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CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF *Syzygium polyanthum AND Syzygium aromaticum*

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ABSTRACT

The essential oils of the leaves of two Syzygium species commonly used as spices in Indonesia (*Syzygium polyanthum* and *S. aromaticum*) were analyzed by gas chromatography/mass spectrometry (GC/MS) and evaluated for their minimum inhibitory concentration (MIC) against five food-borne microorganisms. The essential oils of both plants were obtained by hydrodistillation method. The major constituents ofessential oil of*S. polyanthum* were cis-4-decanal (43.489%), 1-decyl aldehyde (19.752%), and capryl aldehyde (14.092%), while the major constituents ofessential oil of*S. aromaticum* were p-eugenol (75.190%) and β-caryophyllene (18.364%). Beta-caryophyllene, α-humulene, α-farnesene and caryophyllene oxide were detected in both essential oilsof *S. polyanthum* and *S. aromaticum*. Both essential oils strongly inhibited *Bacillus subtilis* growth. Essential oil of *S. aromaticum* showed a stronger inhibitory activity against *Staphylococcus aureus, Salmonella typhimurium* and *Vibrio cholera* than that of *S. polyanthum*. Both essential oils did not inhibit the growth of *Escherichia coli*.

Keywords: Essential oils, *S.polyanthum, S. aromaticum,* chemical constituents, MIC

INTRODUCTION

Food-borne illness is a growing public health problem in many countries.[1] In Indonesia, *Vibrio cholera* and *Salmonella typhimurium* are the common causes of food-borne illness along with food poisoning caused by *Escherichia coli*, *Bacillus* spp., and *Staphylococcus aureus.*[2–4]Active substances of spices as plant derived products are considered to be safer and more effective against the pathogens, and thus be useful to control of food-borne illness.[5–8]*Syzygium polyanthum* and *S. aromaticum* are spices from Syzygium genus commonly used in Indonesian culinary. *S. polyanthum* (Indonesian bay leaf, *salam* in Bahasa Indonesia) has been empirically used for antiulcer, antidiabetes, anti-inflammatory, and antidiarrhea treatment. It has been proven possessing antidiarrheal, antimicrobial, antihypertensive, and antioxidant activities in in-vitro studies.[9–12]*S. aromaticum* (clove, *cengkih* in Bahasa Indonesia) is indigenous to Indonesia and recently grown in India, Tanzania, Sri Lanka, Brazil, and Madagascar. It is used as an expectorant and to cure upset stomach. The essential oil of clove is used for treatment of diarrhea, hernia, intestinal gas, nausea, vomiting and bad breath.[13,14] Essential oil of*S. aromaticum* had been known widely as a potent antimicrobial against various bacteria and fungi, with eugenol as the active ingredient.[15–18]In this study, we compared chemical constituents of essential oils of *S. polyanthum* and *S. aromaticum* and evaluated their minimal inhibitory concentration (MIC) against the common food-borne microorganisms in Indonesia.

EXPERIMENITAL

The leaves of *S. polyanthum* and *S. aromaticum* were purchased from a local market at Purwokerto, Central Java, Indonesia. The leaves were dried under direct sunlight.

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Hydrodistillation of Essential Oils

Hydrodistillation of essential oils of leaves of *S. polyanthum* and *S. aromaticum* were conducted as previously reported.[19]

Microorganisms

Five bacterial strains were obtained from the American type culture collection (ATCC; Rockville, MD, USA) as well as the culture collection of the Assessment Service Unit, Airlangga University, Surabaya, Indonesia. They were *Bacillus subtilis* ATCC 6633*, Escherichia coli* ATCC 8739*, Staphylococcus aureus* ATCC 6538*, Salmonella enterica typhimurium* ATCC 14028, and *Vibrio cholera* ATCC 9027. All microorganisms were stocked in appropriate conditions and regenerated before used.

