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GC-MS Analysis and Antibacterial Activity of Essential Oils of Five *Syzygium* Species Leaves

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ABSTRACT. The essential oil can inhibit pathogenic bacterial activities, which can be developed to be a natural preservative for food. This research aimed to evaluate the antibacterial activity of the essential oils from five species Syzygium on *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica sv Typhimurium*. The research results show that five *Syzygium sp.* Essential oils have moderate antibacterial properties with a minimum inhibitory concentration (MIC) value of 250 – 500 µg/mL. *S. polyanthum* essential oils have the highest antibacterial activity than the rest species on *B. cereus* at 250 µg/mL. Meanwhile, the essential oil of *S. polycephalum* also showed the highest antibacterial activity with a MIC value of 250 µg/mL against *L. monocytogenes*. The chemical component analysis using GC-MS shows the main constituents farnesol, nerolidol, and n-decanal, presenting the antibacterial effect.

INTRODUCTION

Syzygium is a genus of the family Myrtaceae consisting of about 1200 species. This plant is widely distributed in Southeast Asia, South Asia, Australia, and New Caledonia. Some plant species are also found in Africa and the Pacific islands (Raj *et al*., 2016). Traditionally, some of the species have been used by people to treat some diseases, such as *S. aqueum* (itch) (Manaharan *et al*., 2013), *S. samarangense* (fever) (Simirgiotis *et al*., 2008), *S. polyanthum* (diarrhea/dysentery, skin infection, diabetes) (Kusuma *et al*., 2011), *S. jambos* (rheumatism, diabetes diarrhea/dysentery) (Sharma *et al*., 2013), *S. guineense* (diarrhea/dysentery) (Djoukeng *et al*., 2005), *S. caryophyllatum* (diarrhea/dysentery) (Raj *et al*., 2016), *S. cordatum* (diarrhea/dysentery) (Sidney *et al*., 2015), *S. cumini* (diarrhea/dysentery, inflammation) (Shafi *et al*., 2002), *S. jambolanum* (skin infection) (Chandrasekaran *and* Venkatesalu, 2004), *S. malaccense* (diabetes) (Arumugam *et al*., 2016) and diuretic (*S. aromaticum*) (Pandey *and* Singh, 2011). The presence of essential oil content in this plant species is one of the reasons for its pharmacological properties.

The liquid extracts of aromatic plants, known as essential oils, are used in a variety of different sectors. One example is natural food preservatives against pathogenic bacteria, including *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum*, *Cronobacter sakazakii*, and *Salmonella spp* (Abebe *et al*., 2020, Jesseberger *et al*., 2020; Bintsis, 2017). Essential oil is one of the natural preservatives for food that keeps developing because of pharmacological activity and economic reasons (Chouhan *et al*., 2017). Clove oil, produced by the Syzygium aromaticum plant, is an example of the essential oils from *Syzigium sp*. created for food preservation (Hu *et al*., 2018). Therefore, the essential oil of the *Syzygium* plant has the potential to be further studied as a natural antibacterial, especially against food-borne pathogens.

The exploration of the antibacterial properties of essential oils Syzygium is continuously carried out. Essential oil made of the leaves of *S. aromaticum* is reported to have antibacterial activity with MIC value 1.36-

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2.72 mg/mL on some pathogenic Gram-negative bacteria such as *Serrati sp, Salmonella sp*., *Kluyvera sp*., *Klebsiella sp*., and *E. coli* F5 (Selles *et al*., 2020). Essential oil made of *S. cumini* leaves is reported to have inhibitory activity on some tested bacteria with a diameter value of inhibitory capacity of 12–14 mm at 10 µL against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Bacillus subtilis*, and *Enterococcus faecalis* (Mohamed *et al*., 2013). Essential oil made of the leaves of *S. travancoricum* has an inhibitory capacity with a diameter value of 10 – 12 mm at 5 µL against *E. coli*, *S. aureus*, *P.aeruginosa*, *B. subtilis*, *B. sphaericus, and S. typhimurium* bacteria (Shafi *et al*., 2002). Therefore, this research evaluated the antibacterial property of essential oil derived from some species of Syzgium against some pathogenic bacteria, namely *B. cereus*, *E. coli*, *L. monocytogenes*, *S. aureus*, and *S. enterica sv Typhimurium*. To our knowledge, there is no report on the antibacterial property made of the essential oil of *S. polycephalum.* In addition, no one has reported the antibacterial properties of the essential oil from the plant Syzygium sp against *L. monocytogenes* bacteria.

