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RESEARCH ARTICLE

Antibacterial activity of plant extracts commonly used as folk medicines collected from National Parks in Jambi, Indonesia

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Abstract

The local communities in Jambi used medicinal plants collected from national parks, such as *Calamus manan, Helminthostachys zeylanica and Zingiber montanum,* to treat illnesses. This study aims to evaluate the antibacterial activity of *C. manan, H. zeylanica and Z. montanum* against reference strains of *Escherichia coli* [ATCC 25922], *Pseudomonas aeruginosa* [ATCC 15442] and *Staphylococcus aureus* [ATCC 6538]. The assessment was conducted using disc and agar well diffusion methods, where all plant extracts showed antibacterial activity against the tested bacteria. Chloroform extract of *C. manan* (CMC) stems had the most significant growth inhibition zone against all reference strains for both methods. Specifically, the disc and agar well diffusion values of CMC extract observed against *S. aureus* were 17.67 mm and 22.00 mm respectively. MIC determination also indicated that CMC extract possesses the most significant antibacterial activity, with the lowest value observed against *S. aureus* at 0.312 mg/mL. The SEM results also showed the damage to the morphology and structure of *S. aureus* cells, especially the damaged cytoplasmic cell walls and membranes. The GC-MS analysis identified 30 different compounds from the CMC extract, with 4,5-dimethyl-1-hexene and terpineol-4 being the main components. These compounds are known for their potential as antimicrobial, antioxidant and anti-inflammatory agents. Considering these results, it was concluded that Jambi medicinal plants, particularly *C. manan*, exhibit antibacterial activities and contain secondary metabolites that may serve as a potential source of antibacterial compounds.

Keywords: cell damage; secondary metabolites; SEM; traditional medicines

Introduction

Indonesia is a mega-biodiversity country with a long history of using medicinal plants in traditional medicine and disease prevention (1). As the trend towards using natural, plant-derived drugs continues, conventional medicine is gaining popularity as an alternative treatment option (2). One of the reasons for this growing interest is the lack of side effects and affordability compared to most conventional medicines (3). As a biodiversity hotspot, ethnobotanical studies conducted in Indonesia hold significant potential for discovering secondary metabolites for novel antibacterial compound development. Jambi province is located in the centre of Sumatra Island of Indonesia. Its tropical climate and low altitude are optimal for vegetation growth, leading to the vast area of tropical rainforest national parks. The local communities around national parks in Jambi have been using various medicinal plants as traditional medicines to treat illnesses. Previous ethnobotanical studies had reported the medicinal plants used by the Suku Anak Dalam, Penguluh, Kerinci and Orang Serampas people (4 – 7). This study focuses on three species, namely *C. manan*, *H. zeylanica* and *Z. montanum* due to their extensive use for treating bacteria-related illness.

Several ethnobotanical studies have supported the use of the aforementioned medicinal plants to treat various illnesses. Stem and the sap of *C. manan* are used to treat asthma, stomachache, mouth ulcers and cough and fever (4, 7, 8). Its fruits are also consumed to relieve abdominal pain and to cure oral candidiasis (9). Roots of *H. zeylanica* had been traditionally applied as medicine for whooping cough (pertussis), dysentery and respiratory tract infections, while the rhizome are used as an anti-inflammation agent and pulmonary disease treatment (10, 11). The rhizome of *Z. montanum* had been used for traditional medicines to treat fever, skin infections and digestive tract disease (12, 13). In Indonesia, the rhizome is also commonly used as a colic reliever for infants, as it is rubbed on the abdomen (14). These studies showed the potential of those 3 herbs as the source of antibacterial compounds.

Previous studies of the three medicinal plants have reported antibacterial activity against several bacterial strains. Ethanolic extract *of C. manan* fruit showed inhibition against *E. coli*, *Streptococcus mutans*, *Vibrio cholerae and Staphylococcus epidermidis* (9, 15). Similar findings on

C. manan seed extract also reported growth inhibition on *Salmonella typhi* (16). Several studies on various parts of *H. zeylanica* exhibited growth inhibition against *Bacillus cereus, Bacillus subtilis, S. aureus and Listeria monocytogenes* (17, 18). Meanwhile, ethanolic extract of *Z. montanum* leaves reported growth inhibition against *P. aeruginosa*, while ethanolic extract of the rhizome showed growth inhibition of *B. cereus* and *Klebsiella pneumonia* (19, 20). Furthermore, the essential oils of *Z. montanum* rhizome also showed antibacterial activity against *B. cereus*, *E. coli* and multidrug-resistant (MDR) strain *Acinetobacter baumannii* (21, 22).

The antibacterial activity of the plant extracts are attributed to the presence of various phytochemical compounds with bioactive properties. The fruits and seeds of *C. manan* have been reported to contain flavonoids, saponin, tannin, alkaloids and triterpenoids (9, 15). Several studies on *H. zeylanica* rhizome have successfully isolated flavonoid compounds, such as ugonin J, ugonin M, ugonin T and quercetin (11, 23, 24). The rhizome of *Z. montanum* reported to contain alkaloid, steroid, flavonoid, terpenoid and phenylbutenoid (20, 25). Essential oils of *Z. montanum* rhizome produce had been reported to contain sesquiterpenes, such as zerumbone and kaempferol and monoterpenes, majorly terpineol-4 (26, 27).

These reports supported the ability of the 3 selected medicinal plants to inhibit bacterial growth. However, the most used plant organs based of ethnobotanical studies, such as *C. manan* stems and *Z. montanum* rhizomes, have not thoroughly been investigated. The antibacterial activity of the commonly used parts of the 3 selected medicinal plants, namely *C. manan* stems, *H. zeylanica* roots and *Z. montanum* rhizomes, need to be evaluated. Therefore, this study was aimed to assess the antibacterial activity of polar and non-polar extracts from the most used plant organs of 3 selected medicinal plants. It investigated the methanolic and chloroform extracts of *C. manan* stems, *H. zeylanica* roots and *Z. montanum* rhizomes for their antibacterial activities against bacterial strains, along with the identification of the chemical constituents by GC-MS analysis.

