

# Plant essential oils potency as natural antibiotic in Indonesian medicinal herb of "jamu"

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**Abstract.** The main purposes of this study are to compile antibacterial activity data of essential oils from Indonesian's plants in order which can be used as a natural antibiotic in "jamu" to increase potential Indonesian medicinal herb. By using Agar Diffusing method, Bioautography and Gas Chromatography Mass Spectrum, respectively, antibacterial activity and chemical compounds of 12 plants essential oils were studied in the Natural Product Chemistry Laboratory, Department of Chemistry, Faculty of Science and Mathematics, Satya Wacana Christian University, Salatiga since 2007 until 2015. The results of this studies showed that all of the essential oils have a medium to a strong antibacterial activity which are in the range of 30 – 2,500 µg and 80-5,000 µg. Further on, the essential oils analyzed by GCMS showed that each essential oils have different dominant compounds. These data can be used as basic doses in the usage of essential oils as natural antibiotics.

## 1. Introduction

One of Indonesian culture heritage is to drink "jamu" and another traditional herbal medicine to maintain health and disease preventive. Doctors also believed that herbal medicine has a good bioactivity and also safe. But one of the problems of Indonesian herbal medicine is they do not have enough clinical test to support medicinal treatment.

As a tropical country, Indonesia very close to many kinds of infectious diseases, so antibiotic using in medicine to be a basic need. Nowadays, around 96% medicine basic commodity were imported from China and India, and the large quantity is used for antibiotics; the amounts reached millions USD.

Medicinal and aromatic plants are used on a large scale in medicine against drug-resistant bacteria, which are considered one of the most important reasons for the lack of success of treatment in infectious diseases. Unresponsible antibiotic utilizing give the facility to form resistant super bacteria to antibiotic and will become a boomerang for the human shortly.

In 2007 a lot of countries decided to stop antibiotic production because the complicated process and the benefit which provide is not in proportion to the danger in the future. So what are we have to do to handle a lot of infections around us? Essential oils may be one of the alternatives to answer this problem, because of its ability as an antibacterial compound

As a tropical country, Indonesia has a lot of aromatic plants, in fact, 40-50 kinds of essential oils trading in the world came from Indonesia. [1] One of the important compounds in Indonesian herbal is essential oils, a volatile oil from plants, this oil is composed of many phenolic compounds which are



responsible for the strong antibacterial effect. [2] This nature gives an opportunity to essential oils to be used as an antibiotic substitution, especially in "jamu" Indonesian traditional medicine utilizing.

The aims of this research are to equip data of essential oils dose which showed the antibacterial effect against Gram-positive and negative bacteria and to give information about dominant chemical compounds which responsible to antibacterial activity to be used optimally as an antibiotic substitution.

## 2. Material and method

### 2.1. Material

Essential oils of twelve species plants were used as samples, which are: Limo (*Citrus amblycarpa* (Hassk) Ochs), Lime (*C. aurantifolia* Swingle), Lemon (*C. limon* (L.) Burm.), Kefir lime (*C. hystrix* D.C.), Masoi (*Cryptocarya massoia* (Oken) Kosterm.), Surinam cherry/ Dewandaru (*Eugenia uniflora* L.), Anise (*Foeniculum vulgare* Mill.), Star anise (*Illicium verum* Hook. E.), Kragean (*Litsea cubeba* (Lour.) Pers.), Torch Ginger/Kecombrang (*Nicolaia speciosa* Horan), Basil (*Ocimum basilicum* Linn.) and Legetan (*Spilanthes paniculata* Wall), respectively.

Isolates of Gram-positive bacteria: *Bacillus subtilis* ATCC 6051, *B. cereus* FNCC 0057, *Staphylococcus aureus* FNCC 0047 and Gram-negative bacteria: *Escherichia coli* IFO 0091; *Pseudomonas cepacia* FNCC 0063; and *P.aeruginosa* FNCC 0063 were obtained from Laboratory of Microbiology, Faculty of Science and Mathematics, Satya Wacana Christian University, Salatiga.

All chemical reagents made by E-Merck, Jerman: toluene, ethyl acetate, ethanol, H<sub>2</sub>SO<sub>4</sub>, PEG, silica gel 60 F254, vanillin, NaCl, Nutrient Broth. Mueller- Hinton Agar (PA, Oxoid, Inggris), tetracyclin (PA, Oxoid, Inggris) and paper disc (PA, Whatman, Inggris),.

Steam distillation apparatus with clevenger, micropipette (Biohit), analytical balance (Mettler H80), oven (WIB-Binder), spectrophotometer (UV-Vis Mini Shimadzu U-1240), Gas Chromatography – Mass Spectrophotometer (Shimadzu QP2010S).

