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ESSENTIAL OIL COMPOUND EXTRACT OF SIX TEMU-TEMUAN (*Curcuma spp*) PLANTS AS ANTIBACTERIAL AGENT

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Abstract

The objective of this research was to see the components of six temu-temuan Plants (*Curcuma spp)* essential oil extract: temu giring (*Curcuma heyneana*)/TG, temu Ireng (*Curcuma aeruginosa*)/TI, temu kunci (*Boesenbergiia pandurata*)/TK, temu lawak (*Curcuma xanthorrhiza*)/TL, temu mangga (*Curcuma mangga*)/TM, and temu putih (*Curcuma zeodaria*)/TP as antibacterial agents. The bacteria used were *Staphylococcus epidermidis*, *Staphylococcus Aureus,* and *E. Colli*. Essential oil with high yield was separated with chromatography column for fractionation. Each active fraction was determined and analyzed by GC-MS. The result shows that each yields from TG, TI, TK, TL, TM, and TP 1.00%, 0.47%, 1.33%, 0.47%, 1.00%, and 0.47%, MIC on *Staphylococcus epidermidis* for TK and TG 1000 mg/ml, TI, TM, TP 500 mg/ml, and TL 125 mg/ml. The most active MIC is TL with 125mg/L, and MBC is TG with 1000 mg/ml concentration. TK was the highest for essential oil yield from the six *curcuma sp*. plants. All *Curcuma spp* essential destilation extracts were tested for antibacterial activity with micro plate method. All *Curcuma sp* essential oil destilation extracts with minimum inhibitory concentration (MIC) gave the following results: TK and TG 1000 mg/ml, TI, TM, and TP 500 mg/ml, and TL 125mg/ml. TG were the most active with MIC 1000 mg/ml and minimum bactericidal concentration (MBC) 1000 mg/ml. All volatile oil *curcuma spp* were obtained by water distillation. Chemical components were determined using gas chromatography and mass spectrometry (GC-MS). From the positive results of MIC and MBC, the TG main components of volatile oil were obtained as follows: 1.8-Cineole, Camphene, Camphor, Borneol L, 1-β-Pinene, β-elemene, Methoxymethyl, Xanthorhizol, Germacrene, 1-α-terpineol, caryophyllene oxide, 6-methylxanthotoxin, critonilide, 2-methoxymethyl-4,4-dimethyl-5-phenyldihydro pyran.

Keywords : *Essensial oil, antibacterial, Curcuma heyneana, Curcuma aeruginosa, Boesenbergiia pandurata, Curcuma xanthorrhiza, Curcuma mangga, Curcuma zeodariam, Staphylococcus epidermidis.*

INTRODUCTION

Indonesia is an archipelago country and the second highest in biodiversity (high number of indigenous medicinal plants, soil) in the world, producing medicinal plants commodity that has the future prospect as export commodity. Based on WHO data, request for herbal products all over Europe in 1999-2004 is estimated almost 66% from all over the world (Wardana *et al*. 2002). One of the examples is spice plants, which people know as property and from generation to generation, especially *Curcuma spp* (Salvi *et al*., 2000; Shirgurkar *et al*. 2000)

Curcuma spp is one of the *Zingiberaceae* family members, which grows in tropical land and is used as spices, perfumes, medicine, cosmetic material, dye for food, and ornamental flowers (Heywood, 1985). Active compounds of *Curcuma spp* is usually found as essensial oil. Ketaren (1985) said that essential oil is one of oil group that has fragrant characteristic and is volatile in room temperature. Essential oil is generally divided into two components: hydrocarbon and group of oxygenation hydrocarbon (Robinson, 1991; Soetarno, 1990). Heyne (1987) has reported that a derivative compound from oxygenation hydrocarbon (phenol) has the potency to be strong antibacterial agent. Distillation by water is one of methods for extracting essential oil. This method makes the material not easily damaged by steam for materials such as roses and ginger. Essential oils extraction uses matching solvent for separating flower oil that is not stable and can be damaged by the heat of water steam (Kahol, 1984). Curcuma is a genus from the Zingiberaceae family, division: Magnoliophyta, sub-division: Spermatophyte, class: Liliopsida, order: Zingiberales (Kress *et al*. 2002). In this research, the six *Curcuma spp* used are temu giring (*Curcuma heyneana/* TG), temu ireng (*Curcuma aeruginosa/TI*), temu kunci (*Curcuma boesenbergia pandurata/TK*), temu lawak (*Curcuma xanthorriza species*/TL), temu mangga (*Curcuma magga*/TM), and temu putih (*Curcuma zeodaria*/TP).