Material and Methods

Plant collection

Plant samples, namely *C. manan* stems, *H. zeylanica* roots and *Z. montanum* rhizomes, were collected from Dua Belas National Park (TNBD) and Kerinci Seblat National Park (TNKS), Jambi Province (Fig. 1). All plant samples were stored according to the established standard protocols. Finally, the Laboratory of Plant Systematics, Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada authenticated the species determination of collected plant materials.

Sample preparation

All plant samples were washed under running tap water, after which the parts were chopped into smaller pieces and air-dried. The dried samples were then grinded into coarse powders using a food blender and mechanical grinder. Finally, the powdered form are stored at room temperature until further usage.

Extraction

The powdered samples were extracted by serial maceration methods using 100 % chloroform followed by 100 % methanol. The solvent solution was added to each plant powder in a ratio of 1:10 (w/v) and left for 3 days with constant homogenization. Subsequently, the extracts were collected and the resulting liquid was filtered and vaporized in maceration chambers. The residue was re-macerated in different solvents using the same ratio for 3 more days. The resulting liquid was filtered and left vaporized in maceration chambers. Finally, the crude extract pastes were preserved at -4 °C until further usage.

Bacterial strains

The evaluation of antibacterial activity involved the utilization of reference bacterial species, specifically the American type of cell culture (ATCC) of *E. coli* [ATCC 25922], *P. aeruginosa* [ATCC 15442] and *S. aureus* [ATCC 6538]. These strains were obtained from the MBRIO Food Laboratory (MBRIO). The bacteria used for the antibacterial assay was inoculated in Nutrient Broth (Merck) with adjusted turbidity of 0.5 McFarland standard.

Disc diffusion method

The disc diffusion method was performed according to CLSI (Clinical and Laboratory Standards Institute) guidelines with modification (28). Using a glass rod spreader, 100 µL of bacterial suspension (1.5×10^8 CFU/mL) was inoculated on Mueller Hinton agar (Oxoid). Subsequently, 10 µL of plant extracts (100 mg/mL) dissolved in DMSO 10 % (Vivantis) was transferred onto a 6 mm sterile paper disc (Oxoid). To detect the solvent effect, 10 µL of ciprofloxacin (1 µg/mL) was used as a positive control and 10 µL of DMSO 10 % as a negative control. All plate were then incubated at 37 °C and left for 18 hr. Finally, the diameter of inhibition was measured using millimetre blocks. All experiments were conducted in triplicate.

Agar well diffusion method

The agar well diffusion method was performed according to CLSI guidelines with some modifications (29). Using a glass rod spreader, 100 µL of bacterial suspension (1.5×10^8 CFU/mL) was inoculated on Mueller Hinton agar (Oxoid). Subsequently, an 8 mm diameter hole was punched in the ager the agar using a cork borer and each was filled with 20 µL of plant extracts (100 mg/mL). Ciprofloxacin and DMSO 10 % were used as positive and negative controls, respectively, in this study. All plate were incubated at 37 °C and left for 18 hr. Finally, the diameter of inhibition was measured by using millimetre blocks. All experiments were performed in triplicate.

MIC microdilution assay

A serial microdilution assay was performed with modifications to determine the Minimal Inhibitory Concentration (MIC) of plant extracts (30). Subsequently, 100 µL of plant extract in the range of 0.312 - 10 mg/mL were added to 100 µL of bacterial suspension in Muller Hinton broth (10^4 CFU/mL) in 96-well microplates and incubated at 37 °C for 18 hr. The inhibition of bacterial growth was measured at wavelength of 595 nm using microplate reader. The MIC was determined as the lowest

