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Research Article
Antioxidant, Antimicrobial Activity and Phytochemical Screening of *Syzygium cumini* L. Leaves in Tropical Region from Surabaya, East Java, Indonesia

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Abstract

Background and Objective: *Syzygium cumini* is a plant of the Myrtaceae family. This plant has multifunctionality, due to its utilization as medicine, food, dye and building. This study aimed to determine the activities of antioxidant, antimicrobial, bioactive compounds and secondary metabolites contained in *S. cumini* leaves. **Materials and Methods:** The powder of *S. cumini* leaves was extracted with n-hexane, ethyl acetate and methanol as solvents. Three extracts were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and phytochemical screening to determine the type of bioactive compound and secondary metabolite. Antioxidant activity was tested with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method and the antimicrobial test by diffusion method. **Results:** The results showed that n-hexane, ethyl acetate and methanol extracts of *S. cumini* contained 69, 57 and 64 bioactive compounds. Phytochemical screening displayed flavonoids, terpenoids and steroids from n-hexane extracts. Ethyl acetate extracts showed only flavonoid and methanol extracts contained flavonoid and saponin. N-hexane extracts of *S. cumini* had very strong antioxidant activity content with 23.79% of IC_{50} when compared with the other two extracts. **Conclusion:** The results of the diffusion test exhibited three types of extracts that could inhibit the microbial pathogen such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231.

Key words: Antioxidant, antimicrobial, Indonesia, phytochemical screening, *Syzygium cumini*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants have secondary metabolites and bioactive compounds which provide benefits for human health such as antioxidants, antimicrobials, anticancer, anti-inflammatory and antidiuretic[1-4]. Medicinal materials of the plant have the advantage for easy to obtain, cheap, safe, efficient and rarely causing side effects[5].

One type of plant that has potential as a medicinal ingredient is *Syzygium cumini*. The local name of this plant is Juwet which is also a member of the Myrtaceae family. The presence of this plant in the community is increasingly difficult to find, due to not many cultivating it. The shape of *S. cumini*'s stem is round, branched and rooted. Moreover, its leaves are opposite which are generally oval, the base of the leaves is peg-shaped or round with the tips of the leaves that are blunt, pointed and rounded, then the texture of the leaves is smooth and the colour is green, light green, dark green. The fruit of this plant is buni type, oval in shape, purple in colour, black to dark purple in the skin fruit and purple to whitish purple in flesh. The texture of fruit provides smoothness, sweet taste and sour taste[6].

People use this plant as a shade plant, fruit producer, building material and medicinal ingredient for antidiabetic, anti-inflammatory, antidiarrheal and wound. However, little information has been revealed about the content of secondary metabolites and bioactive compounds from leaf extract of *S. cumini*. Information about its potential as an antioxidant and antimicrobial has also not been widely reported. Therefore, the objective of this study was to identify the bioactive compounds and secondary metabolites contained in n-hexane, ethyl acetate and methanol extracts of *S. cumini* and evaluate the antioxidant and antimicrobial activities of three extracts.

MATERIALS AND METHODS

Plant collection and extraction preparation: The leaves of *S. cumini* were collected from Surabaya, Indonesia. This plant was identified at the Plant Physiology Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlanggga from April to November, 2021. Leaves with healthy ones, free of pests and disease were used as research material. The leaves are washed with tap water, then dried and made into powder. Each leaf powder of *S. cumini* weighing 70 g was extracted with 900 mL of n-hexane (Fulltime, China), ethyl acetate (Fulltime, China) and methanol (Fulltime, China). Extraction was done by the maceration method. The extraction time for each solvent was 3 days and repeated 3 times.

Phytochemical screening and GC-MS analysis: The extract was treated by a phytochemical screening method to identify the type of secondary metabolites. Moreover, the alkaloid test used Mayer, Wagner and Dragendorff reagents. The terpenoid test utilized acetic anhydride and sulfuric acid. Flavonoid test with hydrochloric acid and magnesium (Mg) band. The saponin test used hot water and hydrochloric acid. Identification of the number and types of bioactive compounds were analyzed by GC-MS (Agilent Technologies 7890A, United States) and software GC-MS D5975C for data analysis.