RESEARCH METHODS

Plant Material

The species Syzygium used in this research were *S. samarangense* (Semarang rose-apple), *S. myrtifolium* (*pucuk merah*), *S. aqueum* (watery rose apple), *S. polycephalum* (*jambu gowok*), and *S. polyanthum* (Indian baywatch). The leaves of the five Syzygium sp. were obtained from the garden collection of the Faculty of Biology, Jenderal Soedirman University, and determined at the Laboratory of Plant Taxonomy of the Faculty of Biology, Jenderal Soedirman University.

Essential Oil Distillation

As much as one kg of unprocessed natural ingredients of the leaf of Syzygium sp. were each put in a round-bottom flask for a water vapor distillation process according to the procedure conducted by Fareza *et al*. (2019). The essential oils obtained were added with anhydrous Na_2SO_4 and stored in a dark bottle at 4 ℃ until analysis.

GC-MS Analysis of Essential Oils

The analysis of the chemical components of essential oil for each Syzygium sp. was conducted using GC-MS (Shimadzu QP 2010 Ultra). The essential oil was injected for 0.2 µL into column RTX®-5-MS (diphenyl dimethyl polysiloxane), length 30 m, diameter 0.25 mm, column temperature 60 − 270 ℃ (increment 8 ℃/minute), injector temperature 280 ℃, detector temperature 250 ℃, carrier gas helium (He), ionizing type EI (Electron Impact), and ionization energy 0.8 kV. The essential oil components were identified based on retention time and fragmentation pattern. Spectrum patterns were compared using Wiley 7 database, with a similarity index (SI) ≥ 90%.

Antibacterial Assay

Bacterial Culture

A series of bacterial strains were available in the stock culture collection of the Microbiology Laboratory, Universitas Padjajaran: *B. cereus* ATCC 11778, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 25923 and *S. enterica sv Typhimurium* ATCC 14028. Amoxicillin was used as a positive control for antibacterial activity.

Sample Preparation

The test solution was made by making a solution stock of each essential oil of Syzygium sp. 1000 µg/mL in dimethyl sulfoxide/DMSO (EMSURE®). The concentration series of test solution was made by diluting it in a 96-well microplate with a concentration ranging from 1000–31.25 µg/m.

Determination of Antibacterial Activity

The test on antibacterial activity was conducted using the microdilution method based on the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2017). The microbial suspension was made by growing microbes in NaCl solution 0.9% (b/v) and equalized with standard 0.5 Mc Farland (10^5 CFU/ml). Microbes were grown 24 hours at 37 ℃ in an incubator (Memmert INB 500). The minimum inhibitory concentration (MIC) value was determined by inserting 200 µl liquid media Mueller Hinton Broth/MHB (Oxoid™ CM0337) into each well. The

first well was added with 100 µl test solution 1000 µg/mL. The second solution was filled with 100 µl test solution of the first well, and the second was taken for 100 µl and put into the third well, and the same was conducted until the eighth well; thus, the solution in each well was 200 µl. Furthermore, 10 µl microbial suspension was put into each well. The first control solution well was filled with 200 µl liquid media and 10 µl microbial suspension (growth control), while the second control wells were only filled with liquid media (sterility control). Microplate was incubated at 37 ℃ for 24 hours. Microbial growth was determined using a universal microplate reader at a wavelength of 600 nm. The determined MIC value shows the lowest concentration, which may inhibit microbial growth. This is marked with an equal absorbance value between the treatment and the second control solution. The control solution was culture media added to the test sample solution to compare solution clarity (Goyal *et al*., 2007). The antibacterial test on the samples and control was conducted with two repetitions.