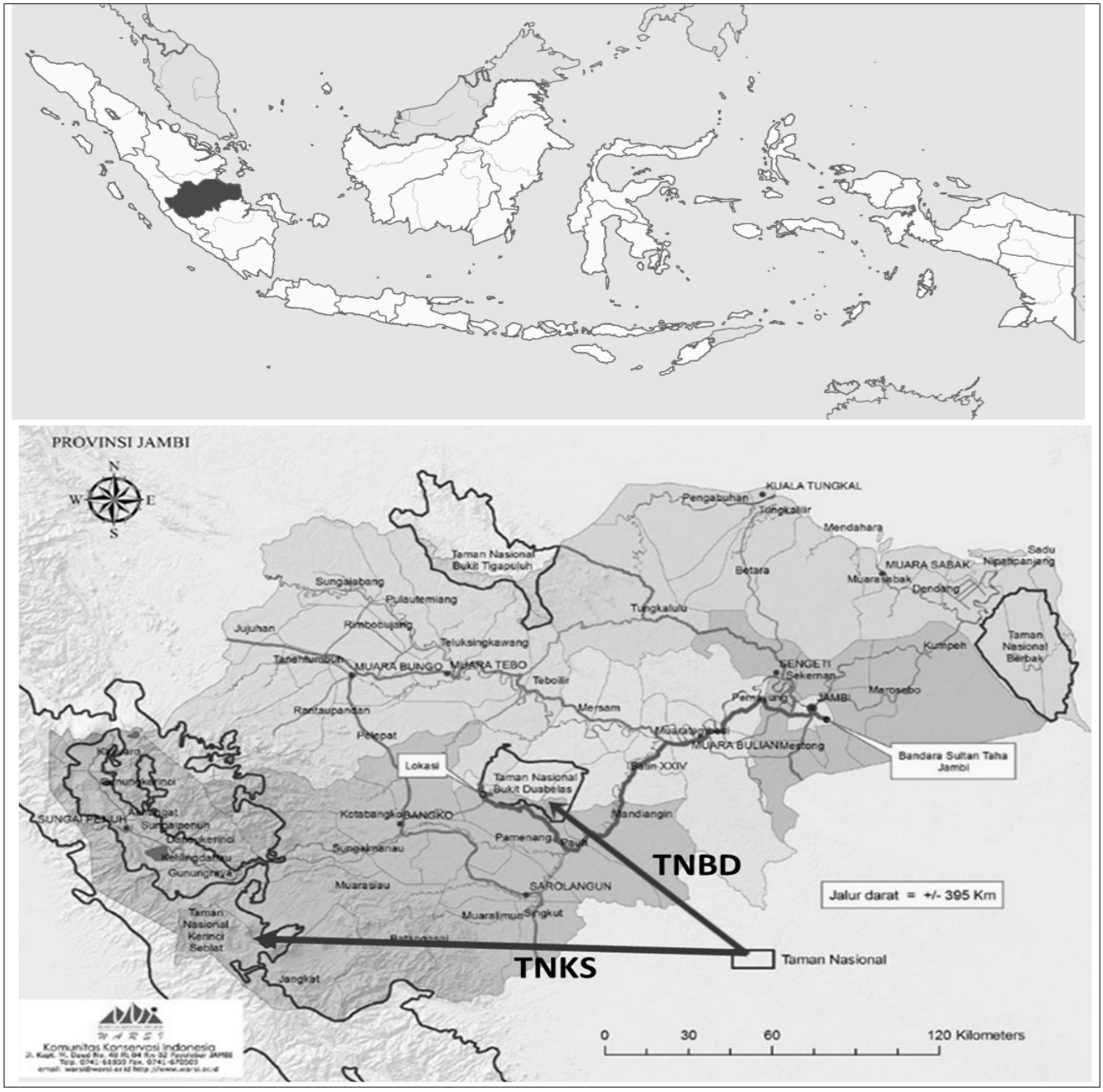

Fig. 1. Collection sites of plant samples in Jambi Province, Indonesia.

concentration at which bacteria growth was 50 % inhibited. Each test was performed in triplicate using Ciprofloxacin as the positive control and DMSO 10 % as blank.

SEM analysis

Scanning Electron Microscopy (SEM) was performed to examine the morphological change in the cell wall of *S. aureus* when treated with a potential extract of plant samples. To observe the treated *S. aureus* culture, potential plant extract at MIC value was added and incubated at 37 °C for 24 hr. Finally, the bacteria samples were sent to the Research Unit for Natural Product Technology - National Research and Innovation Agency (BRIN) for SEM analysis according to laboratory protocol.

GC-MS analysis

GC-MS (Gas Chromatography-Mass Spectrometry) identified secondary metabolites at the Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Science, Universitas Gadjah Mada. 1 µL of the dissolved plant extract in solvent was filtered and injected into a gas chromatograph (Shimadzu GC-MS-QP2010S). The instrument was operated using a glass column with dimensions of 30 m in length, 0.25 mm in diameter and 0.25 µm in thickness. The oven was programmed with initial and final temperatures of 60 °C and 300 °C with an increase rate of 5 °C/min, a helium carrier gas at a pressure of 16.5 kPa, a total rate of 30.5 mL/min and a split ratio of 1:49. The mass spectra then compared with the NIST (National Institute of Standard and Technology) library to identify the compound based on similarity.

Statistical analysis

The data obtained from the measurement of inhibition zones' diameter was analyzed using Statistical Packages for Social Sciences (SPSS) software version 25. Each value was expressed in mean and standard deviation (SD) from 3 replicates. The statistically significant difference between

means was tested using Duncans' New Multiple Range Test, with p-values < 0.05 indicating an important difference.

Results

Disc diffusion method

Table 1 shows the results of the antibacterial activity of plant extracts using disc diffusion method. The antibacterial activity was observed by inhibition zones around the disc paper, as shown in Fig. 2. The results indicate that all plant extracts exhibited antibacterial activity against at least 2 bacterial strains using the disc diffusion method. Furthermore, the chloroform extract of *C. manan* (CMC) showed the broadest spectrum of action. On the other hand, none of the methanolic extracts from the 3 plants showed any observable inhibition zone against *E. coli.*

Comparison of different agar diffusion methods

Table 2 shows further results of plant extracts' antibacterial activity using agar well diffusion methods. The results indicate that the plant extracts were antibacterial against all reference strains. Overall, antibacterial activity using disc and agar well diffusion methods showed similar results for

Table 1. Antibacterial activity of plant extracts by disc diffusion method

Extract	Diameter of inhibition (mm)		
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***
CMC	17.67 ± 1.25^{b}	15.33 ± 2.87^{b}	15.67 ± 1.70^{b}
CMM	10.00 ± 1.41^{c}	8.67 ± 0.94^{c}	0
HZC	9.67 ± 0.94^{c}	8.00 ± 0.82^{c}	8.33 ± 0.94^{c}
HZM	11.67 ± 0.94^{c}	9.00 ± 0.82^{c}	0
ZMC	0	7.67 ± 0.47^{c}	7.67 ± 0.47^{c}
ZMM	9.33 ± 1.70^{c}	7.67 ± 0.82^{c}	0
Ciprofloxacin	36.00 ± 0.82^{a}	38.00 ± 1.41^{a}	31.33 ± 1.25^{a}
DMSO 10 %	0	0	0

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

All values are expressed in mean ± SD (n = 3)

Means within a column with different letters significantly differ by Duncans' test at p < 0.05.

