Chemical compositions and antimicrobial potential of *Actinodaphne macrophylla* leaves oils from East Kalimantan

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Abstract. Essential oils producing plants comprises about 160-200 species, one of which belongs to Lauraceae family. *Actinodaphne macrophylla* is a plant of the Lauraceae family and widely spread on Kalimantan island. For humans, essential oils are used in cosmetics industry, food industry, and pharmaceutical industry. This research aimed to analyze the characteristics of essential oil and potential of antimicrobial activity from *A. macrophylla* leaves oils. Essential oils were obtained by steam distillation method. Antimicrobial activity was assayed using agar diffusion method which compared with two synthetic standards including chlorhexidine and chloramphenicol. Four microorganisms were used in this study were *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus sobrinus*. The obtained oil was determined for its characteristics including the yield, refractive index, and chemical components. The attained components were analyzed using GC-MS. The results of this study showed that essential oils of *A. macrophylla* leaves contained 0.1051% of yield, clearless, and refractive index was 1.425. Based on GC-MS analysis result, it showed chemical components including spathulenol, 2-monopalmitin, (+)-sabinene, copaen, camphene, and β-pinene. This plant potentially can inhibit the growth of *S. aureus*, *C. albicans*, *S. sobrinus*, and *S. mutans* with inhibition zones of 17.22, 20.89, 22.34 and 22.89 mm, respectively.

1. Introduction

Family of Lauraceae contains approximately 45 genera and 2250 species. The genus Actinodaphne belongs to the family Lauraceae. This genus distributes mostly in tropical and subtropical Asia and is an important component of tropical forest. Indonesia, Malaysia, East Asia, and North America (a few) are where the genus mainly distributes, and there are about 70 species of evergreen tree and shurbs there [1]. It is locally known as wuru (Indonesia) or medang kuning and medang kunyit (Malaysia) [2].

Several parts of this genus are pharmaceutically important. The decoction of *A. angustifolia* leaf is used to treat the kidney stones. The seed oil obtained from *A. hookeri* is used in rheumatic pain treatment. The *A. obovata* bark is used to treat fracture. The *A. lancifolia* is very useful for treating arthritis, edema, overexertion, and stomacheache [3,4,5].

The genus Actinodaphne has been reported to contain isoquinoline alkaloids compounds (aporphines, oxoaporphines) [6], lactones [7], lignans [8], and phenolic amides [9]. The alkaloids are important for pharmacologists as antitumor, antibacterial, and antifungal [10]. In some areas, the plant of the genus Actinodaphne is used as an ornamental plant.

Research on the essential oil of family Lauraceae showed that there are several classes of secondary metabolite compounds such alkaloids, phenyl propanoid, flavonoids, 2-pyrron derivatives,

and benzyl-esters [11]. However, there are variations in the components of essential oil derived from different growing sites even within the same species [3].

Infection disseases is one of main health problems in the develop countries include Indonesia. Some infection diseases are invading skin tissue, mouth ducts, respiratory and digestion. In the mouth and respiratory canal can occur infection of tooth tissue (dental caries) and lung. *Streptococcus sobrinus* and *S. mutans* are found in the high quantities by the form of biofilm in patients with tooth decay. The growth and development of *Streptococcus sobrinus* and *S. mutans* occur after both of them paste on the tooth and decay it [12]. *S. mutans* is also associated with the severity of periodontal disease in adults [13]. *Staphylococcus aureus* and *Eschericia coli* are a major cause of various humans infections. The first causes skin and soft tissues infections, surgical site infections, and bone and joint infections [14]. *Candida* species are major human fungal pathogens that cause both mucosal and deep tissue infections [15]. *Candida* are known to be aetiological agents of human infection, however, more than 90% of invasive infection are caused by *Candida albicans*, *C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei* [16].

There are no literature reports on the chemical composition and biological activities of the essential oils from the leaves of *Actinodaphne macrophylla* grown in East Kalimantan. Therefore, this present study used steam distillation to extract the leaf oil of this species and analyzed the chemical components of oil using a GC-MS, also its antimicrobial activity by agar well diffusion.

