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Antimicrobial activity of *Kaempferia galanga* against plant pathogen on rice

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Abstract. *Suharti WS, Tini EW, Istiqomah D. 2023. Antimicrobial activity of* Kaempferia galanga *against plant pathogen on rice. Biodiversitas 24: 1320-1326. Rhizoctonia solani* J.G.Kühn and *Xanthomonas oryzae* are two main pathogens in rice plants that cause sheath blight and bacterial leaf blight disease. Controlling these two diseases using plant extracts is an alternative, environmentally friendly method. Plant extracts are known to have the ability to inhibit microbial growth. The purpose of this study was to determine the ability of *Kaempferia galanga* L. (aromatic ginger) extract to inhibit the growth of *R. solani* and *X. oryzae* in-vitro and to determine the antimicrobial compounds contained in the extract. First, *K. galanga* was extracted using a maceration technique with 96% ethanol. Next, followed by an antifungal test on *R. solani* with a diffusion well technique and an antibacterial test with a disc agar technique against *X. oryzae* using two types of solvents (water and 96% ethanol) to obtain a concentration gradient (2, 4, 6, 8, and 10%). Then, the content of *K. galanga* compounds was examined using GC-MS. The results showed that *K. galanga* could inhibit the growth of pathogenic fungi and bacteria, as indicated by the formation of an inhibition zone. Chemical compounds such as 2-Propenoic acid, 3-(4-methoxyphenyl)-, and ethyl ester are the dominant compound in *K. galanga*. In addition, several compounds with antimicrobial activity were found in the *K. galanga* extract, including Germacrene-D, 1,8-Cineol, Borneol, Caryophyllene, Jasmone, and Heptadecane.

Keywords: Chemical compound, *Kaempferia galanga*, *Rhizoctonia solani*, *Xanthomonas oryzae*

INTRODUCTION

Rice is a staple food for several countries in the world, especially Asia. The rice demand in the Asian region is known as the highest in the world due to the people's preference for rice (Mohanty 2013). Efforts to increase production have been carried out, including innovation in cultivation technology such as a new superior varieties technology, planting technology, integrated planting calendar, and integrated crop management (Faisal et al. 2019). However, the presence of plant diseases is a limiting factor in cultivation influencing rice production.

Sheath blight disease is one of the plant diseases that can affect the quality and quantity of rice yield. According to Nuryanto (2018), sheath blight disease is an important disease of rice plants that can potentially be economically detrimental. The fungus *Rhizoctonia solani* AG1-1A J.G. Kühn causes sheath blight disease which develops in subtropical and tropical areas (Ramos-Molina et al. 2016). Sheath blight disease was first reported in Japan in 1910 and has spread worldwide. Yield losses due to sheath blight disease can reach more than 50% in susceptible varieties and suitable environmental conditions (Milati et al. 2021). Initial symptoms due to *R. solani* infection are greenish-gray and water-soaked lesions on the leaf sheath. Symptoms may develop in the presence of grayish-white lesions with dark brown edges. The lesions enlarge, coalesce, and cover the stem. The leaf sheath turns yellow, wilted, and rots; then, whole leaves may dry up (Singh et al. 2016).

Another disease in rice plants is bacterial leaf blight. This disease is caused by *Xanthomonas oryzae*. Bacterial pathogens infect at all stages of growth, from seeding to harvesting through wounds on the leaf surface or through natural holes, such as stomata (Kadir 2011). Infections caused by bacterial leaf blight can decrease yield by up to 12%, whereas in severe conditions, it can suppress rice production by up to 50% (Muneer et al. 2007). In addition, *X. oryzae* infection produces lesions with wavy margins, which appear from the leaf tips. The lesions will enlarge, turn yellow, and can cause plant death (Nino-Liu et al. 2006).

Several control techniques can be applied to control these diseases, such as biological control, inducing plant resistance, and cultural control (Yasmin et al. 2017; Bahtiar et al. 2021, Suharti and Leana 2021; Adnan et al. 2022). However, the general control carried out by farmers is using synthetic pesticides. The application of synthetic pesticides has high effectiveness but poses risks. Synthetic chemical control that is commonly used negatively impacts plants, human health, and the environment (Nicolopoulou-Stamati et al. 2016). Bio-pesticides from plant materials can potentially be developed as a control technique. In addition to being environmentally friendly, plant materials are inexpensive, easy to obtain, and do not leave residues on plants.

