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Short Communication: In vitro antibacterial activity of essential oils from twelve aromatic plants from East Kalimantan, Indonesia

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Abstract. *Kuspradini H, Putri AS, Egra S, Yanti. 2019. Short Communication: In vitro antibacterial activity of essential oils from twelve aromatic plants from East Kalimantan, Indonesia. Biodiversitas 20: 2039-2042.* In the aim of this works was to investigate the antibacterial activity of twelve essential oils against *Streptococcus mutans* and *Streptococcus sobrinus*, oral pathogen causing dental caries. The essential oils were collected by a steam distillation method. Refractive index was measured by using a refractometer. The antibacterial activity of essential oils was determined using an agar well diffusion method. The yields of essential oils varied with the plant species. The steam distillation yielded clear to yellowish essential oils. Refractive indexes of oil were found to be in the range of 1.344 to 1.500. *Syzigium* sp. appeared to be more abundant in oil (1.54%) than the other plant species, while *C. odorata* was poorer (0.04%). All of the essential oils tested showed a varied level of inhibition zone (0-53.65 mm) against *S. mutans* and *S. sobrinus*. The oil from leaves of *Cymbopogon citratus* had the highest activity against *S. sobrinus* and *S. mutans* (53.15 and 52.85 mm, respectively). On the other hand, the *Magnolia x alba* oil showed the lowest activity against *S. mutans* and *S. sobrinus* (10.50 and 11.65 mm, respectively). The research results demonstrated that the essential oil in this study has the potency for development of dental health products for preventing and treating oral infections.

Keywords: Essential oils, *Streptococcus mutans*, *Streptococcus sobrinus*, East Kalimantan

INTRODUCTION

Essential oil is one of the natural ingredients that can be used to treat bacterial infections. Essential oils from several plants were able to control the growth of microorganisms causing skin infections, dental caries, and food spoilage. Essential oils have a potential application in the management of dental caries (Lang and Buchbauer 2012; Lobo et al. 2014). Several oral products have incorporated essential oil-derived antimicrobials such as eucalyptus, lavandula, and rosmarinus (Allaker et al. 2009). People have traditionally used essential oils such as calamus, citronella, clove, eucalyptus, lemongrass, lime, cananga (ylang-ylang), piper for various purposes in different parts of the world.

Acorus calamus has many biological activities as insecticidal, antifungal, antibacterial properties (Phongpaichit et al. 2005). The essential oils of *A. calamus* have been found to possess an antibacterial activity (Şerban et al. 2015; Kasture et al. 2015). Various ethnomedicinal and ethnobotanical uses have been ascribed to the rhizomes of the plant (Manikandan et al. 2010). Cloves (*Syzygium aromaticum* (Linn.) (Syn. *Eugenia aromatica*) have been used by humans for medicinal applications to alleviate toothache (Chaieb et al. 2007; Hema et al. 2010; Duke 1985). In Thailand and Indonesia, *Zingiber cassumunar* has been used as traditional medicine (Waranee et al. 2012). In Indonesia, *Z. cassumunar* has been traditionally used to relieve colic in children (Ong 2008). The young shoots of *Macaranga triloba*, *M. pruinosa*, and *M. gigantea* are used to treat fungal infections, while decoctions of their leaves are known to treat stomach aches (Fiala et al. 1989; Grosvenor et al. 1995). *Cananga odorata* (ylang-ylang) oil can be found in various cosmetic and households products such as the massage oils, moisturizing creams, perfumes, and even scented candles, and also safety as a food ingredient. Ylang-ylang essential oils have been investigated covering the antibacterial, antifungal, amebicidal, and cytotoxic activities (Hern et al. 2015; Burdock and Carabin 2008; Saedi and Crawford 2006). *Eucalyptus deglupta* is used to a limited extent for firewood and charcoal. The genus *Eucalyptus* essential oils are widely used all over the word as spices, flavors, perfumes industrial raw materials, and pharmaceutical (Dellacassa et al. 1990; Boland et al. 1991). The essential oil of *Citrus hystrix* has been used as flavor and fragrance agents, as well as in perfumery and medicinal preparation (Doreen et al. 2011). Lime oil from Indonesia contains abundant bioactive compounds and shows antimicrobial and antioxidant activity (Lawrence et al. 1971).