### 2.2. Method

#### 2.2.1. Essential oil extraction [3]

Essential oil from fresh, clean, weighed aerial parts, fruit peel, bark, fruit, seed, flower and leaves and extracted by hydro-steam distillation using Clevenger apparatus were collected and store. The oil was filtered through anhydrous Na<sub>2</sub>SO<sub>4</sub> to dry yielded essential oil, keep in tightened vials and stored in a refrigerator.

Physicochemistry test was done by rendement calculation, colour, transparent and aroma. GCMS analysis essential oil was done in Organic chemistry laboratory, Faculty of MIPA Universitas Gadjah Mada Yogyakarta.

#### 2.2.2. Direct bioautographic test [4]

The essential oil was evaluated in vitro by Thin-layer Chromatography (TLC) plate, before using the plate was activated in 105°C 10 minutes. The plate of silica gel F254 4x10 cm (Merck) as a solid phase and toluene: ethyl acetate 93:7 as a mobile phase were used. Afterward, plate was sprayed with bacterial suspension in Mueller- Hinton Broth (MHB), and plate was stored in a water-vapor chamber at 37°C 24 hours. Iodonitrotetrazolium chloride 5 mg/ml was used to visualize the antibacterial spot.

#### 2.2.3. Antimicrobial disk diffusion test [5]

One ose bacteria was inoculated to Nutrient Broth (NB), incubated at 30 oC for 24 hours. Dilute inoculum by using physiological solution ( NaCl 0,9%) to match a 0,5 Mc Farland standard. The bacterial suspension was diluted and measured by UV-Vis Spectrophotometer to obtain Optical Density (OD) 0,4-0,5 at 550 nm.

A paper disc was dropped 20 µl essential oil in certain concentration and put the disc in a petri dish with medium content bacteria inside. The petri dish was incubated at 30 ° C 24 hours. Inhibition Area Diameter (IAD) was measured as a middle line start from the clear spot around the disc. The lowest concentration which shows the clear spot around the paper disc is the Minimum Inhibitory Concentration (MIC).

#### 2.2.4. Isolation and identification antibacterial compound of essential Oil

The essential oil was evaluated in vitro by using Thin Layer Chromatography (TLC) plate. 4x10 cm silica gel F254 sheets (Merck) as a solid phase and toluene: ethyl acetate 93:7 as a mobile phase. The isolated spot was analyzed by GCMS in Laboratory of Organic Chemistry, Fakultas MIPA Universitas Gadjah Mada Yogyakarta. The Mass Spectrum of every peak from the chromatogram was compared to database.

#### 2.2.5. Data analysis [6]

Antibacterial activity data of each plant were analyzed by using Randomized Completely Block Design (RCBD) Sub-Sampling, five treatments, three subsamples, and five replications. As the blocks are the analysis time.

### 3. Result and discussion

Table 1 demonstrated that the rendement of each essential oil has variation in amount but in general, the amount of essential oil is relatively less than 3 %, and the oil content distributed in a different part of a plant. The small amounts of essential oil have already enough to do the function as a self-protecting to the prey, as a fascinating compound. The application essential oil in human life for example as a flavor in food, cosmetic, medicine, toiletries [2, 7, 8]

**Table 1.** Plants and their families, partly used and rendement

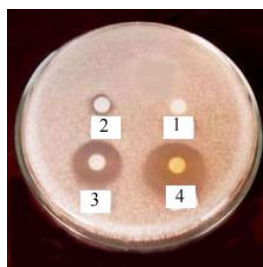
Scientific name	Plant family	Part used	Rendement(%) w
<i>Citrus amblycarpa</i> (Hassk.) Ochs (Limo)[9]	Rutaceae	Peel	0.92
<i>C. histrix</i> D.C. (Kefir lime)[9]	Rutaceae	Peel	0.72
<i>Cryptocarya massoiya</i> (Oken) Kosterm. (Masoi) [10]	Lauraceae	Bark	1.40
<i>Illicium verum</i> Hook F. (Star anise) [11]	Illiciaceae	Flower	2.11
<i>Litsea cubeba</i> (Lour) Pers. (Krangean) [12]	Lauraceae	Bark	0.73
<i>Nicolaia speciosa</i> Horan ( Torch Ginger) [13]	Zingiberaceae	Flower	1.34
<i>Ocimum basilicum</i> L. (Basil) [14]	Lamiaceae	Leaf	1.53

Table 2 demonstrated the strength of antibacterial activity at Minimum Inhibitory Concentration (MIC). Low (L) if the Inhibitory Area Diameter (IAD) less than 0.7 cm; Medium (M) the IAD 0.7-0.8 cm; and Strong (S) the IAD > 0,8 cm [15]. There are three big groups of essential oils have antibacterial activity in medium and strong levels, the first group has MIC less than 100 µg, the second group has MIC 100 - 1000 µg; and the last group with MIC > 1,000 µg. The higher the concentration of the essential oil, the lower the antibacterial activity obtained. Limo, kefir lime, star anise and torch ginger all these species usually used in food, they have antibacterial activity with MIC > 1,000 µg, whereas masoi and krangean usually used in traditional herb medicinal showed a medium to strong antibacterial activity with MIC less than 100 µg. All the essential oils samples indicated broad antibacterial spectrum because of its show antibacterial activity against Gram- positive and negative bacteria. Figure 1 showed the Inhibitory Area Diameter (IAD) as a clear spot around the paper disc.

