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Phytochemical Constituents and Antimicrobial Activity of *Elaeocarpus sphaericus* Schum Seed Extract

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ABSTRACT

The use of medicinal plants in health care services has tremendously increased. Medicinal plants contain bioactive compounds that are very important in biological activities and may possess antioxidant, anticancer, and antimicrobial properties. Therefore, this study was aimed at identifying the phytochemical constituents of *Elaeocarpus sphaericus* Schum (genitri) seed extract and determining its antibacterial activity. *E. sphaericus* seeds were obtained, dried, and prepared into powder form. The seed powder was extracted with methanol. Phytochemical analysis was conducted on the methanol seed extract using the Liquid Chromatography-Mass Spectrometry (LC-MS) technique. Antibacterial sensitivity testing was carried out with the agar disk diffusion method. The result of the phytochemical screening revealed that *E. sphaericus* seed extract contains 72 compounds which include dicarboxylic acid, aromatic acid, ester, glucose, coumarin, alkaloid, flavonoid, tannin, glycoside, steroid, terpenoid, quinone, and coumestan. Each phytochemical compound varies in composition, with caffeic acid (3.12%) being the highest. The antibacterial sensitivity testing of the 15 and 40% *E. sphaericus* seed extracts against *E. coli* indicated inhibition zone diameters of 7.25 and 7.75 mm, respectively, while values of 7.5 and 9 mm, respectively were recorded for *L. acidophilus*. Also, the antibacterial activity of the seed extract was found to be concentration-dependent. The findings of this study reveal that *E. sphaericus* seed extract contain several phytochemical compounds and has antibacterial activity against *E. coli* and *L. acidophilus*, thereby making it a potential antibacterial agent.

Keywords: Antibacterial, *Elaeocarpus sphaericus* Schum, Metabolites, Phytochemicals.

Introduction

Biodiversity provides plants that can be used for medicinal purposes. For ages, traditional medical therapies in many countries have used plants to cure a variety of illnesses.[1-3] The World Health Organization (WHO) showed that about 75% of the world's population uses herbal extracts for medicine.[2,4] Furthermore, phytochemical compounds found in plants have the potential to be used as drugs.[5,6] Several studies have been conducted to investigate their activities in medicinal plants and treatments.[6,7] Most of the plants that can be used as antioxidants, anticancer, and detoxifying agents, have secondary metabolites such as alkaloids, glycosides, terpene, tannins, steroids, and flavonoids.[8-10] Meanwhile, the rhizome extract of *Nelumbo nucifera* can be used as an antidiabetic and anti-inflammatory agent because it contains asteroidal triterpenoid compounds.[9] Panduratin A is a phytochemical in the extract of *Boesenbergia rotunda* and it exhibits anti-SARS-CoV-2 activity.[11] In essence, various phytochemical compounds have been investigated for treating various infections such as compounds that function as antimicrobials.[12-14] Genitri (Javanese) holds a taxonomic name from the Elaeocarpidae family. This plant originated from Java island, and it is currently distributed across Indonesia,[15] with the complete species name *Elaeocarpus sphaericus* Schum.

Several previous studies reported that parts of genitri (seeds, fruits, and leaves) have antidiabetic, antiviral, and antimalarial activities.[16-19] There is a dearth of information on the antibacterial activity of seeds of genitri. The seeds contain more complex secondary metabolites than the leaves and fruits and have biologically active phytoconstituents.[20-23]

E.coli is the most common gram-negative pathogenic bacteria causing a diverse range of clinical diseases that affect all age groups. The pathogen has the potential to invade many tissues and cause infection in any age group.[24] Meanwhile, *L. acidophilus* is a gram-positive bacterium which is widely used as a probiotic for humans and animals.[25,26] Agar disc-diffusion bioassay is widely used in many micro- and nanobiology laboratories. The advantages of the disc diffusion bioassay are ease of use, low cost, capacity to test a large number of microorganisms, and the ability to understand the findings.[27,28] Furthermore, there are limited reports on the phytochemical composition of the genitri seed extract. The phytochemical studies performed on the Elaeocarpaceae family, particularly on the seed extract were qualitative test.[23] The qualitative test can not reveal the spesific phytochemical constituents of the extract. The present study was therefore conducted to perform a phytochemical screening and investigate the antibacterial properties of *Elaeocarpus sphaericus* Schum seed extract.

