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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. *Syzygium* 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, lavender, 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 (Serban 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 (Fia¹ 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 world 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 121°C for 15 minutes. Bacteria were cultured in sterile nutrient agar (NA) and incubated at 37°C 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. *Syzygium* 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.

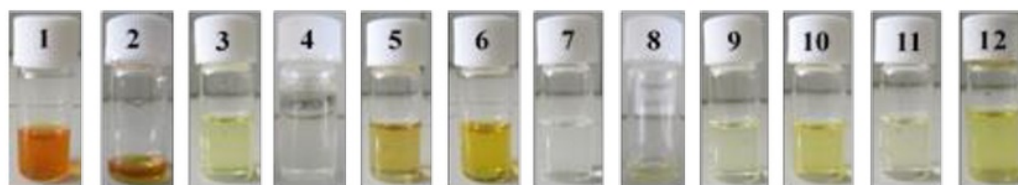


Figure 1. Varying color of essential oils extracted from various plant species: (1) *M. alba*, (2) *A. calamus*, (3) *Z. cassumunar*, (4) *P. odorata*, (5) *S. aromaticum*, (6) *E. deglupta*, (7) *M. gigantea*, (8) *C. odorata*, (9) *Syzygium* sp., (10) *C. hystrix*, (11) *C. nardus*, (12) *C. citratus*.

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
<i>Magnolia x alba</i>	Magnoliaceae	0.28	Orange	1.344
<i>Acorus calamus</i>	Acoraceae	0.35	Brownish Yellow	1.482
<i>Zingiber cassumunar</i>	Zingiberaceae	0.25	Yellow	1.500
<i>Piper odorata</i>	Piperaceae	0.06	Colorless	1.492
<i>Syzygium aromaticum</i>	Myrtaceae	0.22	Yellow	1.459
<i>Eucalyptus deglupta</i>	Myrtaceae	0.40	Yellow	1.485
<i>Macaranga gigantea</i>	Euphorbiaceae	0.34	Colorless	1.369
<i>Cananga odorata</i>	Annonaceae	0.04	Pale Yellow	1.496
<i>Syzygium</i> sp.	Myrtaceae	1.54	Pale Yellow	1.485
<i>Citrus hystrix</i>	Rutaceae	1.26	Yellow	1.452
<i>Cymbopogon nardus</i>	Poaceae	0.26	Pale Yellow	1.367
<i>Cymbopogon citratus</i>	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 *)	
<i>Magnolia x alba</i>	10.50 ± 0.28	16.70 ± 11.81	0.63 ± 0.02
<i>Acorus calamus</i>	14.65 ± 0.49		0.88 ± 0.03
<i>Zingiber cassumunar</i>	18.65 ± 6.15		1.12 ± 0.37
<i>Piper odorata</i>	20.85 ± 5.87		1.25 ± 0.35
<i>Syzygium aromaticum</i>	22.15 ± 1.20		1.33 ± 0.07
<i>Eucalyptus deglupta</i>	23.35 ± 0.49		1.40 ± 0.03
<i>Macaranga gigantea</i>	23.65 ± 0.92		1.42 ± 0.06
<i>Cananga odorata</i>	43.50 ± 9.19		2.60 ± 0.55
<i>Syzygium</i> sp.	50.85 ± 1.20		3.04 ± 0.07
<i>Citrus hystrix</i>	51.70 ± 0.00		3.10 ± 0.00
<i>Cymbopogon nardus</i>	52.15 ± 0.21		3.12 ± 0.01
<i>Cymbopogon citratus</i>	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 *)	
<i>Magnolia x alba</i>	11.65 ± 0.49	17.70 ± 0.00	0.66 ± 0.03
<i>Macaranga gigantea</i>	13.50 ± 0.71		0.76 ± 0.04
<i>Acorus calamus</i>	17.80 ± 2.12		1.01 ± 0.12
<i>Piper odorata</i>	18.35 ± 0.92		1.04 ± 0.05
<i>Syzygium aromaticum</i>	20.00 ± 0.00		1.13 ± 0.00
<i>Eucalyptus deglupta</i>	34.50 ± 0.28		1.95 ± 0.02
<i>Zingiber cassumunar</i>	47.65 ± 7.57		2.69 ± 0.43
<i>Syzygium</i> sp.	50.00 ± 0.00		2.82 ± 0.00
<i>Cananga odorata</i>	51.30 ± 2.83		2.90 ± 0.16
<i>Citrus hystrix</i>	52.35 ± 0.49		2.96 ± 0.03
<i>Cymbopogon nardus</i>	52.50 ± 0.28		2.97 ± 0.02
<i>Cymbopogon citratus</i>	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-Schrieks 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*. Geraniol 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 antimicrobial activity since Geraniol (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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REFERENCES