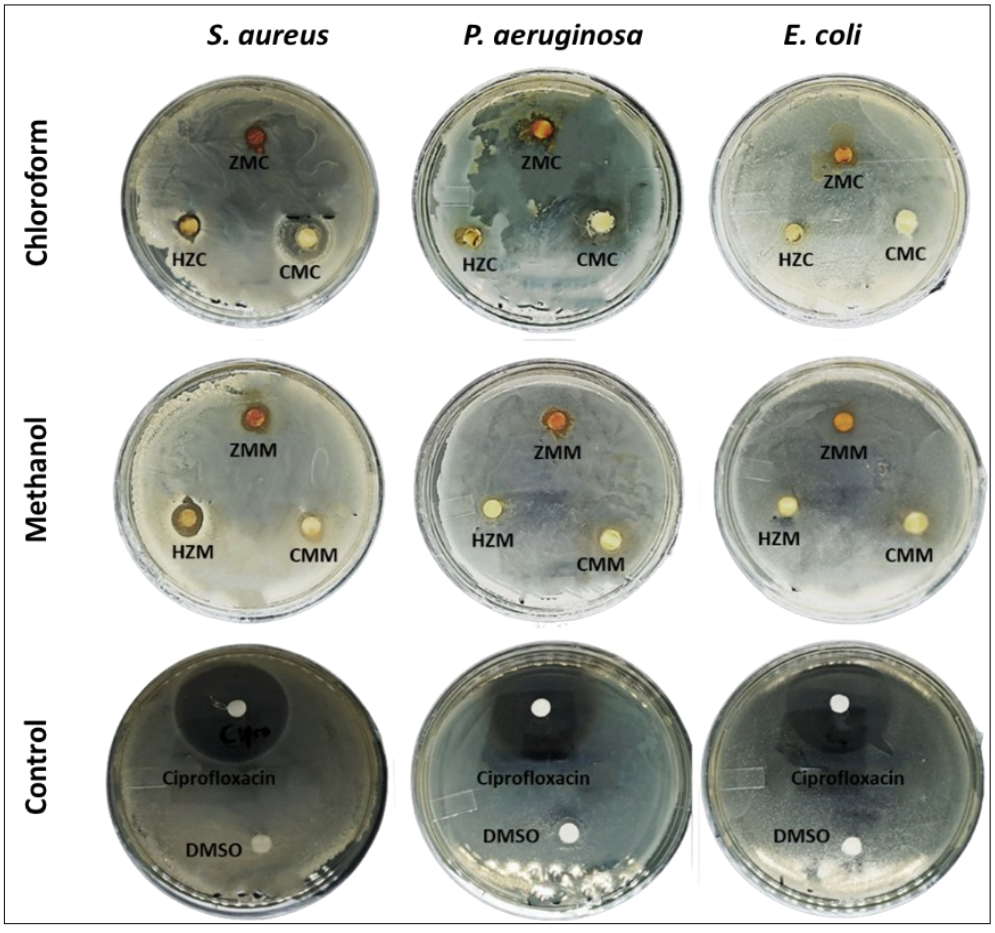

Fig. 2. Zone of inhibition by disk diffusion methods of 3 medicinal plants extract.

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

Table 2. Antibacterial activity of plant extracts by agar well diffusion method

Extract	Diameter of inhibition (mm)		
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Escherichia coli***
CMC	21.33 ± 2.49^{b}	22.00 ± 0.00^{b}	18.67 ± 0.94^{b}
CMM	14.67 ± 0.94^{cd}	17.33 ± 0.94^{c}	14.00 ± 1.63^{c}
HZC	9.33 ± 0.94^{e}	10.67 ± 2.49^{e}	12.00 ± 0.00^{cd}
HZM	16.67 ± 0.94^{c}	15,33 ± 3.40^{cd}	17.33 ± 1.89^{b}
ZMC	12.00 ± 1.63^{de}	12.67 ± 0.94^{de}	8.67 ± 0.94^{e}
ZMM	10.67 ± 2.49^{e}	9.33 ± 0.94^{e}	10.00 ± 0.00^{de}
Ciprofloxacin	52.67 ± 2.49^{a}	34.00 ± 1.63^{a}	31.67 ± 0.47^{a}
DMSO 10 %	0	0	0

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

All values are expressed in mean ± SD (n = 3)

Means within a column with different letters significantly differ by Duncans' test at $p < 0.05$.

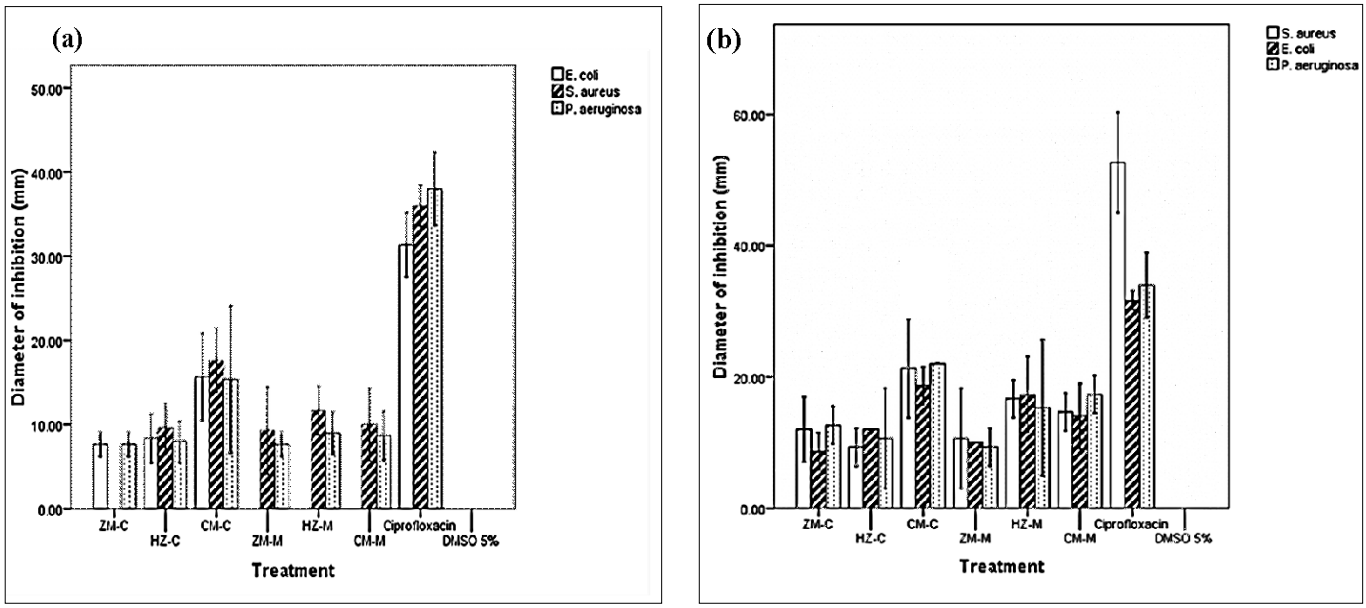

Fig. 3. Antibacterial activity of plant extracts by (a) disc diffusion method, (b) agar well diffusion method.

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

all plant extracts, as shown in Fig. 3. Furthermore, the CMC extract showed the largest inhibition zone compared to the other plant extracts.