Antioxidant activity: Antioxidant activity assay using DPPH (1,1-diphenyl-2-picrylhydrazyl) (Himedia, India) method with variant concentration at 6.25, 10, 12.5, 15, 25, 35, 50, 75 and 100 ppm. The percentage of antioxidant activity was calculated using formula[7]:

$$\text{Antioxidant activity (\%)} = \frac{\text{Control absorbance-Sample absorbance}}{\text{Control absorbance-Sample}} \times 100$$

Calculation of inhibition concentration 50% (IC_{50}) was the concentration of the extract that inhibited 50% of DPPH free radicals. The IC_{50} value was calculated using the linear regression equation:

$$y = ax+b$$

where, the x-axis was the concentration value (ppm) and the y-axis was the value of antioxidant activity (%).

Antimicrobial activity: This study also used three types of microbes such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 each grown on Eosin Methylene Blue (EMB) (Oxoid, United Kingdom), Mannitol Salt Agar (MSA) (Oxoid, United Kingdom) and Potatoes Dextrose Agar (PDA) (Oxoid, United Kingdom) mediums. The paper blank disc (Oxoid, United Kingdom) was used to determine antimicrobial activity with Mueller Hinton Agar (MHA) medium (Oxoid, United Kingdom). The microbial suspension was prepared by determining the turbidity of microbial suspension at optical density (OD) 0.1 with the wavelength of 625 nm for bacteria and OD 0.1 with the wavelength of 600 nm for fungi.

A total of test microbial suspensions was put into the sterile petri dish and then 15 mL of MHA medium was added to the dish to be homogenized. A total of three sterile paper blank discs with a diameter of 6 mm and the same thickness were placed on the agar surface at an equal distance apart. In the paper disc, 25 µL of each extract was injected with the concentration of 0, 250, 500, 750 and 1000 ppm. Growth inhibition was indicated by the formation of a clear inhibition area (Halo) around the paper disc. The diameter of the inhibition zone was measured with a calliper (LC = 0.05 mm).

RESULTS

Identification of secondary metabolites and bioactive compounds on n-hexane, ethyl acetate and methanol of *S. cumini* leaves extracts: Table 1 showed that the extracts of n-hexane, ethyl acetate and methanol contain flavonoids. Terpenoids were only present in n-hexane extract. Saponins are only present in methanol extract. Meanwhile, Table 2 showed that the n-hexane extract of *S. cumini* leaves identified 69 compounds with two dominating compounds, namely 3-Buten-1-ol, 3-methyl and Furan, tetrahydro-3-methyl. While, Table 3 showed that 57 compounds were identified in the ethyl acetate extract of *S. cumini* leaves with the two compounds having the highest (%) area being 2E, 4E, 14E-N-Isobutylicosa and squalene. Table 4 shows that the methanol extract of *S. cumini* leaves has 64 compounds with 3 dominating compounds, namely acetic acid, prolol 92,1,5-cdoindolizine-1-crab and delta tocopherol.

Antioxidant activity observation: Table 5 shows that n-hexane extract of *S. cumini* leaves has the highest OD value at concentration 6.25 µg mL^{-1} with 0.333 and the highest IC (%) at 100 µg mL^{-1} with 80.65611%. While in the ethyl acetate extract of *S. cumini* leaves, a concentration of 15 µg mL^{-1} has the highest OD value (0.439) and in concentration of 100 µg mL^{-1} has the highest IC (%) with 29.63801% and the lowest OD value with 0.311. The concentration of 100 µg mL^{-1} in the methanol extract of *S. cumini* leaves has the highest IC (%) with 59.27602%, while a concentration of 50 µg mL^{-1} in the methanol extract of *S. cumini* leaves has the highest OD value (0.283). Table 5 also shows that n-hexane extracts of *S. cumini* leaves have the highest antioxidant activity with IC_{50} 23.79 (very strong) compared between ethyl acetate and methanol extracts of *S. cumini* with IC_{50} 171.53 (weak) and 64.44 (strong), respectively.

Antimicrobial activity evaluation: Table 6 shows that all various extracts of *S. cumini* leaves can inhibit *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 in all concentrations with an inhibition diameter was 6 mm, except for a concentration 750 ppm in n-hexane extract with an inhibition diameter were 6.7 mm. While the growth of *E. coli* ATCC 25922 can be the best inhibited by n-hexane extracts of *S. cumini* with the highest inhibition diameter being 8.8 mm at a concentration of 250 ppm. Meanwhile, in ethyl acetate extract of *S. cumini* the highest inhibition diameter was 8.1 mm at a concentration of 1000 ppm. The highest inhibition diameter at the methanol extract of *S. cumini* was 7.6 mm at a concentration of 250 ppm.

