Research article

Comparative Study of Antibacterial Activity of *Elephantopus scaber* Linn. and *Elephantopus mollis Kunth*. Extract

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Abstract

Keywords

Elephantopus scaber L.;
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antibacterial activity;
agar diffusion method;
broth microdilution method

Elephantopus scaber Linn. and *E. mollis* Kunth. are medicinal plant that are traditionally used in Indonesia. This research aimed to determine and compare the antibacterial activity of leaf, stem, and root extracts of the two Elephantopus species against various pathogen bacteria strains. The leaves, stem, and roots of *E. scaber* Linn. and *E. mollis* Kunth. were extracted using a Soxhlet apparatus. The disk diffusion method for screening antibacterial activity was conducted with a concentration of 50 mg/mL. The activities of the extracts were determined by Minimum Inhibitory Concentration (MIC) assay using broth microdilution method at a concentration range of 2500 to 1.2 µg/mL against nine human pathogenic bacteria. The results showed that all tested extracts demonstrated antibacterial activitied at varying degrees on all pathogen bacteria strains used in this study. The n-hexane and ethyl acetate extracts from both plants were potent antibacterials with MIC values of 19-156 µg/mL against *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25 175, *Vibrio cholerae* Inaba, and *Pseudomonas aeruginosa* ATCC 27853. The present study also revealed that among the bacteria tested, *S. mutans* ATCC 25175 was the most susceptible to antibacterial properties of *E. scaber* Linn. and *E. mollis* Kunth., especially in ethyl acetate fractions (MIC 19 and 39 µg/mL, respectively). The findings suggested that the leaves of both plants hold promise as sustainable bioresources for the

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development of antibacterial agents. Additionally, ethyl acetate and n-hexane extracts were found to be particularly effective for obtaining natural antibacterial agents from these plants. However, further optimization of the extraction process is needed.

1. Introduction

Infectious diseases are one of the significant problems in developing countries, and rank third as primary causes of death in the world after cardiovascular disease and cancer [1, 2]. These infections can be caused by bacterias, viruses, fungus, and parasites [3]. Antibiotics have been the most effective drugs against bacterial infections, but somehow they have slowly lost their efficacy due to resistance [4]. Therefore, searching for new naturally sourced compounds with antibacterial activity has attracted the interest of researchers [5].

One of the potential sources of antibacterial compounds is the Elephantopus plant. Elephantopus genus has 32 species worldwide, and two species are in Indonesia, namely *E. scaber* Linn. and *E. mollis* Kunth [6]. Morphologically, *E. scaber* Linn. and *E. mollis* Kunth. have similar characteristics. The differences between them lie in leaf shape and the color of the flower. *Elephantopus scaber* Linn. has cauline leaves and purple flowers, whereas *E. mollis* Kunth. has rosulate leaves and white flowers [7].

In Indonesia, *E. scaber* Linn. is more well-known than *E. mollis* Kunth. even though they are called by the same traditional name, "Tapak Liman". The Indonesian Herbal Pharmacopeia has established the extract and simplicia monographs of *E. scaber* Linn. for quality standards, offering thorough documentation for their use as herbal raw materials. However, a similar level of documentation and standardization has not yet been developed for *E. mollis* Kunth.[8]. *Elephantopus scaber* Linn., a traditional medicine, has been reported to be used to treat asthma [9], and as antimicrobial [10], wound healing [11], and hepatoprotective agents [12]. Moreover, the herb has been used as a febrifuge, brain and cardiac tonic, analgesic, diuretic, laxative, and as a treatment for inflammations and bronchitis [13]. On the contrary, relevant information about *E. mollis* Kunth. is very limited. However, some traditional uses of *E. mollis* Kunth. in Indonesia have been reported. Its leaves and roots have traditionally been used as a tonic, antipyretic, expectorant, anti-catarral, emollient, healing, anti-rheumatic, astringent, and diuretic. It has also been utilized externally for the treatment of elephantiasis and bruises. An infusion of the leaves is believed to reduce kidney stones, whooping cough, and bronchitis [13].

Previous studies reported that 35 compounds were isolated from *E. scaber* Linn., including four sesquiterpene lactones, five flavones, and nine triterpenes. Research conducted on *E. scaber* Linn. showed that its extracts and compounds possessed antibacterial, antiviral, and cytotoxic properties [14]. Among the bioactive compounds, sesquiterpene lactones were of particular interest due to their hepatoprotective and anti-inflammatory effects [15]. As for *E. mollis Kunth.*, around 35 compounds were reported [16]. Previous bioactivity literature also reported that its extracts or compounds had anti-inflammatory, antioxidant, cytotoxic, apoptoxic, anti-α-glucosidase and antimicrobial activities [16-18]. In this research, we compared and evaluated the antibacterial activity of *E. scaber* Linn. and *E. mollis* Kunth. from leaves, stem, and roots of *E. scaber* Linn. and *E. mollis* Kunth. against several pathogenic bacteria strains. It is hoped that this study will contribute to the identification and development of compounds that can be utilized in the creation of novel and highly effective antimicrobial drugs derived from natural sources.