Even though, there are several studies on antimicrobial activity of these plant species in Indonesia, studies on the essential oils of different species from a specific part of the plant in East Kalimantan have not been extensively

conducted. This study was done to investigate and compare the antibacterial activity of the essential oil derived plants locally available in East Kalimantan against oral bacteria and to search for the most effective essential oil from these plants.

MATERIALS AND METHODS

Study area

Leaves of aromatic plants were collected from Samarinda, East Kalimantan. *Magnolia x alba, Acorus calamus, Zingiber cassumunar, Piper odorata, Syzygium aromaticum, Eucalyptus deglupta, Macaranga gigantea, Cananga odorata, Syzygium* sp., *Citrus hystrix, Cymbopogon nardus, Cymbopogon citratus* were the selected aromatic plants.

Procedures

Steam distillation method

The essential oils extraction process was conducted using the steam distillation method (Kuspradini et al. 2016). The result of the steam distillation process was two layers solution containing oil and water. The oils were collected and separated using the separatory funnel. Na_2SO_4 was added to the oils and incubated for 24 hours to get pure essential oils. The volume of oils was presented according to the yield.

Antibacterial assay

The antibacterial activity was assayed by the agar diffusion method (Kuspradini et al. 2016) and replicated two times. *Streptococcus mutans* and *Streptococcus sobrinus* were used in this study. Sterilization of all used materials was conducted by using an autoclave at 121ºC for 15 minutes. Bacteria were cultured in sterile nutrient agar (NA) and incubated at 37ºC for 24 hours. 25 µl of the bacterial suspension was then spread all over the surface of solid media plates three times by sterile swab. After that, wells were made by using a sterile cork borer of 6 mm in diameter. Twenty microliters of the pure essential oils were added into the wells, then were incubated at 37˚C for 18-24 hours. In this study, Chlorhexidine was used as an antibiotic for positive control at the concentration of 10µg $well^{-1,}$ and acetone was used as a negative control. The negative control showed no inhibitions in preliminary studies. After incubation, the plates were observed for the formation of a clear inhibition zone around the well, indicating the presence of antibacterial activity. The Inhibition Zone (IZ) was determined by measuring the diameters of the inhibition zone around the well (in mm, taken with a ruler), and the Activity Index (AI) was calculated using the formula below:

Activity Index = (Inhibition Zone of essential oil sample/Inhibition Zone of antibiotic)

Data analysis

The results of steam distillation, including yields and color of oil, were presented in Tables and Figure. The antibacterial assay was made in duplicates, and the data were reported as mean ± SD for (n = 1 x 2) presented in Tables 1, 2, and 3.

RESULTS AND DISCUSSION

All essential oils were obtained from steam distillation process. The yields of essential oils varied with plant species. The steam distillation yielded clear and yellowish essential oils (Figure 1). Refractive indexes of oil were found to be in the range of 1.340 to 1.500. The results of the yields and refractive indexes are presented in Table 1. *Syzigium* sp. appeared to be more abundant in oil (1.54 %) than the other plant species, while *C. odorata* was poorer (0.04%). The Syzygium species of the family Myrtaceae are well known for their aromatic nature. In this study, several species of the family Myrtaceae had a higher yield (0.22-1.54%) compared to that obtained by Rameshkumar et al. (2015) and Muthumperumal et al. (2016). The leaf essential oil yield was between 0.10-0.33%. Our results indicated that the species of the family Myrtaceae in East Kalimantan had oil yields higher than previously reported.