MIC determination of plant extract

The results of the agar diffusion tests showed that all plant extracts exhibited antibacterial activity against the tested bacteria. Subsequently, the MIC of each extract was determined to ascertain the lowest concentration required to inhibit bacterial growth by 50 % using a microdilution assay. Table 3-5 present the optical density at a wavelength of 595 nm for *E. coli, P. aeruginosa and S. aureus*. Table 6 summarizes the MIC of each plant extract against the tested bacterial strains. MIC microdilution assay also indicated that the CMC extract possess the greatest antibacterial activity.

SEM analysis results

The CMC extract had showed the highest antibacterial activity according to diffusion methods and MIC microdilution assay. Therefore, SEM analysis was performed to observe the changes in morphology and structure of bacterial cells, specifically *S. aureus,* under the presence of CMC extract. Fig. 4 presents the outer cells structure of untreated *S. aureus* compared to *S. aureus* treated with *C. manan* chloroform extract. The results showed the damage to the morphology and structure of *S. aureus* cells, specifically to the cytoplasmic cell walls and membranes.

GC-MS analysis results

Fig. 5 shows the chromatogram with separate peaks based on retention time (RT) and percentage area of *C. manan* chloroform extract. The GC-MS analysis identified a total of 30 different compounds, with 4,5-dimethyl-1-hexene and terpineol-4 accounting for nearly 20 % of the combined main proportion. Table 7 lists the phytoconstituents discovered in

Table 3. MIC determination of plant extracts against *E. coli*

Treatment (mg/mL)	Absorbance λ_{595} nm					
	CMC	**CMM**	**HZC**	**HZM**	**ZMC**	**ZMM**
DMSO 10 %	0.123 ± 0.010^{c}	0.397 ± 0.003^{c}	0.123 ± 0.010^{c}	0.397 ± 0.003^{c}	0.123 ± 0.010^{c}	0.397 ± 0.003^{c}
0,312	0.093 ± 0.091^{c}	0.334 ± 0.084^{c}	0.074 ± 0.000^{bc}	0.323 ± 0.016^{bc}	0.092 ± 0.007^{bc}	0.273 ± 0.022^{c}
0,625	0.042 ± 0.030^{b}	0.268 ± 0.090^{bc}	0.075 ± 0.013^{bc}	0.338 ± 0.051^{bc}	0.074 ± 0.001^{b}	0.377 ± 0.022^{c}
1,25	0.033 ± 0.008^{b}	0.144 ± 0.071^{ab}	0.025 ± 0.028^{ab}	0.199 ± 0.126^{ab}	0.069 ± 0.016^{b}	0.191 ± 0.027^{b}
2,5	0.043 ± 0.042^{b}	0.115 ± 0.005^{a}	0.023 ± 0.094^{ab}	0.105 ± 0.013^{a}	0.058 ± 0.004^{b}	0.122 ± 0.011^{a}
5	0.023 ± 0.070^{ab}	0.107 ± 0.033^{a}	0.010 ± 0.105^{a}	0.078 ± 0.003^{a}	0.011 ± 0.026^{a}	0.096 ± 0.010^{a}
10	0.005 ± 0.000^{a}	0.075 ± 0.016^{a}	0.007 ± 0.068^{a}	0.090 ± 0.076^{a}	0.004 ± 0.043^{a}	0.107 ± 0.012^{a}
Ciprofloxacin	0.003 ± 0.007^{a}	0.093 ± 0.007^{a}	0.003 ± 0.007^{a}	0.093 ± 0.007^{a}	0.003 ± 0.007^{a}	0.093 ± 0.007^{a}

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

All values are expressed in mean ± SD (n = 3); Bold values indicate *Minimum Inhibitory Concentration* (MIC)

Means within a column with different letters significantly differ by Duncans' test at $p < 0.05$.

Table 4. MIC determination of plant extracts against *P. aeruginosa*

Treatment (mg/mL)	Absorbance λ_{595} nm					
	CMC	**CMM**	**HZC**	**HZM**	**ZMC**	**ZMM**
DMSO 10 %	0.314 ± 0.088^{b}	0.511 ± 0.044^{d}	0.314 ± 0.088^{c}	0.511 ± 0.044^{d}	0.314 ± 0.088^{c}	0.511 ± 0.044^{cd}
0,312	0.269 ± 0.037^{b}	0.445 ± 0.000^{d}	0.209 ± 0.009^{bc}	0.442 ± 0.114^{cd}	0.219 ± 0.003^{bc}	0.668 ± 0.186^{d}
0,625	0.196 ± 0.119^{a}	0.340 ± 0.000^{c}	0.245 ± 0.015^{bc}	0.264 ± 0.174^{bc}	0.213 ± 0.140^{bc}	0.472 ± 0.077^{cd}
1,25	0.073 ± 0.024^{a}	0.279 ± 0.000^{c}	0.246 ± 0.026^{bc}	0.231 ± 0.028^{ab}	0.228 ± 0.138^{bc}	0.351 ± 0.050^{bc}
2,5	0.141 ± 0.081^{a}	0.106 ± 0.006^{b}	0.112 ± 0.017^{ab}	0.181 ± 0.028^{ab}	0.273 ± 0.225^{bc}	0.194 ± 0.071^{ab}
5	0.098 ± 0.004^{a}	0.067 ± 0.006^{ab}	0.174 ± 0.134^{ab}	0.081 ± 0.010^{ab}	0.115 ± 0.063^{ab}	0.072 ± 0.010^{a}
10	0.115 ± 0.072^{a}	0.012 ± 0.012^{a}	0.109 ± 0.023^{a}	0.031 ± 0.010^{a}	0.125 ± 0.104^{ab}	0.022 ± 0.009^{a}
Ciprofloxacin	0.104 ± 0.008^{a}	0.067 ± 0.005^{a}	0.104 ± 0.008^{a}	0.067 ± 0.005^{a}	0.104 ± 0.008^{a}	0.067 ± 0.005^{a}

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

All values are expressed in mean ± SD (n = 3); Bold values indicate *Minimum Inhibitory Concentration* (MIC)

Means within a column with different letters significantly differ by Duncans' test at $p < 0.05$.