2. Materials and Methods

2.1 Chemicals and reagents

All chemicals used in the study were of analytical grade. Methanol, ethyl acetate, *n*-hexane, Nutrient Agar (NA) (Merck®), paper disk (Whatman®), dimethyl sulfoxide (DMSO) (Merck®) and Chloramphenicol (Sigma®) were used in this study.

2.2 Plant samples

Samples of two Elephantopus plants (*E. scaber* Linn. and *E. mollis* Kunth.) were collected from the uncultivated areas in Andalas University, Padang, West Sumatera, Indonesia. The plants were identified and authenticated by a taxonomist in Andalas University Herbarium (ANDA), Padang, West Sumatera, Indonesia, and the specimens samples were submitted at the herbarium (No327/K-ID/ANDA/XII/2016).

2.3 Preparation of crude extract and solvent fractions

The leaves, stems, and root of *E. scaber* Linn. L. and *E. mollis* Kunth. were dried in an oven at the temperature of 50°C for 48 h. The dried leaves, stems, and roots were powdered mechanically using a commercial electrical stainless-steel blender (Phillip®). Crude methanolic extracts of *E. scaber* L. and *E. mollis* Kunth. were prepared using a Soxhlet apparatus. Twenty-five grams of the powdered sample materials (leaves, stems, and roots) were extracted using 250 mL of methanol. Then, the extracts were filtered through a funnel with Whatman No 1 filter paper. Each extract was concentrated under low pressure at 55°C. Each 25 g powdered sample materials (leaves, stem, and root) were also extracted in a Soxhlet apparatus by sequential extraction using solvents of increasing polarity, starting from non-polar (hexane, 250 mL), followed by semipolar (ethyl acetate, 250 mL and polar (methanol, 250 mL) solvents, respectively (boiling point range 60-80°C) for 8 h. The solvents were removed using a rotary vacuum evaporator at a temperature not exceeding 55°C (solvent fraction). The obtained residues were stored at a desiccator to be used later, and all residues were kept in the tightly stoppered bottle until further use for the antibacterial test [19].

$$\text{Percentage of Yield Extract} = \frac{\text{Mass of Extract}}{\text{Mass of dry Simplicia}} \times 100\%$$

2.4 Phytochemical screening

Secondary metabolites from crude methanol extract of leaves, stems and roots of both species were identified qualitatively using standard analytical procedures with slight modification.

2.4.1 Test for alkaloids

Each extract (0.5 g) was stirred with 5 ml H_2SO_4 2 N on a steam bath and then filtered. A few drops of Mayer's reagent (potassium mercuric iodide) were used to treat 1 mL of the filtrate. The presence of alkaloid was signified by turbidity and/or white colored precipitation [20].

2.4.2 Test for phenolics

Each extract (0.5 g) was dissolved with aquabidest and then filtered. A few drops filtrates were treated with $FeCl_3$ 1%. The formation of a blue-colored precipitate indicates the presence of phenolics [21].

2.4.3 Test for flavonoids

Each extract (0.5 g) was dissolved with aquabidest and then filtered. A few drops filtrates were treated with concentrated hydrochloric acid and Mg powder. The formation of a red or orange colored indicates the presence of flavonoids [22].

2.4.4 Test for saponins

Each extract (0.5 g) was dissolved in distilled water in a test tube, and it was mixed vigorously. Froth that remained when the mixture is heated, is preliminary evidence for saponins [22].

2.4.5 Test for steroid and terpenoid

Each extract (0.5 g) was treated with chloroform and filtered using norit as an absorbent. The filtrate was treated with a few drops of acetic anhydride and concentrated H_2SO_4. Formation of a blue colored precipitate indicates the presence of steroid, a red-colored precipitate indicates the presence of terpenoid, while a purple-colored precipitate indicates the presence of terpenoid and steroid [23].

2.5 Antibacterial activity

2.5.1 Tested bacteria

Antibacterial activity was tested against nine species of bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Streptococcus mutans* ATCC 25175, *Vibrio cholerae* Inaba, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella thypi* ATCC 19430, and *Salmonella tiphimurium* ATCC 14028. All bacteria were obtained from the Indonesian Food and Drug Administration (BBPOM) Padang, West Sumatera, Indonesia. All bacterial cultures were stored in solid NA medium at a temperature of -17°C and were re-cultured before being tested.