Materials and Methods

Sample collection

Genitri seeds were picked up from the plants collected from the forest edge area of Poncol Village, Magetan city, East Java, Indonesia, in February 2020. The seeds were identified at the Taxonomy Laboratory of the Universitas PGRI Madiun. They were assigned the following

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identification number: 0023/Taxo-Plant/Biology/IV/2021. Genitri fruit was drained, peeled, and separated between the flesh and seeds. Then, the genitri seeds were dried by aeration and crushed by pounding. It was filtered through a sieve until a homogeneous powder was obtained.

Preparation of Elaeocarpus sphaericus seed extract
Each genitri seed powder was weighed (0.5 – 2 g) and macerated with 95% methanol in a ratio of 1:5 (sample: methanol) for 24 h at cold temperature in a closed bottle. Then, the sample solutions were filtered using an Erlenmeyer vacuum filter to obtain the filtrate and dregs. These steps were repeated three times. Subsequently, all the obtained filtrates were mixed and evaporated using a rotary evaporator to separate and obtain the methanol solvent and the semi-viscous extract. Meanwhile, the extract was dissolved using methanol solvent to a concentration below 0.1 mg/mL and homogenized using a vortex tube to obtain a homogeneous solution. To separate the solids, the solution was centrifuged at 8000 rpm for 10 minutes. An aliquot of 2 mL of supernatant was used in the protein precipitation stage. The supernatant was mixed with 3 mL of acetonitrile, acidified with 0.2% formic acid, and centrifuged at 8000 rpm for 30 seconds. Furthermore, the supernatant obtained was then used for analysis.

Fractionation of Elaeocarpus sphaericus seed extract
The extract of *Elaeocarpus sphaericus* seeds was purified through solid-phase extraction using C18 Sep-Pak, and the cartridge column C18 was acclimatized with a 1 mL solution of 80% acetonitrile. Also, an aliquot of 0.5 ml of the solution was put in a container and 1 ml of the protein precipitate was added into the Sep–Pak column. Then, 0.5 ml of the resulting solution was collected. About 0.25 ml of 200 mM ammonium formate in a 50:50 solution (acetonitrile: methanol) was added into the Sep-Pak column. Then, 0.5 ml of the resulting solution was obtained and 0.2 ml of 25:75 solution (acetonitrile: buffer) was added to 25 mM ammonium formate (pH 4.5). Furthermore, the solution was ready for injection in liquid chromatography-mass spectrometry (LC-MS). The solution was filtered using a 0.45 m cellulose acetate filter membrane and the degassing procedure was conducted. Also, the sample was injected into the LC-MS system and analyzed.

Antimicrobial sensitivity testing
Escherichia coli and *Lactobacillus acidophilus* were collected from the Microbiology Laboratory, Biology Department, Universitas PGRI Madiun, Indonesia. This study used 40 and 15% concentrations of genitri seed extract. Details for each concentration were 4 g of powdered genitri in 10 mL of water (40%) and 1.5 g of powder of genitri in 10 mL of water (15%). Previous studies showed that these concentrations can be used as an antibacterial test solution.[29] In addition, a sheet of filter paper was placed on the surface of the half-solidified nutrient agar (NA) medium and incubated at 37°C for 24 hours. The antibacterial activity was measured by the diameter of the clear zone on NA medium that had been inoculated with *E. coli* or *L. acidophilus*. The procedure involved the addition of 1 ml of bacterial culture from 10^{-8} dilution and 1 ml of chloramphenicol solution to NA medium in a petri dish and allowed to solidify. After solidifying, a paper disc that had been soaked in the sample (for two hours) was taken with tweezers and placed on the medium. Each medium contained six paper discs, and the cultures were later incubated upside down for 18-24 hours at 37 ^{o}C.[30] The size of the antibacterial inhibition zone was determined by measuring the clear zone on a petri dish and subtracting the diameter of the paper disc. These measurements were done by a caliper. The inhibition index was calculated using the following formula.[31]
Inhibition index =

$$\frac{Control\ clear\ zone\ diameter - Treatment\ clear\ zone\ diameter}{Disc\ paper\ diameter}$$

From the results of these measurements, the inhibitory effectiveness value can then be calculated based on the following equation:[32,33]

$$E = \frac{D}{Da} \times 100\%$$

Where E: Inhibitory effectiveness (%); D: Diameter of inhibition zone of plant material (mm); Da: Diameter of antibiotic inhibition zone (mm).