Table 5. MIC determination of plant extracts against *S. aureus*

Treatment (mg/mL)	Absorbance λ_{595} nm					
	CMC	**CMM**	**HZC**	**HZM**	**ZMC**	**ZMM**
DMSO 10 %	0.240 ± 0.018^{d}	0.725 ± 0.019^{d}	0.240 ± 0.018^{c}	0.725 ± 0.019^{e}	0.240 ± 0.018^{c}	0.725 ± 0.019^{e}
0,312	0.093 ± 0.005^{c}	0.802 ± 0.014^{d}	0.249 ± 0.001^{c}	0.713 ± 0.000^{e}	0.183 ± 0.026^{c}	0.702 ± 0.004^{e}
0,625	0.064 ± 0.007^{bc}	0.620 ± 0.014^{c}	0.221 ± 0.020^{bc}	0.546 ± 0.004^{d}	0.166 ± 0.026^{bc}	0.615 ± 0.007^{d}
1,25	0.032 ± 0.009^{ab}	0.371 ± 0.001^{b}	0.154 ± 0.008^{bc}	0.362 ± 0.003^{c}	0.090 ± 0.027^{ab}	0.417 ± 0.000^{c}
2,5	0.030 ± 0.028^{ab}	0.382 ± 0.016^{b}	0.080 ± 0.017^{ab}	0.359 ± 0.017^{c}	0.029 ± 0.015^{a}	0.356 ± 0.011^{bc}
5	0.033 ± 0.092^{ab}	0.324 ± 0.018^{b}	0.027 ± 0.042^{a}	0.320 ± 0.004^{bc}	0.044 ± 0.003^{a}	0.323 ± 0.008^{b}
10	0.013 ± 0.005^{a}	0.334 ± 0.017^{b}	0.011 ± 0.039^{a}	0.269 ± 0.007^{ab}	0.030 ± 0.021^{a}	0.292 ± 0.030^{ab}
Ciprofloxacin	0.003 ± 0.007^{a}	0.240 ± 0.018^{a}	0.003 ± 0.007^{a}	0.240 ± 0.018^{a}	0.003 ± 0.007^{a}	0.240 ± 0.018^{a}

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

All values are expressed in mean ± SD (n = 3); Bold values indicate *Minimum Inhibitory Concentration* (MIC)

Means within a column with different letters significantly differ by Duncans' test at $p < 0.05$.

Table 6. MIC of plant extracts against tested bacteria

Extract	MIC (mg/mL)		
	Escherichia coli	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
CMC	0.625	0.625	0.312
CMM	1.25	2.5	1.25
HZC	1.25	2.5	2.5
HZM	1.25	1.25	1.25
ZMC	1.25	5	1.25
ZMM	1.25	2.5	1.25

CMC: *C. manan* chloroform extract; HZC: *H. zeylanica* chloroform extract; ZMC: *Z. montanum* chloroform extract;

CMM: *C. manan* methanol extract; HZM: *H. zeylanica* methanol extract; ZMM: *Z. montanum* methanol extract.

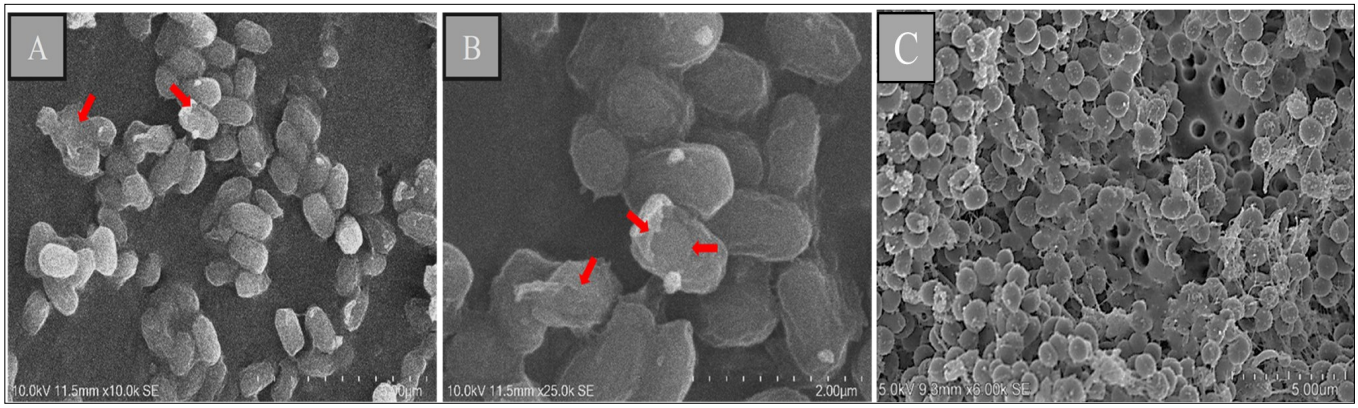

Fig. 4. SEM micrograph of *Staphylococcus aureus* cells treated with chloroform extract of *Calamus manan* (CMC) with magnification 10,000x (A) and 25,000x (B); Untreated *S. aureus* cells with magnification 6000x (C).

