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The phytochemistry of *Cymbopogon winterianus* essential oil from Lombok Island, Indonesia and its antifungal activity against phytopathogenic fungi

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Abstract. *Andila PS, Hendra IPA, Wardani PK, Tirta IG, Sutomo, Fardenan D. 2018. The phytochemistry of* Cymbopogon winterianus *essential oil from Lombok Island, Indonesia and its antifungal activity against phytopathogenic fungi. Nusantara Bioscience 10:* 232-239. *Cymbopogon winterianus* Jowitt ex Bor [Sereh Wangi] is a plant producing essential oil that has been extensively used in perfumery, mosquito repellent, medicinal, and cosmetic industry. In Indonesia, the accession of *C. winterianus* plant had been collected from natural forest in Sembalun, Lombok, West Nusa Tenggara (NTB) Province. However, the phytochemical properties and its biological potential of this accession have never been reported. The aim of this study was to determine the phytochemical content of essential oil of *C. winterianus* from Lombok and to explore its potential as a bioantifungal against several phytopathogen fungi. The essential oils were extracted from fresh stem and leaf of *C. winterianus* by hydrodistillation. The chemical composition of them was determined by chromatography-mass spectrometry (GC-MS). A total of 26 and 20 compounds were obtained from the stem and leaf oil with the dominant compounds of essential oil in stem were Torreyol (17.89%), Oxirane, octyl- (15.84%), (-)-Isopulegol (14.44%), Isopulegol 2 (12.18%), and Geranyl Acetate (5.89%) while in leaf were (-)-Isopulegol (22.69%), Oxirane, octyl- (16.64%), Cyclohexanol, 5-methyl-2-(1-methylethenyl)- (14.21%), "KW3 AUS Epiglobulol" (9.47%), and Cyclohexene, 1-methyl-4-(1-methylethenyl)- (7.14%). The presence of these contents as dominant chemical compounds in *S. winterianus* was reported for the first time. The highest content of oils was identified as monoterpene (38.01% and 45.78% in stem and leaf oil) and sesquiterpene (27.67% and 17.78 % in stem and Leaf oil). The biological study showed that leaf essential of *C. winterianus* expressed positive antifungal activity against *Fusarium solani, Aspergillus niger*, dan *Cladosporium* sp.

Keywords: Hydrodistillation, GC-MS, leaf oil, phytochemical, stem oil

INTRODUCTION

Cymbopogon is an aromatic perennial herb of the *Poaceae* family, with the height up to and above 1 m with leaf shape showing narrow and long green. *Cymbopogon* consists of more than 144 species, and It is widely distributed in the tropical and subtropical regions of Africa, Asia, and America (Avoseh et al. 2015). Traditionally, several *Cymbopogon* species are used for various purposes in different countries, such as flavouring agent (Bhattacharya 2016), medicinal purposes as diuretics and treating gastrointestinal disorders (Hasim et al. 2017), anti-inflammatory, analgesic, flu control, antioxidant, antiseptics, malaria therapy, and also to cure rheumatism and fever (Avoseh et al. 2015).

Several bioassays have shown pharmacological evidence of some Cymbopogon species. *C. citratus* was found to express pharmacologic effects as cytotoxicity (Kpoviessi et al. 2014), insecticidal (Pinto et al. 2013), antitrypanosomal (Kpoviessi et al. 2014), anti-diabetic (Bharti et al. 2013), antihypertensive (Dzeufiet et al. 2014), antioxidant (Somparn et al. 2014), anti-inflammatory (Tavares et al. 2015), hepatoprotective (Rahim et al. 2014), antifungal (Amornvit et al. 2014), and antimalaria (Chukwuocha et al. 2016). Biological assay of *C. martinii* extracts revealed a potential of this species as a strong antioxidant (Andrade et al. 2014a), and anti-inflammatory (Andrade et al. 2014b). *C. flexuosus* showed positive activities as antibiofilm against rapidly growing mycobacteria (Rossi et al. 2017), mosquito repellent (Carreño Otero et al. 2018), and anti-inflammatory (Han and Parker 2017). *C. schoenanthus* exhibited positive anticancer activity by promoting melanogenesis in B16 melanoma cells and human epidermal melanocytes (Villareal et al. 2017) and positive antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, methicillin-sensitive (MSSA) and *Klebsiella pneumoniae* (Hashim et al. 2017). *C. flexuosus* had potential as an anti-inflammatory (Han and Parker 2017) and antimicrobial activity (Adukwu et al. 2016). While *C. nardus* was reported for representing positive antifungal activities against *Candida albicans* (Trindade et al. 2015, Kandimalla et al. 2016; De Toledo et al. 2016) and *Microsporum canis* (Capoci et al. 2015), as a mosquito repellent (Ilahi and Yousafzai 2017). *C. jwarancusa* was found to have a flukicidal activity which used as alternative sources of treatment for trematodes infection (Shafiq et al. 2015). *C. validus* (Stapf) Stapf ex Burtt Davy had potential as an anti-inflammatory (Rungqu et al. 2016), and *C. winterianus* was found exhibiting potential as a mosquito repellent, bioinsecticide (Deletre et al. 2015; Sajo et al. 2015; Silva et al. 2018), and as an orofacial antinociceptive (Santos et al. 2015).