Analysis of Chemical Constituents

The volatile composition of essential oils were analyzed using GC-MS system (Agilent 6980N GC System coupled to Agilent 5973 inert MSD detector), equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The condition of separation and identification followed previously reported method.[19]

Determination of Antimicrobial Activity

The Minimum Inhibitory Concentration (MIC) was examined by broth dilution method in nutrien broth (NB) using a method previously described with modification.[20] Briefly,active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks and inoculated in NB and incubated at 37 °C for 24 h. The cultures were diluted with NB to achieve an optical density of 10^7 CFU/mL at the wavelength of 600 nm by UV/Vis Spectrophotometer. Essential oils were diluted to get the final concentration ranging from 0 to 1000 µg/mL in NB. Finally, 20 µL inoculums of each bacteria strain were inoculated and the tests were performed at a final volume of 5.0 mL. The plates were incubated at 37 °C for 24 h. The lowest concentration of the test samples which did not show any visual growth of tested organisms after macroscopic evaluation was determined as MIC, which was expressedin µg/mL.

RESULTS AND DISCUSSION

In this study, the rendement of essential oils of *S. polyanthum* and *S. aromaticum* was 0.075 and 0.75 %, respectively. The rendement ofessential oil of*S. polyanthum* is higher than the previously reported data (0.05%).[21]The chemical constituents of essential oils of *S. polyanthum* and *S. aromaticum* are shown in Table-1.

Table-1: Chemical constituents of essential oils of *S. polyanthum* and *S. Aromaticum*

S. No.	Compound name	Retention time (min)	Percentage (%)	
			S. polyanthum	*S. aromaticum*
1	Capryl aldehyde	9.416	14.092	-
2	n-Nonaldehyde	14.812	0.483	-
3	Cis-4-decenal	20.520	43.489	-
4	1,2,3,3a,4,6a-Hexahydro-pentalene	20.574	2.062	-
5	1-Decyl aldehyde	21.193	19.752	-
6	2,6-Octadienal	23.096	0.990	-
7	Geranial	25.021	1.681	-
8	α-Cubebene	29,367	-	0.800
9	α-Copaene	30.378	0.941	-
10	p-Eugenol	30.436	-	75.190
11	Eugenyl acetate	30.551	-	0.427
12	Geraniol acetate	31.127	0.562	-
13	β-Caryophyllene	32.301	1.734	18.364
14	α-Bergamotene	32.816	0.412	-

15	α-Humulene	33.409	0.388	2.729
16	β-Farnesene	33.664	0.206	-
17	1(5).6-Guaiadiene	34.120	-	0.090
18	α-Curcumene	34.429	2.271	-
19	α-Selinene	34.673	0.712	-
20	α-Zingiberene	34.834	1.375	-
21	α-Muurolene	34.902	0.347	-
22	β-Bisabolene	35.223	1.590	-
23	α-Farnesene	35.358	0.857	0.230
24	β-Sesquiphellandrene	35.651	1.407	-
25	δ-Cadinene	35.671	-	0.696
26	Cadina-1,4-diene	35.908	-	0.087
27	2-Hydroxy-4,6-cyclooctadien-1-one	36,435	-	0.135
28	Caryophyllene oxide	37.261	0.997	1.130
29	α-Bisabolene	37.631	0.821	-
30	3,4-Dimethyl-3-cyclohexen-1-carboxaldehyde	37.890	1.587	-
31	Naphthalene	37.956	-	0.121
32	Tumerone	39.472	0.180	-
33	Ethyl-(2E)-3-(4-metoxyphenyl)-2-propenoate	41.497	0.454	-
34	Farnesyl acetate	43.309	0.235	-
35	6,10,14-Trimethyl-2-pentadecanone	43.362	0.372	-

The major constituents of essential oil of *S. polyanthum* were cis-4-decanal (43.489%), 1-decyl aldehyde (19.752%), capryl aldehyde (14.092%), α-curcumene (2.271%), and 1,2,3,3a,4,6a-hexahydropentalene (2.062%) (Fig.-1). Our result was different than previously reported data. *S. polyanthum* obtained from Malang, Indonesia was mainly consisted of cis-4-decenal (27.12%), octanal (11.98%), α-pinene (9.09%), farnesol (8.84%), β-ocimene (7.62%), and nonanal (7.60%).[22]

