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Article

Phytochemical Composition and Antibacterial Activity of *Zingiber Cassumunar* Roxb. against Agricultural and Foodborne Pathogens

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Abstract: *Zingiber cassumunar* Roxb. is a powerful medicinal plant that has been used as traditional medicine to cure respiratory problems, pain, and inflammation in China, Indonesia, Thailand and other Asian countries by using the crude extracts. The objective of this research is to identify phytochemical composition of *Z. cassumunar* Roxb. and to analyze antibacterial activity of crude extract, purified compounds, and their microencapsulated products of Rhizome *Z. cassumunar* Roxb. Identification of phytochemical composition in crude extract of rhizome *Z. cassumunar* Roxb. was achieved by chromatography-mass spectrophotometer. The major phytochemical composition in crude extract of *Z. cassumunar* Roxb. is essential oils, including terpinen4-ol (37.7%), β-pinene (20.8%), and (*E*)-1-(3,4-dimethoxyphenyl)but-1-ene (13.3%). Crude extract of *Z. cassumunar* Roxb. was purified with silica gel flash column chromatography, resulting two purified compounds. The antibacterial activity of crude extract, purified compounds, and their microencapsulated products of Rhizome *Z. cassumunar* Roxb. were evaluated against agricultural and foodborne pathogens by using disc agar diffusion and broth microdilution techniques. All of the samples studied (crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb.) were effective against all the bacteria. Based on the results of the disc-diffusion assay suggested that amongst the samples studied, purified compounds (compound 1 and 2) and microencapsulated purified compounds (compound 1 and 2) exhibited more effective against all the bacteria compared to the crude extracts. Antibacterial activity of the rhizome of *Z. cassumunar* Roxb. was contributed mainly by the essential oils components as the active compounds. Gram-negative bacteria (*X. oryzae, X. translucens, Pseudomonas* spp, *E. coli,* and *S. typhimurium*) appeared to the most resistant to the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. compared to the gram-positive bacteria (*S. aureus, B. cereus,* and *L. monocytogenes*). Microencapsulated of the tested samples (crude extract, purified compound 1, and purified compound 2) of the rhizome *Z. cassumunar* Roxb. exhibited high antibacterial activity with no significantly different with the tested samples without microencapsulation. These results suggest potential antibacterial properties of *Z. cassumunar* Roxb., which useful for agricultural plant health, food preservation, natural therapies, and pharmaceuticals.

Keywords: *Zingiber cassumunar* Roxb.; essential oils; microencapsulated products; antibacterial activity; agricultural pathogens; foodborne pathogens; gram-positive bacteria; gram-negative bacteria

1. Introduction

Zingiber cassumunar Roxb. is a perennial and herbaceous plant and belongs to the family Zingiberaceae. *Z. cassumunar* Roxb. is known as “Bangle” in Indonesia, “Bulei” in

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China, and "Plai" in Thailand, which is a popular traditional medicinal plant that has been used to treat pain caused by menstrual, musculoskeletal, gastrointestinal distress, sprains, rheumatisms, asthma, arthritis, skin trouble, and respiratory problems. Based on the World Health Organization (WHO) [1], most of the world's population relied on traditional medicine for their healthcare. In the Southeast Asian countries, the aqueous extracts and powdered forms of the rhizome *Z. cassumunar* Roxb. is commonly used as medicines, spices, dyes, and flavouring agents [2,3,4]. The primary part of the plant that usually use for medicinal purposes is the rhizomes. Curcuminoids, essential oils, and phenylbutenoids have been identified as the phytochemical compounds which majority found in the rhizome of *Z. cassumunar* Roxb. [5]. Many studies reported that the rhizome of *Z. cassumunar* Roxb. exhibited diverse medicinal properties, including antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, and cosmeceutical activities [5-8]. Bioactive compounds associated with those medicinal properties need to be explored from the rhizome of *Z. cassumunar* Roxb.