Table 1: Phytochemical screening test of *S. cumini* leaves extracts in various solvents

Types of test	n-hexane extract	Ethyl acetate extract	Methanol extract
Alkaloid			
Mayer	-	-	-
Wagner	-	-	-
Dragendorff	-	-	-
Flavonoid	+	+	+
Saponin	-	-	+
Terpenoid	+	-	-

Table 2: Identification compound of n-hexane extract of *S. cumini* leaves

Retention time	Area (%)	Compounds
3.023	0.01	Pentane, 2 methyl
3.126	0.08	Pentane, 2 methyl
3.425	2.58	Furan, tetrahydro-3-methyl
3.469	2.01	Furan, tetrahydro-3-methyl
3.563	2.34	Furan, tetrahydro-3-methyl
3.617	5.87	Furan, tetrahydro-3-methyl
3.663	2.92	Decane, 2,2,3-trimethyl
3.706	4.08	Furan, tetrahydro-3-methyl
3.802	2.36	Furan, tetrahydro-3-methyl
3.884	3.61	Borinic acid, diethyl

Table 2: Continue

Retention time	Area (%)	Compounds
3.891	2.60	Borinic acid, diethyl
3.919	3.88	Borinic acid, diethyl
3.970	1.64	Furan, tetrahydro-3-methyl
4.013	5.76	Furan, tetrahydro-3-methyl
4.065	2.26	Furan, tetrahydro-3-methyl
4.100	4.64	Furan, tetrahydro-3-methyl
4.182	4.08	3-Buten-1-ol, 3-methyl
4.246	4.18	Borinic acid, diethyl
4.269	1.43	Furan, tetrahydro-3-methyl
4.289	2.63	3-Buten-1-ol, 3-methyl
4.335	2.37	Furan, tetrahydro-3-methyl
4.365	3.76	3-Buten-1-ol, 3-methyl
4.462	6.19	3-Buten-1-ol, 3-methyl
4.539	2.06	Cyclopropane, 1-ethyl-1-methyl
5.071	0.02	Furan, tetrahydro-2,5-dimethyl
5.205	0.03	Furan, tetrahydro-2,5-dimethyl
6.200	0.01	Succinic acid
30.870	0.03	Acetamide
32.317	0.03	1-Octadecene
33.084	0.02	Neophytadiene
33.758	0.01	Neophytadiene
34.414	0.01	Hexadecanoic acid
34.745	0.01	Benzenepropanoic acid
35.315	0.02	Sulfurous acid
35.411	0.02	Fumaric acid
36.611	0.01	5-oxoheptanoic acid
36.597	0.02	1-Octadecanol
36.755	0.02	9,12Octadecadienoic acid
36.844	0.04	9,12,15 Octadecatrienoic acid
37.034	0.61	Phytol
37.130	0.03	Octadecanoic acid
37.396	0.03	9-Octadecanoic acid
37.478	0.03	9,12-Octadecanoic acid
37.593	0.02	1-Bromoheptadec-13-yne
37.689	0.04	1-Bromo-3,5,10-pentadecatriene
37.770	0.03	Stigmasterol
37.955	0.06	di-alpha-Tocopherol
38.154	0.37	di-alpha-Tocopherol
38.969	0.04	Dammaran-3-ol, (3.beta.)
39.022	0.02	9,19-Cycloanost-24-en-3-ol
39.078	0.03	Iron, tricarbonyl
39.123	0.03	5 phenyl-4,5-dihydro
39.170	0.04	9.19-Cyclolanost-24-en-3-ol
39.221	0.02	9.19Cyclolanost-24-en-3-ol
39.302	0.03	Ergosta-8,25-dien-3-one
39.524	0.01	Sesquirosefuran
39.920	0.01	Lanosterol
40.127	0.02	9.19-Cyclolanost-24-en-3-ol
40.236	0.01	9.19-Cyclolanost-24-en-3-ol
40.288	0.02	9.19-Cyclolanost-24-en-3-ol
40.597	0.07	Squalene
40.708	0.08	Tocopherol
41.254	0.03	Alpha Tocospiro B
41.391	0.01	Eicosyl isopropyl ether
41.824	0.28	Gamma-sitosterol
42.097	0.01	Adamantane,1-isothiocyanato
42.197	0.01	Heptacosane, 1-chloro
42.451	0.01	Ergost-5-en-3.beta,ol
42.478	0.01	Ergost-5-en-3.beta,ol