Cymbopogon winterianus locally called "Sereh Wangi" (Java, Bali, Lombok) is a member of genus *Cymbopogon* with wide distribution area. This plant had been used traditionally in several countries in the world, such as Brazil, India, and Southeast Asia (Avoseh et al. 2015). The pharmacological study confirmed that this species had enormous potency as Molucidal, Larvicidal (Rodrigues et al. 2013), and as an antifungal against *Candida albicans* (Cavalcanti et al. 2017). *C. winterianus* contains various plant metabolites such as Geraniol, Citronellal, Citronellol (Rodrigues et al. 2013) Linalool and Camphene (Wany et al. 2013) with have potential for various purpose, including as bio-fungicidal agent. In Indonesia, traditionally, this species has been used for many purposes, such as treatment for various diseases, mosquito repellent, and as a flavoring agent, but the information about its phytochemical properties and its biological function, especially as an antifungal agent was still limited. The aim of this study was to explore the phytochemical composition of *C. winterianus* essential oil collected from Sembalun, Lombok Island, Indonesia and to investigate its biofungicide potential against plant pathogenic fungi.

MATERIALS AND METHODS

Study area

Sampling sites of *C. winterianus* are in the natural forest of Sembalun Sub-district, East Lombok District (Lombok Island), West Nusa Tenggara Province, Indonesia.

Plant material and sample preparation

Cymbopogon winterianus was obtained from plants collection of "Eka Karya"-Bali Botanic Garden in March 2017. This plant was collected from natural forest in Sembalun, Lombok Island, West Nusa Tenggara in 2014,

Figure 1. Location of natural forest in Sembalun, Lombok Island, West Nusa Tenggara, Indonesia indicating the sampling sites of *Cymbopogon winterianus*

and planted at Bali Botanic Garden in 2015. Bali Botanical Garden was located in dry highland in the Bedugul Region, North Bali with temperature ranging between 18-20^{0}C, 70-90% humidity and altitude of 1250-1450 m above sea level ((Peneng and Andila 2017). The plant was identified by a taxonomist of Bali Botanic Garden, Ida Bagus Ketut Arinasa, of 56 herbaria of this species were deposited in Herbarium of Tabanan Hortus Botanicus Baliense (THBB). Fresh materials (stems and leaves) of *C. winterianus* were chopped and dried without direct sun irradiance for several days until the materials were completely dry.

Isolation of essential oil

Two hundred gram dry material of *C. winterianus* was extracted by hydrodistillation for five hours using a Pudak Scientific apparatus. The essential oil was separated by a Duran Schott separator and produced a yellowish viscous essential oil.

Determination of Extraction Yield (% yield)

The essential oil yielded (%, w/w) from all dry materials was calculated according to modification of Negreiros et al. (2015) method, as follows:

$$Yield\ (\%) = \frac{W1\ \times 100}{W2}$$

Where W1 is the weight of the essential oil after hydrodistillation, and W2 is the weight of the fresh plant material.

GC-MS analytical conditions and Identification of essential oil component

The chemical components of *C. winterianus* essential oil were analyzed using GC-MS equipment model Shimadzu GC-MS-QP2010 with an Rtx 5ms capillary column (60.0 m x 25 mm with 0.25 µm thickness). Carrier gas was UHP Helium with the conditions of GC setting was column oven at temperature of 50 ^{0}C (±5 minutes to 280ºC, injection temperature at 280ºC, injection mode: split, total program time: 50 minute, flow control mode : Linear velocity, pressure : 101.0 KPa, Total Flow : 46.5 mL/Min, Column Flow: 0.85 mL/min, linear velocity: 23.7 cm/sec, purge flow : 3.0 ml/min, split ratio : 1: 50,total sample injection : 1 µL. The MS settings were ion source temperature: 200^{0}C, interface temperature: 280^{0}C, solvent cut time: 1.5 min, and detector temperature 280^{0}C.