Many previous studies have contributed to identify the phytochemical compounds and their biological activities of the rhizome *Z. cassumunar* Roxb. [6,9,10]. Curcuminoids in the rhizome *Z. cassumunar* Roxb. consist of curcumin [11]; cassumunins A-C [12]; (1E,4E,6E)-1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one [13]; and bisdeoxycurcumin [14]. Curcumin as the major compound with yellow pigment. Essential oils in the rhizome of *Z. cassumunar* Roxb. have been identified by using gas chromatography-mass spectrometry (GC-MS). The major constituents of essential oils in the rhizome of *Z. cassumunar* Roxb. were identified as monoterpenoids, terpinene-4-ol, and sabinene [15].

Various techniques have been used to isolate phenylbutenoids in the rhizome of *Z. cassumunar* Roxb., including recrystallization [16,17,18], silica gel [19], thin-layer chromatography [20], and high-performance liquid chromatography [21,22]. Many studies identified phenylbutenoid as (E)-1-(30,40-dimethoxyphenyl)but-1,3-diene; (E)-4-(30 ,40 -dimethoxyphenyl) but-3-en-1-yl palmitate; (E)-4-(30 -40 -dime-thoxyphenyl)but-1-ene; (E)-4-(20 ,40 ,50 -trime thoxyphenyl)but-1-ene; phenylbutenoid dimers, trans-3-(20 ,40 ,50 -trimethoxyphenyl)-4-[(E)-3000,4000-dimethoxystyryl]cyclohexl-ene; cis-1,2-bis[(E)-3,4-dimethoxystyryl]cyclobutene; cis-3-(20 ,40 ,50 -trimethoxyphenyl)-4-[(E)-3000,4000-dimethoxystyryl] cyclohex-l-ene; (E)-4-(20 ,40 ,50 -trimethoxyphenyl)but-1,3-diene; trans-3-(20 ,40 ,50 -trimethoxyphenyl)-4-[(E)-2000,4000,5000-trimethoxystyryl]cyclohex-lene; trans-3-(30 ,40 -dimethoxyphenyl)-4-[(E)-2000,4000,5000-trimethoxystyryl]cyclohex-1-ene; and (trans-3-(3,4-dimethoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1- ene in the rhizome of *Z. cassumunar* Roxb. [6,19,21,23-30]. The essential oils including terpinene-4-ol as the major volatile terpenes. These essential oils contribute to the effectiveness of extract *Z. cassumunar* Roxb. The essential oils of *Z. cassumunar* Roxb. showed antimicrobial activity against gram-negative and gram-positive bacteria, fungi, and yeasts (Chongmelaxme et al., 2017). (E)-1-(3,4-dimethoxyphenyl)but-1-ene 2) as the essential oils in *Z. cassumunar* Roxb. also demonstrated anti-infammatory activity.

The extracts (i.e. methanol, ethanol, ether, hexane, chloroform, and ethyl acetate) and purified compounds of the rhizome *Z. cassumunar* Roxb. have been reported showed various bioactivities, such as antioxidant, anti-inflammatory, antibacterial, and antifungal. Major compound in the rhizome *Z. cassumunar* Roxb. which exhibited high antibacterial activity was essential oils that against gram-positive and gram-negative bacteria with minimum bactericidal concentrations 0.62 to 2.5 vol %. The gram-positive bacteria, including *Bacillus subtilis, Streptococcus pyogenes, Staphylococcus aureus, Streptococcus epidermis, Staphylococcus epidermis, Propionibacterium acnes,* and *Streptococcus mutans* [31,32]. Furthermore, the gram-negative bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Proteus vulgaris* [33,34].

Objectives of this study are to identify phytochemical composition of the rhizome *Z. cassumunar* Roxb. and to analyze antibacterial activity of crude extract and purified compounds of the rhizome *Z. cassumunar* Roxb. against agricultural and foodborne pathogens. Bacterial diseases which attack main crops (i.e. rice and wheat) and also chickens potentially cause the yield loss as high as 75%. The majority of the agricultural pathogens are

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Xanthomonas oryzae (gram-negative) causes rice bacterial blight, *Xanthomonas translucens* (gram-negative) causes bacterial leaf streak, *Pseudomonas* spp (gram-negative) cause bacterial leaf spot, and *Staphylococcus aureus* (gram-positive) causes bumblefoot in chickens [35,36]. Additionally, the most popular bacteria that responsible for foodborne pathogens of contaminated products *Escherichia coli* (gram-negative), *Salmonella typhimurium* (gram-negative), *Bacillus cereus* (gram-positive), and *Listeria monocytogenes* (gram-positive) [37,38]. Minimizing agricultural and foodborne pathogens with chemical control has been ineffective due to safety concerns and bacterial resistance [39,40]. Controlling the agricultural and foodborne pathogens with natural compounds like extracts of rhizome *Z. cassumunar* Roxb. expected to be more effective and safe.