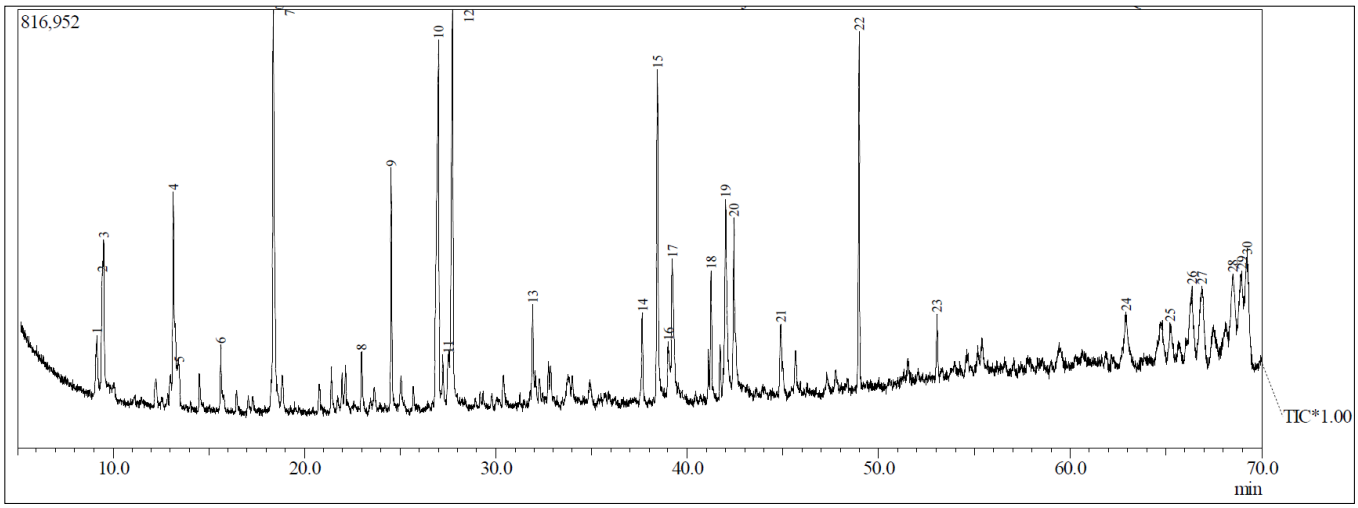

Fig. 5. GC-MS Chromatogram of chloroform extract from *Calamus manan* stem.

Table 7. Compounds identified in chloroform extract of *C. manan* stem by GC-MS

Peak no	Retention time	Area %	Compound name	Formula	SI
1	9.165	1.22	Hexylene glycol	$C_6H_{14}O_2$	90
2	9.425	2.10	Sabinene	$C_{10}H_{16}$	80
3	9.512	2.08	Sabinene	$C_{10}H_{16}$	85
4	13.158	5.30	Eucalyptol	$C_{10}H_{18}O$	90
5	13.450	1.20	Eucalyptol	$C_{10}H_{18}O$	80
6	15.634	0.98	Isopinocampheol	$C_{10}H_{18}O$	84
7	18.378	9.80	Terpineol-4	$C_{10}H_{18}O$	92
8	22.997	1.15	Ethylbutyl acetylene	C_8H_{14}	86
9	24.542	3.78	Z-2 hexenyl acetate	$C_8H_{14}O_2$	84
10	27.000	10.88	4,5-dimethyl-1-hexene	C_8H_{16}	82
11	27.508	1.23	Diamyl ketone	$C_{11}H_{22}O$	78
12	27.726	8.34	Diamyl ketone	$C_{11}H_{22}O$	82
13	31.926	1.63	b-tumerone	$C_{15}H_{22}O$	73
14	37.661	1.53	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	92
15	38.448	5.93	Octadecanoic acid	$C_{18}H_{36}O_2$	91
16	39.017	1.17	3-(3',5'-dimethoxy 4'hydroxyphenyl)-E-2-propenal	$C_{11}H_{12}O_4$	64
17	39.225	3.35	3-octen-5-yne, 2,2,7,7 tetramethyl	$C_{12}H_{20}$	66
18	41.234	1.72	11-octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	90
19	41.995	5.07	9-octadecanoic acid	$C_{18}H_{34}O_2$	90
20	42.438	3.29	Octadecanoic acid	$C_{18}H_{36}O_2$	90
21	44.883	1.55	Methyl ricinoleate	$C_{19}H_{36}O_3$	88
22	48.973	5.60	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	92
23	53.047	1.27	Cholesta-8,24-dien-3-ol, 4-methyl-, (3 beta, 4 alpha)	$C_{28}H_{46}O$	78
24	62.923	1.61	4-pentadecyne, 15-chloro	$C_{15}H_{27}Cl$	74
25	65.234	1.07	Nodosin	$C_{20}H_{26}O_6$	63
26	66.361	1.33	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	60
27	66.884	1.71	Methyl 5,8,11-eicosatrienoate	$C_{21}H_{36}O_2$	56
28	68.508	4.18	Simiarenol	$C_{30}H_{50}O$	75
29	68.958	4.23	Cycloeucalenol	$C_{30}H_{50}O$	73
30	69.241	5.68	Gynolutone	$C_{21}H_{30}O_2$	76

the potential CMC extract and their chemical formula, as determined by GC-MS peak reports of total ion chromatogram.

Discussion

The antibacterial activity of 3 medicinal plants used by the local communities in Jambi was evaluated against bacterial strain using disc and agar well diffusion methods, yielding relatively similar results for all extracts. In this study, the highest antibacterial activity was observed in the chloroform extract of *C. manan* (CMC) against all tested bacteria. CMC extract showed the largest zone of inhibition compared to others for all reference strains. The largest diameter for disc diffusion being observed was against *S. aureus* (17.67 mm) and for agar well diffusion being observed against *P. aeruginosa* (22.00 mm), both were treated with CMC extract. Both zone of inhibition diameters are interpreted as intermediate sensitivity against *S. aureus* and *P. aeruginosa* (31).

All extracts were subjected to MIC microdilution assay to evaluate their antibacterial activity further. Table 6 shows that CMC extract exhibited the lowest concentration capable of inhibiting bacterial growth by 50 % for all strains. These results supported the outcome of the previous agar diffusion assays, which identified CMC extract for having the highest antibacterial activity compared to other tested plant extracts. This result was similar to the last investigation, which showed the growth inhibition activity of *C. manan* fruits and seeds extract against *E. coli* and *S. mutans* (9). Another study on *Calamus ornatus* fruit extract also reported growth inhibition on *E. coli* due to the presence of alkaloids (32).

