or rosette pattern around the epidermis.

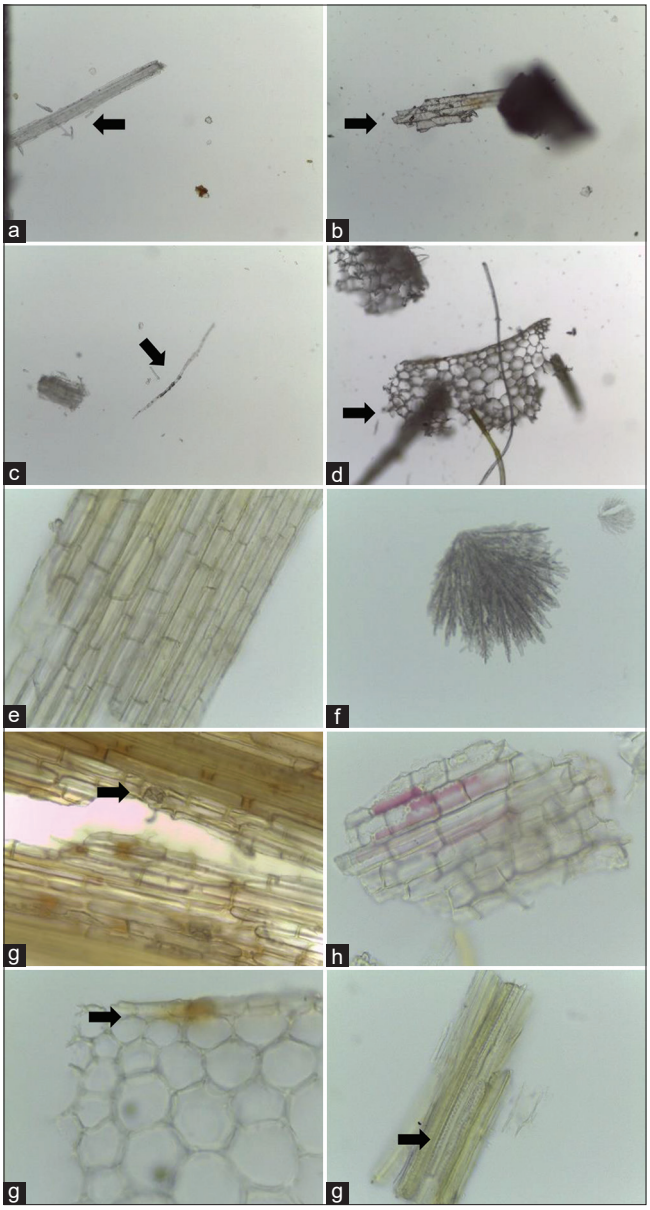

Figure 2: Microscopic identification of Cymbopogon winterianus stem: (a) trichome (100×); (b) parenchyma (elongated appearance) (100×); (c) sklerenchyma fiber (100×); (d) cortex (100×); (e) upper epidermis (400×); (f) oxalate calcium (rosette) (400×); (g) lower epidermis with stomata (gramineae type) (400×); (h) collenchyma (400×); (i) stem pith (400×); and (j) trachea (ladder and spiral thickening) (400×)

The outermost protective tissue, the lower epidermis cells with Gramineae type stomata, was identified at 400× magnification using chloral hydrate. This type of stomata had two dumbbell-shaped guard cells that are flanked by two parallel subsidiary cells [20]. After the epidermal cells, collenchyma was arranged in a cylindrical shape with thickened cell walls to sustain the plant. In addition, polygonal-shaped stem pith consisting of oil cells which resulted from metabolism seen. There was a trachea with a secondary wall thickening in the form of a ladder and a spiral in the deeper region. The results of microscopic observations of *C. winterianus* are not much different from *C. nardus*, listed in Indonesian Materia Medica (1989), consisting of a slightly rounded upper epidermis and cover hairs. There are stomata in the upper epidermis and more in the lower epidermis. *C. nardus* had parenchyma cells that contains oil with collenchyma tissue scattered between the parenchyma [18].

Soluble extractive values

Water-soluble and ethanol-soluble extractive values are crucial in the evaluation of crude drugs [21] and the result shows in Table 2. The water-soluble extract content of *C. winterianus* dried stems was examined to provide an initial description of the levels of polar chemical components and an indication of the number of medicinal ingredients extracted by water solvents. The cold maceration technique was used to keep volatile compounds from degradation using a saturated chloroform solvent. Because of the pressure differential, immersion broke down the cell walls, allowing the secondary metabolites *C. winterianus* to dissolve in the saturated chloroform solvent [22], [23], [24].