2. Materials and Methods

2.1. Plant Material

The rhizomes of *Zingiber cassumunar* Roxb. were collected in Salatiga, Indonesia, and were identified by botanist Soenarto Notosoedrmo (Satya Wacana Christian University, Salatiga, Indonesia) as well as by identification based on the data from literature.

2.2. Phytochemical Composition Analysis

Phytochemical composition of *Z. cassumunar* Roxb.were identified by gas chromatography-mass spectrophotometer (GC-MS). GC analysis was carried out on a Shimadzu GC 14A capillary chromatograph equipped with a FID detector (a DB-5 with 30 m – 0.25 mm, 0.25 film thickness) capillary column. The rhizome of *Z. cassumunar* were dissolved in hexane and were injected in split mode by using pressured controlled nitrogen as carrier gas at a linear velocity of 50 cm/s. Detector and injector temperature was maintained at 250 °C. The oven temperature was programmed at 75 °C for 10 minutes to 230 °C at 3 °C/minute. The relative amounts of individual components are based on peak areas obtained. Peak areas and retention times were measured by electronic integration.

GC-MS analyses were performed by using a Hewlett Packard GC-MSD 5890 series, EI electron impact ion source, 70 EV using a BPX5 (30 m – 0.25 mm, 0.25 mm film thickness) capillary column. The rhizome of *Z. cassumunar* Roxb. were dissolved in hexane and were injected in split mode by using pressured controlled nitrogen as carrier gas at a linear velocity of 50 cm/s. Detector and injector temperature was maintained at 250 °C. The oven temperature was programmed at 75 °C for 10 minutes to 230 °C at 3 °C/minute. Identification of the phytochemical components in *Z. cassumunar* Roxb. was based on the comparison of their mass spectral data with the existing National Institute of Standards and Technology (NIST) mass spectral database library, also confirmed by comparison with data published in the literature. The composition of the components were reported as relative percentage of the total peak area, calculated by using this calculation: Relative % of peak area = (Area of the peak/Total peak area) x 100.

2.3. Extraction and Isolation

The dried rhizomes of *Z. cassumunar* Roxb. (500 g) were extracted with MeOH (3 – 2 l) at room temperature, overnight. The MeOH solvent was evaporated to get a concentrated MeOH extract, and then suspend in water (200 ml). The extract was partitioned with n-hexane (200 ml), $CHCl_3$ (200 ml), and n-BuOH (200 ml), subsequently. Next, the n-BuOH extract (5 g) was separated by a silica gel flash column chromatography (f 5.5 cm; 230–400 mesh, 500 g; Merck, Germany) with a mobile phase with a gradient of CH_2Cl_2-MeOH-H_2O (9 : 1 : 0.05 →1 : 1 : 0.1, 5 l each). The thin-layer chromatographic (TLC) analysis was carried on Kieselgel 60 F254 with silica gel, 0.25 mm layer thickness (Merck, Germany) and RP-18 F254s plates (Merck, Germany). The results of TLC analysis were visualized under UV-light (254–365 nm) and sprayed with 10% (v/v) sulfuric acid, then heat for 5 minutes at 120 °C.

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2.4. Microencapsulation of Crude Extract and Purified Compounds of Z. cassumunar Roxb. by Spray Drying using Maltodextrin

Crude extract and purified compounds of *Z. cassumunar* Roxb. were microencapsulated by spray drying using maltodextrin as wall materials. The microencapsulation process consist of three steps, including preparation emulsion, homogenization emulsion, and spray-drying emulsion. In preparation emulsion step, the maltodextrin was dissolved in double-distilled water with constant agitation 650 rpm at temperature 50 °C for 24 hours. Next, 60 g of crude extract and purified compounds, respectively and 1.20 g of tween 20 surfactant were mixed, respectively. The mixtures were homogenized until stable. Then, the emulsions were sprayed by a YC-1500 laboratory spray dryer (Yacheng experimental Co. Ltd.) with a 0.70 mm diameter rotating atomizer. The optimal conditions for microencapsulation with inlet drying air temperature of 185 °C, feed rate of 6 ml/minute, and pressure of compressed air 0.2 MPa. The microencapsulated crude extract and purified compounds were harvested and stored in 4 °C fridge.