2. Materials and methods

2.1. Plant Material

Fresh leaves of *Actinodaphne macrophylla*, Lauraceae, were collected in 2015 from Education Forest of Forestry Faculty, Mulawarman University, Samarinda, East Kalimantan. The voucher specimen was deposited in the Dendrology Laboratorium of Mulawarman University. Before extraction, the leaves were air dried at room temperature protected from the light for 24 hours.

2.2 Isolation of essential oils

The essential oil of *A. macrophylla* leaves (10 kg) were extracted using steam distillation method [17]. After extraction, the volume of oil obtained was measured and stored in vial bottle sealed with cap, covered with alumunium foil to protect the contents from light, and kept in the dark storage until used. The volume of leaf oil was presented in the percentage of yield.

2.3 GC-MS analysis

The essential oil was analyzed by GC-MS (Shimadzu-QP-5050A) in the Hasanuddin University, Makassar, with the following setting: column: HP-5 MS, 60 m x 250 µm ID x 0.25 µm film thickness; temperature program: from 70°C to 290°C (40 minutes) at 15°C.minute^{-1}; and injection temperature: 290°C. The injection port temperature was 290°C, and the detector temperature was 250°C. The injection mode was split (50:1), and the inlet pressure was 18.03 psi. The carrier gas was helium with a flow rate of 1 ml. minute^{-1}. The mass spectrometer conditions were as follows: ionization voltage: 70 eV; MS source temperature at 250°C; MS quadruple temperature at 150°C; interface temperature at 290°C; and electron ionization mass spectra were acquired over the mass range of 40-800 m/z. The compositions were reported as a relative percentage of the total peak area.

2.4 Antimicrobial activity

The in vitro antimicrobial activity of the oil against human oral pathogen were evaluated by agar diffusion method using Nutrient agar for bacteria and fungi [17]. The microbial included three bacterias are *Streptococcus mutans*, *Streptococcus sobrinus*, and *Staphylococcus aureus*, and one fungus is *Candida albicans*. The final concentration used in this test were 100% (pure oil), 10%, and 1% which dilluted in 40% ethanol. Two kinds of antibiotic standard as a positive control were used in this study, Chlorhexidine for bacteria and Chloramphenicol for fungi. The 40% ethanol was served as a negative control. After incubation at 32^{o}C for 18-24 hours, the antimicrobial activity values were

determined. The diameter of inhibition zones and activity index were measured [18] and calculated [19] in this study. All experiments were performed in triplicate.

3. Results and discussions

Steam distillation of *A. macrophylla* leaves produced a yellowpale-colored oil (figure 1). The oil from *A. macrophylla* leaves obtained 0.11% of a yield (Table 1). The refractive index was measured by hand refractometer was 1.425.

Table 1. Characteristics of *A. macrophylla* leaves oil

Name of plant	*Actinodaphne macrophylla*
Family	Lauraceae
Yield	0.11%
Color	Yellow pale
Refractive index	1.425

Figure 1. The essential oil from leaves of *A. Macrophylla*

Thirty five compunds have been evaluated by GC-MS. The chemical components were identified comparing by retention time, molecular formula, molecular weight, and percentage of area (Table 2). The leaf oil of *A. macrophylla* contained sesquiterpenes were predominant (49.3%), followed by monoterpenes (25.05%), aliphatic and mixed compounds (19.02%), and benzene (0.60%). Among the sesquiterpenes, copaen (7.01%), spathulenol (11.58%), and caryophyllen oxide (5.82%) were the major compounds, and of the monoterpenes, camphene (4.23%), (+)-sabinene (8.49%), β-pinene (4.73%) were the chief components. Among the aliphatic and mixed compounds, 2-monopalmitin was the main component. This is the first study on the chemical characterization from the leaf oil of *A. macrophylla* grown in East Kalimantan.

