Poster:

Antibacterial activity of secondary metabolite compounds from leaf of *Eclipta alba* L. Hassk

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Abstract. Two secondary metabolite compounds of steroid and triterpenoid derivates had been extracted from the leaf of *Eclipta alba* L. Hassk. The extraction of the compounds was carried out by maceration and then isolated by column chromatography. Structural elucidation was established using GC-MS. Both of the compounds showed antibacterial activity against *S. mutans*, *A. viscosus*, and *L. kaesal* at the concentration of 5%, 10%, and 25%.
Keywords: *Eclipta alba* L. Hassk, antibacterial activity, steroid and triterpenoid derívates.

Introduction

Plants have been widely used by Indonesian as traditional medicines to address a variety of health problems. Although the formal use of the herb has not became entrenched due to the limitations of scientific study, but the use of traditional medicines is promising because of its low price, raw materials availability, can be grown, and can be prepared by consumers themselves.

One of the plants that has been used by people as a traditional medicine is urang-aring (*Eclipta alba* L. Hassk). It is a small and branched annual herb with white flower heads inhabiting tropical and subtropical regions of the world like America, Asia, and Africa (Sharma *et al.*, 2001). *Eclipta alba* L. Hassk's leaves have been used in the treatment of toothache, cavities and gum swelling (Mardisiswojo and Harsono, 1985; Soegihardjo, 1986; Heyne, 1987; Rossie and Donatus, 1996). The leaves of this herb, combined with coconut oil, were applied on the sore tooth (Mardisiswojo and Harsono, 1985; Heyne, 1987).

Dental cavities or caries is a dental-hard-tissue disease from the email to the dentin, infiltrates the pulp tissue that would eventually lead to periodontal disorders by the activity of microorganisms on fermented carbohydrates. It is shown by demineralization of dental hard tissues, followed by the damage of organic materials. Consequently, it leads to the invasion of bacteria and pulpal death and the spread of the infection to the tissue which causes pain on periapeks (Pelczar and Chan, 2005).

Rossie and Donatus (1996) reported the alcohol extract of urang-aring contains wedelolaktone and dimetilwedelolaktone compounds with hepatoprotective activity in rats. Urang-aring leaves also contain nikotine (Sirait *et al*., 1983). Hasballah *et al*., (2001) also reported that the ethanol extract of urang aring leaves at the concentration of 15% showed antimicrobial activity against dental-caries-cause bacteria, including *Lactobacillus kaesal*, *Streptococcus mutans*, and *Actinomyces viscosus*. The zone of inhibition produced after 24 hours of treatment were 17,6 mm, 9,3 mm, and 11,3 mm, respectively. Until now, there has been no report on the types of compounds in the leaves extracts of urang-aring with antimicrobial potential against bacteria causing dental caries. Based on the above background, this study is intended to isolate the active antimicrobial compounds from the leaves extract of urang-aring guided by *in vitro* assays against *Lactobacillus kaesal*, *Streptococcus mutans* and *Actinomyces viscosus* bacteria. Active compounds obtained were characterized using chemical and spectroscopic methods of Gas Chromatography-Mass Spectroscopy (GC-MS). The results obtained are expected to support the scientific data about urang-aring leaves so it can be upgraded to phytopharmacotherapy.

Materials and Methods

Plant Materials

The leaves of *Eclipta alba* L. Hassk were collected from Kuta Alam region, Banda Aceh, Indonesia, and identified at Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

Extraction and Isolation

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The dried ground leaves (1.4 kg) were extracted successively with methanol. The methanol extract was evaporated by vacuum rotary evaporator. The semisolid phase was fractionated with chloroform and the extract components were separated by vacuum liquid chromatography over silica gel GF_{254} (stationary phase) and eluted with different mixture of ethyl acetate-n-hexane (mobile phase). The fractions obtained were analyzed by Thin Layer Chromatography (TLC). The fractions with the same spot pattern were combined, produced 12 fractions. The obtained fractions were then evaluated for their antibacterial activity.

The active fractions (1.2 gram) were separated by Gravitation Column Chromatography over silica gel G_{60} (stationary phase) with dichloromethane (CH_2CL_2): methanol (mobile phase) gave 48 fractions. Fractions with the same spot pattern were combined and further fractionation by silica gel column gave 12 groups of combined fractions. J Fraction eluted in CH_2Cl_2 100%, yielded triterpenoid compound and steroid compound (a white needle crystal solid phase). After it was purified by Gravitation Column Chromatography and eluted in CH_2Cl_2-methanol (88:12) yielded 51 mg stigmasterol as white needle crystal.

GC-MS m/z:(% intensity): 412 ($M^{\cdot+}$), 27%, 395 (2.7%), 255 (2.7%), 215 (5.4%), 173 (100%), 159 (4%), 133 (16,7%), 119 (22.2%), 105 (29.7%), 95 (16.7%), and 81 (19.4%).