2.5. Bacteria Used

The most common bacteria which responsible for agricultural pathogens, including *Xanthomonas oryzae* (gram-negative) causes rice bacterial blight, *Xanthomonas translucens* (gram-negative) causes bacterial leaf streak, *Pseudomonas* spp (gram-negative) cause bacterial leaf spot, and *Staphylococcus aureus* (gram-positive) causes bumblefoot in chickens were used in all antibacterial assays. The most popular bacteria that responsible for foodborne pathogens of contaminated products were also used in all antibacterial assays. These bacteria including *Escherichia coli* (gram-negative), *Salmonella typhimurium* (gram-negative), *Bacillus cereus* (gram-positive), and *Listeria monocytogenes* (gram-positive). All of these bacteria were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Bacterial strains were grown in Mueller Hinton broth (Moltox, Sycamore Life Science, Houston, TX, USA) and incubated at 37 °C.

2.6. Determination of Antibacterial Activity

The antibacterial activities of the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. against the agricultural and foodborne pathogens were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, diameter of zone of inhibition and minimum inhibitory concentration (MIC) values by using disc agar diffusion technique and broth microdilution method.

2.6.1. Disc Agar Diffusion Technique

Antibacterial activity of the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. was determined with disc agar diffusion technique according to the standard methods of the National Community for Clinical Laboratory Standards (NCCLS). A suspension of the tested bacteria (100 µl of 10^6–10^8 CFU/ml) was swabbed on Mueller Hinton Agar plates uniformly by using sterile cotton swabs. Sterile discs of 6 mm in diameter were individually impregnated with 15 µl of each crude extracts (diluted in MeOH solvent), purified compounds (diluted in MeOH solvent), and microencapsulated (diluted in MeOH solvent) of *Z. cassumunar* Roxb. and placed onto the inoculated agar plates. The plates were incubated at 37 °C for 24 hours. Antibacterial activity was evaluated by measuring diameter of the resulting zone of inhibition against the tested bacteria in millimeters. Tetracycline (30 µg/ml) as the positive control and 100% MeOH (15 µl per blank sterile disc) as the negative control. This antibacterial assay was performed in triplicate.

2.6.2. Broth Microdilution Method

The crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. that exhibited significant antibacterial activity in the disc agar diffusion technique were chosen for determination MIC by using the broth microdilution method against the

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same bacteria. Broth microdilution method were performed in Mueller Hinton Broth. The crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. were dissolved in DMSO and diluted to a concentration of 10%. Dilution of the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. were performed in a 96-well microplate (Nunc, Copenhagen, Denmark) over the range of 0.02 µg/µl to 33.30 µg/µl. This was performed by firstly filling all wells to be used with 200 µl of media Mueller Hinton Broth. Then, 100 µl of 10% the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb., respectively were transferred to the first well and mixed well. The three-fold serial dilution was performed by transferring 100 µl of the mixture in the first well into the next consecutive wells, until the end of the row. Next, 10 µl of bacterial culture was transferred into all wells of the column. The 96-well microplate was incubated at 37 °C for 24 hours. The bacteria growth was determined by using a universal microplate reader by interpreting the growth curve in each well by the presence of turbidity and a pellet on the bottom of the well. MIC is defined as the lowest concentration of the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. at which the bacteria does not grow. This test for all samples, positive and negative control were performed in triplicate.

2.7. Statistical Analysis

Results were reported in the form of mean, standard deviation and percentage value, which was calculated by using the Microsoft Excel 2020 software and SAS 9.4 software.

