ANTIBACTERIAL ACTIVITY OF FRACTIONATED SANDALWOOD OILS

A.T. Karossi, H. Agustina and L. Sutedja

R & D Centre for Applied Chemistry - LIPI
Jalan Cisitu Sangkuriang - Bandung 40135 - INDONESIA

ABSTRACT

Sandalwood oil was prepared through water distillation of sandalwood (Santalum album L) sawdust. The inhibitory activity of the oil was tested against Staphylococcus aureus and Bacillus cereus. This antibacterial active oil was further fractionated through column chromatography into five fractions. Larger antibacterial activity, expressed as inhibitory diameter (ID), was observed in the prepared sandalwood oil and its fractions compared to sandalwood oil originated from Kupang and santalol from International Flavors and Fragrance (IFF). The inhibitory diameter of the isolated sandalwood oil against S. aureus and B. cereus were 8.75 and 8.20 mm respectively. While the ID of sandalwood oil from Kupang and santalol IFF against S. aureus were 7.20 and 7.23 mm, and against B. cereus 6.62 and 7.35 mm respectively. The ID of the sandalwood oil fractions against S. aureus ranged between 7.32 - 9.93 mm, and the largest inhibition was shown by fraction -2. Against B.cereus the ID ranged between 7.64 - 11.12 mm., and the largest inhibition was shown by fraction - 1. Suggested possible structures for sandalwood oil fractions were based on the infra red spectra of the oils and sandalwood oil components.

INTISARI

Minyak cendana diperoleh dari hasil destilasi air serbuk gergaji kayu cendana (Santalum album L), kemudian difraksinasi melalui kromatografi kolom menjadi lima fraksi. Aktivitas antibakteri minyak cendana diuji terhadap Staphylococcus aureus dan Bacillus cereus. Hasil uji aktivitas antibakteri yang dinyatakan dalam diameter inhibisi (ID) menunjukkan bahwa minyak cendana hasil isolasi dan fraksi-fraksinya mempunyai aktivitas antibakteri yang lebih besar dibandingkan dengan minyak cendana yang berasal dari Kupang dan santalol dari International Flavors and Fragrance (IFF). Diameter inhibisi minyak cendana hasil isolasi terhadap S. aureus dan B. cereus masing-masing adalah 8.75 dan 8,20 mm, sedangkan ID minyak cendana Kupang dan santalol IFF terhadap S. aureus adalah 7,20 dan 7,25 mm, dan terhadap B. cereus adalah 6,62 dan 7.35 mm. Diameter inhibisi fraksi-fraksi minyak cendana terhadap S. aureus berkisar antara 7,32 - 9,93 mm. Inhibisi terbesar di tunjukkan oleh fraksi - 2, sedangkan terhadap B.cereus, ID fraksi-fraksi berkisar antara 7,64 - 11,12 mm. Inhibisi terbesar ditunjukkan oleh fraksi - 1. Struktur dari senyawa-senyawa yang mungkin terdapat dalam masing-masing fraksi diperkirakan berdasarkan spektra infra merah.

INTRODUCTION

Santalol oil belongs to the group of essential oils which play important role as an export commodity of Indonesia. It is widely utilized as raw material in the production of perfumes, cosmetics and medicines. During the early part of the twentieth century a large portion of the sandalwood oil produced in the world was used for medicinal purposes. Natives in Asia use the oil for selftreatment of certain deseases such as asthma, wounds and urinary tract infections (1,2,3).

Santalol oil is usually prepared from the root and heartwood of the sandalwood tree (*Santalum album* LINN) by water distillation (1). It differs in chemical composition and odor, depending on the age and species of sandalwood tree. The roots yield up to 10 % of oil, while the intermediary layers between soft and hard wood give only about 2.5% (1,4).

Reported major component of sandalwood oil is santalol (90% of the oil), a mixture of two primary sesquiterpene alcohols $C_{15}H_{24}O$, that is α - and ß - santalol, in which the α - form predominates. Besides santalol, the presence of other compounds such as aldehydes, ketones, acids, sesquiterpene hydrocarbons were detected in the oil (1,5).