Mass spectra fragmentation patterns identified the compounds. Identities of compounds were approved by comparing each retention time indices and the spectral data with those from computer library WILEY7.LIB and free-published literature.

Antifungal assay

The antifungal assay of *C. winterianus* essential oil was carried out by the disc diffusion assay method (Diánez et al. 2018). Four plant pathogenic fungi obtained from the Laboratory of Mycology, Udayana University, *Fusarium solani*, *Aspergillus niger*, *Colletotrichum* sp. were used for the antifungal assay. The Fungi cultures collection were stored on an agar slant of Difco TM Potatoe Dextrose Agar at 40ºC and re-grown every two months. The mature fungi cultures (about 7 days of incubation) on agar slant were diluted in sterile distilled water to obtain the spores fro experiment (Broekaert et al. 1990). The sporangial suspension (1 mL) was inoculated into petri disc, poured with dilute PDA (medium temperature of about 45-50ºC), and homogenized. The plates were left for 40 minutes at temperature of 27°C to allow the mixture of PDA and spores to compact. Afterward, one paper disc (Whatman paper disc with 6 mm in diameter) were put on the center of PDA plate surface, and impregnated with 20 µl of the fruit essential oil of *Z. avicennae* (0-5% concentration). Petri dishes were incubated at room temperature around 27-30ºC for 24-48 hours and the antifungal activity was observed by measuring minimal inhibitory concentration (MIC) (Kacem et al. 2016). MIC is the lowest concentration of essential oil, which inhibits the visible growth of plant pathogenic fungi during the incubation period. These treatments were carried out in triplicate. The antifungal activity was determined by the formation of a clear zone around the paper discs as an antifungal activity indicator (after 48-72 hours of incubation). A paper disc impregnated with acetone was served as the negative control.

RESULTS AND DISCUSSION

The essential oil extracted from aerial parts (stem and leaf) of *C. winterianus* yielded 0.6-0.67% (w/w). A total of 26 and 20 volatile substances were identified in the stem and leave essential oil of *C. winterianus*, accounting for 97.4% and 94.5% of the oil. The identified phytochemical components and their relative concentration were summarized in Table 1 and Table 2. While the GC-MS chromatograms were performed in Figure 2.

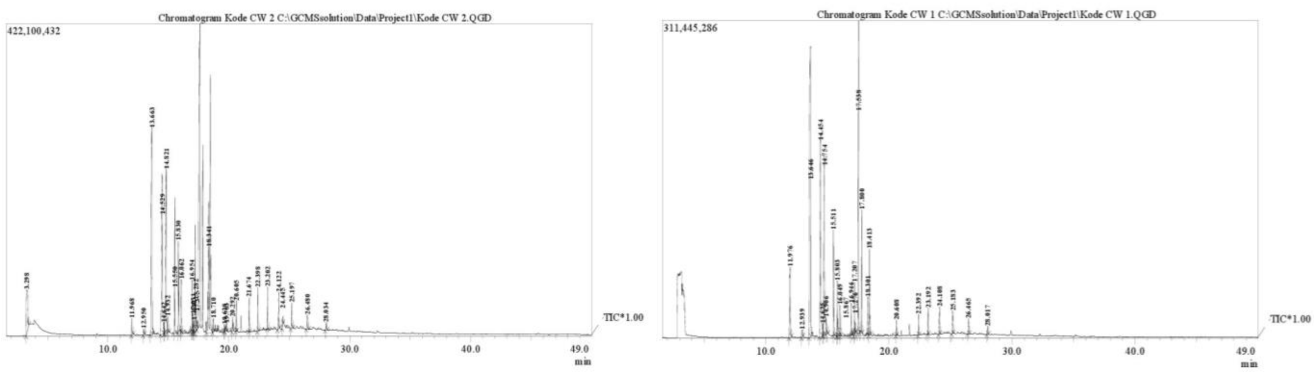
As shown in Table 1, the major components found in the stem essential oil of *C. winterianus* in the present study were Torreyol (17.89%), Oxirane, octyl- (15.84%), (-)-Isopulegol (14.44%), Isopulegol 2 (12.18%), and Geranyl Acetate (5.89%). While the main compounds of essential oil of *C. winterianus* leaf (Table 2) were Isopulegol (22.69%), Oxirane, octyl- (16.64%), Cyclohexanol, 5-methyl-2-(1-methylethenyl)- (14.21%), "KW3 AUS Epiglobulol" (9.47%), and Cyclohexene, 1-methyl-4-(1-methylethenyl)- (7.14%). In this species, a high amount of terpenoids were identified as dominant compounds with details 67.45% of the total stem oil and 71.12% of the total leaf oil. The volatile oil of *S. winterianus* stem consisted of monoterpene 38.01%, sesquiterpene 27.67%, and another terpene 1.18%. Whereas, the leave essential oil comprised monoterpene 45.78%, sesquiterpene 17.78%, and another terpene 7.14%.