Table 2: Physicochemical parameters of *C. winterianus* stem

Parameters	Values ± SD (%w/w)	Indonesian Materia Medica Guideline for *C. nardus* (*C. winterianus* not listed yet)
Water content	7.16 ± 0.72	Not more than 10%
Water-soluble extractive value	12.15 ± 0.003	No <4.5%
Ethanol-soluble extractive value	12.29 ± 0.76	No <3%
Distillation yield	6.46 ± 0.50	–

The polarity (semipolar-nonpolar) of the active compound content was estimated by ethanol-soluble extract analysis. The analysis was obtained by the maceration method for 24 h to provide optimum solute and solvent contact, so that more extract was produced [22], [24], [25]. The ethanol-soluble extract in *C. winterianus* stem resulted in 0.19% higher compared to water-soluble extract. Purba *et al*., (2020) also discovered a 0.14 percent higher ethanol-soluble extract on *C. citratus* leaves as compared to water-soluble extracts [26]. This indicates that the active ingredient in *Cymbopogon* stems was extracted more strongly in ethanol, or that it was semi-polar and nonpolar. The water-soluble and ethanol-soluble extractive content of *C. winterianus* fulfilled the RI standard requirements for *C. nardus*, which is included in Indonesian Materia Medica. The extraction values for water-soluble and ethanol-soluble extracts must be >4.5% and <3%, respectively [18].

Essential oils distillation

The distillation procedure was carried out using the steam distillation method, which involved applying high-pressure steam to the sample and passing the steam through a condenser [27]. The distillate was obtained in the form of a clear liquid which was a mixture of essential oils and ethanol. The results of the distillate did not undergo phase separation, because the citral derivatives were soluble in ethanol. Citronellal came out first to condenser because of the lower boiling

point, then citronellol and geraniol came out last [28]. The cloudy pale yellow color was obtained under the standard of SNI 06-3953-1995 after the separation process.

The amount of essential oil obtained after evaporation was 29–33 mL (Figure 1c); the difference could be due to the length of the process in reducing the sample size so that oil evaporation occurs, the condenser did not work optimally so that condensation did not run perfectly, and there was a leak in the tool so that the essential oil evaporated more quickly. The test findings showed that the volatile oil content obtained was 4.77% higher than the 1.69% found in a study conducted by Hamzah *et al*. (2014) on *C. nardus* extracted by steam distillation using water solvent [29]. Andila *et al*. (2018) investigation on *C. winterianus* utilizing hydrodistillation method was 5.79% lower than this study [13].

Chemical composition

Table 3 shows the results of the *C. winterianus* GCMS analysis, and Figure 3 shows the mass spectra. Citral derivatives, 6-Octen-1-ol, 3, 7-dimethyl-, formate were detected at the first peak. This typical compound was carboxylic ester, known as citronellyl formate. This fragrant compound was also found in geranium rose essential oil [30] and had antibacterial activity in both positive and negative gram bacteria [31]. Another essential oil compound was detected as second peak, trifluoroacetyl-lavandulol with the IUPAC name (5-methyl-2-prop-1-en-2-ylhex-4-enyl) 2, 2, 2-trifluoroacetate. This compound was reported as an essential oils component in lemon [32], orange fruit peel [33], lavender [34], and *Cymbopogon citrates* [35].

Table 3: GCMS analysis of C. winterianus stem distillate oil

RT	Area (%)	Chemical substances	Molecular Formula	Molecular Weight (g/mol)
10.879	0.91	6-Octen-1-ol, 3,7-dimethyl-, formate	$C_{11}H_{20}O_2$	184.27
11.232	0.53	Trifluoroacetyl-lavandulol	$C_{12}H_{17}F_3O_2$	250.2
21.589	20.52	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
23.390	31.57	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.4
24.161	0.34	Oxacyclotetradecane-2,11-dione, 13-methyl-	$C_{14}H_{24}O_3$	240.34
24.379	3.80	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282.5
24.797	12.07	Glycidyl palmitate 1	$C_{19}H_{36}O_3$	312.5
24.932	3.22	Glycidyl palmitate 2	$C_{19}H_{36}O_3$	312.5
25.857	0.63	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.4
26.315	16.30	Glycidyl palmitoleate 1	$C_{19}H_{34}O_3$	310.5
26.447	2.03	Glycidyl palmitoleate 2	$C_{19}H_{34}O_3$	310.5
27.676	1.17	1,4-Bis (trimethylsilyl) benzene 1	$C_{12}H_{22}Si_2$	222.47
28.530	0.90	1,4-Bis (trimethylsilyl) benzene 2	$C_{12}H_{22}Si_2$	222.47
29.923	0.40	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46