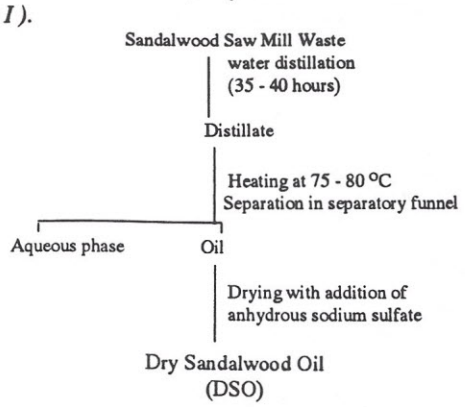
Regarding its contribution in the medical field, biological activity of sandalwood was investigated against *Staphylococcus aureus* and *Bacillus cereus*. These are gram positive pathogenic bacteria which could cause respiratory and gastrointestinal infections (6). Fractionation of sandalwood oil was carried out in the purpose to identify the fractions or components responsible for the antibacterial activity of the oil, as reported in this paper.

MATERIALS AND METHODS

Sandalwood sawdust was purchased from Bali, Indonesia. A sample of sandalwood oil was also obtained from "Tropical Oil" Sandalwood Distillary, Kupang, Nusa Tenggara Timur, Indonesia, while santalol was obtained from International Flavors and Fragrance Inc. (IFF), Indonesia. Solvents used for fractionation were redistilled from technical grade solvents. Microbiological assay used chemicals from Difco and E.Merck of analytical grade.

Isolation of sandalwood oil

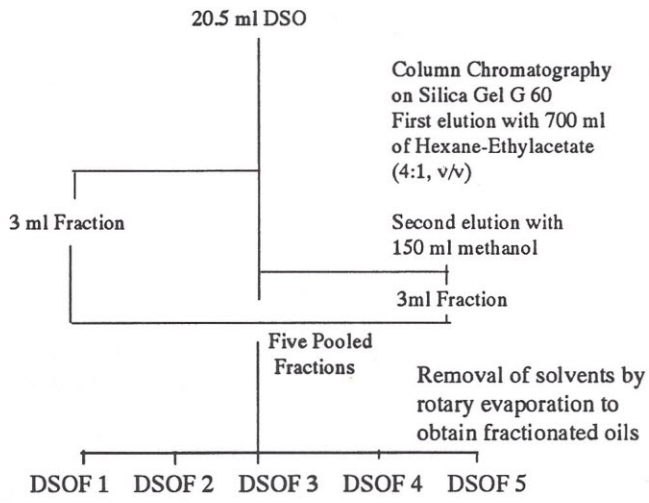
Sandalwood oil was prepared from sandalwood saw mill waste by water distillation for 35-40 hours. After separation from water in the distillate at 75-80°C, followed by drying with anhydrous sodium sulfate, dry sandalwood oil was obtained *(Scheme I)*.



Scheme I : Preparation of Sandalwood Oil.

Fractionation of sandalwood oil

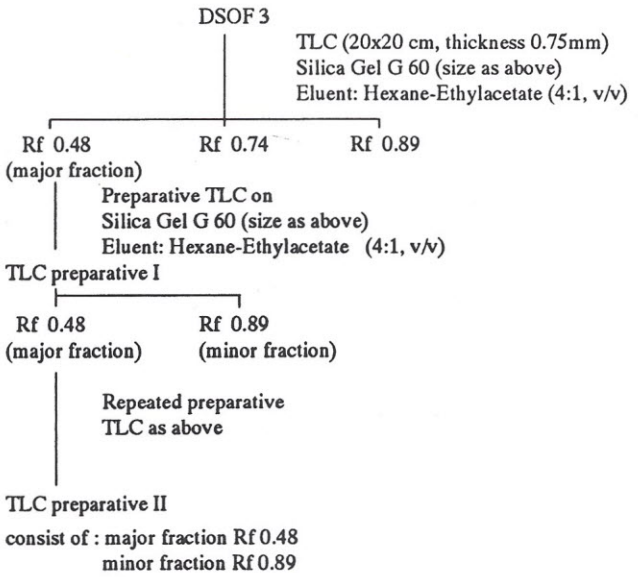
Sandalwood oil was fractionated by column chromatography using Silica Gel G60 (230-400 mesh) as adsorbent. Elution was conducted by hexane - ethylacetate (4:1,v/v) followed by methanol. Fractions with the same Rf value in the thin layer chromatography performance, were pooled together then concentrated by evaporation *(Scheme II)*.