Table 1. The result of GCMS analyses obtained from essential oil of *Cymbopogon winterianus* Jowitt ex Bor stem

Peak	Name	R. time	Relative conc%
1	1,1-dimethyl diborane-D6	3.298	5.76
2	1-P-mentha-1,8-diene	11.968	0.77
3	Alpha.-Terpinolene	12.950	0.41
4	(-)-Isopulegol	13.663	14.44
5	Oxirane, octyl-	14.529	15.84
6	Isopulegol 2	14.821	12.18
7	Z-Citral	14.932	0.82
8	Citronellyl acetate	15.550	4.68
9	Geranyl Acetate	15.830	5.89
10	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-	16.062	1.48
11	Germacrene D	16.954	1.54
12	alpha.-Muurolene	17.031	0.47
13	Spathulenol	17.082	0.41
14	Elemol	17.282	4.95
15	Torreyol	18.341	17.89
16	Farnesol	18.710	0.93
17	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	19.628	0.30
18	(-)-Spathulenol	19.783	0.59
19	Palmitic acid	20.292	0.78
20	Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-	20.605	0.76
21	Tricosane	21.674	0.84
22	Docosane	23.202	1.48
23	Eicosane	24.122	1.58
24	M-N-undecyl phenol	24.445	0.39
25	Pentatriacontane	25.197	1.32
26	Hexatriacontane	28.034	0.53
			97.03
	Terpene		67.45 %
	Monoterpene		38.01%
	Sesquiterpene		27.67%
	Another terpene		1.18%

Table 2. The result of GCMS analyses obtained from essential oil of *Cymbopogon winterianus* Jowitt ex Bor leaf

Peak	Name	R. time	Relative conc%
1	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	11.976	7.14
2	Alpha.-Terpinolene	12.939	0.63
3	(-)-Isopulegol	13.646	22.69
4	Oxirane, octyl-	14.454	16.64
5	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	14.754	14.21
6	Z-Citral	14.906	1.16
7	Citronellyl acetate	15.511	3.55
8	Geranyl acetate	15.803	2.13
9	Eugenol	15.867	1.41
10	2,4-diisopropenyl-1-methyl-1-vinyl-cyclohexane	16.049	1.00
11	Germacrene D	16.946	1.12
12	delta.-Cadinene	17.207	1.63
13	Elemol	17.276	0.47
14	"KW3 Aus Epiglobulol"	17.538	9.47
15	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-	17.800	4.27
16	Alpha.-Cadinol	18.413	4.09
17	Nerolidol Z and E	20.608	0.42
18	Tricosane	23.192	0.92
19	Pentatriacontane	24.108	1.15
20	Heptacosane	28.017	0.55
			94.65%
	Terpene		71.12 %
	Monoterpene		45.78 %
	Sesquiterpene		17.78 %
	Another terpene		7.14 %



A B

Figure 2. GC-MS chromatograms of the stem (A) and leaf (B) essential oil of *Cymbopogon winterianus* Jowitt ex Bor from Lombok, West Nusa Tenggara

By comparing these results with previous studies, completely different chemical compositions were obtained. The presence of these contents as dominant chemical compounds in *S. winterianus* was reported for the first time. Previous research studies mentioned that the dominant contents of *S. winterianus* were citronella, citronellol, and geraniol (Quintas-Junior et al. 2008; Katiyar et al. 2011; Pinheiro et al. 2013). Several publications about phytochemical contents in *S. winterianus* was shown in Table 3.