Oxacyclotetradecane-2, 11-dione, 13-methyl- was volatile component with common name 13-methyloxacyclotetradecane-2. The previous research showed this component extracted from *Angelica biserrata*, used as traditional Chinese medicine. It was also a major component from *Terminalia chebula* fruit aqueous extract [36] and mangrove *Sonnetaria caseolaris* bark hexane extract [37]. Meanwhile, 1, 4-bis(trimethylsilyl) benzene or trimethyl-(4-trimethylsilylphenyl)silane was aromatic hydrocarbon [38], showed as a major bio compound of *Dillanea scarbrella* extract [39], *Pteridium aquilinum* extract [40], *Eclipta alba* extract [41] in GCMS analysis. Cyclotrisiloxane, hexamethyl- or dimethylsiloxane cyclic trimer known as a surfactant for cosmetics, softening, and brightening agent. This component had therapeutical properties such as antimicrobials, antioxidants, and antidiabetics. This component was reported in *Punica granatum* rind extract, *Jatropha curcas* leaves extract, *Turbinaria decurrens* extract, and *Bauhinia acuminata* leaves extract [42], [43], [44], [45].

Ethanol as a solvent in the steam distillation method could dissolve nonpolar compounds such as fatty acid. n-Hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, palmitic acid vinyl ester, glycidyl palmitate, glycidyl palmitoleate, and 9,17-octadecadienal, (Z)- were fatty acid [46]. The great abundance was 9, 12-octadecadienoic acid (Z,Z)- or known as alpha-linoleic acid, with IUPAC name octadeca-9,12-dienoic acid. A doubly unsaturated fatty acid occurring widely in plant glycosides. It is an essential fatty acid in mammalian nutrition and is used in the biosynthesis of prostaglandins and cell membranes. This fatty acid was reported that had anti-inflammatory [47], hepatoprotective, antimicrobial, anticancer, anti-arthritic, anti-asthma, and diuretic activities [48]. Meanwhile, n-hexadecanoic acid or palmitic acid with IUPAC name octadeca-9,12-dienoic acid was found naturally in palm oil and palm kernel oil, as well as in butter, cheese, milk, and meat. n-Hexadecanoic acid reported had nematicide, pesticide, antioxidant, hypocholesterolemic [46], [47], anti-inflammatory activities [49], antifibrinolytic, hemolytic, 5-alpha reductase inhibitor, and anti-alopecic activities [46], [50].

Palmitic acid vinyl ester or ethenyl hexadecanoate (IUPAC) was found as phytocompound in *Cinnamomum zeylanicum* barks extract [51] and *Simarouba glauca* leaves. This fatty acid was used in throat disorders, anti-pruritic, anti-migraine, anti-convulsant, anti-epileptic, anti-asthma, and anti-psoriatic [52]. Another palmitic derivate, glycidyl palmitate was essential in the preparation of lysophosphatidic acids which inhibit apoptosis [53], used as anticancer, larvicidal, nematicide, and pesticide [54]. This compound with IUPAC name oxiran-2-ylmethyl hexadecanoate was found in *Azaridiachta indica* leaf extract. Whereas, glycidyl palmitoleate with IUPAC name oxiran-2-ylmethyl hexadec-9-enoate had the therapeutic potential to augments neuron level, manipulate a subset of enzymes that control eCB signaling. The eCB signaling system regulates a wide array of physiological processes in the central nervous system, especially in cannabinoid signaling [55]. 9,17-Octadecadienal, (Z)- was long-chain unsaturated aldehyde that identified as the major constituent of *Jacaranda*