The plant that has the potential to be used as bio-pesticides from the Zingiberaceae group is *Kaempferia*

galanga (aromatic ginger*). Kaempferia galanga*, a tropical plant that grows in many areas in Indonesia, is known as a medicine for treating various health problems due to its compounds. *Kaempferia galanga* contains essential oils that function as antimicrobials. In addition, *K. galanga* rhizome contains several active compounds, including: alkaloids, saponins, flavonoids, steroids, quinones, polyphenols, tannins, monoterpenes, and sesquiterpenes (Hasanah et al. 2011; Latifah 2015).

The ability of *K. galanga* extract as an antimicrobial against human and animal pathogens is well known (Fahrinda et al. 2018; Alsalhi et al. 2020). However, the role of *K. galanga* extract against plant pathogens is limited and needs to be developed. Therefore, research on the antimicrobial activity of *K. galanga* plant extract in suppressing the growth of the fungus *R. solani* and the bacteria *X. oryzae* in vitro is necessary. In addition, this study also examined the potential of *K. galanga* as an antimicrobial compound by determining the chemical compounds by GC-MS.

MATERIALS AND METHODS

The research was conducted at the Laboratory of Plant Protection, Faculty of Agriculture, Universitas Jenderal Soedirman. The search for isolates of *R. solani* and *X. oryzae* around Banyumas, Central Java, Indonesia preceded it. Furthermore, the *K. galanga* used was the variety of Galesia 1, organically grown in Cipanas, West Java, Indonesia

Kaempferia galanga extract preparation

The *K. galanga* extract preparation was used for the antimicrobial test and GC-MS analysis, followed by extract preparation for phytochemical screening carried out by Mohammed et al. (2023) with modifications. First, the rhizome of the *K. galanga* plant was washed under running water, then sliced and dried until the water content of the rhizome was stable. Next, moisture content was measured using a moisture meter with a moisture value of less than 10%. Then, the rhizome of *K. galanga* was crushed to make powder with a grinder machine and sieved through a 40-mesh sieve for further storage in glass jars.

The *K. galanga* powder was macerated using 96% 1: 10 (w/v) ethanol as solvent. The maceration solution was allowed to stand for three days, filtered using Whatman filter paper no 2, and evaporated with a rotary evaporator at a temperature of 78°C for 1 hour to produce a thick extract. According to Mekala et al. (2022), a temperature around 78°C is the boiling point of ethanol. Therefore, the temperature setting on the rotary evaporator aligned with the solvent boiling point is expected to separate the solvent from the extract completely. Research by Sánchez et al. (2019) explained that a rotary evaporator is used to manufacture solvent-free extract by separating the solvent and extract. After maceration, filtering, and evaporation with a rotary evaporator, the *K. galanga* extract produced as much as 44.4 g of concentrated extract. The thick extract of *K. galanga* rhizome was stored in bottles in a refrigerator at 5°C until used. The extract was then made in various concentrations with water and 96% ethanol as solvents. Both solvents are commonly used in the extraction process to obtain secondary metabolites from plant extracts (Sultana et al. 2009; Reis et al. 2012).

Antifungal activity against *Rhizoctonia solani*

The antifungal activity test used the well-diffusion method according to Magaldi et al. (2004) with modifications. Cultures of *R. solani* were taken and placed in the middle of a petri dish containing 10 mL of Potato Dextrose Agar (PDA), which had been solidified. Four holes were made around the culture using a cork borer. The holes were filled with 20µL of *K. galanga* plant extract solution with a concentration of 2, 4, 6, 8, 10% dissolved in sterile water and *K. galanga* plant extract with a concentration of 2, 4, 6, 8, 10% dissolved in 96% ethanol. Cultures were incubated for two days, and zones of inhibition around the wells were observed.