Isopulegol is a monoterpene alcohol which is present in the essential oils of various plants. The existence of (-)-Isopulegol in EO of *S. winterianus* had been reported by Quintas-Júnior et al. (2008), Pinheiro et al. (2013), and Kakaraparthi et al. (2014), but in low relative concentration (in concentration series of 0.60%, 1.53% and 0.30-0.50%). Torreyol is classified as sesquiterpenoid alcohol produced by many plants, animal and microorganisms. The presence of Terreyol in the *S. winterianus* essential oils was also detected by Oliveira et al. (2011) and by Pinheiro et al. (2013) with relative concentration 1.65% and 1.44%. Several compounds commonly existed in *C. winterianus* essential oil was also found in this present study, but in lower concentration, i.e. geranyl acetate (5.89% in the stem EO and 2.13% in the leaf EO, Citronellyl acetate (4.68% in the stem EO and 3.55% in the leaf EO, Elemol (4.95% in the stem EO and 0.47% in the leaf EO . The difference in the chemical composition of essential oils in a species of plant from different places is due to various factors among others such as differences of geographical location, season, and climate (Mahzooni-Kachapi et al. 2014; Shams et al. 2016; Sanli et al. 2017), genetic diversity (Purushothamhkjan and Ravi 2013), various of plant parts (stem, flower, fruit) (Faidi et al. 2014), and differences stage of plant growth (Flamini et al. 2013; Faidi et al. 2014) and other factors.

A study of the antifungal activity of essential oil compounds of *C. winterianus* against several plant pathogenic fungi was reported in Table 4. This study was conducted to observe the effect of variation of essential oil concentration towards *Fusarium solani, Aspergillus niger,* and *Colletotrichum* sp. The results showed that essential oil of *C. winterianus* expressed significant antifungal activity against *Fusarium solani* (MIC 3% and inhibition zone 1.75 ± 0.21 cm), *Aspergillus niger* (MIC 0.25% with inhibiting zone 1.67 ± 0.39 cm), and *Colletotrichum* sp. (MIC 1% with inhibit zone 1.75 ± 0.22 cm).

Table 3. The results of major phytochemical contents in *S. winterianus* essential oil base on the previous literature

No.	Species	Material	Dominant compounds	Origin	Literature
1	*S. winterianus*	The fresh material of leaves	Geraniol (28.62%), citronellal (23.62%), citronellol (17.10%)	Espírito Santo State, Brazil (an altitude of 250 m.)	(Pinheiro et al. 2013)
2	*S. winterianus*	The oven-dried material of leaves	geraniol (40.06%), citronellal (27.44%), citronellol (10.45%)	at the Research Station ‘‘Campus Rural da UFS’’ of the Universidade Federal de Sergipe, Brazil	(Quintans-Junior et al. 2008)
3	*S. winterianus*	Not mentioned	citronellal (31.1-35.4%), geraniol (22.4-30.2%), citronellol (7.4-11.0%)	Andhra, Pradesh, Assam, Gujarat, Jammu and Kashmir, Tamilnadu, Uttar Pradesh and Uttarakhand (India)	(Wany et al. 2013)
4	*S. winterianus*	Not mentioned	geraniol (50.1%), citral (21.8%), citronellal (11.8%)	Southern and northern sub-Himalayan region of India	(Wany et al. 2013)
5	*S. winterianus*	The fresh material of the aerial part of plants	citronellal (35.9%), geraniol (20.9%)	Viamão in the state of Rio Grande do Sul, in Southern Brazil.	(Cassel and Vargas 2006)
6	*S. winterianus*	The fresh material of leaves	citronellal (29.15%), geraniol (22.52%), citronellol (7.43%)	Kanpur, India	(Singh and Kumar 2017).
7	*S. winterianus*	The air-dried material of leaves	citronellal (26.5%), geraniol (16.2%), elemol (14.5%), citronellol (7.3%)	Sao Luıs, Maranhao, Brazil	(Rodrigues et al. 2013)
8	*S. winterianus*	The fresh material of the aerial part of plants	citronellal (24-34.2 %), geraniol (16.4-33.3%), geranylacetate (2.2-16.0%), citronellol (4.5-12.5%), elemol (2.6-9.5%)	Hyderabad, Andhra Pradesh, India.	(Kakaraparthi et al. 2014)
9	*S. winterianus*	The fresh material of leaves	linalool (27.4%), citronellol (10.9%), geraniol (8.5%)	at the slopes of Usambara mountains in Mombo, Tanzania	(Malele et al. 2007)
10	*S. winterianus*	Not mentioned	citronellal (23.59%), geraniol (18.81%), citronellol (11.74%)	Brazil (Specific place was not mentioned)	(Oliveira et al. 2011)

Table 4. The result of the antifungal activity of the leaf essential oil of *C. winterianus* [Sereh Wangi] from Lombok Island against plant phytopathogenic fungi