Table 2. Chemical compositions of *A. macrophylla* leaves oil

No	RT[a] (minutes)	Name of Compounds	MF[b]	MW[c] (g/mol)	Area (%)
1	3.062	Camphene	$C_{10}H_{16}$	136.24	4.23
2	3.193	(+)-Sabinene	$C_{10}H_{16}$	136.24	8.49
3	3.252	β-Pinene	$C_{10}H_{16}$	136.24	4.73
4	3.542	meta-Cymene	$C_{10}H_{14}$	134.22	2.29
5	3.581	D-Limonene	$C_{10}H_{16}$	136.24	1.39
6	3.616	Eucalyptol (1,8-Cineole)	$C_{10}H_{18}O$	154.25	0.85
7	4.010	(+)-Linalool	$C_{10}H_{18}O$	154.25	1.97
8	4.409	(+)-Camphor	$C_{10}H_{16}O$	152.24	1.74
9	4.600	4-Terpineol	$C_{10}H_{18}O$	154.25	1.62
10	5.223	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	$C_{12}H_{19}ClO_2$	230.73	0.60
11	5.608	α-Cubebene	$C_{15}H_{24}$	204.36	2.82
12	5.811	Copaene	$C_{15}H_{24}$	204.36	7.01

No	RT[a] (minutes)	Name of Compounds	MF[b]	MW[c] (g/mol)	Area (%)
13	5.871 6.472	γ-Muurolen	$C_{15}H_{24}$	204.36	3.04
14	6.629	(-)-δ-Cadinol	$C_{15}H_{26}O$	222.37	1.89
15	6.629	β-Cadinene	$C_{15}H_{24}$	204.36	1.04
16	6.752	(-)-Calamenene	$C_{15}H_{22}$	202.34	1.36
17	6.941	(-)-Spathulenol	$C_{15}H_{24}O$	220.35	1.04
18	6.988	Longipinocarveol, trans-	$C_{15}H_{24}O$	220.35	0.78
19	7.141	Spathulenol	$C_{15}H_{24}O$	220.35	10.54
20	7.187	Caryophyllene oxide	$C_{15}H_{24}O$	220.36	5.82
21	7.246	Epiglobulol	$C_{15}H_{26}O$	222.37	1.15
22	7.325	Humulene oxide	$C_{15}H_{24}O$	220.35	3.10
23	7.385	Cubenol	$C_{15}H_{26}O$	222.37	3.73
24	7.455	(-)-δ –Cadinol	$C_{15}H_{26}O$	222.37	2.07
25	7.507 7.546	Cadala-1(10),3,8-triene	$C_{15}H_{22}$	202.34	3.24
26	7.609	Longiverbenone	$C_{15}H_{22}O$	218.34	1.56
27	7.859	2-Cyclohexen-1-one, 4-butylidene-3-methyl-5-propyl-	$C_{10}H_{16}O$	152.24	1.09
28	7.923	Acetamide, N-tricyclo[4.3.1.1(3,8)]undec-3-yl-	$C_{12}H_{19}NO$	193.29	0.79
29	8.085	Acetamide, N-(3,4-dichlorophenyl)-N- methoxy-	$C10H11Cl_2NO_2$	248.11	0.90
30	8.148	22,23-dibromostigmasterol acetate	$C_{31}H_{48}Br_2O_3$	628.53	0.77
31	8.222	Boronia butenal	$C_{14}H_{22}O$	206.32	1.46
32	8.342	Platambin	$C_{15}H_{26}O_2$	238.37	1.06
33	8.442	Stigmasterol	$C_{29}H_{48}O$	412.70	3.87
34	8.583	Dihydro-β-ionone	$C_{13}H_{22}O$	194.32	1.05
35	9.440	2-Monopalmitin	$C_{19}H_{38}O_4$	330.50	11.23

[a]RT was Retention Time
[b] MF was Molecular Formula
[c]MW was Molecular Weight

From the results presented above, the leaf oil contituents of *A. macrophylla* were primarily sesquiterpenoids. According to the study of Salleh and Ahmad [20], they have been evaluated the chemical compositions of the *A. macrophylla* leaf oil were collected from Simpan Bangi Forest, Selangor, Malaysia, and extracted by hydrodistillation, their main components were germacrene B (16.8%), globulol (16.10%), t-muurolol (7.2%), δ-cadinene (6.7%), cis-cadin-4-en-10-ol (5.6%), and epi-cubenol (4.1%). It is proved that the chemical differences may depend on the procedure of extraction, the season, and the distinct habitat in which the plant was collected.