The *C. manan* chloroform extract exhibited the lowest MIC value against *S. aureus* at 0.312 mg/mL. However, nearly all extracts showed weaker antibacterial activity against *P. aeruginosa,* with MIC values ranging from 1.25 - 5 mg/mL. This is related to the differences between Gram-positive bacteria (*S. aureus)* and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) in the aspect of constituent components of the cell wall. Gram-negative bacteria possess lipopolysaccharide (LPS) as the outer layer of the cell wall, making them more complex than their Gram-positive counterpart. This outer layer comprises phospholipids, polysaccharides and proteins, which limit the entry of chemical substances into the cell wall. In addition to that, it also possesses enzymes capable of degrading extracellular molecules in the periplasmic space. All aforementioned cell wall properties are attributed to the higher MIC value of all tested extracts against *P. aeruginosa* compared to other reference bacterial strains. The difference between Gram-positive and Gram-negative bacteria also reported on other antibiotics. It was reported that vancomycin showed weaker inhibition against the Gram-negative bacteria than against the Gram-positive bacteria due to the absence of the outer membrane and periplasm in the Gram-positive bacteria, leading to higher permeability (33). Other study also reported higher case of multidrug resistance on Gram negative bacteria, including *A. baumannii*, *Enterobacter cloacae*, *Proteus mirabilis* and *Morganella morganii*, compared to only *Enterococcus faecalis* for Gram-positive bacteria (34).

SEM results showed observable morphological changes between the treated and untreated *S. aureus* cells. The untreated cells had standard shape and no observable damage on the cell wall. Conversely, those cells treated with *C. manan* chloroform extract had cell shrinkage and leakage on the cell wall (Fig. 4). The damage resulted in the bacterial cell appearing hollow. Furthermore, the leakage is caused by the interaction between antibacterial compounds in *C. manan* chloroform extract and chemical constituents of bacterial cell walls. Several plant secondary metabolites, such as phenolics and terpenoids, were reported to disrupt membrane integrity and inhibit cell wall synthesis. Some phenols, including flavonoid, tannin and polyphenols, bind to adhesin in the cell wall and form an enzyme complex that inhibits synthesis (35). Alternately, terpenoids such as eugenol and thymol induce the formation of hydrogen bonds with enzymes that leads to enzyme inactivation, cell membrane disruption and induce changes on ion channels (36).

The high antibacterial activity observed in the chloroform extract of *C. manan* could be attributed to secondary metabolites. The GC-MS analysis identified a total of 30 different compounds, with 4,5-dimethyl-1-hexene and terpineol-4 accounting for nearly 20 % of the combined main proportion. Previous phytochemical test showed that *C. manan* fruit extract contains alkaloids, tannins and triterpenoids (37). Another part that has been investigated was the young stem of *C. manan, which contains* flavonoid, glycoside, saponin and tannin (38). However, there was no prior investigation regarding the antibacterial activity of *C. manan* stem extract. Therefore, this study was the first to report its antibacterial and identify its phytochemical constituents.

Based on GC-MS results, 4,5-dimethyl-1-hexene is the dominant compounds identified from *C. manan* chloroform extract. This alkene contains methyl groups and has been reported to have a fungistatic effect against *Penicillium digitatum,* the cause of postharvest green mold disease on citrus fruit. In that report, 4,5-dimethyl-1-hexene was one of the 3 volatile compounds identified from the gas phase of *Enterobacter cloacae*, with it being the most predominant (39). However, there is currently no further investigations regarding its antibacterial activity or toxicity.

Terpineol-4 is a terpenoid from the monoterpene group present in essential oils. Terpineol-4 has been reported to possess antibacterial, antioxidant and anti-inflammatory properties, as well as its role as a plant metabolite (40). Furthermore, it exhibited antibiofilm activity against some biofilm-forming species, making it a promising candidate for clinical application. The antibacterial activity of terpineol-4 has been reported to affect both positive and negative Gram bacteria. Terpineol-4 has shown strong antibacterial activity against *S. aureus, Listeria innocua, E. coli, Serratia liquefaciens and Salmonella typhimurium* (41).

The lipophilic properties enable terpineol-4 to accumulate in the lipid bilayer of the plasma membrane, resulting in membrane expansion, increased membrane

fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration and alteration of ion transport processes. Terpineol-4 can also inhibit bacterial growth by interfering with cell wall formation and nucleic acid metabolism. This phytoconstituent probably targets penicillin-binding protein 2 (PBP2a), thereby disrupting peptidoglycan crosslinking during the bacterial cell wall synthesis (42). Furthermore, terpineol-4 was reported to inhibit purine and pyrimidine metabolism, leading to growth inhibition and the decrease of eDNA in the biofilm of methicillin-resistant *S. aureus* (MRSA) (43).

In addition to *C. manan,* the current study has identified the comparably high antibacterial activity of the methanolic extract of *H. zeylanica* against all reference strains, as determined by the agar well diffusion method. It showed the highest antibacterial activity against *E. coli* while moderately inhibiting *S. aureus* and *P. aeruginosa*. Similar results also reported that ethanolic extract of *H. zeylanica* had a significant inhibitory effect against *B. cereus* (17). Similar research also reported the antibacterial activity of polar extract of *H. zeylanica* against *Propionibacterium acne* (44). Several studies identified flavonoid compounds in *H. zeylanica* polar extracts, mainly quercetin and ugonin (24, 45). The presence of flavonoids indicated the antibacterial activity of *H. zeylanica* extract could be due to its ability to inhibit DNA gyrase activity of bacterial cells (46).

Conclusion

This study showed the antibacterial properties of 3 medicinal plants used by the local communities in Jambi, namely *C. manan, H.* zeylanica and *Z. montanum,* extracted using different solvents. Additionally, it reported the first result of significant antibacterial activity of chloroform extract of *C. manan* stems compared to other extracts, as evidenced by *S. aureus* cell leakage and shrinkage observed through SEM analysis. GC-MS analysis discovered 4,5-dimethyl-1-hexene and terpineol-4 as the primary compounds in *C. manan* chloroform extract. Therefore, this extract could be used as a potential source for developing new and effective antibacterial compounds.

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Authors' contributions

MHA designed and conceptualized the study, curated the data, performed the experiments and statistical analysis and wrote the original draft. ER designed and conceptualized the study, provided critical revision and edited the final version of the manuscript. YAP designed and conceptualized the study provided critical revision and edited the final version of the manuscript. LHN designed and conceptualized the study, supervised the research and revised the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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