Concentration % EOL of *C. winterianus*	Inhibition zone of EOL versus plant phytopathogenic fungi (cm)			
	Fusarium solani	*Aspergilus niger*	*Cladosporium* sp.	Control (Aceton)
5	2.67 ± 0.18	NG	2.17 ± 0.28	-
3	1.75 ± 0.21*	3.43 ± 0.48	1.73 ± 0.13	-
1	-	1.98 ± 0.20	1.75 ± 0.22*	-
0.5	-	1.80 ± 0.03	-	-
0.25	-	1.67 ± 0.39*	-	-
0.125	-	-	-	-

Note: NG stands for "Not Growing" mean that there was no pathogen fungal growing in PDA media at all. * means that the inhibitory zone formed on the MIC (the minimum inhibitory concentration)

This is an encouraging result showing that essential oil of *C. winterianus* has potential as a bio-fungicide agent. Plant pathogenic fungi play an important role in the success of agricultural production in terms of both qualitative and quantitative yields. It had been predicted that the inability to control plant fungal disease has caused the disadvantage in agricultural fields reaching millions of dollars per year (Shuping and Eloff 2017). Several fungal diseases such as downy mildew and botrytis are difficult to eradicate, outbreaks of plant fungal diseases can continue to last several seasons, and have the potential to cause damage the horticultural crops (Wightwick et al. 2010). To control this damage, farmers have used chemical fungicides for years. The disadvantages of this approach are contamination of the environment (land, air, and water), potential human exposure to fungicide, and deposition of residue on the plants (crops, fruits).

Similarly, they have been the main cause of plant pathogenic fungi resistance (Sales et al. 2016). Due to these factors, adopting the plant extracts as biofungicide would be useful due to low toxicity to mammals, reduce the environmental exposure to synthetic chemical fungicides, and avoid the resistance effects (Seiber et al. 2014). To improve the resistance evolution control of plant pathogenic fungi, FRAC (Fungicide Resistance Action Committee) had given recommendations to use fungicides mixtures to broaden the spectrum of disease control or to make use of synergistic interactions leading to more potent fungal diseases control and higher flexibility. This recommendation is appropriate for the biofungicide action. Plant metabolite contains several antimicrobial compounds which can reduce the development of pathogens resistance due to the variation of the metabolic process (Shuping and Eloff 2017).

The research on the use of *C. winterianus* the fields of pharmacology, perfume, aromatherapy, and insect repellent has been widely studied, but the use of *C. winterianus* in biopesticides is still limited to be studied, especially as a biofungicide agent. Previous studies have reported that essential oil *C. winterianus* has insecticidal activity against *Frankliniella schultzei* and *Myzus persicae* (Pinheiro et al. 2013) and ethanol extract from *C. winterianus* are insecticidal to *Tetranychus urtica* (VicentiniI et al. 2015). In Thailand, the mixture of crude extract of leaves of *C. winterianus* and *Azadirachta indica* A.H.L. Juss. and rhizome of *Alpinia galanga* (L.) Wild. were used as bio-insecticide in vegetable and citrus orchard (Westphal and Jansen 1989). In this study, the screening of essential oil of *C. winterianus* as antifungal against plant pathogenic fungi is reported for the first time.

In the antifungal action of essential oil components, monoterpenes have taken an important role by inhibiting the fungal growth (Marei et al. 2018). The previous study showed that treatment of monoterpene inhibited the mycelial growth of several plant pathogenic fungi, including *Rhizoctonia solani*, *Fusarium oxysporum*, *Penicillium digitatum*, and *Aspergillus niger* which cause collar rot, fusarium wilt, green, and black mold, respectively (Marei et al. 2012). Other studies showed that A new monoterpene lactone has strong antifungal activity against Botrytis cinerea and Phytophthora nicotianae (Xu et al. 2016), Furthermore, carvacrol and thymol (phenolic monoterpenes) possessed fungicidal effects against *Phytophthora capsici*, *P. nicotianae*, *Alternaria solani*, *Botrytis cinerea*, *F. oxysporum*, *Pyricularia grisea*, *Rhizoctonia solani*, *P. capsici*, and *P. nicotianae* (Wang et al. 2018).

Based on the results of this study, it can be concluded that the essential oil of stem and leaves of C. *winterianus* from Sembalun, Lombok, West Nusa Tenggara, Indonesia have different composition due to their phytochemical contents compared with previous researches. The antifungal assay of its leaf essential oil exhibited biofungicidal potential against pathogen fungal *F. solani*, *A. niger*, and *Cladosporium* sp.

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