2.5.2 Disk diffusion method

The disk diffusion method was used to screen the antibacterial activity. Each bacterial suspension (0.5 mL) was adjusted to 0.5 McFarland (1.5×10^8 CFU/mL in NaCl 0.9% solution). The standardized bacterial inoculum was spread homogeneously on NA medium. Each sterile paper disk (6 mm) was dripped with 10 µL of sample extract (50 mg/mL in DMSO), negative control (only DMSO) and positive control (chloramphenicol; 3 mg/mL in DMSO) and then placed on the surface of each agar plate. Plates were incubated for 24 h at 37°C. A clear zone in the media indicated the antibacterial activity measured in millimeters (mm). The determination of this assay was conducted in triplicate [24]. Chloramphenicol was used as the reference standard or positive control in this antibacterial activity assay because chloramphenicol is a broad-spectrum antibiotic that is effective against a variety of susceptible and serious infection of Gram-positive and Gram-negative bacteria [25]. Harun *et al.* [26] stated that antibacterial activity can be categorized into three levels. A high inhibition level is indicated by the antibacterial compound exhibiting a diameter of the inhibition zone more than 16 mm.

Moderate inhibition is indicated when a diameter of the inhibition zone ranges from 11-15 mm, and weak inhibition is indicated by the diameters ranging from 6-10 mm. Resistance or no activity is indicated if the diameter of inhibition is less than 6 mm or no inhibitory zone.

2.5.3 Broth microdilution method

The antibacterial activity of the potential extracts was determined by Minimum Inhibitory Concentration (MIC) assay, using microdilution method and Nutrient Broth (NB) as culture media. The assay was done in a 96-well plate. Sample extracts (50 mg/mL) were dissolved firstly in DMSO as stock solutions and then serially diluted to concentrations ranging from 2500 to 1.2 µg/mL. Each sample solution (10 µL) was added by 90 µL NB. A negative control (NB + DMSO) and a positive control (chloramphenicol) were also used as a comparison. The wells were inoculated with 5 µL of bacterial suspension (1.5 x 10^8 CFU/mL in NaCl 0.9 % solution). All experiments were performed in duplo, and the microdilution plate was incubated at 36ºC for 18 h in a shaker incubator. Determination of MIC was carried out by the addition of 20 µL of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride solution (INT) (0.5 mg/mL in ethanol). Then, the microdilution plate was incubated again at room temperature for 30 min. The change of color by INT from yellow to purple indicated bacterial growth. MIC was defined as the lowest concentration that completely inhibited bacterial growth. The results were expressed in milligrams per milliliter (mg/mL) [24].

2.5.4 Statistical analysis

The results were reported as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was employed to determine statistical significance between groups, followed by Duncan Multiple Range Test. The differences were considered statistically significant at $p<0.05$.

3. Results and Discussion

3.1 Extraction yield

Since many antibiotic-resistant and unexpected side effects of antibiotic usage have been demonstrated, we hoped to find alternative new antibacterial compounds derived from plants. In this research, we compared the antibacterial activity of the crude methanol extract, n-hexane, ethyl acetate, and methanol fractions of root, stem, and leaf of the two Elephantopus species, *E. mollis* Kunth. and *E. scaber* Linn.

The objective of the extraction process was to optimize the yield of target compounds and achieve the greatest biological activity in the resulting extracts. Moreover, the extraction technique employed, and solvent used can significantly impact both the extraction yield and the biological activity of the resulting extract [27]. The present study examined two extraction methods using Soxhlet apparatus; universal solvent (crude methanol extract), and sequential extraction, i.e., using solvents of increasing polarity, starting from non-polar (hexane fraction), followed by semipolar (ethyl acetate fraction) and polar (methanol fraction). In Figure 1, the determination of the yield of extract of the two species showed varying amounts of yields in each part of the plant when different extraction solvents were applied. The highest yield for all parts of the plant used was from the crude methanol extract, followed by the methanol fraction, n-hexane fraction, and ethyl acetate fractions in decreasing orders of % yield.

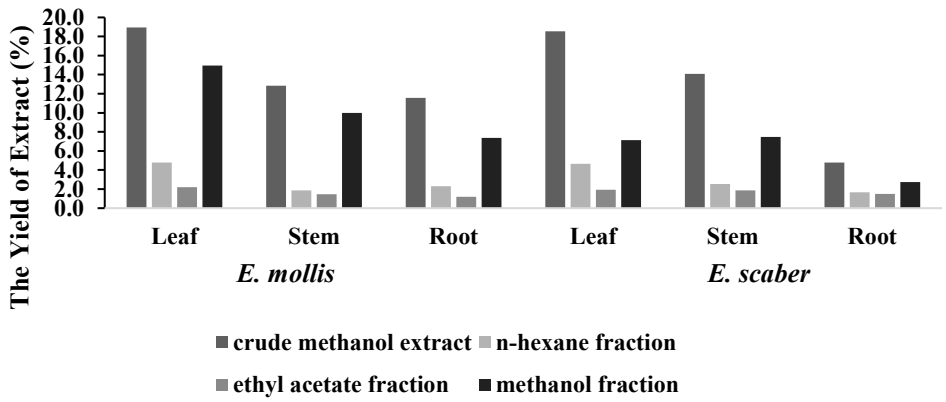

Figure 1. The yield of extract of parts from *E. mollis* Kunth. and *E. scaber* Linn.