Results and Discussion

The results of the phytochemical screening of genitri seed extract using the LC-MS method are shown in Figure 1 and Table 1. Caffeic acid has the highest concentration in genitri seed extract with a value of 3.12%. LC-MS spectrum analysis identified and classified 72 compounds which include dicarboxylic acid, aromatic acid, ester, glucose, coumarin, alkaloids, flavonoids, tannins, glycosides, steroids, terpenoids, quinones, and coumestans. Secondary metabolites are known to have a specific function as antibacterial agents. This observation is in agreement with a study conducted by Tripathy et al.,[20] where they reported that genitri seeds contain secondary metabolites such as alkaloids, phenols, phytosterols, amino acids, flavonoids, and terpenoids. The ethanol/ ether extract of genitri contains secondary metabolites of alkaloids, flavonoids, carbohydrates, proteins, and tannins. In contrast, the aqueous extract contains carbohydrates, proteins, tannins, indoleizidine alkaloids, isoelaeocarpine, epiisoelaeocarpiline, epielaeocarpiline, alloelaeocarpiline, and pseudoepiisoelaeocarpilline.[18] The antibacterial activity of the seed extract was observed due to the presence of several compounds such as flavonoids, saponins, tannins, and alkaloids.[20,21]
The results (Table 2 and Figure 2) of the antibacterial activity test of the 15 and 40% aqueous extract of genitri seeds, as well as 50 mg/mL chloramphenicol (positive control), revealed the presence of inhibitory zone formed around the filter paper in the cultures. Inhibition zone diameters of 7.25 and 7.75 mm were observed in the *E. coli* culture when the 15 and 40% of *E. sphaericus* seed extract were respectively tested. For the *L. acidophilus* culture, inhibition zone diameters of 7.5 and 9 mm were recorded for the 15 and 40%, respectively of the *E. sphaericus* seed extract. Meanwhile, there was no inhibition zone observed in the negative control, P1 (distilled water). Table 3 shows the inhibitory effectiveness values of the genitri seed extract on the growth of *E. coli* and *L. acidophilus*. The results indicated that the antimicrobial activity of the seed extract was concentration-dependent. In the *E. coli* culture, a higher value of antibacterial effectiveness was obtained for the 40% concentration (83.78%) compared to the 15% concentration (78.37%). A similar observation was made in the antibacterial culture of *L. Acidophilus*, where values of 87.80% and 73.17% were recorded for the 40 and 15% concentrations, respectively.
The secondary metabolites in genitri seed extract possess inhibitory activities against bacterial growth. Flavonoids are bioactive compounds that have antibacterial properties, and they inhibit cell membrane function by forming a complex with extracellular proteins. They can also damage the bacterial cell membranes to release intracellular compounds.[34,35] The seeds of genitri contain tannin compounds, which have antibacterial properties.[36-39] The mechanism of tannin compounds in inhibiting bacterial cells is by denaturing the proteins of the bacterial cells, disrupting the function of the transport system, and synthesis of nucleic acid.[40] Furthermore, the saponins in the seed extract interact with the bacterial cells and affect the permeability of the cell walls to be ruptured.[41,42] Moreover, saponins inhibits the activities of enzymes and disrupt bacterial metabolism.[43] Alkaloids induced stress in bacteria cells to inhibit their growth.[44-46]
There are several reports which indicate that the formation of inhibition zones on bacterial growth cultures was induced by flavonoids, glycosides, steroids, alkaloids, saponins, and tannins contents in genitri seeds.[47-50] In the present study, the results of the experiments for the two test bacteria showed a significant effect. The diameter and average inhibition zone obtained revealed that the zone of inhibition of *L. acidophilus* was greater than that of *E. coli*. This observation was because *L. acidophilus* is a gram-positive bacteria with a thicker peptidoglycan layer on the cell wall.