Scheme II : Fractionation of DSO (Dry Sandalwood Oil)

Further purification of sandalwood oil fraction was carried out by preparative thin layer chromatography.

The sample was separated on a Silica Gel G 60 plate of 20 x 20 cm, with a thickness of 0.75 mm. Hexane - ethylacetate (4:1,v/v) was used as eluent. The spots were identified by comparing with colored spots after spraying with 0.5 % phosphomolybdic acid in ethanol.The separated fractions were extracted from the adsorbent by hexane - ethylacetate (4:1,v/v) then concentrated by evaporation *(Scheme III)*.



Scheme III : Purification of DSOF 3 through Preparative TLC Technique.

Spectrometric analysis

Infrared absorption spectra of sandalwood oil fractions were obtained on a Shimadzu Spectrophotometer FTIR - 4300, using CCl_4 as solvent.

Microbiological assay

The microbiological assay was performed according to Merck (7). *S.aureus* and *B.cereus* were cultured on nutrient agar composed of meat extract, 0.3%; peptone, 0.3%; NaCl, 0.5%; and agar, 1.5% at 37°C for 24 hours. The cultures were activated in nutrient broth composed of meat extract, 2.3%; and peptone, 0.3% at 37°C for 24 hours on a rotary shaker until 25% transmittance was obtained at 580 nm. Then 0.1 ml of these suspensions were transferred into sterile petri dishes. A test medium composed of yeast extract, 0.28%; peptone, 1.56% amylum, 0.4%; glucose, 0.1%; gelatine, 0.4%; NaCl, 0.3%; bromcresol purple, 0.0016%; and agar, 1.0%, was maintained at 42°C and dispensed at 25 ml perplate. Sterile paper discs of 4 mm diameter were applied to the solidified seeded agar. The sample was dropped at 4 microliter per disc. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition of growth were measured. The zones of inhibition were detected as clear zones in which no bacterial growth was observed. The diameter of the inhibition zones was indicated as inhibitory diameter (ID).

The minimum inhibitory concentration (MIC) was determined as described by Morris *et al* (5). The samples were diluted with ethanol, which was also used as a blank. The smallest concentration which still showed zones of inhibition was taken as the minimum inhibitory concentration.

RESULTS AND DISCUSSION

Sandalwood oil

Dry sandalwood oil prepared from sandalwood saw mill waste was yellow in color with a yield of 5%, specific gravity 0.9660 (27°C) and refractive index 1.5057 (20°C).

Fractionation of sandalwood oil

Five fractions, namely DSOF 1, DSOF 2, DSOF 3, DSOF 4 and DSOF 5, were collected from fractionation of sandalwood oil by column chromatography. These fractions were impure as indicated in the thin layer chromatogram shown in *Figure 1*. Using Silica Gel G 60 as adsorbent and hexane-ethylacetate (4:1, v/v) as eluent; DSOF 1, DSOF 2, DSOF 3, DSOF 4 and DSOF 5 consisted of at least two, four, three, two and one spot(s) respectively.