Table 3: Identification compound of ethyl acetate extract of *S. cumini* leaves

Retention time	Area (%)	Compounds
3.013	0.03	Thiirane
5.466	0.38	Desulphosinigrin
5.829	0.46	Propanoic acid
6.635	0.01	Toluene
8.596	0.01	Ethylbenzene
8.778	0.04	Benzene, 1,3 dimethyl
9.391	0.01	Benzene, 1,3 dimethyl
19.730	0.01	Octadecanoic acid
23.165	0.01	Copaene
24.339	0.01	Caryophyllene
25.210	0.01	Humulene
25.840	0.02	Benzene1-(1,5 dimethyl-4 hexenyl)
26.130	0.01	Pentadecane
26.343	0.01	1,3 Benzodioxole
26.530	0.02	Beta.bisabolene
26.947	0.01	Piperine
29.804	0.01	Benzenemethanol. alpha.phenyl
30.827	0.04	5-(2-Thienyl)pentanoic acid
32.279	0.06	1-Octadecane
32.606	0.01	1,3-Benzenediol,2-methyl
33.053	0.09	Neophytadiene
33.158	0.01	Benzyl (dideuterated)methylester
33.440	0.01	Neophytadiene
33.725	0.04	3,7,11,15 Trimethyl-2-hexadecen
34.072	0.01	Hexadeadiene-1-ol acetat
34.206	0.01	2-Thiophenemethanethiol
34.380	0.03	Hexadecanoic acid
34.714	0.06	Benzenepropanoic acid
35.032	0.01	2-Methyl-Z,z-3,13Octadecadienol
35.281	0.01	1H-indene,5-butyl-6-hexyloctahydr
35.342	0.02	Hexadecanoic acid
35.749	0.04	Delta-tocopherol
35.937	0.05	Delta-tocopherol
36.068	0.05	Delta-tocopherol
36.563	0.05	3-Octadecene
36.720	0.05	Octadecadienoic acid
36.788	0.02	1,1-Octadecenoic acid
36.989	0.47	Phytol
37.227	0.11	2E,4E,14E-N-Isobutylicosa
37.448	0.17	2E,4E,14E-N-Isobutylicosa
37.563	0.32	2E,4E,14E-N-Isobutylicosa
38.131	0.32	2E,4E,14E-N-Isobutylicosa
38.353	0.55	2E,4E,14E-N-Isobutylicosa
38.549	0.73	2E,4E,14E-N-Isobutylicosa
38.713	1.31	2E,4E,14E-N-Isobutylicosa
39.537	0.59	Piperidin
39.613	0.35	Piperidin
39.731	0.15	Piperidin
39.885	0.02	(E)-pent-2-en-3-yl hexanoate
40.288	0.03	1H-Indene, 5-butyl-6-hexyloctahydr
40.624	1.07	Squalene
40.958	0.01	2(1H)Naphthalenone, octahydro
41.042	0.01	Pyridine-3-carboxamide, oxime
41.208	0.06	Gamma-tocopherol
41.356	0.01	3-Amino-2-methyl-5-nitro-6-phenyl
42.138	0.07	1-Hexacosene
42.573	0.03	Marrubine