Antibacterial activity against *Xanthomonas oryzae*

The antibacterial activity was conducted by the disc diffusion method, according to Bakht et al. (2011), with modifications. First, Nutrient Agar (NA) media was heated until melted and poured into a 10 mL petri dish. Next, the inoculum of *X. oryzae* bacteria cultured in an NA medium was diluted. A total of 100 L of the diluted *X. oryzae* culture was taken and poured over the solid NA, leveled with an L glass. Next, Whatman filter paper No. 1 was shaped into a circle with a perforator and immersed in various concentrations of *K. galanga* extract. Next, the Whatman paper was placed in a petri dish containing NA which had been inoculated with *X. oryzae*. Finally, the cultures were incubated for 1 x 24 hours**.**

The content of chemical compounds of *Kaempferia galanga* extract with GC-MS

The *K. galanga* extract's chemical compounds were analyzed using GC-MS Shimadzu QP2010SE. Samples were taken from plant extracts that had been macerated and evaporated with a rotary evaporator to obtain a concentrated extract, according to Mohammed et al. (2023). Furthermore, 96% ethanol extract was added to make a 6% concentration, followed by centrifugation at 9,000 rpm for 3 minutes. Finally, the 0.1 µL supernatant was injected into GC-MS. The injection time was 70 minutes with a temperature of 250°C injector, 260°C detector, and 100°C column. The carrier gas in this study was helium with a pressure of 73.1 kPa, with a constant flow rate of 1 mL/minute. The chemical compound was identified based on retention time, peak area, height, and mass spectral patterns with the database from Wiley 229, NIST 12, and NIST 62.

RESULTS AND DISCUSSION

Antifungal activity against *Rhizoctonia solani*

The antifungal test of *K. galanga* extract against *R. solani* shows that *K. galanga* extract can inhibit *R. solani*. That was indicated by forming an inhibition zone around the well-diffusion containing 20 µL of *K. galanga* extract. In treating various concentrations of *K. galanga* extract with water as a solvent, the higher the concentration, the greater the inhibition formed. Water is a suitable polar solvent for extracting medicinal plants (Abubakar and Haque 2020). The zone of inhibition in treating various concentrations with water solvent was still formed. That indicates the *K. galanga* extract has an antifungal ability against fungal pathogens. The diameter of the highest inhibition zone was in the treatment of 10% *K. galanga* extract concentration in water solvent (Figure 1).

In the treatment of extract with ethanol solvent, increasing the concentration of *K. galanga* extract increased its inhibitory ability against pathogenic *R. solani*. However, the 2% *K. galanga* extract treatment with ethanol solvent had a higher inhibition than the ethanol solvent *K. galanga* extract with 4 and 6% concentrations. It suggested that the amount of ethanol in the 2% *K. galanga* extract treatment is higher than in other treatments. Ethanol can affect the development of pathogens by reducing microbial growth (Yan et al. 2015).

In the inhibition zone observation, the *K. galanga* dissolved in ethanol had a higher inhibitory ability than the extract dissolved in the water. Therefore, ethanol is a good solvent and filter for the extraction method. Furthermore, according to Widyaningrum et al. (2020), ethanol is suitable for extraction due to its ability to dissolve natural ingredients. Therefore, the *K. galanga* extract can release several active compounds which have antimicrobial activity.

Kaempferia galanga extract inhibits the growth of various plant pathogenic fungi. This follows the research conducted by Yendi et al. (2015), which stated that *K. galanga* extracts with a concentration of 10% (v/v) was effective in suppressing the growth and development of the fungus *Colletotrichum musae* (Berk. & M.A.Curtis) Arx in vitro. Another study stated that *K. galanga* extract was effective in inhibiting the growth of the fungus *Phytophthora infestans* (Mont.) de Bary in vitro (Simanjuntak 2018). In addition, *K. galanga* extract has a fungicidal activity to kill the vegetative forms of the fungus *Fusarium oxysporum* Schltdl. (Dissanayake 2014).

Antibacterial activity of the *Kaempferia galanga* extract against *Xanthomonas oryzae*

Inhibition zones in the treatment of *K. galanga* extract against *X. oryzae* were formed at all treatment concentrations with two different types of solvents. Water and ethanol used as solvents could influence the ability of the *K. galanga* extract to produce inhibition zones. Based on the results of this study, it was found that the higher the concentration of *K. galanga* extract, the larger the inhibition zone formed. For example, the diameter of the highest inhibition zone was shown at 10% concentration treatment with 96% ethanol solvent (Figure 2). Therefore, it is suggested that *K. galanga* extract has antibacterial compounds that can inhibit the growth of plant pathogenic bacteria. Moreover, *K. galanga* has been widely used as a plant material containing antibacterial compounds that control pathogens in humans and food (Fahrinda et al. 2018; Wang et al. 2020).