**Table 2.** Minimum Inhibitory Concentration, Strength of Essential Oil Antibacterial Activity

Scientific name	Part used	Minimum Inhibitory Concentration (MIC) µg.					
		<i>B. cereus</i> FNCC 0057	<i>B. subtilis</i> ATCC 6051	<i>S. aureus</i> FNCC 0047	<i>P. aeruginosa</i> FNCC 0063	<i>P. cepasia</i> FNCC 0063	<i>E. Coli</i> 0091 IFO
<i>Citrus amblycarpa</i> (Hassk.) Ochse (JerukLimau)[9]	Peel	-	2,500(M)-5,000 (S)	-	2,500(M) - 5,000(S)	-	2,500(M)-5,000(S)
<i>Citrus histrix</i> D.C (JerukPurut)[9]	Peel	-	2.500(M)-5.000 (S)	-	2.500(M)-5.000(S)	-	5.000(M)-7.500(S)
<i>Cryptocarya massoiya</i> (Oken) Kosterm (Mesoyi)[10]	Bark	-	30(M)-80(S)	-	-	30(M)-120(S)	30(M)-80(S)
<i>Illicium verum</i> Hook. F.(AdasBintang)[11]	Flower	-	3,000(M)-4,000(S)	3,000(M)-3,500(S)	4,000(M)-6,000(S)	-	3,500(M)-5,000(S)
<i>Litsea cubeba</i> (Lour) Pers.(Krangean)[12]	Bark	75(M)-100(S)	-	75(M)-100(S)	>200(S)	-	100(M)-150(S)
<i>Nicolaia speciosa</i> Horan (Kecombrang)[13]	Flower	-	1,700(M)-2,100(S)	-	-	-	1,700(M)-2,100(S)
<i>Ocimum basilicum</i> L. (Kemangi)[14]	Leaf	-	2,500(M)-5,000(S)	2,500(M) 5,000(S)	-	-	7,500(M)-10,000(S)

Note : – no test (M) : medium ( IAD 0,7-0,8cm ) (S) : Strong (IAD > 0,8 cm )



**Figure1.** MIC Essential oil of Limo peel Against *B. subtilis* ATCC 6051 : 1. Negative control, paper disc and solvent, 2. Limo essential oil 2,500 µg, 3. Limo essential oil 5,000 µg, 4. Tetracyclin



**Figure 2.** Bioautography test: A.Antibacterial compound in white spot. B.The isolated antibacterial compound was Rechecked.

Table 3 informed five main compounds of each essential oil. The essential oil of Citrus group has many similar compounds e.g. limonene, pinene, terpineol, however, the essential oil content is different. The main compound of essential oil and its amount are responsible for the characteristic aroma of the plant. Beside five main compounds, there are a lot of different compounds which is synergy to give antibacterial activity.

**Table 3 :** Five Main Compounds of each Essential Oil

No	Scientific name	5 main Compounds
1	<i>Citrus amblycarpa</i> (Haask.) Ochse (Limo), peel	limonena 26.85 %, $\beta$ -pinena 20.52 %, Sitronellal 11.69 %, $\alpha$ -terpineol 10.68 %, Geranilasetat 6.91 %.
2	<i>C. histryx</i> D.C(kefir lime), peel	$\beta$ – pinena 35.00 %, sitronelal 18.24 % , terpineol 8.11 % , limonena 6.29 %, geranilasetat 6.1 %.
3	<i>Cryptocarya massoia</i> (Oken) Kosterm (Mesoyi/masoi), bark	5,6-dihydro-6-pentil-2H-pyran-2-one 80.51 %, 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl) ester 6.99 %, benzybenzoat 5.64%, ethan, 1,1-dicyclopentyl 1.52 %, nonana 1.34%.