3. Results

3.1. Phytochemical Composition of Z. cassumunar Roxb.

GC-MS analysis of the rhizome *Z. cassumunar* Roxb. identified the presence of 34 compounds (Table 1), which sesquiterpenes (98%) dominating the compounds and followed by monoterpenes (2%). The major compounds in the rhizome *Z. cassumunar* Roxb. were terpinen4-ol (37.7%), β-pinene (20.8%), and (*E*)-1-(3,4-dimethoxyphenyl)but-1-ene (13.3%). Meanwhile, the rest of the compounds were only present in trace amounts. The phytochemical composition possess strong aromatic fragrances and characteristic yellow color appearance in the rhizome of *Z. cassumunar* Roxb. According to Kamazeri et al. [41], the rhizome of *Z. cassumunar* Roxb. contained the highest essentials oils (0.30% w/w) compared to the others Zingiberaceae family plants, including *Curcuma aeruginosa* (0.19% w/w) and *Curcuma manga* (0.12% w/w). Several less-polar components isolated from the Zingiberaceae plants have been reported to exhibit biological activities, including antibacterial, antioxidant, antifungal, and anti-inflammatory activities [2,42].

Table 1. Phytochemical composition of the rhizome *Z. cassumunar* Roxb.

Compounds	Percentage	Retention Index
α-thujene	0.5	927
α-pinene	0.9	937
Sabinene	0.3	970
β-pinene	20.8	979
myrcene	1.0	990
α-phellandrene	0.1	1003
δ-3-carene	2.4	1009
α-terpinene	1.7	1015

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p-cymene	0.8	1026
β-phellandrene	0.4	1030
γ-terpinene	5.1	1055
cis-linalool oxide	0.6	1075
terpinolene	0.9	1089
linalool	0.6	1099
isopulegol	0.6	1138
camphor	0.1	1149
citronellal	0.3	1153
terpinen-4-ol	37.7	1178
α-terpineol	0.7	1188
cis-piperitol	0.3	1196
decanal	0.4	1205
δ-elemene	0.2	1335
β-caryophyllene	0.3	1415
γ-elemene	0.3	1433
α-humulene	0.1	1453
α-zingiberene	0.3	1494
β-bisabolene	0.8	1505
β-sesquiphellandrene	0.2	1520
(*z*)-nerolidol	0.2	1534
(*E*)-nerolidol	1.0	1560
(*E*)-1-(3,4-dimethoxyphenyl)but-1-ene	13.3	1616
(*E*)-1-(3,4-dimethoxyphenyl)butadiene	4.7	1710
zerumbone	0.4	1733
methyl m-methoxy carbonylcinnamate	1.1	1786

3.2. Antibacterial Activity of Crude Extract, Purified Compounds, and Microencapsulated Compounds from the Rhizome Z. cassumunar Roxb.

Results obtained from the negative controls indicated that the solvents (MeOH and DMSO) had no effect on the bacteria which responsible for agricultural pathogens and foodborne pathogens (Table 2 and 3) after 24 hours of incubation. Meanwhile, the positive controls showed that the tetracycline exhibited high selectivity against all the bacteria based on the zone of inhibition and minimum inhibitory concentrations. The presence of zone of inhibition in the positive control suggested that all the bacteria used in the study were sensitive against the antibacterial agents. All of the samples studied (crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb.) were effective against all the bacteria. The zone of inhibition and minimum inhibitory concentrations varied in the samples studied and bacteria used suggesting the varying degree of efficacy and different phytocompounds on the target bacteria. Based on the results of the disc-diffusion assay (Table 2) suggested that amongst the samples studied, purified compounds (compound 1 and 2) and microencapsulated purified compounds (compound 1 and 2) exhibited more effective against all the bacteria compared to the crude extracts. These purified compounds (compound 1 and 2) and microencapsulated purified compounds (compound 1 and 2) giving larger zone inhibition and lower minimum inhibitory

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concentrations compared to the crude extracts. This indicates that purified compounds are the active component to the antibacterial activity. Considering the large number of different groups of phytochemical compounds present in the crude extract of the rhizome *Z. cassumunar* Roxb., probably producing antagonism effects on each other of the compounds present in the rhizome *Z. cassumunar* Roxb. that reduce the antibacterial activity.

Table 2. Effect of crude extract, purified compounds, and microencapsulated compounds from the rhizome *Z. cassumunar* Roxb. on the growth of agricultural and foodborne pathogens.