Antibacterial assay

The five fractions of chloroform extract fractionated by Vacuum Liquid Chromatography (A, C, I, J, and L) were tested against *S. mutan*, *L. kaesal*, dan *A. viscosus* using disc diffusion method of Kirby-Bauer over Muller Hinton Agar (MHA). The agar was autoclaved at 121°C for 15 minutes. 0.1 ml suspension containing 1-2 x 10^8 CFU/ml (EUCAST, 2009; Franco *et al.*, 2006) was pipetted out into the plate. The cooled agar (45°C) was dispensed at 12 ml per petridish and let to solidify. The discs containing the diluted fractions were placed on the agar medium at appropriate arrangement. The plates were incubated at 37°C for 24 – 48 hours. Then, the zone of growth inhibition around the discs was measured. Methanol extract of urang-aring leaves (120 gram) followed by chloroform fractionation yielded 80.75 gram of chloroform extract. The chloroform extract (7 gram) was separated by Vacuum Liquid Chromatography and gave 12 combined fractions (Figure 1). Of the obtained fractions, five were selected for antibacterial activity, while the rest of the fractions were not adequate for the assay.

The result of antibacterial activity of the tested fractions against dental-caries-cause disease such as *S. mutan*, *L. kaesal*, dan *A. viscosus* is shown in Table 1. All tested fractions were found to be active against the target bacteria compared to chloroform extract, which was only active against *A. viscosus* (minimum inhibitory concentration 20%, zone of growth inhibition 12 mm). Among the tested fractions, J fraction at the concentration of 10% exhibited the strongest antimicrobial activity after 48 hours of treatment, followed by L and I fractions, while A and C were found to be less active. Wijaya Kusuma (1992) reported wedelolaktone, a coumarine derivate, as a component contained in urang-aring leaves; however a further study on structural elucidation of a compound based on spectroscopy data such as GC-MS is crucial.

Results and Discussion

J fraction eluted with ethyl acetate 100% was obtained in a larger amount and based on TLC analysis it became a purer fraction. Chemical characterization showed that the compound contains steroid as main compound and triterpenoid as minor compound.

Stigmasterol was isolated from the chloroform extract of urang-aring leaves by column chromatography. This compound was obtained as white needle crystal from fraction eluted with CH_2Cl_2 100%. Figure 2 present the chromatogram of Gas Chromatography and Figure 3 present the spectrum of Mass Spectroscopy obtained for J fraction of urang-aring leaves. The GC-MS spectra showed that the molecular ion peak at m/z 412, consisted of the molecular formula: $C_{29}H_{48}O$ with double-bond equivalent (DBE) = 6. Since the molecular formula indicated six units of saturation, this compound was concluded to be stigmasterol (Figure 4). In the GC-MS spectra retention time of the peak was 32.23 and displayed molecular ions at m/z 412 (M^+), 395 (M^+-$^{\cdot}OH$), 255: (M^+-H_2O-$C_{10}H_{19}$). This fragmentation patterns supported that the compound was stigmasterol.

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Figure 1. TLC chromatogram of the five fractions of chloroform extract fractionated by Vacuum Liquid Chromatography

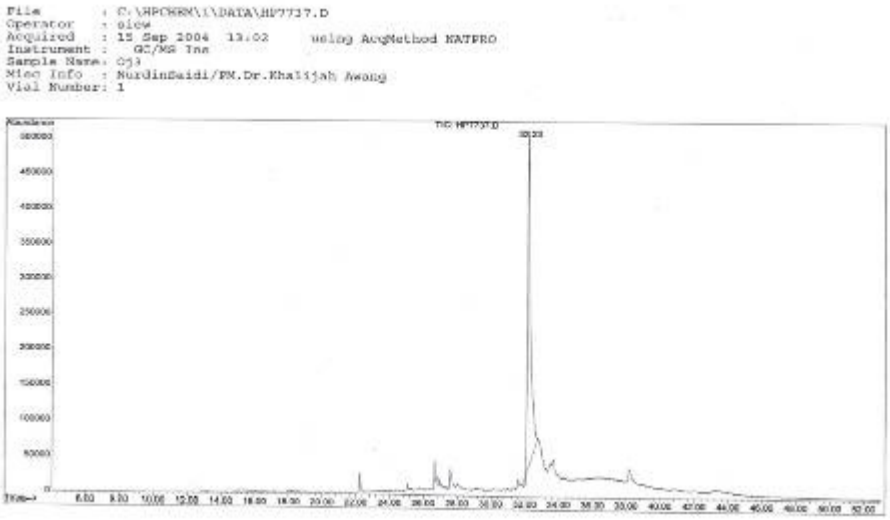

Figure 2. The chromatogram of J fraction of urang-aring leaves obtained from Gas Chromatography (GC)