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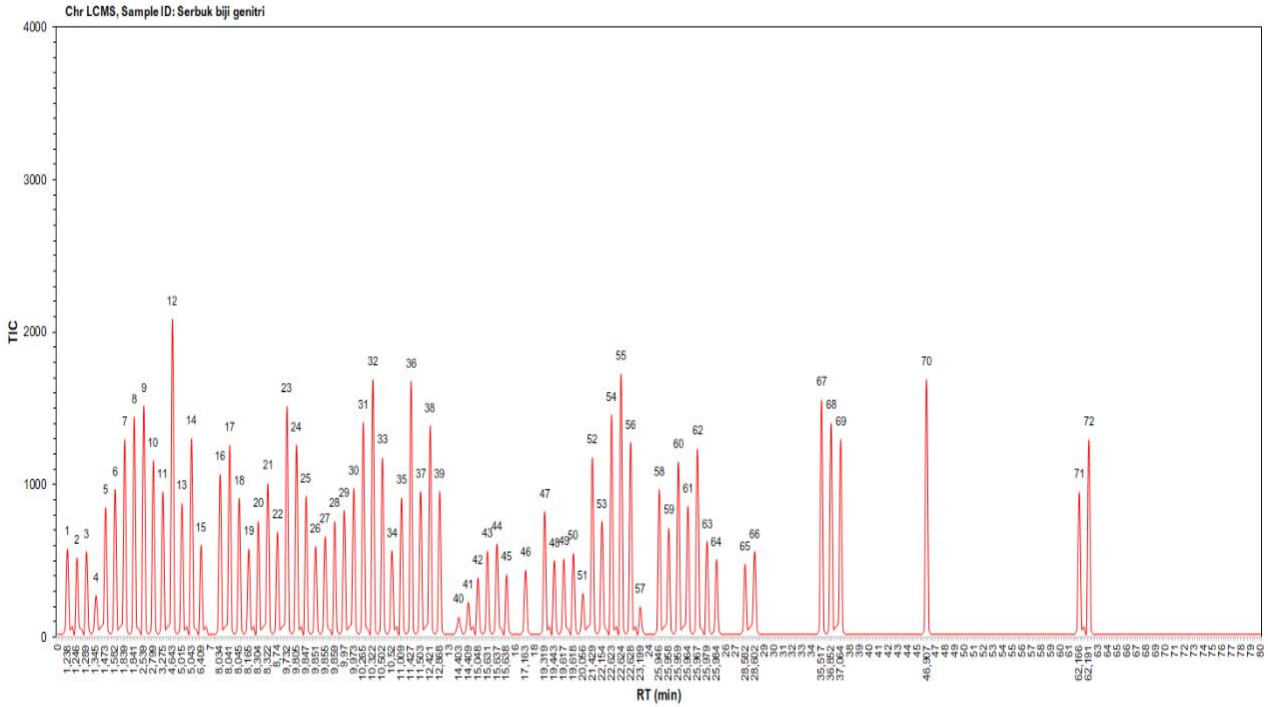

Figure 1: Liquid chromatography-mass spectrometry (LC-MS) spectra of *Elaeocarpus sphaericus* seed extract

Table 1: Phytochemical constituents of *Elaeocarpus sphaericus* seed extract

Peak	Composition (%)	Mass	Formula	Compound	Class
1	0.86	116.0110	$C_4H_4O_4$	Fumaric acid	Dicarboxylic acid
2	0.78	118.0266	$C_4H_6O_4$	Succinic acid	Dicarboxylic acid
3	0.84	122.0368	$C_7H_6O_2$	Benzoic acid	Aromatic acid
4	0.41	116.0837	$C_6H_{12}O_2$	Ethyl butyrate	Ester
5	1.27	134.0215	$C_4H_6O_5$	Malic acid	Dicarboxylic acid
6	1.45	148.0524	$C_9H_8O_2$	Cinnamic acid	Aromatic acid
7	1.94	164.0473	$C_9H_8O_3$	p-coumaric acid	Aromatic acid
8	2.16	164.0685	$C_6H_{12}O_5$	Rhamnose	Glucose
9	2.27	150.0528	$C_5H_{10}O_5$	Xylose	Glucose
10	1.73	168.0423	$C_8H_8O_4$	Vanillic acid	Aromatic acid

Peak	Composition (%)	Mass	Formula	Compound	Classs
37	1.43	306.0740	$C_{15}H_{14}O_7$	epigallocatechin	Tannin
38	2.07	354.0951	$C_{16}H_{18}O_9$	Chlorogenic acid	Tannin
39	1.43	372.1420	$C_{17}H_{24}O_9$	Syringin	Glycoside
40	0.19	402.3498	$C_{27}H_{46}O_2$	δ-tocopherol	Streoid
41	0.34	416.3654	$C_{28}H_{48}O_2$	γ-tocopherol	Streoid
42	0.58	400.3705	$C_{28}H_{48}O$	Campesterol	Terpenoid
43	0.85	412.3705	$C_{29}H_{48}O$	Isofucosterol	Terpenoid
44	0.91	412.3705	$C_{29}H_{48}O$	24-methylene pollinastanol	Steroid
45	0.61	412.3705	$C_{29}H_{48}O$	stigmasterol	Steroid
46	0.66	414.3862	$C_{29}H_{50}O$	β-sitosterol	Steroid