Samples	Zone of inhibition (mm) (mean±SD)							
	Agricultural pathogens			Foodborne pathogens				
	X. oryzae	*X. translucens*	*Pseudomonas* spp	*S. aureus*	*E. coli*	*S. typhimurium*	*B. cereus*	*L. monocytogenes*
Crude extract	17 ± 0.1	19 ± 0.4	16 ± 0.7	24 ± 0.2	18 ± 0.5	19 ± 0.4	27 ± 0.2	27 ± 0.6
Compound 1	22 ± 0.3	25 ± 0.2	23 ± 0.5	29 ± 0.4	25 ± 0.2	23 ± 0.7	28 ± 0.1	29 ± 0.3
Compound 2	24 ± 0.4	21 ± 0.1	22 ± 0.4	31 ± 0.7	26 ± 0.4	22 ± 0.1	30 ± 0.3	31 ± 0.5
Microencapsulated crude extract	18 ± 0.5	21 ± 0.3	17 ± 0.6	23 ± 0.8	19 ± 0.1	21 ± 0.3	28 ± 0.5	26 ± 0.5
Microencapsulated compound 1	23 ± 0.2	27 ± 0.4	23 ± 0.1	29 ± 0.3	26 ± 0.5	24 ± 0.2	27 ± 0.4	30 ± 0.2
Microencapsulated compound 2	22 ± 0.4	23 ± 0.6	21 ± 0.8	29 ± 0.2	27 ± 0.4	21 ± 0.1	28 ± 0.1	29 ± 0.3
Tetracycline	35 ± 0.3	37 ± 0.1	36 ± 0.6	41 ± 0.4	34 ± 0.8	36 ± 0.4	41 ± 0.1	40 ± 0.5
MeOH	NZ*	NZ	NZ	NZ	NZ	NZ	NZ	NZ

* NZ = No Inhibition Zone

Table 3. The minimum inhibitory concentrations of crude extract, purified compounds, and microencapsulated compounds from the rhizome *Z. cassumunar* Roxb. on the growth of agricultural and foodborne pathogens.

Samples	Minimum inhibitory concentrations (µg/µl) (mean±SD)							
	Agricultural pathogens			Foodborne pathogens				
	X. oryzae	*X. translucens*	*Pseudomonas* spp	*S. aureus*	*E. coli*	*S. typhimurium*	*B. cereus*	*L. monocytogenes*
Crude extract	28 ± 0.1	25 ± 0.4	27 ± 0.6	32 ± 0.5	29 ± 0.2	27 ± 0.5	31 ± 0.3	30 ± 0.5
Compound 1	19 ± 0.4	21 ± 0.7	17 ± 0.3	26 ± 0.2	18 ± 0.7	19 ± 0.3	28 ± 0.6	26 ± 0.1
Compound 2	17 ± 0.3	19 ± 0.1	16 ± 0.5	25 ± 0.4	19 ± 0.4	21 ± 0.2	29 ± 0.4	28 ± 0.4
Microencapsulated crude extract	26 ± 0.2	23 ± 0.4	28 ± 0.7	30 ± 0.5	27 ± 0.9	25 ± 0.5	29 ± 0.7	28 ± 0.4
Microencapsulated compound 1	18 ± 0.6	21 ± 0.4	17 ± 0.9	24 ± 0.6	19 ± 0.9	20 ± 0.5	26 ± 0.2	24 ± 0.4
Microencapsulated compound 2	15 ± 0.7	16 ± 0.2	17 ± 0.7	24 ± 0.5	17 ± 0.7	20 ± 0.2	27 ± 0.6	26 ± 0.3
Tetracycline	0.05 ± 0.02	0.07 ± 0.04	0.05 ± 0.01	0.16 ± 0.02	0.10 ± 0.04	0.09 ± 0.03	0.20 ± 0.02	0.18 ± 0.03