3.2 Phytochemical screening

Extraction with a universal solvent has the advantage that the solvent can dissolve a wide range of polar, semipolar, and non-polar secondary metabolites. Hence, a higher yield of extract was obtained with the universal solvent. The results suggested that the different polarities of solvent affected the compound solubilities in each part of the plant. The yield determination based on the solvent polarity of the two species showed that the extracts of the two plants were dominated by polar compounds that were likely phyto-compounds. Secondary metabolites of plants are separated using sequential solvent extraction based on their polarity and solubility. For instance, nonpolar solvents like hexane and chloroform are effective in extracting alkaloids, coumarins, fatty acids, and terpenoids. On the other hand, polar solvents such as ethyl acetate, ethanol, methanol, and water have the ability to extract saponins, tannins, flavones, polyphenols, terpenoids, anthocyanins, polypeptides, and lectins from plants [28]. The phytochemistry analysis in this study showed that both plants had the same chemical constituents of their leaves, stems, and roots, which were phenolics, flavonoids, terpenoids, and steroids (Table 1).

Table 1. Phytochemical screening of crude methanol extract of *E. mollis* Kunth. and *E. scaber* Linn.

Crude Methanol Extract	Phytochemical Screening					
	Alkaloid	Phenolic	Flavonoid	Terpenoid	Steroid	Saponin
***Elephantopus mollis* Kunth.**						
Leaves	-	+	+	+	+	-
Stem	-	+	+	+	+	-
Root	-	+	+	+	+	-
Elephantopus scaber L.						
Leaves	-	+	+	+	+	-
Stem	-	+	+	+	+	-
Root	-	+	+	+	+	-

3.3. Antibacterial activity

Antibacterial activity was tested using the disk diffusion method against human pathogenic bacteria S*taphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922*, Streptococcus mutans* ATCC

25175, *Vibrio cholerae* Inaba, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella thypi* ATCC 19430, and *Salmonella tipimurium* ATCC 14028 at a concentration of 50 mg/mL. According to Kirby-Bauer disc diffusion method, the size of the inhibition zone provides a measure of the compound's effectiveness, i.e., the greater the inhibition zone is, the more effective the compound becomes [29]. Each extract from both Elephantopus plants was active against the Gram-positive and Gram-negative bacteria with a range of inhibitory zones between 7 and 21 mm (Table 2, Figure 2). The leaves had the most active antibacterial activity, followed by stem and root. The stem and root extracts also showed antibacterial activity but did not show significant differences between their n-hexane, ethyl acetate, and methanol extracts. Based on the polarity of the solvent on the leaves, n-hexane fraction showed the best antibacterial activity, followed by ethyl acetate and methanol fractions. The optimal solvent plays a significant role in the extraction of the bioactive compounds contained in plant materials due to the differing solubility properties of plant compounds [27].