Chemical components of *Kaempferia galanga* extract and potential as antimicrobial

Analysis using GC-MS produced a chromatogram, as shown in Figure 5. GC-MS is a gas chromatographic analysis technique that can separate and analyze a mixture of several chemical components in a sample. The results are chromatograms and mass spectra of the compounds with the lowest concentrations (Al-Rubaye et al. 2017). More than 700 chemical compounds were detected.

The most dominant compound in *K. galanga* extract dissolved in 96% ethanol were 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester at a retention time of 26.22 minutes with an area percentage of 78.46%. The next largest components were Pentadecane with an area percentage of 9.02%, 2-Propenoic acid 3-phenyl-, ethyl ester with an area percentage of 2.5%, and 7-Heptadecene, 1-chloro- with an area percentage of 1.62% (Figure 3)

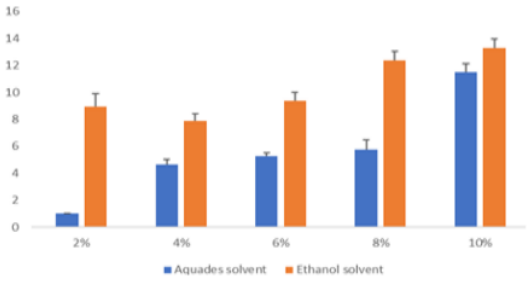

Figure 1. Diameter of inhibition zone of *Kaempferia galanga* extract against *Rhizoctonia solani* (mm)

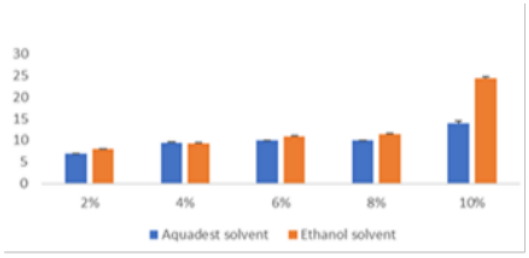

Figure 2. Diameter of inhibition zone of *Kaempferia galanga* extract against *Xanthomonas oryzae* (mm)

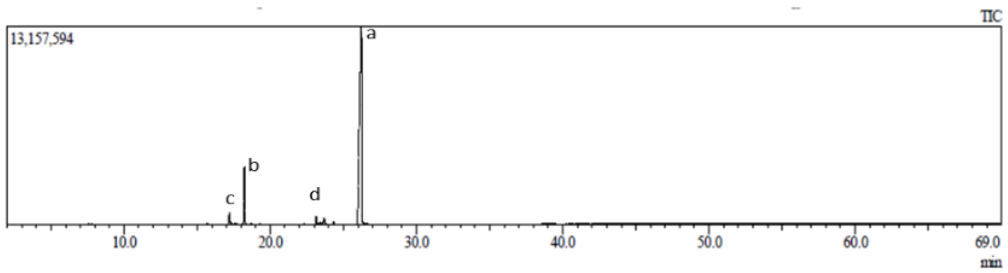

Figure 3. Chromatogram analysis of *Kaempferia galanga* extract with GC-MS a. 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester; b. pentadecane; c.2-Propenoic acid 3-phenyl-, ethyl ester, d. 7-Heptadecene, 1-chloro-

The compound 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester is the main compound found in *K. galanga* rhizome oil (Bhuiyan et al. 2008). The formula for 2-Propenoic acid, 3-(4-methoxyphenyl), ethyl ester is C14H18O4 with a chemical structure based on Pubchem (2022), as shown in Figure 4. Based on research by Shamsol et al. (2021), in addition to 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester, the content of *K. galanga* rhizome is dominated by ethyl cinnamate. Although, this Shamsol's research is different from the results of this research that has been done. It is suspected that samples are present in rhizome oil. Therefore, the ethanol maceration technique gave different results on reading the GC-MS chromatogram.