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DMSO	-	-	-	-	-	-	-	-

***-** = No Activity

Antibacterial activity of the rhizome *Z. cassumunar* Roxb. may be due to the presence of various active compounds in their rhizome [43]. Extract of the rhizome *Z. cassumunar* Roxb. was reported to possess antibacterial activity that was associated with phytochemical composition, including essential oils, tannins, alkaloids, flavonoids, saponins, and glycosides compounds. The antibacterial activity of the rhizome *Z. cassumunar* Roxb. extracts may therefore be attributed to the presence of those phytochemicals in the crude extracts, purified compounds, and microencapsulated compounds. The antibacterial activity may be due to the different modes of action of the total phytochemical components in rhizome of *Z. cassumunar* Roxb. towards the bacteria. Rhizome of *Z. cassumunar* Roxb. rich in phytochemical compositions which have antibacterial potential due to their characteristics which allow them to reach with proteins in the bacteria to form stable water soluble constituents thereby killing the bacteria by damaging the bacteria cell membrane [44]. The relative antibacterial activity of the total phytochemical components in the rhizome of *Z. cassumunar* Roxb. could not be easily associated with the individual compound. Many studies indicated that the antibacterial activities of the rhizome of *Z. cassumunar* Roxb. were probably due to their respective major compounds, such as terpinen4-ol (37.7%), β-pinene (20.8%), and (*E*)-1-(3,4-dimethoxyphenyl)but-1-ene (13.3%) (Table 1). Based on this study, it may be that the antibacterial activity of the rhizome of *Z. cassumunar* Roxb. was contributed mainly by the essential oils components as the active compounds. The antibacterial activity of essential oil components due to the lipophilic characteristic of the hydrocarbon skeleton and hydrophilic characteristic of the functional groups, which can damage the bacteria [45]. Many studies also reported that the essential oils as the active compounds against bacteria [41].

Based on the zone of inhibition and minimum inhibitory concentrations (Table 2 and 3), among the bacteria tested, the gram-negative bacteria (*X. oryzae, X. translucens, Pseudomonas* spp, *E. coli,* and *S. typhimurium*) appeared to the most resistant to the crude extracts, purified compounds, and microencapsulated of *Z. cassumunar* Roxb. by showing smaller zone of inhibition. Meanwhile, the gram-positive bacteria (*S. aureus, B. cereus,* and *L. monocytogenes*) were the most sensitive bacteria to all of the tested samples, since produced bigger zone of inhibition. Many studies also reported that the gram-positive bacteria showed more sensitive towards the phytochemical compounds compared to the gram-negative bacteria [41,46]. The gram-negative bacteria are more resistant to the action of phytochemical compounds of the rhizome *Z. cassumunar* Roxb. since they have a very restrictive outer membrane surrounding the cell wall, that restricts the diffusion of hydrophobic compounds through the lipopolysaccharide membrane [46].

Microencapsulated of the tested samples (crude extract, purified compound 1, and purified compound 2) of the rhizome *Z. cassumunar* Roxb. exhibited high antibacterial activity with no significantly different with the tested samples without microencapsulation (Table 2 and 3). This microencapsulation used to increase the stability and masking the odor or taste of the phytochemical compounds of the rhizome *Z. cassumunar* Roxb. that protected from the deteriorating effects of oxygen and extreme temperature [47]. Microencapsulation also very useful for the commercial products. The antibacterial properties of the rhizome *Z. cassumunar* Roxb. is particularly relevant to their commercial use as a major ingredient in the traditional herbal drink known as 'Jamu'. Accordingly, this implies the inhibition of agricultural and foodborne pathogens. In the agriculture, microencapsulated (crude extract, purified compound 1, and purified compound 2) of the rhizome *Z. cassumunar* Roxb. potentially used as natural materials to against serious diseases, such as rice bacterial blight caused by *X. oryzae*, bacterial leaf streak caused by *X. translucens*, bacterial leaf spot caused by *Pseudomonas* spp, and bumblefoot in chickens caused by *S. aureus*. These microencapsulated compounds also potentially use for food preservation to

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against foodborne pathogens, including *E. coli*, *S. typhimurium*, *B. cereus*, and *L. monocytogenes.*

4. Conclusions

The results of this study suggest that the phytochemical compounds of the rhizome *Z. cassumunar* Roxb. have potent antibacterial properties that may useful in many applications, including agricultural plant health, food preservation, natural therapies, and pharmaceuticals. However, further studies are needed to extract, isolate, purified, and characterize the phytochemical compounds in the rhizomes of *Z. cassumunar* Roxb. and study their action mechanisms to develop new antibacterial materials to against agricultural and foodborne pathogens.

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