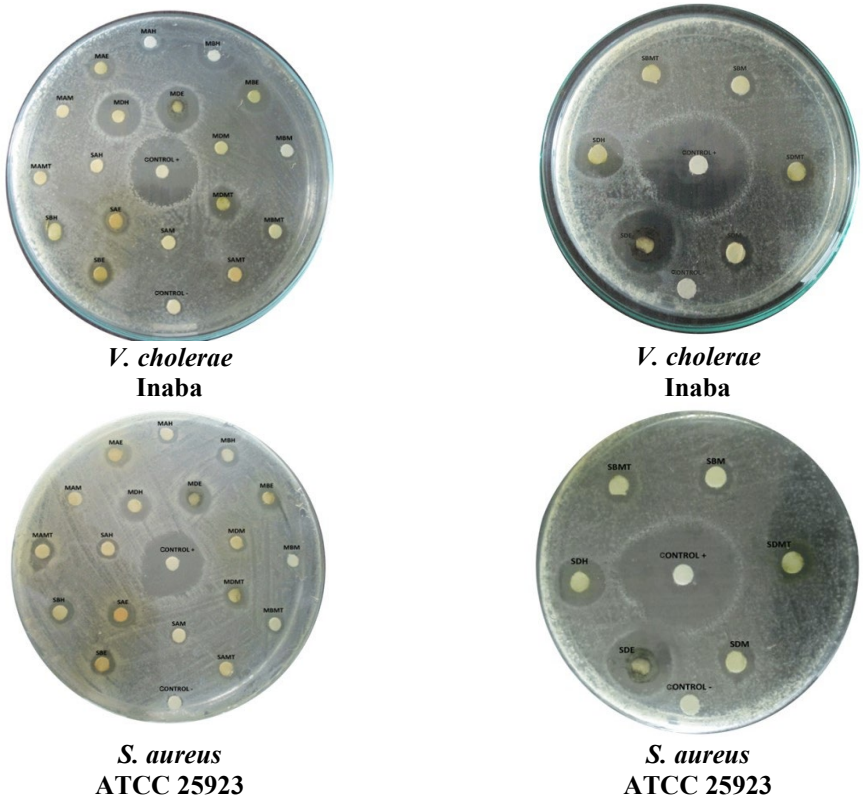

Figure 1. Antibacterial screening by the disk diffusion method.
Elephantopus mollis Kunth; leaves = crude methanol extract (MDMT); n-hexane (MDH); ethyl acetate (MDE); methanol (MDM); Stem = crude methanol extract (MBMT); n-hexane (MBH); ethyl acetate (MBE); methanol (MBM); Root = crude methanol extract (MAMT); n-hexane (MAH); ethyl acetate (MAE); methanol (MAM). *Elephantopus scaber* L: leaves = crude methanol extract (SDMT); n-hexane (SDH); ethyl acetate (SDE); methanol (SDM); Stem = crude methanol extract (SBMT); n-hexane (SBH); ethyl acetate (SBE); methanol (SBM); Root = crude methanol extract (SAMT); n-hexane (SAH); ethyl acetate (SAE); methanol (SAM).

Table 2. Inhibitory zone of part of *E. mollis* Kunth. and *E. scaber* Linn.