Table 4: Identification compound of methanol extract of *S. cumini* leaves

Retention time	Area (%)	Compounds
2.648	0.18	Ethane, 1-bromo-2-chloro
3.265	0.14	Dimethylphosphine
3.395	0.06	2-Hydroxyethyl isobutyl sulfide
3.517	1.62	2-Hydroxyethyl isobutyl sulfide
5.045	2.10	Methanamine
5.478	0.45	Methanamine
5.889	0.11	Hydrazine, 1-1-dimethyl
6.315	0.12	Silanediol, dimethyl
17.039	0.12	2,3 Dihydro-3,5,-dihydroxy-6-methyl
23.210	0.14	Alpha-Cubebene
24.385	0.30	Caryophyllene
25.256	0.21	Humulene
25.890	0.47	Benzene,1-(1,5-dimethyl-4-hexenyl)
26.180	0.23	1,3,5 Benzenetriol
26.354	0.33	Piperidine
26.571	0.45	Beta,-Bisabolene
26.992	0.31	Isonicotinic acid
27.399	0.23	Benzenetriol
28.580	0.14	Santrolinatriene
29.484	0.14	9-Octadecanoic acid
29.725	0.80	Benzenemethanol, alpha phenyl
30.141	0.27	Pentadecanoic acid
30.264	0.25	3-Heptadecene
30.416	0.20	6-Tridecene
30.862	0.15	Cyclohexanepropanoic acid
32.311	0.24	Cyclohexanepropanoic acid
32.608	0.19	Piperidine
33.081	0.70	Neophytadiene
33.189	0.07	2-Methyl-7-nonadecene
33.470	0.21	2-Pentadecylfuran
33.637	0.07	7-Octenal,3,7-dimethyl
33.755	0.38	2-Pentadecylfuran
33.940	0.20	Cyclohexapropanol
34.400	0.80	Hexadecanoic acid
34.524	0.08	Isoaromadendrene epoxide
34.744	0.19	Benzenepropanoic acid
34.999	1.26	N-Hexadecanoic acid
35.312	0.27	Cyclohexane
35.816	7.42	Delta tocopherol
36.009	8.52	Delta tocopherol
36.588	0.70	1-Octadecene
36.750	0.71	9,12 Octadecadienoic acid
36.836	0.78	Hexadecatrienoic acid
36.902	0.34	2-Hexadecen
37.001	4.11	Phytol
37.123	1.02	Heptadecanoic acid
37.408	2.83	2-Cyclohexene-1-carboxylic acid
37.626	0.67	Octadecanoic acid
38.013	2.89	Di-alpha tocopherol
36.415	0.39	Acetic acid
38.943	0.15	Oleic acid
39.093	0.73	Heptadecane
39.304	0.34	Z,Z-10,12-Hexadecadien-1-ol acetat
39.780	0.78	14, beta-H-pregna
39.908	0.64	Mono(2-ethylhexyl)phthalate
40.321	0.05	Glycidyl (Z)-9-nonadecanoate
40.580	4.30	Squalane
40.688	2.54	Beta-Tocopherol
40.996	0.13	Spathulenol
41.222	4.55	Gamma-Tocopherol
41.706	7.67	1,4-naphthalenediacetonitrile
41.954	11.82	Acetic acid
42.134	17.74	Prolol 92,1,5-cdoindolizine-1-crab
42.205	3.97	Nonacosane

Table 5: Antioxidant activity of n-hexane, ethyl acetate and methanol extracts from *S. cumini* leaves

	n-hexane		Ethyl acetate		Methanol	
Concentration (μg mL^{-1})	OD	IC (%)	OD	IC (%)	OD	IC (%)
100	0.086	80.65611	0.311	29.63801	0.180	59.27602
75	0.096	78.28054	0.339	23.30317	0.219	50.56561
50	0.079	82.23982	0.404	8.597285	0.283	35.97285
35	0.078	82.46606	0.407	8.031674	0.217	50.90498
25	0.121	72.62443	0.436	1.470588	0.226	48.9819
15	0.293	33.82353	0.439	0.678733	0.249	43.66516
12.5	0.273	38.23529	0.425	3.959276	0.264	40.27149
10	0.324	26.69683	0.421	4.864253	0.267	39.70588
6.25	0.333	24.77376	0.434	1.923077	0.270	38.91403
IC_{50}	23.79		171.53		64.44	
	Very strong		Weak		Strong	

Table 6: Diameter of inhibition zone in n-hexane, ethyl acetate and methanol extracts of *S. cumini* leaves

		Diameter of inhibition zone (mm)		
Types of solvent	Concentration (ppm)	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
Methanol	1000	6.0	7.2	6
	750	6.0	7.4	6
	500	6.0	7.3	6
	250	6.0	7.6	6
Ethyl acetate	1000	6.0	8.1	6
	750	6.0	7.5	6
	500	6.0	7.5	6
	250	6.0	7.4	6
n-hexane	1000	6.0	8.1	6
	750	6.7	8.1	6
	500	6.0	8.1	6
	250	6.0	8.8	6

DISCUSSION

According to the results of phytochemical screening, flavonoids were present in three types of extracts (Table 1). Flavonoids are part of polyphenol compounds that have a benzo-γ-pyran structure and generally were found in plants. These metabolites are synthesized by the phenylpropanoid pathway and have various pharmacological activities[8,9]. Flavonoids are a group of phenolic compounds found in all parts of plants, especially in photosynthetic plant cells. These flavonoids have various biological activities such as antioxidants, hepatoprotective, antimicrobial, anti-inflammatory, anticancer and antiviral[10-16].