RESULTS AND DISCUSSION

Chemical Compound Analysis

The essential oils isolated from Syzygium sp. leaves vapor distillation were typically brownish yellow, with *S. samarangense* yielding 2.6 g (0.26%), *S. aqueum* yielding 2.38 g (0.24%), *S. myrtifolium* yielding 4.35 g (0.43%), *S. polycephalum* yielding 1.4 g (0.14%), and *S. polyanthum* yielding 2.4 g (0.24%) as individual yields. As a result, less than 1% of essential oils were produced during this investigation. According to the findings of the GC-MS analysis of the essential oil components of Syzygium sp. leaves, *S. samarangense*, *S. aqueum*, *S. myrtifolium*, *S. polycephalum*, and *S. polyanthum* essential oils all contained different amounts of different compounds. *S. samarangense* contained 79 compounds, *S. aqueum* contained 34 compounds, *S. myrtifolium* contained 74 compounds, *S. polycephalum* contained 71 compounds, and *S. polyanthum* contained 53 compounds. Our findings show that terpenoids such as monoterpene and sesquiterpene often make up the majority of the essential oil's composition (Table 1).

The main constituents of the essential oil of *S. polyanthum* are *n*-decanal (5.51%), *n*-octanal (2.24%), *γ*-cadinene (2.21%), α-muuroline (0.8%), and n-dodecanal (0.34%). These results are different from other research that reports *cis*-4-decanal (43.49%), 1-decyl aldehyde (19.75%), α-curcumene (2.27%), and 1,2,3,3a,4,6a-hexahydropentalene (2.06%) as the main contents of *S. polyanthum* essential oil based on GCMS analysis in the research conducted by Hamad *et al*. (2017). In this research, the yield of essential oils of *S. aqueum* and *S. samarangense* are 0.23% and 0.26%. The essential oils obtained have a similar yield to the essential oils of *S. aqueum* and *S. samarangense* derived from Cairo, Egypt respectively 0.22% and 0.32% (Sobeh *et al*., 2016). The main contents of the essential oil of *S. aqueum* identified are *trans*-caryophyllene (9.25%), β-selinene (4.30%), and α-humulene (1.55%). The essential oil in *S. aqueum* derived from Cairo, Egypt identified using GLC-MS and GLC-FID contains the main components α-selinene (13.85%), β-caryophyllene (12.72%), β-selinene (12.72%), and cuminyl aldehyde (9.82) (Sobeh *et al*., 2016). The contents of essential oil of *S. samarangense* identified in our present research are major compounds, namely *trans*-caryophyllene (12.10%), γ-terpinene (6.81%), d-cadinene (6.03%), p-cymene (5.05%), and γ-cadinene (3.49%).

Our results were compared with essential oils from *S. samarangense* derived from Cairo, Egypt, with the main compounds identified are germacrene D (21.62%), cuminyl aldehyde (10.56%), β-caryophyllene (5.93%), δ-cadinene (5.25%), spathulenol (4.53%), anethole (4.25%), and caryophyllene oxide (3.35%) (Sobeh *et al*., 2016). The yield of essential oils *S. myrtifolium* is 0.435 with the main constituents β-pinene (9.95%), *trans*-caryophyllene (9.27%), linalool (7.46%), and α-terpineol (4.82%). The essential oil of *S. myrtifolium* derived from eastern India was reported to contain δ-cadinol (29.53 %), caryophyllene oxide (26.25 %), and cyclocolorenone (7.7 %) as the main compounds based on GC/MS analysis (Jena *et al*., 2021). The main constituents of essential oils *S. polycephalum* are α-bergamotene (5.57%), nerolidol (10.84%), *trans*-caryophyllene (1.11%), trans-farnesyl acetate (0.72%). In general, the expression of compounds by individual plants of the same species at different location can be influenced by ecological factors (Pilatti *et al*., 2019).

