IDENTIFICATION OF CHEMICAL CONSTITUENTS IN STONE DAMMAR EXTRACTS AND THEIR POTENCIES AS ANTIBACTERIAL AGENTS

Noryawati Mulyono
School of Biology, Faculty of Biotechnology, Atma Jaya Catholic University, Jakarta 12930 Indonesia

Email noryawati@atmajaya.ac.id

ABSTRACT

Stone dammar, resin obtained from *Shorea eximia*, grows well in Kalimantan, Sumatra and Sulawesi. Its wood has been utilized, but the other parts of the plant have no economical value. The aim of this fundamental research was to extract the stone dammar using nonpolar and semipolar organic solvents, and to identify chemical constituents in stone dammar resin using py-GC/MS. Sesquiterpenes and terpene-O were the main compounds in stone dammar extracts using hexane or ethyl acetate. Some predominant sesquiterpenes are α-copaene, δ-cadinene, valencene, β-elemene and alloaromadendrene; and terpene-O were spathulenol and α-cadinol. High content of sesquiterpenes in these extracts indicated their potencies to be used as antimicrobial agent.

KEYWORDS: dammar; sesquiterpene; antibacterial.

1. Introduction

Stone dammar is resin from the bark of *Shorea eximia*, included in *Dipterocarpaceae* family. There are about 600 species of *Dipterocarpaceae* in Indonesia [1]. Research and development of this nontimber forest product is needed to improve its economical value but this is still rare because only wood that has been commercialized [2-3]. Since it is secondary metabolite and it is produced as an immune system, it is potential to be developed as antibacterial agent. It will not only improve its economical value, but also diminish bacteria resistance due to continuous exposure of synthetic antibiotics and suitable to be applied as natural antibacterial in lipophillic products, both food and cosmetics. The aim of this research was to inform chemical constituents in stone dammar resin.

2. Materials and Methods

2.1. Plant Material

The resin was supplied by Research and Development Agency, Indonesia Forestry Department (Bogor). Dry resin was ground into fine powder and extracted at room temperature using hexane and ethyl acetate, separately at 5% (w/v). Each extract was concentrated by vacuum rotary evaporator and dried by vacuum oven. The yields of hexane and ethyl acetate extracts were 25.79 and 37.61% (w/w), respectively.

2.2 Chemical Identification Using Py-GC/MS

Chemical compounds from single extraction were identified using py-GC/MS (QP 2010 Shimadzu). The GC column was a 30 m DB-5 column (J&W Scientific, Folsom, USA) with 0.25 mm i.d. and 0.25 μm film thickness. The temperature conditions (^{o}C): pyrolisis 400, column 50, interface 280, ion source 280. Helium was used as carrier. Mass spectra were automatically compared to database from National Institute of Standards and Technology, Wiley, Pesticide, Flavor and Fragrance Natural and Synthetic Compounds, and Drug libraries, and were used to predict the chemical composition. Percentage composition were calculated based on peak area.

3. Results and Discussion

The chromatogram of hexane soluble fraction of stone dammar showed that there were 45 compounds in that fraction, but only 6 peaks could be predicted with high similarity index (SI>90%) and 15 peaks with SI 80-90%. Extraction using ethyl acetate yielded fraction consisted of 31 compounds, 8 and 17 out of them could be predicted with high and moderate SI, respectively (Figure 1). For all data in table, number in bold means that SI > 90% and in regular means that SI 80-90%. Compounds with a SI <80% were not reported here.