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Peak	Composition (%)	Mass	Formula	Compound	Class
11	1.42	178.0266	$C_9H_6O_4$	esculetin	Coumarin
12	3.12	180.0423	$C_9H_8O_4$	caffeic acid	Aromatic acid
13	1.31	192.0423	$C_{10}H_8O_4$	scopoletin	Coumarin
14	1.95	194.0579	$C_{10}H_{10}O_4$	Ferulic acid	Carboxylic acid
15	0.90	211.1572	$C_{12}H_{21}NO_2$	Elaeokanine C	Alkaloid
16	1.59	257.1416	$C_{16}H_{19}NO_2$	(+)-elaeocarpine	Alkaloid
17	1.88	257.1416	$C_{16}H_{19}NO_2$	elaeocarpenine	Alkaloid
18	1.36	257.1416	$C_{16}H_{19}NO_2$	Isoelaeocarpine	Alkaloid
19	0.86	258.1732	$C_{16}H_{22}N_2O$	Grandisine B	Alkaloid
20	1.14	259.1572	$C_{16}H_{21}NO_2$	isoelaeocarpiline	Alkaloid
21	1.51	261.1729	$C_{16}H_{23}NO_2$	Grandisine D	Alkaloid
22	1.03	267.1735	$C_{17}H_{21}N_3$	elaeocarpidine	Alkaloid
23	2.26	272.0685	$C_{15}H_{12}O_5$	Naringenin	Flavonoid
24	1.89	257.1521	$C_{16}H_{21}NO_3$	isoelaeocarpicine	Alkaloid
25	1.39	276.1838	$C_{16}H_{24}N_2O_2$	Grandisine F	Alkaloid
26	0.89	277.1678	$C_{16}H_{23}NO_3$	Grandisine A	Alkaloid
27	0.98	277.1678	$C_{16}H_{23}NO_3$	Grandisine C	Alkaloid
28	1.14	277.1878	$C_{16}H_{23}NO_3$	Grandisine E	Alkaloid
29	1.24	279.1834	$C_{16}H_{25}NO_3$	Habbemine A	Alkaloid
30	1.46	279.1834	$C_{16}H_{25}NO_3$	Habbemine B	Alkaloid
31	2.11	286.0477	$C_{15}H_{10}O_6$	Luteolin	Flavonoid
32	2.53	286.0477	$C_{15}H_{10}O_6$	Kaempferol	Flavonoid
33	1.76	290.0790	$C_{15}H_{14}O_6$	Catechin	Tannin
34	0.85	290.1994	$C_{17}H_{26}N_2O_2$	Grandisine G	Alkaloid
35	1.37	298.0477	$C_{16}H_{10}O_6$	Trifoliol	Coumestan
36	2.51	302.0427	$C_{15}H_{10}O_7$	Quercetin	Flavonoid
47	1.23	426.3861	$C_{30}H_{50}O$	β-amyrin	Terpenoid
48	0.75	426.3862	$C_{30}H_{50}O$	Cycloeucalenol	Terpenoid
49	0.77	426.3862	$C_{30}H_{50}O$	obtusifoliol	Terpenoid
50	0.82	426.3862	$C_{30}H_{50}O$	cycloartenol	Terpenoid
51	0.43	430.3811	$C_{29}H_{50}O_2$	α-tocopherol	Steroid
52	1.76	432.1058	$C_{21}H_{20}O_{10}$	kaempferol-3-rhamnoside	Flavonoid
53	1.13	440.4018	$C_{31}H_{52}O$	24-methylene cycloartanol	Terpenoid
54	2.18	448.1006	$C_{21}H_{20}O_{11}$	kaempferol-3-O-D-glucoside	Flavonoid
55	2.58	448.1006	$C_{21}H_{20}O_{11}$	kaempferol-7-O-β-D-glucoside	Flavonoid
56	1.91	448.1006	$C_{21}H_{20}O_{11}$	luteolin-7-glucoside	Flavonoid
57	0.29	450.3498	$C_{31}H_{48}O_2$	phylloquinone	Quinone
58	1.45	484.3189	$C_{30}H_{44}O_5$	Elaeocarpucin E	Terpenoid
59	1.07	484.3189	$C_{30}H_{44}O_5$	Elaeocarpucin H	Terpenoid
60	1.72	486.3345	$C_{30}H_{46}O_5$	Elaeocarpucin A	Terpenoid
61	1.28	486.3345	$C_{30}H_{46}O_5$	Elaeocarpucin B	Terpenoid
62	1.85	486.3345	$C_{30}H_{46}O_5$	Elaeocarpucin C	Terpenoid
63	0.94	486.3345	$C_{30}H_{46}O_5$	Elaeocarpucin G	Terpenoid
64	0.76	488.3502	$C_{30}H_{48}O_5$	Elaeocarpucin F	Terpenoid
65	0.72	516.3087	$C_{30}H_{44}O_7$	Cucurbitacin D	Terpenoid
66	0.84	518.3244	$C_{30}H_{46}O_7$	Cucurbitacin F	Terpenoid
67	2.33	610.1534	$C_{27}H_{30}O_{16}$	Rutin	Flavonoid
68	2.09	624.1690	$C_{26}H_{32}O_{16}$	isorhamnetin-3-O-rutinoside	Flavonoid
69	1.94	638.1847	$C_{29}H_{34}O_{16}$	rhamnazin-3-rutinoside	Flavonoid
70	2.53	770.2269	$C_{34}H_{42}O_{20}$	isorhamnetin-3-rutinoside-4'-rhamnoside	Flavonoid
71	1.42	952.0818	$C_{41}H_{26}O_{27}$	Geraniin	Tannin
72	1.94	1110.1033	$C_{47}H_{34}O_{32}$	elaeocarpusin	Tannin