Species of Plant	Part of Plant	Inhibition Zone (mm)±Standard Deviation (SD)								
		Gram-positive					Gram-negative			
		SA	***ML***	***SM***	***BS***	***EC***	***PA***	***STpm***	***VC***	***STpi***
***E. mollis* Kunth.**	**MDMT**	$11\pm0.00^{f,g}$	11 ± 0.00^{e}	$13\pm0.71^{j,k}$	$11\pm0.00^{g,h,i}$	10.3 ± 0.58^{c}	$10\pm0.00^{b,c,d}$	$10\pm0.00^{b,c}$	12.5 ± 0.71^{g}	11 ± 0.00^{f}
	MDH	16 ± 0.00^{j}	15.5 ± 0.71^{i}	21 ± 0.00^{n}	13 ± 0.00^{k}	14.5 ± 0.71^{g}	17 ± 0.00^{k}	14.3 ± 0.58^{h}	16.5 ± 0.71^{i}	15.5 ± 0.71^{i}
	MDE	15 ± 0.00^{i}	15 ± 0.00^{i}	19.5 ± 0.71^{m}	13.5 ± 0.71^{k}	11 ± 0.00^{d}	15 ± 0.00^{j}	$12\pm0.00^{f,g}$	16 ± 0.00^{i}	13 ± 0.00^{h}
	MDM	$10.3\pm0.58^{e,f}$	9 ± 0.00^{c}	$9\pm0.00^{c,d,e}$	7.7 ± 0.58^{a}	8 ± 0.00^{a}	$10.7\pm0.58^{d,e,f}$	$10\pm0.00^{b,c}$	9 ± 0.00^{b}	$8\pm0.00^{b,c}$
	MBMT	11.3 ± 0.58^{g}	10 ± 0.00^{d}	$9\pm1.41^{c,d,e}$	$11.7\pm0.58^{i,j}$	10 ± 0.00^{c}	$11.3\pm0.58^{e,f,g,h}$	$10\pm0.00^{b,c}$	$10\pm0.00^{b,c,d}$	$10.3\pm0.58^{e,f}$
	MBH	11.5 ± 0.71^{g}	13.5 ± 0.71^{h}	$11\pm0.00^{f,g}$	$11\pm0.00^{g,h,i}$	11 ± 0.00^{d}	$12\pm0.00^{h,i}$	$11\pm0.00^{d,e}$	$12\pm0.00^{f,g}$	12 ± 0.00^{g}
	MBE	$11\pm0.00^{f,g}$	12.5 ± 0.71^{g}	$12\pm0.00^{g,h,i}$	$11\pm0.00^{g,h,i}$	12.5 ± 0.71^{e}	$10\pm0.00^{b,c,d}$	$10\pm0.00^{b,c}$	$11.5\pm0.71^{e,f}$	$10\pm0.00^{d,e}$
	MBM	7 ± 0.00^{a}	7 ± 0.00^{a}	n.d	$8.5\pm0.71^{b,c}$	10 ± 0.00^{c}	7.3 ± 0.58^{a}	8 ± 0.00^{a}	$10\pm0.00^{b,c,d}$	n.d
	MAMT	$11\pm0.00^{f,g}$	10 ± 0.00^{d}	$9\pm0.00^{c,d,e}$	$11\pm0.00^{g,h,i}$	11 ± 0.00^{d}	$10.5\pm0.71^{c,d,e}$	12.5 ± 0.71^{g}	$10\pm0.00^{b,c,d}$	$10\pm0.00^{d,e}$
	MAH	$9.5\pm0.71^{c,d}$	11.3 ± 0.58^{e}	21 ± 0.00^{n}	$11\pm0.00^{g,h,i}$	10 ± 0.00^{c}	12.5 ± 0.71^{i}	$10\pm0.00^{b,c}$	12.5 ± 0.71^{g}	$10.5\pm0.71^{e,f}$
	MAE	11.7 ± 0.58^{g}	11 ± 0.00^{e}	$13.3\pm0.58^{i,j,k}$	$11.3\pm0.58^{h,I,j}$	13.3 ± 0.58^{f}	12.5 ± 0.71^{i}	$12\pm0.00^{f,g}$	$12.3\pm0.58^{f,g}$	$10\pm0.00^{d,e}$
	MAM	$9.5\pm0.71^{c,d}$	8 ± 0.00^{b}	n.d	$8\pm0.00^{a,b}$	9 ± 0.00^{b}	$9.7\pm0.58^{b,c}$	8 ± 0.00^{a}	7 ± 0.00^{a}	7.3 ± 0.58^{b}
***E. scaber* Linn.**	**SDMT**	$10\pm0.00^{d,e}$	$9.5\pm0.71^{c,d}$	$7.7\pm0.58^{b,c}$	$10\pm0.00^{e,f}$	11 ± 0.00^{d}	$9.7\pm0.58^{b,c}$	9.3 ± 0.58^{b}	$9.5\pm2.12^{b,c}$	$8\pm0.00^{b,c}$
	SDH	$9.5\pm0.71^{c,d}$	10 ± 0.00^{d}	$11.3\pm0.58^{f,g,h}$	$10.7\pm0.58^{f,g,h}$	$10.5\pm0.71^{c,d}$	$11.5\pm0.71^{f,g,h}$	$11\pm0.00^{d,e}$	$10\pm0.00^{b,c,d}$	$10.7\pm0.58^{e,f}$
	SDE	15 ± 0.00^{i}	10 ± 0.00^{d}	15.3 ± 0.58^{l}	12 ± 0.00^{j}	10 ± 0.00^{c}	$11.7\pm0.58^{g,h,i}$	$12\pm1.41^{f,g}$	14 ± 0.00^{h}	15 ± 0.00^{i}
	SDM	$9\pm0.00^{b,c}$	9 ± 0.00^{c}	7.5 ± 0.71^{b}	$9.7\pm0.58^{d,e}$	10 ± 0.00^{c}	$10\pm0.00^{b,c,d}$	$9.7\pm0.58^{b,c}$	$9.5\pm0.71^{b,c}$	$10\pm0.00^{d,e}$
	SBMT	$9\pm0.00^{b,c}$	8 ± 0.00^{b}	$9\pm0.00^{c,d,e}$	$11.3\pm0.58^{h,I,j}$	8 ± 0.00^{a}	$10\pm0.00^{b,c,d}$	$10.3\pm0.58^{c,d}$	$9.5\pm0.71^{b,c}$	9.3 ± 0.58^{d}
	SBH	$10\pm0.00^{d,e}$	$12\pm0.00^{f,g}$	$14\pm1.41^{k,}$	$11.7\pm0.58^{i,j}$	10 ± 0.00^{c}	$11.5\pm0.71^{f,g,h}$	$11.3\pm0.58^{e,f}$	$10.7\pm0.58^{d,e}$	11.7 ± 0.58^{g}
	SBE	12.5 ± 0.71^{h}	10 ± 0.00^{d}	$10\pm2.38^{e,f}$	$10\pm0.00^{e,f}$	10 ± 0.00^{c}	$11.5\pm0.71^{f,g,h}$	$10\pm0.00^{b,c}$	$10\pm0.00^{b,c,d}$	$10.7\pm0.58^{e,f}$
	SBM	$10\pm0.00^{d,e}$	8 ± 0.00^{b}	n.d	$10\pm0.00^{e,f}$	10 ± 0.00^{c}	8 ± 0.00^{a}	8.3 ± 0.58^{a}	$10.3\pm0.58^{c,d}$	n.d
	SAMT	$10\pm0.00^{d,e}$	11 ± 0.00^{e}	$8.5\pm2.12^{b,c,d}$	$10.3\pm0.58^{e,f,g}$	11 ± 0.00^{d}	$10\pm0.00^{b,c,d}$	$10.3\pm0.58^{c,d}$	$10\pm0.00^{b,c,d}$	$10.3\pm0.58^{e,f}$
	SAH	$10\pm0.00^{d,e}$	$11.5\pm0.71^{e,f}$	$9.5\pm0.71^{d,e}$	$9.0\pm0.00^{c,d}$	10 ± 0.00^{c}	9.5 ± 0.71^{b}	8.0 ± 0.00^{a}	$10\pm0.00^{b,c,d}$	8.3 ± 0.58^{c}
	SAE	12.3 ± 0.58^{h}	$12\pm0.00^{f,g}$	$12.5\pm0.71^{h,I,j}$	$11.5\pm0.71^{i,j}$	10 ± 0.00^{c}	$11\pm0.00^{e,f,,g}$	$10\pm0.00^{b,c}$	$12\pm0.00^{f,g}$	$10\pm0.00^{d,e}$
	SAM	8.5 ± 0.71^{b}	$9.5\pm0.71^{c,d}$	n.d	$8\pm0.00^{a,b}$	7.5 ± 0.71^{a}	9.5 ± 0.71^{b}	8.3 ± 0.58^{a}	9 ± 0.00^{b}	8.3 ± 0.58^{c}