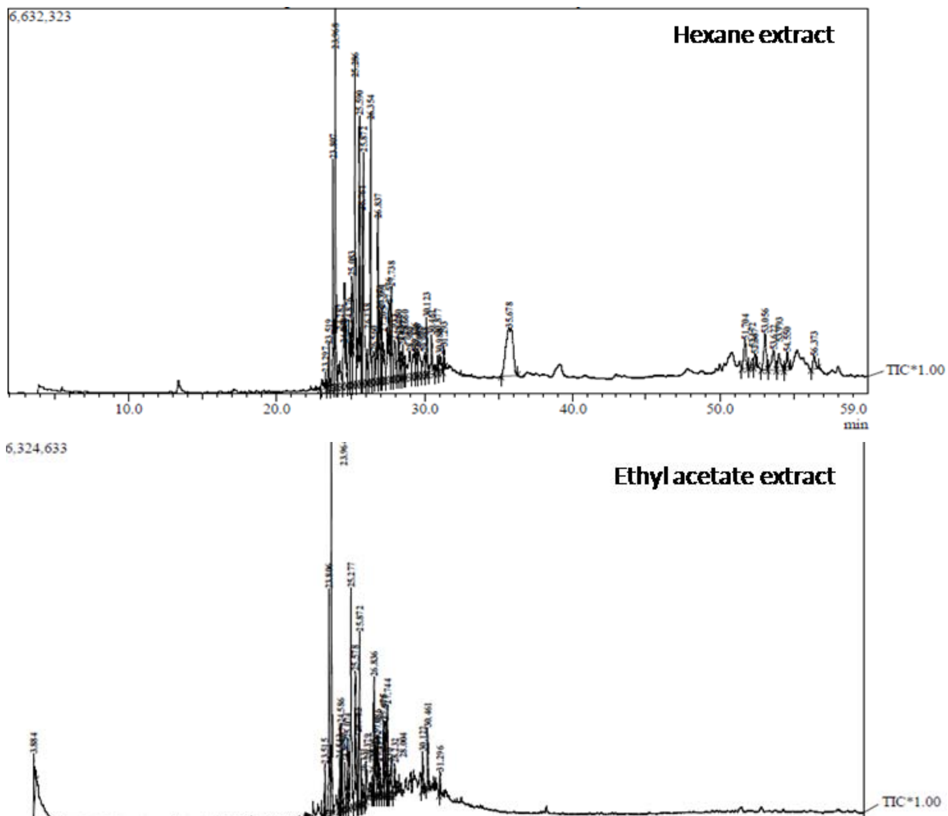

Figure 1. Chromatograms of stone dammar extracts.

There were 14 compounds present in all extracts (Table 1). All of them were sesquiterpenes ($C_{15}H_{24}$), except for (-)-caryophyllene oxide, α-cadinol, platambin and spathulenol, which could be categorized as terpen-O. It seemed that ethyl acetate was better solvent because of its high total relative peak area (70-72% vs 53-56%). Compounds no 1-10 in Table 1 have been reported about their presence in essential oil from medicinal plants such as *Teucrium* sp., *Hypericum* sp, *Nepeta* sp., *Phlomis* sp. and *Ottonia martiana* Miq. [4-10].

Table 1. Chemical compounds in stone dammar extracted by either hexane or ethyl acetate

No	Name	CAS	Formula	Hexane (%)	Ethyl acetate (%)
1.	α -copaene	3856-25-5	$C_{15}H_{24}$	**4.63**	**8.86**
2.	(-)-caryophyllene oxide	1139-30-6	$C_{15}H_{24}O$	**1.81**	**2.44**
3.	δ-cadinene	483-76-1	$C_{15}H_{24}$	**5.93**	**7.45**
4.	α-cadinol	481-34-5	$C_{15}H_{26}O$	5.61	6.74
5.	α-muurolene	31983-22-9	$C_{15}H_{24}$	**0.73**	8.48
6.	Germacrene D	23986-74-5	$C_{15}H_{24}$	3.83	**1.75-3.85**
7.	Valencene	4630-07-3	$C_{15}H_{24}$	8.19	5.39
8.	β-elemene	515-13-9	$C_{15}H_{24}$	**5.67-8.78**	**11.24**
9.	Alloaromadendrene	25246-27-9	$C_{15}H_{24}$	3.83	5.64
10.	Spathulenol	6750-60-3	$C_{15}H_{24}O$	3.52	5.48
11.	1S,cis-calamenene	483-77-2	$C_{15}H_{22}$	5.05	1.88
12.	Longicyclene, (+)-	1137-12-8	$C_{15}H_{24}$	0.78	1.57
13.	Platambin	58556-80-2	$C_{15}H_{26}O_2$	1.48	2.52
14.	γ-gurjunene	489-40-7	$C_{15}H_{24}$	2.04	0.75