Antibacterial Activity

The findings of the study demonstrate that essential oils from *Syzigium sp.* typically have moderate antibacterial activity with MIC values of 250–500 (µg/mL) (Table 2). Antibacterial agents with MIC values of 250 µg/mL are considered to have relatively high antibacterial activity, whereas agents with MIC values of 500 µg/mL and 1000 µg/mL have moderate antibacterial activity and low antibacterial activity, respectively (Adamczak et al.,

2020). A phytochemical product with a MIC value of 100-1000 µg/mL may be classified into antimicrobial agents (Abreu *et al*., 2012). Types of terpenoids in an essential oil affect its antibacterial activity.

Table 1. The identified chemical compound from *Syzygium sp.* essential oils.

No.	Compounds	Relative Abundance (%)				
		S. sam	***S. aq***	***S. myr***	***S. plc***	***S. pla***
1	α-pinene	-	0.10	-	-	-
2	Champene	-	-	0.41	-	-
3	β-phellandrene	0.04	-	-	-	-
4	β-pinene	0.32	-	9.95	-	-
5	β-myrcene	0.39	-	-	-	-
6	*n*-octanal	-	-	-	-	2.24
7	1-phellandrene	0.78	-	-	-	-
8	α-terpinene	0.78	-	0.15	-	-
9	*p*-cymol	-	0.43	-	-	-
10	*p*-cymene	5.05		0.67	-	-
11	*d*-limonene	0.78	-	1.84	-	-
12	*Cis*-ocimene	0.99	-	-	-	-
13	*Trans*-β- ocimene	0.33	-	-	-	-
14	γ-terpinene	6.81	-	0.49	-	-
15	Linalool oxide	-	-	0.16	-	-
16	α -terpinolene	2.56	-	0.45	-	-
17	Linalool	0.53	-	7.46	-	0.24
18	Hotrienol	-	-	0.16	-	-
19	D-fenchyl alcohol	-		0.90	-	-
20	*Trans*-pinocarveol	-	-	0.28	-	-
21	1-terpineol	0.07	-	-	-	-
22	Endoborneol	-	-	1.49	-	-
23	Terpinene-4-ol	-	-	1.90	-	-
24	α -terpineol	-	-	4.82	-	-
25	Myrtenol	-	-	0.48	-	-
26	*n*-decanal	-	-	-	-	5.51
27	Delta-elemene	-	1.13	-	-	-
28	α-cubebene	0.19	0.70	0.16	-	-
29	Nerylacetate	0.07	-	0.26	-	0.24
30	α -ylangene	0.28	-	0.13	-	-
31	α -copaene	1.67	-	0.37	-	-
32	β -elemene	0.13	0.38	-	0.07	-
33	*n*-dodecanal	-	-	-	-	0.34
34	*Cis*-caryophyllene	-	-	0.28	-	-
35	α -gurjunene	1.41	-	-	-	-
36	α-bergamotene	0.81	-	-	5.57	-
37	α-ionone	-	-	-	-	0.30
38	β-cubebene	-	-	0.52	-	-
39	*Trans*-caryophyllene	12.10	9.25	9.27	1.11	0.24
40	Neoalloocimene	1.60	-	1.75	-	-
41	α -humulene	2.15	1.55	1.68	-	-
42	Alloaromadendrene	-	0.25	-	-	-
43	Naphthalene	1.60	-	0.87	-	-
44	β-selinene	1.64	4.30	0.25	0.88	-
45	α-amorphene	-	0.20	-	-	-
46	γ-cadinene	3.49	-	0.98	-	2.21
47	d-cadinene	6.03	0.90	1.66	-	-
48	α- cadinene	-	-	0.27	-	-
49	α-muuroline	1.38	-	-	-	0.80
50	α-caracolene	-	-	-	-	0.22

Note: *S. sam* (*S. samarangense*), *S. aq* (*S. aqueum*), *S. myr* (*S. myrtifolium*), *S. plc* (S. *polycephalum*), *S. pla* (*S. polyanthum*).