The advantages of active compounds from TG has been used for the cooling and cleansing of blood (Heyne, 1987), anthelmintic effects (Asri 2006), alternative to control helminthiasis on Kid Etawah Cross Goat, to increase Superoxide Dismutase (SOD) activity, and to repair pancreatic beta cells damage on diabetes mellitus (Lukiati 2012).

Essential oil extracts of TI has been used as anti-larva, inhibiting larva to consume leaves (Nurhasyim. 1990), antinociceptive, antipyretic, and anti inflammatory agent (Reanmongkol *et al*. 2006), anthelmintic agent (Tamara, O. Sunoko, H. R. 2008). The latest research from Kamazeri *et al* (2012) reported TI as anti-fungi and anti-microbes and that TI can be used as treatment for baldness and placebo control by inhibiting derived 5α-reductase (Pumthong *et al*. 2012).

TK has been reported to be able to inhibit and isolate Orf bacteria (Plant, 2008). TK has been used for rheumatism, muscle pain, febrifuge, gout, gastrointestinal disorders, flatulence, carminative, stomach ache, dyspepsia, and peptic ulcer. In jamu (Indonesian traditional medicine) for women after childbirth, beauty aid for teenage girls, medicine for leucorrhea (Chaudhury and Rafei, 2001). TK was used to against inflammatory diseases, such as dental caries, dermatitis, dry cough and cold, tooth and gum diseases, swelling, wounds, diarrhea, and dysentery, and as diuretic (Chuakul and Boompleng. 2003; Salgeuro. 2003). Riswan and Roenian (2002) has reported TK as antifungal, antiparasitic, and antiscabies agent. Another function of TK is that it can take care of allergies, poisoning, AIDS, and acts as anti-biotic and anti-amoebic agent (Rukayadi Y. et al. 2009). Lastly, TK has been reported as food ingredient, and in ethonomedicinal preparations, used for studies such as drug discovery, polypharmacology, and drug delivery using nanotechnology (Chong T E. *et al*. 2012).

TL has been reported to be able to increase the production of bile sap and to reduce tissue swelling (Liang et al, 1985). One of the essential oil compound in *Curcuma xanthoriza* is camphor, that has been used in medicine plant of big company jamu in Indonesia (Karangjati, 2004). TL has been used as breast cancer cure (Khairinal, 2011). The latest news is that TL is reported as an anthypercholesterolemic agent (Sukandar E.Y, *et al*. 2012).

Bos *et al* (2007) has reported that TM has curcuminoid 0.18% - 0.47%. Tedjo *et al.* (2005) showed that TM has high antioxidant activity. In traditional literature, it is reported as medicine for ulcer, diarrhea, reducing pain reliever on menstruation, white vaginal discharge, acne, blain diseases, shrinking the uterus and enhancing appetite.

The famous name of TP is *Curcuma zedoary* and the patent medicine name is Leilipien and Pao kwun tan (Flanch & Rumawas, 1996). TP has been used as anti-inflammation, anti-cancer, and facilitator for blood circulation, fibrinolytic agent and anti-neoplastic agent. Rita (2010) has reported that triterpenoid on TP as *S. aureus* and *E. coli* antibacterial agent. TP is already shown as antibacterial for *Klebsielle pneumoniae,* the bacteria of pneumonia disease (Nurhayati, 2010). The Health Department of Indonesia showed that TP has an effect as diarrhea and dysentery medicine. As health juice, Nuratmi *et al* (2007) proved that TP can be useful as diarrhea medicine tested in vivo, using white male rats as subjects.