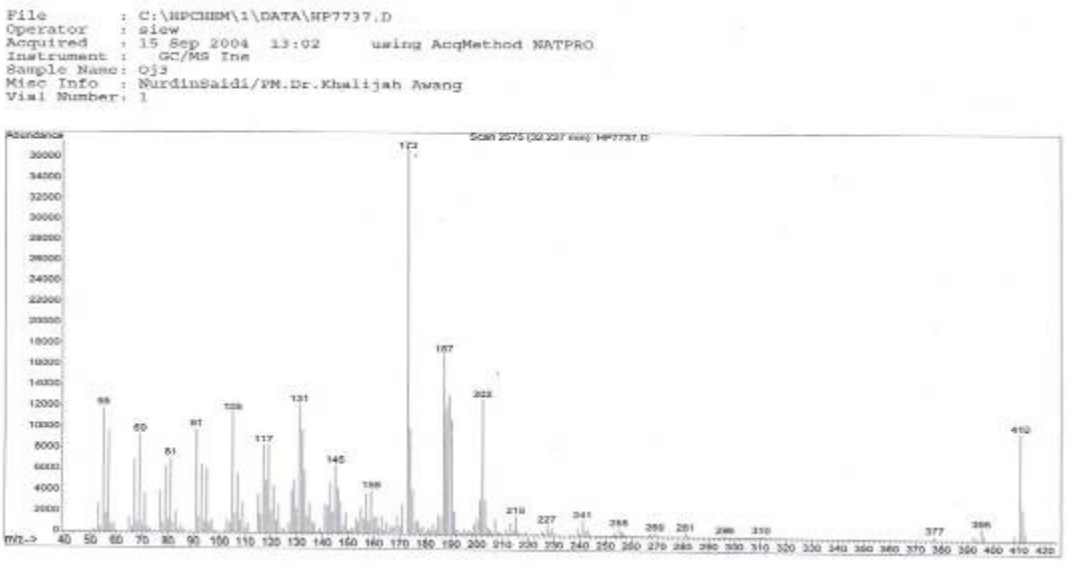

Figure 3. The spectrum of J fraction of urang-aring leaves obtained from Mass spectroscopy (MS)

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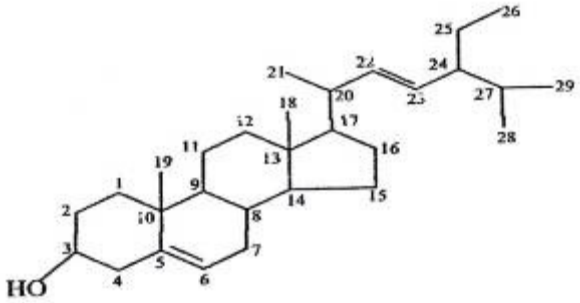

Figure 4. Stigmasterol

Table 1. Antibacterial activity of chloroform extract fractionated by Vacuum Liquid Chromatography

Fractions	Concentrations (%)	Zone of Inhibition Observation (mm)					
		S. mutans		*A. viscosus*		*L. kaesal*	
		24 h	48 h	24 h	48 h	24 h	48 h
A	50 (A_1)	-	-	-	-	-	-
	25 (A_2)	8,0	10,0	-		-	-
	10 (A_3)	-	-	-	-	-	-
	5 (A_4)	-	-	-	-	-	-
	1 (A_5)	-	-	-	-	-	-
C	50 (C_1)	-	11,0	-	-	-	-
	25 (C_2)	-	9,5		-	-	-
	10 (C_3)	-	9,0	-	-	-	-
	5 (C_4)	-	8,0	-	-	-	-
	1 (C_5)	-	6,0	-		-	-
I	50 (I_1)	10,0	15,0	7,0	11,0	-	-
	25 (I_2)	-	12,0	10,0	10,0	-	-
	10 (I_3)	-	8,0	-	9,0	-	-
	5 (I_4)	-	7,0	12,0	17,0	-	-
	1 (I_5)	-	-	-	-	-	-
J	50 (J_1)	9,0	15,0	7,0	8,0	-	-
	25 (J_2)	15,0	17,0	8,0	9,0	-	-
	10 (J_3)	11,0	12,0	10,0	12,0	-	10,0
	5 (J4)	12,0	11,0	17,0	17,0	-	8,0
	1 (J5)	9,0	10,0 _	-	-	8,0	12,0
L	50 (L_1)	9,0	11,0	-	-	-	10,0
	25 (L_2)	-	15,0	-	-	-	-
	10 (L_3)	-	14,0	9,0	10,0	13,0	10,0
	5 (L_4)	-	12,0	-	-	-	15,0
	1 (L_5)	-	9,0	-	-	10,0	13,0

A: combined fractions 1-3; C: combined fractions 6-7; I: combined fractions 13-28;
J: combined fractions 29-34; L: combined fractions 40-41.

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Conclusions

The fractions of chloroform extract of urang-aring leaves exhibited stronger antibacterial activities compared to that of chloroform extract. Chloroform extracts yielded two active antibacterial compounds, including stigmasterol and triterpenoid.

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