- Allaker RP, Douglas CWI. 2009. Novel anti-microbial therapies for dental plaque-related diseases. *Intl J Antimicrobial Agents*, 33 (1): 8-13.
- Boland DJ, Brophy JJ, House PN. 1991. Eucalyptus leaf oils, use, chemistry, distillation, and marketing. Inkata Press, Melbourne.
- Burdock GA, Carabin IG. 2008. Safety assessment of Ylang-Ylang (*Cananga spp.*) as a food ingredient. *Food and Chemical Toxicology* 46 (2): 433-445.
- Burt S. 2004. Essential oils: their antibacterial properties and potential applications in food—a review. *Intl J Food Microbiol* 94: 223-53.
- Chaieb K, Hajlaoui H, Zmantar T, Nakbi KAB, Rouabhia M, Mahdouani K, Bakhrout A. 2007. The chemical composition and biological activity of essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. *Phytother Res* 21 (6): 501-506.
- Cristianne D, Silva SG, Vanessa W, Elfrides ES. 2008. Antifungal activity of lemongrass oil and citral against *Candida* spp. *Brazilian J Infect Dis* 12: 63.
- Dellacassa E, Menedez P, Moyna P. 1990. Chemical composition of *Eucalyptus* essential oils grown in Uruguay. *Flavour Fragrance J* 5: 91-95.
- Doreen SH, Rose LC, Suhaimi H, Mohamad H, Rozaini MZH, Tai M. 2011. Preliminary evaluation on the antibacterial activities of *Citrus hystrix* oil emulsions stabilized by tween 80 and span 80. *Intl J Pharm Pharmaceut Sci* 3: 209-211.
- Duke JT. 1985. *Handbook of Medicinal Herbs*. CRC Press, New York.
- Fiala B, Maschwitz U, Tho YP, Helbig AJ. 1989. Studies on a South East Asian ant-plant association: protection of *Macaranga* trees by *Crematogaster borneensis*. *Oecologia* 79: 463-470.
- Grosvenor PW, Supriano A, Gray DO. 1995. Medicinal plants from Riau province, Sumatra, Indonesia. Part 2: antibacterial and antifungal activity. *J Ethnopharmacol* 45 (2): 97-111.
- Guenther E. 2006. *The Essential Oils Vol I*, Translated in Indonesian by S. Ketaren. UI Press, Jakarta. [Indonesian]
- Heri LT, Chan CK, Lee LH, Goh BH. 2015. Traditional uses, phytochemistry, and bioactivities of *Cananga odorata* (ylang-ylang). *Evid-Based Compl Altern Med*. DOI: 10.1155/2015/896314
- Hema R, Kumaravel S, Sivasubramanian C. 2010. Study on the potentials of *Syzygium aromaticum*. *Researcher* 12: 1-4.
- Kasture A, Patel S, Chauhan J, Krishnamurthy R. 2015. *In vitro* antimicrobial effect of essential oil from leaf and rhizome of various accessions of *Acorus calamus* Linn., and its phytochemical screening. *European J Med Plants* 9 (2): 1-13.
- Kuspradini H, Putri AS, Sukaton E, Mitsunaga T. 2016. Bioactivity of essential oils from leaves of *Dryobalanops lanceolata*, *Cinnamomum burmannii*, *Cananga odorata*, and *Scorodocarpus borneensis*. *Agric Agric Sci Procedia* 9: 411-418.
- Lang G, Buchbauer G. 2012. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review. *Flavour Fragrance J* 27: 13-39.
- Lawrence BM, Hogg JW, Terhune SJ, Podimuang V. 1971. Constituents of the leaf and peel oils of *Citrus hystrix* D.C. *Phytochemistry* 10 (6): 1404-1405
- Lobo PL, Fonteles CS, Marques LA, Jamaru FV, Fonseca SG, de Carvalho CB, de Moraes ME. 2014. The efficacy of three formulations of *Lippia sidoides* Cham. essential oil in the reduction of salivary *Streptococcus mutans* in children with caries: A randomized, double-blind, controlled study. *Phytomedicine* 21: 1043-1047.
- Manikandan SR, Sheela D, Srikumar R, Thangaraj R, Ayyappan R, Jegadeesh R, Hariprasath L. 2010. In-Vitro Antibacterial Activity of Aqueous and Ethanolic Extracts Of *Acorus Calamus*. *Intl J Appl Biol Pharmaceut Technol* 1 (3): 1072.
- Muthumperumal C, Stalin N, Das AK, Swamy PS. 2016. Chemical profiling of leaf essential oil, antioxidant, and antibacterial activity of *Syzygium lanceolatum* (Lam.) Wt. & Arn. (Myrtaceae). *Free Radic Antioxidants* 6 (1): 13-22.
- Oliveira MMM, Brugnara D, Cardoso MG, Alves E, Piccoli R. 2010. Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. *Food Control* 21: 549-553.
- Ong HC. 2008. Rempah-ratus: Khasiat makanan dan ubatan. Utusan Publications, Kuala Lumpur. [Malaysian]
- Phongpaichit S. 2005. Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn. *Songklanakarin J Sci Technol* 27: 517-523.
- Rameshkumar KB, Aravind AAP, Vinodkumar TG. 2015. Leaf essential oil composition of six *Syzygium* species from the Western Ghats, South India. *Rec Nat Prod* 9 (4): 592-596.
- Saedi N, Crawford GH. 2006. Botanical briefs: ylang-ylang oil—extracts from the tree *Cananga odorata*. *Cutis* 77 (3): 149-150.
- Sastrohamidjojo H. 2004. *The Essential Oils Chemistry*. Gajah Mada University, Yogyakarta. [Indonesian]
- Serban ES, Ionescu M, Matinca D, Maier CS, Bojita MT. 2011. Screening of the antibacterial and antifungal activity of eight volatile essential oils. *Farmacia* 59: 440-446.
- Waranee P, Sirthunyalug J, Okonogi S. 2012. Comparison of antibacterial activity against food-borne bacteria of *Alpinia galanga*, *Curcuma longa*, and *Zingiber cassumunar*. *Chiang Mai Univ J Nat Sci* 11 (2): 177-186.

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