There are 7 compounds could be extracted using hexane, but absent in ethyl acetate extract (Table 2). Only 2 of them had been reported about their antibacterial activities, i.e. α-cubebene and α-ylangene in essential oil from *Ottonia martinata* and *Teucrium montanum* [8-9].

Table 2. Chemical compounds present in hexane extract but absent in ethyl acetate extract

No	Name	CAS	Formula	%
1.	α-cedrol	77-53-2	$C_{15}H_{26}O$	3.17
2.	α-cubebene	17699-14-8	$C_{15}H_{24}$	**0.69**
3.	α-ylangene	14912-44-8	$C_{15}H_{24}$	3.83
4.	β-cubebene	13744-15-5	$C_{15}H_{24}$	1.38
5.	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	6892-80-4	$C_{15}H_{26}O$	1.69
6.	1,4-Diiodooctahydropentalene	92617-51-1	$C_{15}H_{24}O$	3.52
7.	Longifolenaldehyde	19890-84-7	$C_{15}H_{24}O$	1.49

Table 3 showed that there were other sesquiterpenes present in ethyl acetate extract and some of them had biological activities. Together with δ-cadinene and β-selinene in essential oil of *Teucrium montanum,* caryophyllene and α-calacorene had antibacterial activities [9,11]. Palustrol was present in essential oil of *Hyptis suaveolensis* and this plant has been used for the treatment of respiratory track infections, anticancer, antifungal and antibacterial activities [12]. Ethanol extract of *Cistus salvifolius* contained aromadendrene, methylpentenol, trimethoxy quercetin, benzeneacetic acid and phenylindolizine strongly inhibited *Staphylococcus aureus*, *Klebsiella pneumoniae, Enterobacter cloacae,* and also inhibited *Acinetobacter baumannii* weakly [13].

Table 3. Predicted compounds present in ethyl acetate extract but absent in hexane extract

No	Prediction	CAS	Formula	%
1.	α-amorphene	23515-88-0	$C_{15}H_{24}$	**9.96**
2.	ε-muurolene	30021-46-6	$C_{15}H_{24}$	2.70
3.	1-Naphthalenamine, 4-bromo-	2298-07-9	$C_{15}H_{24}O$	0.74
4.	Caryophyllene	87-44-5	$C_{15}H_{24}$	**2.80**
5.	Driminol	19078-37-6	$C_{15}H_{26}O$	2.90
6.	Naphthalene, decahydro-1,6-bis(methylene)-4-(1-methylethyl)-	54932-90-0	$C_{15}H_{24}$	2.70
7.	Palustrol	5986-49-2	$C_{15}H_{26}O$	0.75
8.	Aromadendrene	489-39-4	$C_{15}H_{24}$	4.19
9.	α-calacorene	21391-99-1	$C_{15}H_{20}$	1.38
10.	7,7-Dichlorobicyclo(3.2.0)hept-2-enone	5307-99-3	$C_7H_6Cl_2O$	1.64

Conclusion and suggestion

Stone dammar extract may be potential to be developed as antibacterial agent. Based on their yield and total relative peak area, ethyl acetate soluble fraction seemed to be more potential as antibacterial agent than hexane one. Further research to determine the antibacterial spectrum of resin as well as their MICs and MBCs are needed before purifying the active compounds or doing applied research in such a product model.

Acknowledgments

The author would like to thank Faculty of Biotechnology Atma Jaya Catholic University which supported for research funding and Research and Development Agency, Indonesia Forestry Department (Bogor) for supplying the dammar resin. I am also indebted to Mukti Wahyuningjati as Laboratory Technician and my colleagues at the Faculty.