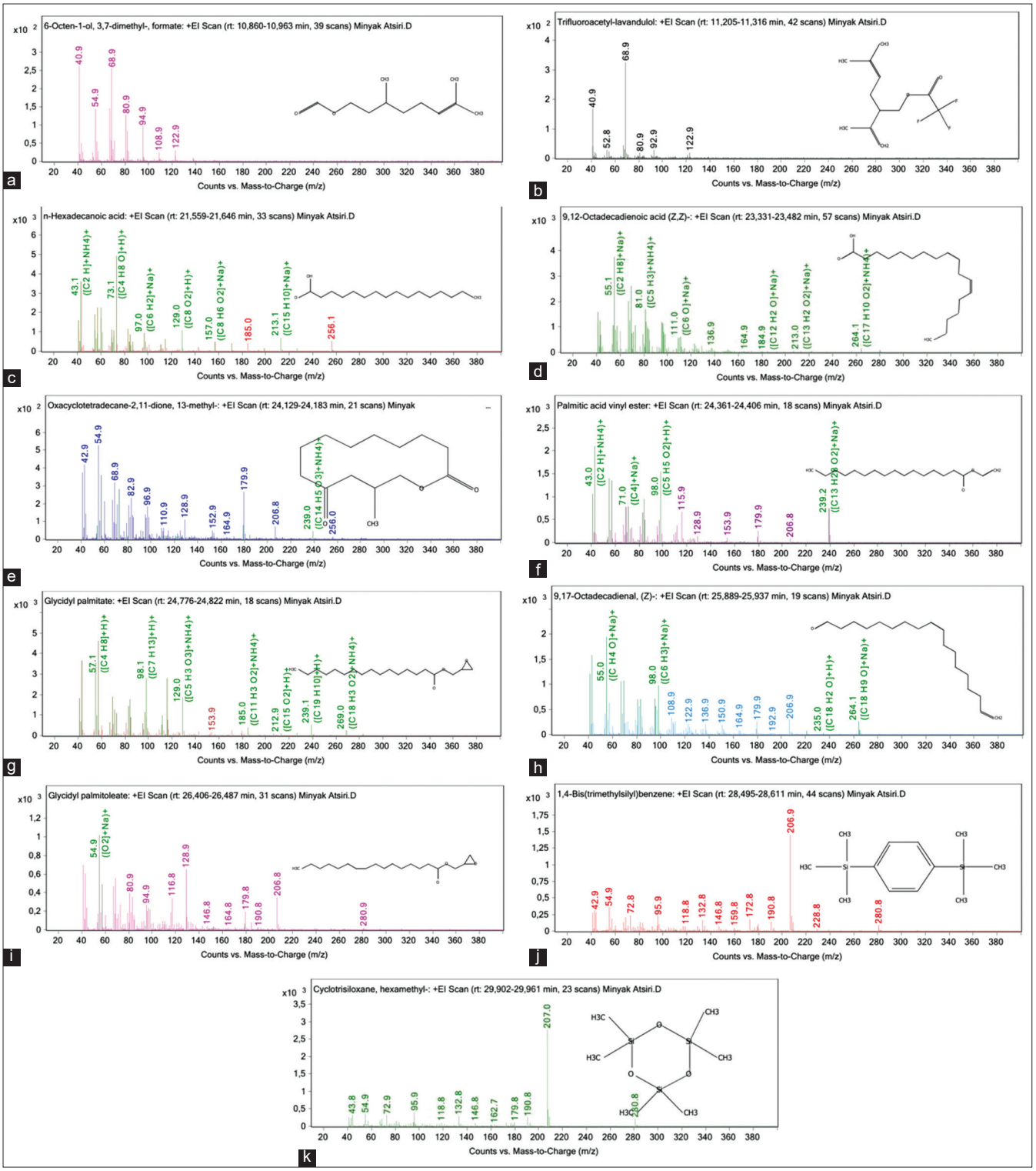

Figure 3: Mass spectra of (a) 6-Octen-1-ol, 3,7-dimethyl-, formate (b) trifluoroacetyl-lavandulol (c) n-Hexadecanoic acid (d) 9,12-Octadecadienoic acid (Z,Z)- (e) Oxacyclotetradecane-2,11-dione, 13-methyl- (f) Palmitic acid vinyl ester (g) Glycidyl palmitate (h) 9,17-Octadecadienal, (z)- (i) Glycidyl palmitoleate (j) 1,4-Bis(trimethylsilyl)benzene (k) Cyclotrisiloxane, hexamethyl-

puspidifolia branch essential oils [56], the compound of *Solena amplexicaulis* tuber extract [57], and *Crateva andasonii* leaves methanolic extract [58]. This compound with (9Z)-octadeca-9,17-dienal IUPAC name was reported to possess antimicrobial properties [57]. In this research, it was found that ethanol steam distillation produced citronellyl formate as the main essential oil component from citral derivatives, whereas hydrodistillation had citronellal, geraniol, and citronellol as main compounds [12]. Apart from aromatic oil, this ethanol steam distillation also extracted several fatty acids.

Conclusion

The essential oil constituents and preliminary pharmacognostic evaluation of *C. winterianus* stem can provide useful data for further phytochemical analysis, quality control, and standardization of *C. winterianus*.

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