Table 2. Inhibitory zone of part of *E. mollis* Kunth. and *E. scaber* Linn. (continued)

Species of Plant	Part of Plant	Inhibition Zone (mm)±Standard Deviation (SD)								
		Gram-positive					Gram-negative			
		SA	***ML***	***SM***	***BS***	***EC***	***PA***	***STpm***	***VC***	***STpi***
Chloramphenicol		25±0.00^{k}	28±0.00^{j}	34±0.00^{o}	22±0.00^{l}	25±0.00^{h}	24±0.00^{l}	20±0.00^{i}	30±0.00^{j}	27±0.00^{j}

Note: ***Elephantopus mollis* Kunth.,** leaves = crude methanol extract (MDMT); n-hexane (MDH); ethyl acetate (MDE); methanol (MDM); Stem = crude methanol extract (MBMT); n-hexane (MBH); ethyl acetate (MBE); methanol (MBM); Root = crude methanol extract (MAMT); n-hexane (MAH); ethyl acetate (MAE); methanol (MAM). ***Elephantopus scaber* L.,** leaves = crude methanol extract (SDMT); n-hexane (SDH); ethyl acetate (SDE); methanol (SDM); Stem = crude methanol extract (SBMT); n-hexane (SBH); ethyl acetate (SBE); methanol (SBM); Root = crude methanol extract (SAMT); n-hexane (SAH); ethyl acetate (SAE); methanol (SAM). SA= *Staphylococcus aureus* ATCC 25923, ML= *Micrococcus luteus* ATCC 10240, SM= *Streptococcus mutans* ATCC 25175, BS= *Bacilus subtilis* ATCC 6633, EC= *Escherichia coli* ATCC 25922, PA= *Pseudomonas aeruginosa* ATCC 27853, STpm= *Salmonella tipimurium* ATCC 14028, VC= *Vibrio cholerae* Inaba, STpi= *Salmonella tyhpi* ATCC 19430. Data are expressed as Mean±SD of three replicates; Statistical significance was determined using one-way ANOVA. Data with different superscript (corresponding to each species) are significantly different ($p<0,05$) on the same bacteria; n.d: not detected - no activity; 6-10 mm: weakly activity; 11-15 mm: moderately inhibited; >16 mm: highly inhibited

The result showed that antibacterial activity of the crude methanol extracts of *E. mollis* Kunth. and *E. scaber* Linn. was moderately inhibitory (the inhibition zones ranged between 7.7 and 13.5 mm) (Table 2). Interestingly, after sequential extraction using n-hexane, ethyl acetate, methanol solvents, each part of both plants demonstrated an increased antibacterial activity. In the case of the leaves of *E. mollis* Kunth, the n-hexane fraction produced inhibition zones in the range of 13-21 mm and the ethyl acetate fraction produced zones in the range of 11-19.5 mm. The leaves of *E. scaber* Linn showed that the ethyl acetate fraction produced inhibition zones in the range of 10-15.3 mm. Meanwhile, the methanol fractions did not significantly demonstrate antibacterial activity on the leaves, stems, and roots of the two species. Those results were determined by bioactive compounds that may have been affected by the polarity of the solvent after the fractionation process and the result may have also been influenced the chemical nature of its bioactive constituents [30].

The results of this study were in line with Ganga Rao *et al*. [31], who reported that ethyl acetate and hexane fractions of *E. scaber* Linn. from the whole plants exhibited highly significant inhibition against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa and S. typhimurium*. On the contrary, Jenny *et al*. [32], reported that antibacterial activity of the methanolic extract from the aerial parts of *E. scaber* Linn. exhibited the most effective inhibition against *Staphylococcus aureus, Salmonella paratyphi A, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella sonnei, Escherichia coli* and *Salmonella typhimurium*. As for *E. mollis* Kunth., a semipolar fraction (dichloromethane extract) from leaves of *E. mollis* Kunth. was reported to have antibacterial potential against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes* at a concentration of 200 mg/mL compared with ampicillin ($p<0.05$) [33]. Moreover, the antibacterial activity of ethanol extract of *E. mollis* was demonstrated to be significantly active against tested Gram-negative, and Gram-positive bacteria strains [34]. These results may imply that geographical origin, freshness of plant, and plant parts can play important roles in determining the antibacterial properties of the plants [28].