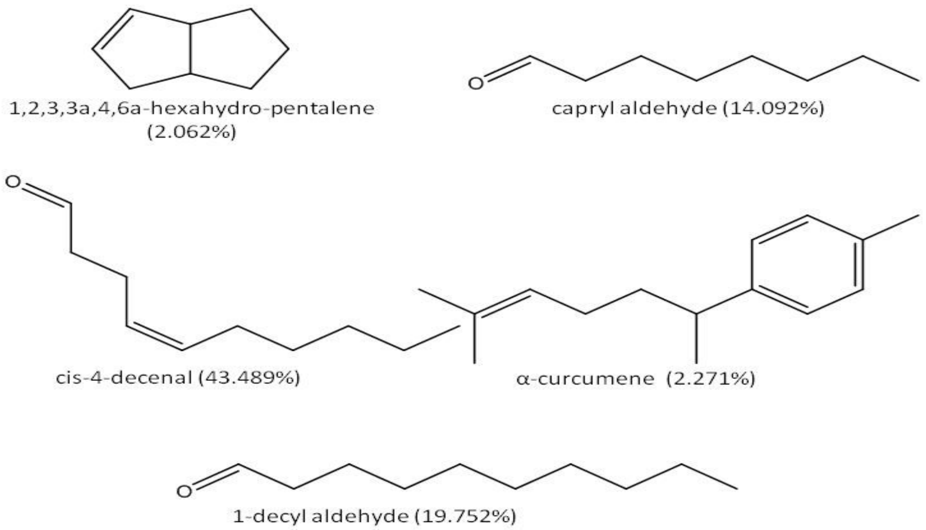

Fig.-1: The major constituents of the essential oil of *S. polyanthum*

The major constituents of essential oil of*S.aromaticum* are p-eugenol (75.190%), β-caryophyllene (18.364%), α-humulene (2.729%), and caryophyllene oxide (1.130%) (Fig.-2). Our result was similar with previously reported data, that essential oil of *S. aromaticum* typically contained p-eugenol, β-caryophyllene and α-humulene as the major constituents. Somehow the concentration of respective constituents in each essential oil was different. The differences of composition of essential oils from a particular species was caused by harvesting seasons and geographical sources.[19,23–25]

There are four constituents detected in bothessential oils of *S. polyanthum* and *S. aromaticum*, they are β-caryophyllene (1.734 and 18.364%), α-humulene (0.388 and 2.729%), α-farnesene (0.857 and 0.230%) and caryophyllene oxide (0.997 and 1.130%). The chemical constituents of essential oils from *Syzygium* genus are varied widely. The major constituents of essential oil of *S. cumini* are α- and β-pinene, β-ocimene, α-terpineol, α- and β-caryophyllene,while the most abundant constituents of essential oilof *S. samarangense* are cis-3-nonen-ol, α-cubebane, β-caryophyllene, caryophyllene oxide, β-carene, α-terpineol, and α-copaene.Beta-caryophyllene is the most common constituents in essential oils of *Syzygium* genus, it is detected in *S. polyanthum*, *S. aromaticum, S. cumini* and *S. samarangense.* Beside found in *S. polyanthum* and *S. aromaticum*, α-humulene and caryophyllene oxide was also detected in *S. cumini*. The constituent detected simultaneously in *S. polyanthum* and *S. cumini*were α-copaene, α-selinene, and α-zingiberene. Alpha-cadinene and cadina-1,4-diene were detected in both *S. aromaticum* and *S. cumini*, while α-cubebene was detected in *S. aromaticum* and *S. samarangense.*[26–31]

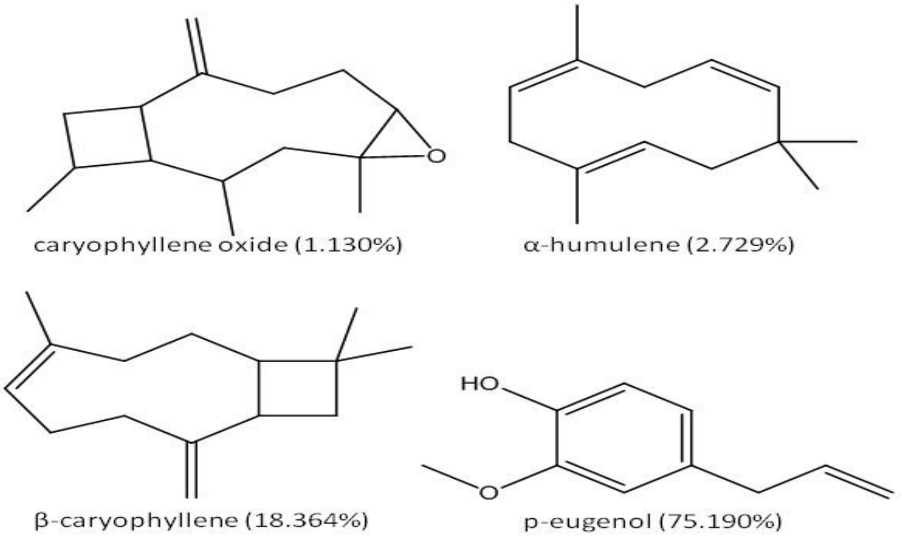

Fig.-2:The major constituents of essential oil of *S. aromaticum*.