In addition to a phytochemical screening test and bioactive compounds were identified using GC-MS. The identification of n-hexane, ethyl acetate and methanol extracts from *S. cumini* were shown in Table 2-4. The n-hexane extracts of *S. cumini* leave contained 69 compounds. This extract contained 37.39% furan compounds which these compounds are known to have antimicrobial activity[17]. The extract also contained 14.27% boronic acid compounds, which have several biological activities such as anticancer, antibacterial and antiviral[18] (Table 2). The ethyl acetate extracts of *S. cumini* contained 57 compounds. One of the dominant compounds was 2E, 4E,14E-N-Isobutylicosa (3.51%). This compound has the potential of anti-inflammatory and anticancer[19]. Another compound found quite a lot was piperine. This compound has antibacterial activity against *Salmonella typhi*[20] (Table 3). The methanolic extracts of *S. cumini* contained 64 compounds. One of the dominant compounds is tocopherol, which is a group of vitamin E. This compound has the potential as an antioxidant[21]. In addition, there are also acetic acid compounds that have the ability as antioxidants and antibacterial agents against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231[22] (Table 4).

Antioxidant activity test of n-hexane, ethyl acetate and methanol extracts using the DPPH method. Value of inhibition concentration 50% (IC_{50}) using DPPH method (1,1-diphenyl-2-picrylhydrazyl). The IC_{50} is the concentration of substrate or sample solution that can reduce the activity of

DPPH (1,1-diphenyl-2-picrylhydrazyl) by 50% which indicates the concentration of extract (ppm) capable of inhibiting the oxidation process[23]. In the DPPH test, antioxidants will react with 1,1-diphenyl-2-picrylhydrazyl (DPPH) which stabilizes free radicals and reduces DPPH. Furthermore, DPPH will react with hydrogen atoms from free radical scavenging compounds to form 1,1-diphenyl-2-picrylhydrazine (DPPH-H) which is more stable. The colour change from purple to yellow will occur due to the reaction of DPPH with antioxidants. The ability of antioxidants will determine the intensity of colour change[23]. The results of the antioxidant activity from n-hexane, ethyl acetate and methanol extracts were shown in Table 5. Based on the results, the antioxidant activity of n-hexane extracts of *S. cumini* was stronger than that of the ethyl acetate and methanol extracts. The IC_{50} of n-hexane extracts was 23.79 ppm, while IC_{50} of silymarin solution was 29.63 ppm. The IC value of n-hexane, ethyl acetate and methanol extracts of *Syzygium cumini* collected from the Bogor Botanical Gardens, Bogor, West Java, Indonesia was 12.58 g mL^{-1}, 48.06 g mL^{-1} and 16.91 µg mL^{-1} [24]. This is also due to the flavonoid content contained in extracts. Flavonoids compound as an antioxidant, the mechanism is to capture reactive oxygen species (ROS) directly, prevent ROS regeneration and indirectly increase the antioxidant activity of cellular antioxidant enzymes[25].

Based on results of antimicrobial activity of n-hexane, ethyl acetate and methanol extracts of *S. cumini* using diffusion test were performed in Table 6. The three types of extracts inhibited *S. aureus*, *E. coli* and *C. albicans*. In general, the inhibition of *E. coli* was better when compared to other microbes. The antibacterial mechanism of flavonoids is to inhibit nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, attachment and biofilm formation, inhibition of porins in cell membranes, change in membrane permeability and attenuation of pathogenicity[26]. Factors affecting the diameter of the inhibition zone were extracts concentration, type of pathogenic microbe, the volume of extracts inserted into the paper disc, the thickness of the agar medium and the diffusion rate of antimicrobial substance through agar. The conclusion of this study is n-hexane, ethyl acetate and methanol extracts from *S. cumini* leaves contain bioactive compounds and secondary metabolites that have antioxidant and antimicrobial activity.

CONCLUSION

It can be concluded that the n-hexane extract of *S. cumini* leaves contains flavonoids and terpenoids with 69 bioactive compounds. The ethyl acetate extract of *S. cumini* leaves only contains flavonoids with 57 bioactive compounds. The methanol extract of *S. cumini* contains flavonoids and saponins with 64 identified bioactive compounds. The n-hexane extract of *S. cumini* leaves has the strongest antioxidant activity with IC_{50} 23.79, it is also the highest of inhibiting the growth of *E. coli* ATCC 25922 with an inhibitory diameter was 8.8 mm at a concentration of 250 ppm.

SIGNIFICANCE STATEMENT

This study discovered the bioactive compounds, antioxidant and antimicrobial activity of *S. cumini* leaves that can be beneficial for providing information in the form of bioactive compounds that have the potential to be used as an alternative to developing drug-based sources in the pharmaceutical field. This study will help the researchers to uncover the critical areas of biological activities of *S. cumini* from Surabaya, Indonesia that many researchers were not able to explore.

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