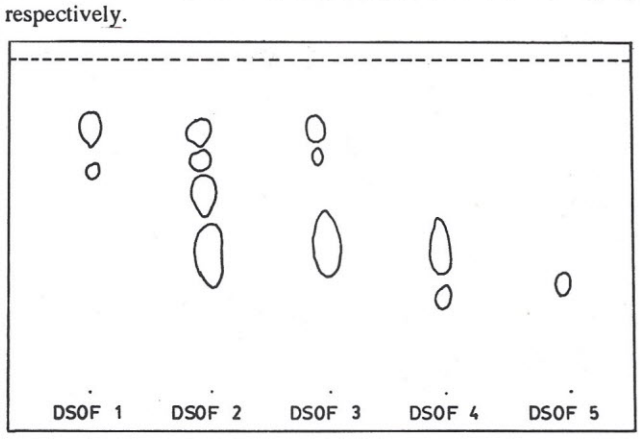


Figure 1. Thin layer chromatogram of sandalwood oil fractions, DSOF 1-5, on Silica Gel G 60. Eluent: hexane - ethylacetate = 4:1, v/v.

Compared to santalol from IFF, DSOF 3 showed similar thin layer chromatograms. Since santalol is the dominant component in sandalwood oil (1), similar thin layer chromatograms were observed between santalol and sandalwood oil *(Figure 2)*. However, more prominent nonpolar spots were observed in the sandalwood oil compared to that of santalol and DSOF 3. Further purification of DSOF 3 showed less and less prominent nonpolar spots.

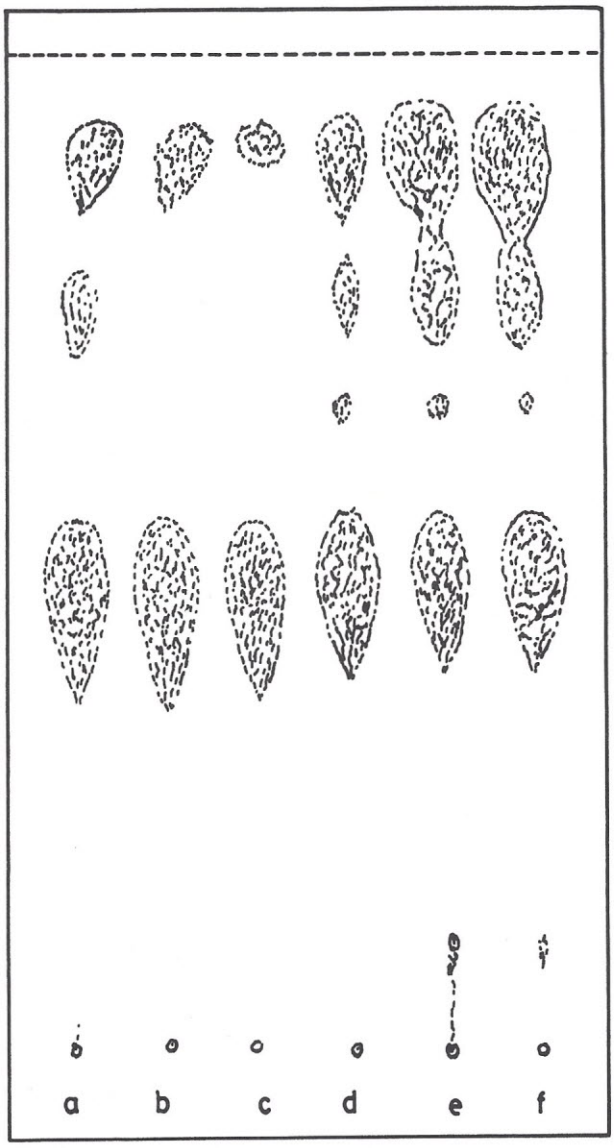


Figure 2. Thin Layer Chromatogram of a. DSOF 3; b. DSOF 3 prep. I; c. DSOF 3 prep II; d. Santalol-IFF; e. Dried Sandalwood oil; f. Sandalwood oil - Kupang.