In the distillation process, filling of the material into the kettle should be arranged in such a way that steam can penetrate and be flattened in the material so that it would produce more yield of oil (Guenther 2006). The refractive index of Zingiber oil (1.500) was higher than the other oils in this study, while the lowest was Magnolia oil (1.340). Sukatta et al. (2009) reported that the range of refractive indexes of *Z. cassumunar* rizhome oil was between 1.5169-1.5386. The refractive index value can be known from the presence of water contained in oil; the higher the water content in oil, the smaller the refractive index. Therefore, higher refractive index values in essential oils are better than lower ones (Sastrohamidjojo 2004).

Two bacteria were investigated for their susceptibility against the essential oils. Although the inhibitory zone of the sample has a lower value than the positive control, it does not mean that the sample has no antimicrobial activity. The clear zones appear on all samples tested in different size (pictures not shown). The clear zone or inhibition zone is the area around the well-containing sample where the bacterial growth is not visible. Tables 2 and 3 showed that all of the essential oil samples tested in this study had the potency to suppress the growth of *S. mutans* and *S. sobrinus*. The inhibition zone range of the 12 essential oils in this study against *S. mutans* and *S. sobrinus* were 10.50-53.65 mm and 11.65-53.15 mm, respectively.

Figure 1. Varying color of essential oils extracted from various plant species: (1) *M. alba*, (2) *A. calamus*, (3) *Z. cassumunar*, (4) *P. odorata*, (5) *S. aromaticum*, (6) *E. deglupta*, (7) *M. gigantea*, (8) *C. odorata*, (9) *Syzygium* sp., (10) *C. histrix*, (11) *C. nardus*, (12) *C. citrates*.

Table 1. The plant species, family, oil yield, and refractive index of extracted oils obtained in the present study.

Plant species	Family	Yield %)	Color	Refractive index
Magnolia x alba	Magnoliaceae	0.28	Orange	1.344
Acorus calamus	Acoraceae	0.35	Brownish Yellow	1.482
Zingiber cassumunar	Zingiberaceae	0.25	Yellow	1.500
Piper odorata	Piperaceae	0.06	Colorless	1.492
Syzygium aromaticum	Myrtaceae	0.22	Yellow	1.459
Eucalyptus deglupta	Myrtaceae	0.40	Yellow	1.485
Macaranga gigantea	Euphorbiaceae	0.34	Colorless	1.369
Cananga odorata	Annonaceae	0.04	Pale Yellow	1.496
Syzygium sp.	Myrtaceae	1.54	Pale Yellow	1.485
Citrus hystrix	Rutaceae	1.26	Yellow	1.452
Cymbopogon nardus	Poaceae	0.26	Pale Yellow	1.367
Cymbopogon citratus	Poaceae	0.46	Yellow	1.385

Table 2. Inhibition zone and activity indices of pure essential oils against *S. mutans*

Plant species (sample)	Inhibition zone (mm)		Activity index
	Sample	Positive control *)	
Magnolia x alba	10.50 ± 0.28	16.70 ± 11.81	0.63 ± 0.02
Acorus calamus	14.65 ± 0.49		0.88 ± 0.03
Zingiber cassumunar	18.65 ± 6.15		1.12 ± 0.37
Piper odorata	20.85 ± 5.87		1.25 ± 0.35
Syzigium aromaticum	22.15 ± 1.20		1.33 ± 0.07
Eucalyptus deglupta	23.35 ± 0.49		1.40 ± 0.03
Macaranga gigantea	23.65 ± 0.92		1.42 ± 0.06
Cananga odorata	43.50 ± 9.19		2.60 ± 0.55
Syzygium sp.	50.85 ± 1.20		3.04 ± 0.07
Citrus hystrix	51.70 ± 0.00		3.10 ± 0.00
Cymbopogon nardus	52.15 ± 0.21		3.12 ± 0.01
Cymbopogon citratus	52.85 ± 1.63		3.16 ± 0.10

* Chlorhexidine (10μg ml^{-1}) was used as a positive control.