The main components in *S. samarangense*, *S. myrtifolium*, *S. aqueum*, *S. polycephalum*, and *S. polyanthum* are consecutively *trans*-caryophyllene (12.10%), *trans*-caryophyllene (9.25%), β- pinene (9.95%), α-bergamotene (5.57%) and n-decanal (5.51%). *Trans*-caryophyllene β-pinene and α-bergamotene are hydrocarbon terpenoids, while n-decanal is oxygenated terpenoids. Not all oxygenated terpenoids have good antibacterial activity. Terpenoids oxygenated by aldehyde and phenol have the highest antibacterial activity. The next lower antibacterial activity was owned by terpenoids oxygenated by alcohol. Meanwhile, terpenoids oxygenated by ketone and ester have the weakest antibacterial activity. Hydrocarbon terpenoids without function group commonly do not have antibacterial activity (Dhifi *et al*., 2016).

Essential oil of *S. polyanthum* has better antibacterial activity than other species, especially against *B. cereus* bacteria. Essential oil of *S. polyanthum* has also been reported to have an antibacterial effect of 31.25 μg/mL on *B. subtilis* (Hamad *et al*., 2017). The reason is that *S. polyanthum* contains sesquiterpene and terpenoid oxygenated by alcohol groups such as linalool. Linalool induces oxidative stress by generating reactive oxygen species (ROS) and initiates lipid peroxidation, leading to bacterial membrane damage (Yang *et al*., 2021). Other research shows that alcohol terpenoids have better antibacterial activity caused by the amount of carbon, hydroxyl, and double bond on terpenoid chains. The longer the chain and configuration of the function group and double bond, the more the bacterial inhibitory effect is (Inoue *et al*., 2004). In addition, the essential oil of *S. polyanthum* also has octanal, decanal, and dodecanal aldehyde groups that expectedly present an antibacterial effect (Faleiro *and* Miguel, 2013). The highest antibacterial activity is shown by *S. polyanthum* of 250 μg/mL against the bacterium *B. cereus* and S. polycephalum of 250 μg/mL against the bacterium *L. monocytogenes*. *B. cereus* and *L monocytogenes* are bacteria that may cause toxicity in food (Jesserberger *et al*., 2020). The essential oil of *S. polyanthum* has also been reported to be a natural antimicrobial in tofu (Hamad *et al*., 2020). Therefore, the essential oil of *S. polyanthum* and *S. polycephalum* can be applied as potential natural preservatives for food.

Table 2. MIC values of Syzygium sp. Essential Oils.

Syzigium sp.	**MIC (μg/mL)**				
	S. aureus	***B. cereus***	***L. monocytogenes***	***E. coli***	***S. thypi***
S. samarangense	500	500	500	500	500
S. aqueum	500	500	500	500	500
S. myrtifolium	500	500	500	500	500
S. polycephalum	500	500	**250**	500	500
S. polyanthum	500	**250**	500	500	500
Amoxicillin	0.24	3.9	3.9	0.97	3.9

CONCLUSION

Monoterpenoids, sesquiterpenoids, and simple aldehydes were found to be the predominant components in the chemical analysis of the essential oils from the five Syzygium species that were studied. These substances are thought to have antibacterial properties. With a MIC value of 250 – 500 g/mL against the tested bacterium, the antibacterial activities of these essential oils demonstrated moderate antibacterial activity. The antibacterial activity of essential oils from *S. polyanthum* and *S. polycephalum* is superior to that of the other species, particularly against *B. cereus* (*S. polyanthum*) and *L. monocytogenes* (*S. polycephalum*), both of which have MIC values of 250 g/mL.

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