Antibacterial activity

Antibacterial activity of sandalwood and its fractions againt *S.aureus* and *B.cereus* expressed as inhibitory diameter (ID) and minimum inhibitory concentration (MIC) are listed in *Table 1.*

Table 1. Antibacterial activity of sandalwood and its fractions against *S.aureus* and *B.cereus.*

Oil Sample	ID (mm) against		MIC(ppm) against	
	S.aureus	*B.cereus*	*S.aureus*	*B.cereus*
Dried sandal wood oil (DSO)	8.75	8.20	1890	1890
DSO Fraction-1	9.18	11.12	3770	3770
DSO Fraction-2	9.93	8.93	3770	3770
DSO Fraction-3	8.22	8.13	1890	1890
DSO Fraction-4	8.75	8.42	1890	1890
DSO Fraction-5	9.70	9.76	7540	7540
DSO Fraction-3 (purified)	7.32	7.64	1890	1890
DSO Kupang	7.20	6.62	3790	3790
Santalol - IFF	7.23	7.35	3800	3800

When one compares the inhibitory effect of the sandalwood oil from Kupang and santalol from IFF to sandalwood oil prepared in this experiment, the latter showed larger inhibition zones, which means larger inhibitory activity against *S.aureus and B.cereus.*

All fractions of sandalwood oil showed antibacterial activity against both bacteria, although their activity varied between one and another. Undiluted fractions DSOF 1, DSOF 2 and DSOF 5 showed larger inhibitory activity. Larger MIC values were also observed compared to the other fractions and the whole sandalwood oil. Inhibitory diameter (ID) observed by paper diffusion method could not be related to MIC, since the ID did not accurately reflect relative antimicrobial effectiveness. The size of ID in this method was dependent on the solubility and rate of diffusion of the sample (5). Undiluted DSOF 3 which showed similar thin layer chromatogram with santalol (IFF), expressed smaller inhibitory activity compared to other fractions as well as to the whole sandalwood oil. Purification of this fraction resulted into even smaller inhibition activity against both bacteria. Other components than that in DSOF 3 might be responsible for larger antibacterial activity.

Infrared spectra assessment

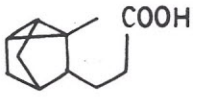
Assessment of infrared spectra of the sandalwood oil fractions, as shown in *Table 2*, indicated that major peaks showed the presence of functional groups OH (3550-3200 cm^{-1}); CH_2, CH_3 (2980-2840 cm^{-1}); C-CH_3 (1454 cm^{-1}); C-H (1375 cm^{-1}) in all fractions. Functional group C=O (1715 + 24 cm^{-1}) in fractions DSOF 1, DSOF 4 and DSOF 5; C=C (1667-1640 cm^{-1}) in fractions DSOF 2 and DSOF 3. Aromatic group was not observed (6, 7). These data indicated that fractions DSOF 1, DSOF 4 and DSOF 5 contained hydroxyl and carbonyl groups, while DSOF 2 and DSOF 3 contained olifenic hydrocarbons as well as hydroxyl groups.

Table 2. Infrared Assessment of Major Peaks of Fractionated Dry Sandalwood Oils

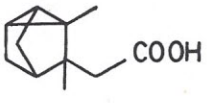
Infrared Absorbances (cm^{-1}) of Fractions of Dry Sandalwood Oil					
DSOF 1	DSOF 2	DSOF 3	DSOF 4	DSOF 5	Indication
3471.6	3409.9	3338.5	3444.6	3404.1	O-H intermolecular 3550-3200 cm^{-1}
2954	2964.4	2954	2954.7	2952.8	C-H stretching: methylene group 2980-2840 cm^{-1}
1712.6	-	-	1710.7	1710.7	C=O carbonyl compounds 1715 cm^{-1} ± 25 cm^{-1}
-	1679.9	1656.7	-	-	C=C olefinic hydrocarbons 1667-1640 cm^{-1}
1458.1	1454.2	1454	1454.2	1454.2	C-H asymmetrical, methyl group near 1450 cm^{-1}
1375.1	1375.1	1375.2	1375.1	1375.2	C-H symmetrical, methyl group occurs near 1375 cm^{-1}
-	1002.9	1001.8	-	-	
877.5	877.5	877.5	877.5	877.5	C-C and C-H alkenes 1000-800 cm^{-1}
-	852.4	852.4	852.4	852.4	

Referring to Demole *et al* (3), who investigated volatile constituents in East Indian sandalwood oil, possible structures for DSOF 1, DSOF 4 and DSOF 5 might be as presented in *Table 3*, and for DSOF 2 and DSOF 3 as presented in *Table 4*.