Table 3. Inhibition zone and activity indices of pure essential oils against *S. sobrinus*

Plant species (sample)	Inhibition zone (mm)		Activity index
	Sample	Positive control *)	
Magnolia x alba	11.65 ± 0.49	17.70 ± 0.00	0.66 ± 0.03
Macaranga gigantea	13.50 ± 0.71		0.76 ± 0.04
Acorus calamus	17.80 ± 2.12		1.01 ± 0.12
Piper odorata	18.35 ± 0.92		1.04 ± 0.05
Syzigium aromaticum	20.00 ± 0.00		1.13 ± 0.00
Eucalyptus deglupta	34.50 ± 0.28		1.95 ± 0.02
Zingiber cassumunar	47.65 ± 7.57		2.69 ± 0.43
Syzygium sp.	50.00 ± 0.00		2.82 ± 0.00
Cananga odorata	51.30 ± 2.83		2.90 ± 0.16
Citrus hystrix	52.35 ± 0.49		2.96 ± 0.03
Cymbopogon nardus	52.50 ± 0.28		2.97 ± 0.02
Cymbopogon citratus	53.15 ± 0.21		3.00 ± 0.01

*Control positive in this study was Chlorhexidine (10 μg ml^{-1})

Measurement of the activity index was necessary to estimate whether the potential antimicrobial activity comparable to a positive control (antibiotic standard). Samples that have an activity index value of 1.00 mean the activity is the same as a positive control. Several essential oils in this study have an activity index higher than one, indicating that these oils exhibited higher antibacterial activity against *S. mutans* and *S. sobrinus* than the chlorhexidine (positive control) which is commonly used for antiseptic and dental setting.

In this study, *Cymbopogon citratus* oil showed the highest activity against *S. mutans* (52.85 ± 1.63 mm) and also against *S. sobrinus* (53.15 ± 0.21). The activity indices of essential oils extracted from *Z. cassumunar, P. odorata, S. aromaticum, E. deglupta, M. gigantea, C. odorata, Syzygium* sp., *C. hystrix, C. nardus,* and *C. citratus,* against *S. mutans* were found to be more than one, while that of *A. calamus, P. odorata, S. aromaticum, E. deglupta, Z. cassumunar, Syzygium* sp., *C. odorata, C. hystrix, C. nardus,* and *C. citratus* were more than one against *S.*

sobrinus. these results indicated that these oils are exhibiting higher antibacterial activity than the chlorhexidine (antibiotic) used against bacterial pathogens, causing dental caries.

In the global oral health, dental caries remains one of the most prevalent diseases in spite of significant improvements (Gemert-Schricks et al. 2008). The main bacterial species responsible for lactic acid production and dental caries are *Streptococcus* (*S. sobrinus* and *S. mutans*) and *Lactobacillus* species (Madigan et al. 2003). This study demonstrated that the essential oils inhibited *S. mutans* and *S. sobrinus* growth, but their effectiveness varied. Oladimeji et al. (2001) and Faleiro (2011) mentioned that the chemical composition of the essential oils is strongly connected to their antimicrobial activity, and therefore, related to their composition, configuration, amount and their possible interaction. The hydrophobicity of essential oils increased cell permeability and consequent leaking of cell constituents (Burt 2004).

Based on our findings, *C. nardus* and *C. citratus* were strongly affecting the growth inhibition of *S. sobrinus*. Geranial was identified as the most abundant compound in *C. citratus* while citronellal was in *C. nardus* (Oliveira et al. 2010). These compounds might be attributed to the anti-microbial activity since Geranial (trans-citral, citral A) can act as a bactericidal and fungicidal agent (Cristianne 2008).

In conclusion, this research showed the antibacterial potency of the twelve essential oils from East Kalimantan in dental hygiene. The pure essential oil (20 µl) of *C. odorata, Syzygium sp., C. hystrix, C. nardus, C. citratus, Z. cassumunar, P. odorata, S. aromaticum, E. deglupta* could inhibit both of *S. mutans* and *S. sobrinus* better than chlorhexidine (10 µg), while *M. gigantea* and *A. calamus* could inhibit better than chlorhexidine against one bacteria only: *S. mutans* and *S. sobrinus*, respectively. Future research is still needed to quantify the active phytochemical compounds.

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