Due to the results of leaf extracts of both plants showing higher antibacterial activity than stem and root, leaf extracts of both plants were further determined for MIC values. Besides, the phytochemical screening of the crude methanol extract of each part in the two species revealed the presence of the same chemical compounds. As MIC was defined as the lowest concentration that completely inhibited bacterial growth, the MIC values were conducted against the selected two Gram-positive (*S. aureus* ATCC 25923, *S. mutans* ATCC 25175) and Gram-negative bacteria (*V. cholerae* Inaba, *P. aeruginosa* ATCC 27853) using the broth microdilution method and reagent solution INT as an indicator of bacterial growth. Our study showed that n-hexane and ethyl acetate fraction of the leaves of *E. mollis* Kunth. and *E. scaber* Linn. had MIC values of 19-156 µg/mL against all bacteria strains (Table 3). On the other hand, contrasting results were seen in methanol fraction of the two species. They did not possess antibacterial activity against all tested bacteria in terms of MIC (MIC values of 1250-2500 µg/mL). The present study also revealed that among the bacteria, S. *mutans* ATCC 25175 was the most susceptible to antibacterial properties of *E. scaber* Linn. and *E. mollis* Kunth., and especially in ethyl acetate fractions (MIC MDE: 19 and MIC SDE: 39 µg/mL, respectively).

The variation in chemical constituents and nature of components in plant extracts is likely responsible for the differences observed in diameter inhibitory zone and MIC [35]. Based on phytochemistry screening, both plants had the same chemical constituents in their leaves, stems, and roots: phenolics, flavonoids, terpenoids, and steroids. Therefore, the bioactive compounds that were responsible for antibacterial activity in the case of *E. mollis* Kunth. were concentrated in the non-polar (terpenoid and steroid) and semipolar solvents (flavonoid and phenolic) whereas for *E.* scaber Linn., they were concentrated in semipolar solvents (flavonoid and phenolic). The inhibition mechanism in antibacterial properties in the plant extract was most probably due to interference by the active chemical constituents present, which caused disruption of the cell walls of bacteria and ultimately loss of rigidity and hence bacteria were killed [29].

Table 3. Minimum inhibitory concentration (MIC) of the leaves of *E. mollis* Kunth. and *E. scaber* Linn.

Part of Plant	MIC (µg/mL)			
	***S. aureus* ATCC 25923**	***S. mutans* ATCC 25175**	***V. cholerae* Inaba**	***P. aeruginosa* ATCC 27853**
***E. mollis* Kunth.**				
MDH	78	156	156	78
MDE	78	19	78	78
MDM	2500	1250	1250	2500
***E. scaber* Linn.**				
SDH	156	78	78	156
SDE	78	39	78	39
SDM	2500	1250	1250	2500
Chloramphenicol (+)	62.5	31.25	31.25	62.5

Note: ***Elephantopus mollis* Kunth.** leaves = n-hexane (MDH); ethyl acetate (MDE); methanol (MDM); ***Elephantopus scaber* L.** leaves = n-hexane (SDH); ethyl acetate (SDE); methanol (SDM);

Elephantopus species comprise sesquiterpene lactones that belongs to the Germacranes groups [16]. The inhibitory effects of sesquiterpenoids on microorganisms involve their ability to destabilize microbial cell membranes. Specifically, lipophilic sesquiterpenoids have the capacity to disrupt the integrity of cell membranes, leading to ion leakage [36]. Another antimicrobial activity proposed for *E. scaber* L. is the inhibition of autolysin, a bacteriolytic enzyme responsible for digesting cell wall peptidoglycan during cell wall turnover [37]. The two species also presented flavonoids, which are a class of polyphenolic compounds reported to exhibit antibacterial activities through a range of mechanisms. Several research studies demonstrated that flavonoids can inhibit nucleic acid synthesis, disrupt cytoplasmic membrane function, and interfere with energy metabolism [38]. Additionally, flavonoids have been observed to decrease bacterial adhesion and biofilm formation, affect porin expression on the cell membrane, alter membrane permeability, and diminish pathogenicity, all of which are vital factors for bacterial growth [39]. This results provide justification for the use of these plants to treat various infectious diseases. Our results may prove to be a prelude to the discovery of a novel and highly effective antimicrobial drugs derived from natural sources. The findings may contribute to the development of new compounds with enhanced antimicrobial properties.

4. Conclusions

From this study, it was observed that different parts of the two species of "Tapak Liman" *E. mollis* Kunth. and *E. scaber* Linn. shared the same groups of secondary metabolites, and both species possessed potential antibacterial activity against Gram-positive and Gram-negative bacteria strains. The findings suggest that the leaves of both plants hold promise as sustainable bioresources for the development of antibacterial agents. The ethyl acetate and n-hexane extracts of these plants were found to be particularly promising sources of natural antibacterial agents. However, further optimization of the extraction process and isolation of the active constituents responsible for the antibacterial properties are required.

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