The pentadecane compound with a percentage area of 9.02% found due to GC-MS analysis of *K. galanga* rhizome is a volatile oil component with the formula C15H32. Pentadecane is an antimicrobial compound that can suppress fungal growth (Hussain et al. 2017). The compound 2-Propenoic acid 3-phenyl-, ethyl ester has the formula C11H12O2 with the chemical structure shown in Figure 4. The synonym of the compound 2-Propenoic acid 3-phenyl-, ethyl ester is ethyl cinnamate, an ester of cinnamic acid and ethanol. According to Belgis et al. (2021) and Shamsol et al. (2021), ethyl cinnamate compound is a major compound in *K. galanga*, which is volatile. Furthermore, Belgis et al. (2021) stated that these compounds have antibacterial properties that can suppress bacterial growth. Another compound with a fairly high area percentage is 7-Heptadecene, 1-chloro, with a chemical structure, as listed in Figure 6. The molecular formula of the compound 7-Heptadecene, 1-chloro is C17H33Cl (Pubchem 2022).

Several other compounds are also found in *K. galanga*. Despite these compounds' low abundance, characterized by a low peak percentage, these compounds have antimicrobial activity. These compounds include Germacrene-D, 1,8-Cineol, Borneo L, trans-Caryophyllene, z-Jasmone, and Heptadecane (Table 1). The chemical structure of these compounds is shown in Figure 5 Germacrene-D is an organic compound found through GC-MS analysis on several herbs such as *Origanum vulgare* subsp. *vulgare* and *Cosmos bipinnatus* Cav. (Şahin et al. 2004; Olajuyigbe and Ashafa 2014). The Germacrene-D compound belongs to the class of sesquiterpenoid germacrene, which has antibacterial properties (Zamora et al. 2018).

The 1,8-Cineol compound known as eucalyptol is the dominant compound in essential oils, such as eucalyptus oil (Moo et al. 2021). This compound can be antimicrobial and synergize to increase the antimicrobial activity of the Chlorhexidine gluconate on several pathogens that infect humans, such as *Staphylococcus aureus* Rosenbach, *Pseudomonas aeruginosa* A, *Escherichia coli* E, *Klebsiella pneumoniae* (Schroeter) Trevisan, *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Bälz and *Candida albicans* (C.P.Robin) Berkhout (Şimşek and Duman 2017).

Borneol and its derivatives are compounds in many Chinese and Japanese traditional medicines. Besides as a disinfectant, Borneol has antibacterial activity. The ability as an antibacterial was known when Borneol was tested by the disc diffusion method on a nutrient agar medium to suppress the development of *C. albicans* (Farhan et al. 2011). Trans-Caryophyllene compounds are abundant volatile compounds found in several types of essential oils. For example, Abdullahi et al. (2020) research shows that the abundance of trans-Caryophyllene in domestic ginger essential oil is 9.64%. The trans-Caryophyllene existence and the abundance of other compounds, such as Eucalyptol and Borneol, promote domestic ginger essential oils as fungicides and bactericides. In addition, the Eucalyptol and Borneol compounds can suppress the growth of phytopathogens.

Based on research by Galovičová et al. (2022), z-Jasmone is the fourth most abundant compound in the essential oil of *Jasminum grandiflorum*. It is noted that 5% of z-Jasmone is contained in *J. grandiflorum* essential oil. On the other hand, the antimicrobial activity test conducted in the study showed the presence of antibacterial ability against several gram-positive and negative bacteria. Heptadecane is a straight-chain alkaline with 17 carbon atoms. This compound is found in several plants, including *Curcuma amada* Roxb., *Cinnamomum verum* J.Presl, and *Alpinia conchigera* Griff. (Pubchem 2022). Heptadecane belongs to the hydrocarbon group and has been reported to have antimicrobial activity (Naeim et al. 2020).