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Table 2: Zone of inhibition of *Elaeocarpus sphaericus* seed extract against test bacteria

Bacterial Type	Data description	Treatment Type			
		P1	P2	P3	P4
E. coli(F1)	Average diameter of inhibition zone (mm)	0	9.25	7.25	7.75
	Inhibition Zone	0	0.85	0.45	0.55
L. acidophilus(F2)	Average diameter of inhibition zone (mm)	0	10.25	7.50	9.00
	Inhibition Zone	0	1.05	0.50	0.80

P1: Sterile aquadest; P2: 5% chloramphenicol; P3: 15% *Elaeocarpus sphaericus* seed extract; P4: 40% *Elaeocarpus sphaericus* seed extract; F1: *E. Coli*; F2: *L. acidophilus*

Table 3: Inhibitory effectiveness of *Elaeocarpus sphaericus* seed extract

Treatment	Effectiveness rate (%)	
	E. coli	***L. acidophilus***
P3 (15%)	78.37	73.17
P4 (40%)	83.78	87.80

P3: 15% *Elaeocarpus sphaericus* seed extract; P4: 40% *Elaeocarpus sphaericus* seed extract

Even though they have a thick cell wall, gram-positive bacteria generally have a simpler cell wall structure containing 90% peptidoglycan, while the other layer is teichoic acid. This causes the cell walls to be easily damaged by antibacterial compounds. In contrast, *E. coli* is a gram-negative bacterium with a complex cell wall structure that prevent quick denaturing.[51-53]

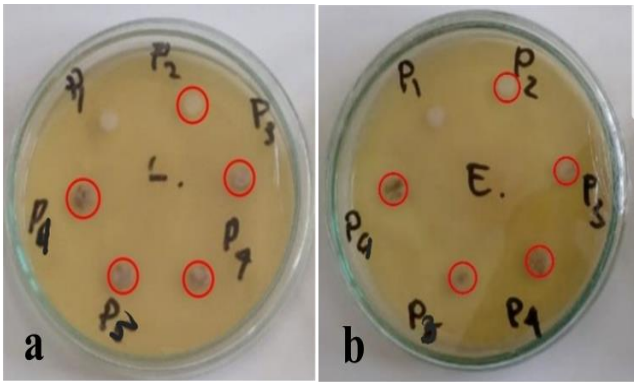

Figure 2: Antibacterial test of *Elaeocarpus sphaericus* seed extract against *L. acidophilus* (a) and *E. coli* (b

Conclusion

The findings of this study indicated that *E. sphaericus* Schum seed extract contains some phytochemical components such as dicarboxylic acid, aromatic acid, ester, glucose, coumarin, alkaloid, flavonoid, tannin, glycoside, steroid, terpenoid, quinone, and coumestan. These compounds have antibacterial activity against *E. coli* and *L. acidophilus*. Therefore, the seed extract of *E. sphaericus* Schum can be potentially used as an antibacterial agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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