The essential oil was screened for their possible antimicrobial activity by using agar diffusion. The results are given in Table 3, indicated that a moderate to strong against all four microbes. The most sensitive microorganisms were *S. mutans,* followed by *S. sobrinus, C. albicans,* and *S. aureus* with the diameter of inhibition zones were 20.89, 22.34, 20.89, and 17.22 mm, respectively. The best inhibition zone on the 1% of oil was against *C. albicans* with the diameter 11.89 mm. While On the 10% of oil, the best inhibition was towards *S. aureus* (15.22 mm).

Table 3. Antimicrobial activity of the leaves essential oil of *A. macrophylla*

Sample	*C. albicans*				*S. aureus*				*S. mutans*				*S. sobrinus*			
Conc.	ZI[a]			AI[b]	ZI			AI	ZI			AI	ZI			AI
10 µg/well[c]	31.67	±	1.53[d]	1.00	15.22	±	0.19	1.00	15.22	±	0.39	1.00	16.33	±	0.39	1.00
100%	20.89	±	0.58	0.66	17.22	±	0.86	1.13	22.89	±	1.51	1.50	22.34	±	0.77	1.37
10%	12.78	±	0.39	0.40	15.22	±	0.86	1.00	11.67	±	1.22	0.77	11.89	±	0.58	0.73
1%	11.89	±	0.19	0.38	10.78	±	0.39	0.71	0.00	±	0.00	0.00	9.44	±	0.77	0.58

[a]ZI was Zone of Inhibition
[b] AI was Activity Index
[c]Concentration of positive control
[d]Chlorhexidine used as positive control against *C. albicans*

The inhibition zones of essential oils against microorganism mainly depend on their chemical composition and the quantity of the major single compounds, concentration of the oil, as well as kind and amount of bacteria [21, 22]. Essential oils primarily destabilize the cellular architecture, leading to the breakdown of membrane integrity and increased permeability, which disrupts many cellular activities. The essential oils pass through the cell wall and cytoplasmic membrane, which may disrupt the arrangement of dissimilar fatty acids, phospholipids bilayers, and polysaccharides molecules. All these events may be responsible for the coagulation of inner cellular components in the cytoplasm and break down of the bonds between the lipid and protein layers [23].

Sokovic et al [24] reported that β-pinene, D-limonene, 1,8-cineol, linalool, and camphor on the concentration of 1.0 µg/ml could inhibited the growth of human pathogen bacterias, especially *Staphylococcus aureus*. The methanolic bark extract of *A. macrophylla* have a good antioxidant activity. Its leaves and bark extract also exhibited moderate the lipoxigenase enzimes inhibitory effect. It has been reported to the antityrosinase, acetylcholinesterase and anti-inflammatory activities of the leaves and bark of *A. macrophylla* [25]. The root bark extracts of *A. lanata* Meissner were potential as antibacterial properties against *Streptococcus pygenes, S. aureus, Bacillus subtilis, Klebsiella penumonia, E. coli,* and *Pseudomonas aeruginosa* [26].

This is the first report describing the effect of *A. macrophylla* leaves oil from East Kalimantan on the human oral pathogen.

4. Conclusion

In conclusion, the results of this study indicated that the essential oil of the *Actinodaphne macrophylla* leaves showed a good antimicrobial activity against all microorganisms. The oil examined in this report are worthy of further study due to their antimicrobial activity. It could be evaluated as one of the alternative natural sources in antimicrobial industry.

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