References

[1]. E.H. Hakim (2002). Oligostilbenoid from Dipterocarpaceous plants. *Bull. Soc. Nat. Prod. Chem.* **2**, 1-19.

[2]. I. Hakim and A. Saiban (1994). Potensi, permasalahan dan prospek pengusahaan hutan damar, di Krui, Lampung Barat. *J. Penel. Pengemb. Kehut.* **9(2)**, 45-52.

[3]. E. S. Sumadiwangsa and Gusmailina (2006). Teknologi budidaya, pemanfaatan dan pengembangan hasil hutan bukan kayu. Forestry Ministry, Bogor.

[4]. M. Pavlovic, O. Tzakou, P. V. Petrakis and M. Couladis (2004). Essential oil composition of *Hypericum perforatum* l., *Hypericum tetrapterum* fries and *Hypericum olympicum* l. growing in Greece. In: 3rd Conference on medicinal and aromatic plants of southeast European Countries. Book of Abstracts, *ed:* M. Haban *et al.,* Slovak University of Agriculture, Nitra, Slovak, pp. 89-90.

[5]. G. Gkinis, I. Fitsiou, O. Tzakou (2004). Volatile metabolites of *Nepeta nuda* ssp. *Nuda* from Greece. In: 3rd Conference on medicinal and aromatic plants of southeast European Countries. Book of Abstracts, *ed:* M. Haban *et al.,* Slovak University of Agriculture, Nitra, Slovak, pp. 105.

[6]. M. Kucuk, C. Gulec, A. Yasar, O. Ucuncu, N. Yayli, K. Coskuncelebi, S. Terzioglu and N. Yayli (2006). Chemical composition and antimicrobial activities of the essential oils of *Teucrium chamaedrys* subsp. *chamaedrys*, *T. orientale* var. *puberulens*, and *T. chamaedrys* subsp. *Lydium. Pharmac. Biol.* **44(8)**, 592-599.

[7]. A. Basta, O. Tzakou, M. Couladis (2004). Essential oil composition of *Phlomis cretica* C. Presl. In: 3rd Conference on medicinal and aromatic plants of southeast European Countries. Book of Abstracts, *ed:* M. Haban *et al.,* Slovak University of Agriculture, Nitra, Slovak, pp. 94.

[8]. M. M. Cunico, A. L. Lopes, L. C. Cocco, C. I. Yamamoto, R. C. B. Plocharski, M. D. Miguel, A. G. Junior, C. G. Auer and O. G. Miguel (2007). Phytochemical and antibacterial evaluation of essential oils from *Ottonia Martiana* Miq. (Piperaceae). *J. Braz. Chem. Soc.* **18(1)**, 184-188.

[9]. N. Vukovic, T. Milosevic, S. Sukdolak, S. Solujic (2007). Antimicrobial activities of essential oil and methanol extract of *Teucrium montanum*. *eCAM* **4(S1)**,17-20.

[10]. N. A. A. Ali, M. Wurster, N. Arnold, U. Lindequist and L. Wessjohan (2008). Chemical composition of the essential oil of *Teucrium yemense* Deflers. *Rec. Nat. Prod*. **2(2)**, 25-32.

[11]. N. Vukovic, T. Milosevic, S. Sukdolak and S. Solujic (2008). The chemical composition of the essential oil and the antibacterial activities of the essential oil and methanol extract of Teucrium montanum. *J. Serb. Chem. Soc.* **73(3)**, 299-305.

[12]. Z. F. Tonzibo, A. B. Florence and G. Bedi (2009). Chemical composition of essential oil of *Hyptis Suaveolensis(L) Poit.* from Côte d'Ivoire. *Eur. J. Sci. Res.* **38(4)**, 565-571.

[13]. K. Bayoub, T. Baibai, D. Mountassif, A. Retmane and A. Soukri (2010). Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogenes* and some other pathogenic strains. *Afric. J Biotechnol* **9(27),** 4251-4258.

KEMBALI KE DAFTAR ISI