The antimicrobial activity of essential oils of *S. polyanthum* and *S. aromaticum* was shown by their MIC against the food-borne microorganisms (Table-2). *B. subtilis* was relatively susceptible to both essential oils, with MIC of 31.25 µg/mL. Essential oil of *S. aromaticum* showed stronger inhibitory activity against *S. aureus, S. typhimurium* and *V. cholera* (MIC 250 µg/mL) than that of essential oil of *S. polyanthum* (MIC>1000µg/mL). *E. coli* was not inhibited by both essential oils. There was limited data on the antimicrobial activity of essential oils of *S. polyanthum.* Somehow, methanol extract of *S. polyanthum* showed a strong antibacterial activity against*B. subtilis* with MIC of 310 µg/mL.[12] Ethyl acetate extract of *S. polyanthum* also inhibited the growth of *B. subtilis, S. aureus,*and *P. aeruginosa.*[32] In addition, *S. polyanthum* water extract was active in reducing the numbers of the oral pathogen *Streptococcus* spp, and chicken meat spoilage bacterium *E. coli.*[21,33] Hence, the antimicrobial activity of *S. polyanthum* is possibly not only due to its essential oils compounds, but also its non-volatile compounds such as tannins and flavonoid as previously reported.[21]

Previously reported MIC of essential oil of *S. aromaticum* evaluated with the same method with that in our study suggested a wide range result. The MIC of essential oil of buds of *S. aromaticum* against tested bacteria was 3260-8600 µg/mL. It relatively inactive against *Listeria monocytogenes*, with MIC of 12500-50000 µg/mL[17,34].In our study, MICs of essential oil of *S. aromaticum* against *E. coli, S. aureus, S. typhimurium* and *V. cholera* were in agreement with those reports. The MIC of essential oil of *S. aromaticum* against *B. subtilis* was considerably lower than those previously reported data.

Essential oil of *S. polyanthum* was mainly consisted of aldehyde (82.074%) and hydrocarbon (13.061%) volatile compounds, while constituent of essential oil of *S. aromaticum* was dominated by phenol (75.190%) and hydrocarbon (23.247%) compounds. Aldehydes in essential oil of *S. polyanthum* (capryl aldehyde, n-nonaldehyde, cis-4-decenal, 1-decyl aldehyde, 2,6-octadienal, and geranial) and phenol in essential oil of *S. aromaticum* (p-eugenol) might be responsible for their respective antimicrobial activity. Aldehydes or phenols were reported possessing the highest antimicrobial activity compared to other constituents of essential oils.[35]

Table-2:MIC of essential oils of *S. polyanthum* and *S. aromaticum* against microorganisms

S.No.	Microorganisms	MIC (µg/mL)	
		S. polyanthum	*S. aromaticum*
1	*B. subtilis*	31.25	31.25
2	*E. coli*	>1000	>1000
3	*S. aureus*	>1000	250
4	*S. typhimurium*	>1000	250
5	*V. cholera*	>1000	250

Our results showed that both essential oils exhibited moderate antimicrobial activity against food-borne microorganisms. However, the antimicrobial activity of those essential oils can be enhanced by combining two essential oils to obtain the synergetic effect. Some succeseful synergetic effect of combination of essential oils have been reported and they are useful to maintain product safety and shelf-life, thereby minimizing the undesirable flavor and sensory changes associated with the addition of high concentrations of essential oils.[35–38]

CONCLUSION

In conclusion, both essential oils of *S. polyanthum* and *S. aromaticum* possessed antimicrobial activity against *B. subtilis*, *S. aureus, S. typhimurium* and *V. cholera*. Those activities was related to their major chemical constituents, aldehydes and eugenol, respectively.

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