4	<i>Illicium verum</i> Hook F. (star anise), flower	anetol 79.65 %, Metil kavikol 6.91% , fenikulin 4.01 %, limonene 3.83 %, linalool 1.10 %.
5	<i>Litsea cubeba</i> (Lour.) Pers. (Krangean), bark	1-8 sineol 29.87 %, sitronelal 17.59 %, 1-limonen 10.74 %, Isopulegol 10.70 %, neoiso (iso) pulegol 5.12 %
6	<i>Nicolaia speciosa</i> Horan (Kecombrang), flower	1-dodecanol 30.26 %, dodecanal 26.17%, dodecanoic acid 18.96%. ester dodecyl 7.84 %, decanal 2.69 %,
7	<i>Ocimum basilicum</i> L. (Kemangi), leaf	Z-sitral 76.35 %, cis- $\alpha$ - bisabolene 5.14%, <i>trans</i> - <i>caryophyllene</i> 3.05 %, trans-caran 4,5-epoksi 2.09 %.

#### 4. Conclusions

According to IAD all of seven essentials oil samples have antibacterial activity in the range of medium to strong level. Especially, Masoi and Krangean showed smaller doses in compared to another, and each essential oil has different dominant compound.

#### References

- [1] Muhtadin AF, Ricky W, Pantjawarni P and Mahfud 2013 Pengambilan Minyak Atsiri dari Kulit Jeruk Segar dan Kering dengan Menggunakan Metode Steam Distillation *Jurnal Teknik Pomits* **2** 1 2301-9271 ISSN 2337-3539
- [2] Burt S. 2004 Essential oils: their antibacterial properties and potential application in foods – a review International. *Journal of Food Microbiology* **94** 223-253.
- [3] Sastrohamidjojo H 2004 Kimia Minyak Atsiri 248 p
- [4] Hamburger MO, Cordell JA. 1987 A Direct Bioautographic TLC Assay for Compounds Possessing Antibacterial activity *J. Nat. Prod* **50** 1 19–22
- [5] Gundidza M. 1993 Antifungal Activity of The Essential Oil from *Artemisia afra* Jacq *Cent. Afr. J. Med* **39** 7 140-142
- [6] Steel R G D, Torrie J H. 1981 Principles and Procedures of Statistic Biometrical Approach 2nd Japan Mc Graw Hill International Book Cop 633
- [7] Elsner P, Maibach I H 2005 Cosmeceuticals and Active Cosmetics 2nd Ed Taylor and Francis New York 675
- [8] Schrader K, Domsch A 2005 Cosmetology-Theory and Practice **3** 62 Verlag fur Chemische Industrie Augsburg
- [9] Soetjipto H, Sulihingsih E and Dewi L 2010 Komposisi Kimia dan Efek Antibakteri Minyak Atsiri Kulit Buah Jeruk Purut (*Citrus hystrix* D.C) dan Jeruk Limau (*Citrus amblycarpa* Hassk) *Prosiding Seminar Nasional Kimia II Bervisi SETS HKI Jawa Tengah Semarang* 117-123
- [10] Soetjipto H, Martono Y and Fitriani IL 2011 Identifikasi Senyawa dan Aktivitas Antibakteri Minyak Atsiri Kulit Masoi (*Cryptocarya massoia* (Oken) Kosterm) *Prosiding Seminar Nasional Kimia dan Pendidikan Kimia III Fakultas Keguruan dan Ilmu Pendidikan Universitas Sebelas Maret Surakarta* 582-588

- [11] Widiastuti P, Soetjipto H and Hastuti SP 2007 Aktivitas Antibakteri dan Identifikasi Komponen Minyak Atsiri Adas Bintang (*Illicium verum* Hook F) *Prosiding Seminar Nasional Aplikasi Sains dan Matematika* Fakultas Sains dan Matematika Universitas Kristen Satya Wacana Salatiga 198-204
- [12] Soetjipto H, Fajarwati D, Timotius KH 2007 Identifikasi Komponen dan Aktivitas Antibakteri Minyak Atsiri Kulit Batang Kragean (*Litsea cubeba* Pers.). *Prosiding Seminar Nasional Aplikasi Sains dan Matematika*. Fakultas Sains dan Matematika Universitas Kristen Satya Wacana Salatiga 190-197
- [13] Soetjipto H, Hastuti SP and Kristanto O 2009 Identifikasi Senyawa Antibakteri Minyak Atsiri Bunga Kecombrang (*Nicolaia Speciosa* Horan). *Prosiding Seminar Nasional Sains dan Pendidikan Sains IV. Fakultas Sains dan Matematika* Universitas Kristen Satya Wacana Salatiga 640-655
- [14] Ristanti WF, Soetjipto H and Timotius KH 2006 Identifikasi Citral dan Aktivitas Antibakteri Minyak Atsiri Daun Kemangi (*Ocimum basilicum* Linn) *Prosiding Seminar Nasional Kimia dan Pendidikan Kimia* Jurusan Kimia Fakultas MIPA- Universitas Negeri Semarang 67
- [15] El Gayyar M, F A Draughon, D A Golden and J R Mount 2001 Antimicrobial Activity of Essential Oils Plants Against Selected Pathogenic and Saprophytic Microorganism *Journal of Food Protection* **64** 7 1019-1024