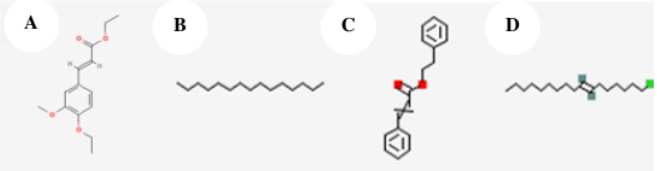

Figure 4. Chemical structure of several dominant compounds found by GC-MS analysis; A. 2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester; B. pentadecane; C. 2-Propenoic acid 3-phenyl-, ethyl ester, D. 7-Heptadecene, 1-chloro-(Pubchem 2022)

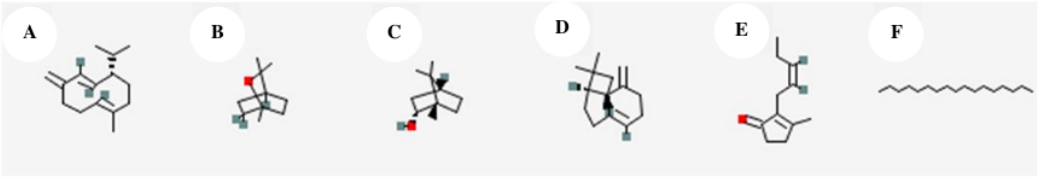

Figure 5. Chemical structure of compound found by GC-MS analysis with antimicrobial activity; A. Germacrene-D, B. 1,8-Cineol, C. Borneol, D. Caryophyllene, E. Jasmone, F. Heptadecane (Pubchem 2022)

Table 1. Compounds found in *Kaempferia galanga* by GC-MS analysis

Compounds	RT	Area %
2-Propenoic Acid, 3-(4-Methoxyphenyl)-, Ethyl Ester	26.217	78.46
Pentadecane (CAS)	18.221	9.02
2-Propenoic Acid, 3-Phenyl-, Ethyl Ester (CAS)	17.203	2.5
7-Heptadecene, 1-Chloro- (CAS)	23.651	1.61
Heptadecane	24.33	0.45
Cis-7-Tetradecen-1-Ol	23.399	0.33
1-Pentadecene (Cas)	17.54	0.14
Naphthalene, 1,2,3,4,4a,5,6,8a-Octahydro-7-Methyl-4-Methylene-1-(1-Methylethyl)-, (1.Alpha.,4a.Beta.,8a.Alpha.)-	18.724	0.11
Triquinacene, 1,4-Bis(Methoxy)-	22.313	0.11
Trans-Caryophyllene	15.662	0.1
1-Tetradecen-3-Yne	23.524	0.1
Germacrene-D	19.291	0.09
Borneol L	7.6	0.07
3-Cyclohexen-1-Ol, 4-Methyl-1-(1-Methylethyl)- (CAS)	7.797	0.06
(-)-.Beta.-Elemene	14.62	0.05
Hexadecanoic Acid, Methyl Ester (CAS)	30.494	0.05
.Alpha.-Gurjunene (Cas)	15.113	0.04
2-Allyl-1,4-Dimethoxy-3-Methyl-Benzene	21.125	0.04
1,8-Cineole	4.135	0.03
Z-Jasmone	7.984	0.03
(Z)-3-Phenyl-2-Propenoic Acid, Methyl Ester	14.476	0.03
African-2(6)-Ene	15.589	0.03
Cyclodecene	17.62	0.03
(-)-.Alpha.-Panasinsen	18.925	0.03
.Beta.-Selinene (Cas)	19.047	0.03
Cyclododecyne	23.985	0.03
Pregn-5-En-20-One, 3-(Acetyloxy)-16-Methoxy-, (3.Beta.,16.Alpha.)- (CAS)	29.186	0.03
(7R)-Trans-Syn-Cis-Tricyclo[7.3.0.0(2,6)]Dodecan-7-Ol	29.22	0.03
Decanal (CAS)	29.396	0.03
Triquinacene, 1,4-Bis(Methoxy)-	32.16	0.03

In conclusion, various concentrations of *K. galanga* extract with water and 96% ethanol as solvents were able to inhibit the growth of rice plant pathogens, *R. solani* and *X. oryzae*. The highest inhibition diameter was in the treatment of 10% *K. galanga* extract with an ethanol solvent. The main chemical compounds of *K. galanga* extract were 2-propenoic acid, 3-(4-methoxyphenyl)-. ethyl ester, pentadecane, 2-propenoic acid 3-phenyl-, ethyl ester, and 7-heptadecene, 1-chloro. The compounds with antimicrobial activity were found in the *K. galanga* extract, i.e., germacrene-d, 1,8-cineol, borneol, caryophyllene, jasmone, and heptadecane.

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