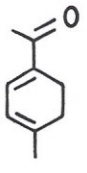
Table 3. Possibile structures for DSOF 1, DSOF 4 and DSOF 5*).



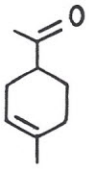
teresantalic acid



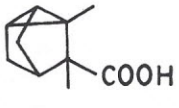
4-methyl cyclohex-3-en-1-yl methyl ketone



4-methyl-cyclohexa-1, 3-dien-1-yl methyl ketone



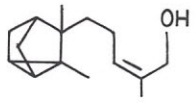
nortricyclo-eka-santalic acid



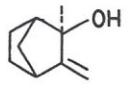
tricyclo-eka-santalic acid

*) Demole, E., C. Demole and P. Enggist, 1976.

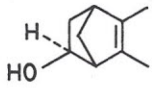
Table 4. Possible structures for DSOF 2 and DSOF 3*).



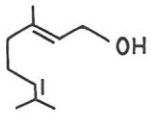
α-santalol



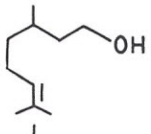
endo-2-methyl-3 methylidene-nonbornan-exo-2-ol



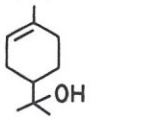
5, 6-Dimethyl-5 non bornen-exo-2-ol



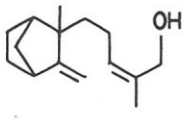
geraniol



citronellol



α-terpineol



ß-santalol

*) Demole, E., C. Demole and P. Enggist, 1976.

As observed from the results obtained, variation in antibacterial activity of sandalwood oil fractions could not be related to the functional groups of each fraction. Further purification will be carried out to identify the component(s) responsible for the antibacterial activity of sandalwood oil.

CONCLUSION

Sandalwood oil prepared from sandalwood sawdust showed antibacterial activity against *Staphylococcus aeureus* and *Bacillus cereus*. The antibacterial activity was larger than that of sandalwood oil from Kupang and santalol from IFF.

All five fractions of sandalwood oil showed antibacterial activity against both bacteria, their activities varied between one another. Larger, similar as well as smaller inhibition activities compared to the whole sandalwood oil were observed .

Terpene-alcohols,- acids, -ketones are the possible compounds analyzed in the sandalwood oil fractions.

REFERENCES

1. Guenther, E., "The Essential Oil", New York, Van Nosstrand, Vol V, 173-187 (1952).
2. Lingga, P., "Resep-resep Obat Tradisional", P.T. Penebar Swadaya, Jakarta, 1987.
3. Intisari, Mei 1987, pp.92.
4. Harris, R., "Tanaman Minyak Atsiri", P.T.Penebar Swadadaya I- Jakarta, 31-33, (1987).
5. Demole, E., C. Demole and P. Enggist, "A Chemical Investigation of the Volatile Constituents of East Sandalwood Oil (*Santalum album* L.), Helvetica Chimica Acta, Vol. 59, Fasc. 3, Nr. 76, 737-747 (1976).
6. Burrows, W., "Textbook of Microbiology", 17th ed.,W.B. Saunders Company, 403-405 (1959).
7. Merck, E., Handbook of Microbiology, first supplement Darmstadt, pp 111-113, (1979).
8. Morris, J.A., A.Khettry and E.W. Seitz, "Antimicrobial Activity of Aroma Chemicals and Essential Oils", Journal of the American Oil Chemists' Society, vol 56, 595-603 (1979).
9. Kemp, W.,"Organic Spectroscopy", The Macmillan Press Ltd., 8-75 (1979).
10. Sudjadi, M.S., "Penentuan Struktur Senyawa Organik", Ghalia Indonesia, 202-256, (1985).