However, of the many advantages of *Curcuma spp* species, until now it has never shown the potential to inhibit or kill skin bacteria. Skin bacteria live in colonies on the surface of dead cells. Most of these bacteria are Staphylococcus (S. *epidermidis* and S. *aureus*). Skin bacteria caused bad body odor. It can be affected by some factors: genetic, emotion, food,

obesity, and clothes material (Jacoeb 2007). Barleet (2007) has discovered that *S. epidemidis* is resistant to penicillin, and meticillin antibiotic. Antibacterial agent is a substance able to exterminate pathogen microbes in human or animals but doesn't affect the host (Gan, 1987). Antibacterial agent work by killing (bactericide) and inhibiting bacteria growth (Schunack 1990). Pelzcar and Chan (1986) said some compounds that have antibacterial characteristic are ethanol, phenol, chlorine, iodine, and oxide ethylene. Characteristics of antibacterial compound can be found in essential oil. Yunilawati (2002) has found that essential oil from betel leaf can inhibit *S. mutants* growth. The latest information from Hidayat (2011) has reported that Kepel leaf (*Stelechocarpus burahol*) has the potential to inhibit and kill *S. epidermidis* bacteria.

One of the efforts to inhibit bacteria is by consuming food that has been proven as bacteria inhibitor or antibacterial agent. *Curcuma spp* is believed to be one of the food species that has potential as antibacterial agent. The objective of this research was to show chemicals compounds from *Curcuma spp* (TG, TI, TK, TL, TM, and TP) compared with GC-MS library profile and the extracts of essential oil compound (distillation with water as solvent) as antibacterial agent.

MATERIALS AND METHODES

Materials

Six species of *curcuma spp* (TG, TI, TK, TL, TM and TP, distilled water, hexane, acetate ethyl, chloroform, DMSO solvent, gel silica, TSB media, bacteria (*Staphylococcus epidermidis, Escherichia coli* and *Bacillus subtilis*), silica gel aluminum plate $G_{60}F_{254}$ by Merck, glass tools, distillation tools, porcelain cup, oven, desiccators, analytical scales, rotary evaporator, container chromatography, column chromatography, autoclave, incubator, 96-well plate, and GC-MS.

Methods

- Sample preparation
  Six of *Curcuma spp* (was take from plantation institution, Cimanggu, Bogor-Indonesia) cleaned with water and were cut into slight pieces. Slight pieces dried under the sun for 3 days.
- Distillation
  150 gram sample were put into distillation flask (capacity 500 gram) and then added by 2 liters distillation water. Flask was put into heat mantel and then connected with condenser. Mixture was boiled for 4 hours. Distillations water was restored at different tube/bottle from essential oil.
- Qualitative identifications of GC-MS
  Qualitative identification was using measurement by GC-MS in Health district laboratory, Jakarta-Indonesia. Directly injection sample was

identified by the following conditions. Instrument: Agilent Tech 6890 Gas Chromatography with auto sampler and 5975N mass selective detector and Chemstation data system, Ionisation Mode: Electron Impact, Electron energy: 70 eV, Column: HP 5 MS. Capilary column length (m): 30x0,25(mm) I.D x 0,25(µm) Film Thickness Oven Temperature: Initial temperature at 60^{o}C was held for 1 minute, rising at 3^{o} C/min to 150^{o} C held for 20 minutes, Injection Port Temperature: 250^{o} C, Ion Source Temperature: 230^{o} C, Interface Temperature: 280^{o} C, Quadrupole Temperature: 140^{o}C, Carrier Gas: Helium, Column mode: Constant flow, Injection Volume: 0,6µL, Split: 250:1, Method File: MGNONWAX

- Antimicrobial inhibitory effects (MIC and MBC) test

***Staphylococcus epidermidis (S. epidermidis)* test**

The organism used on this research was *Staphylococcus epidermidis*. The media used was Trypticase Soy Broth (TSB). 100 µL sterile medium and 40 µL samples were homogenous in DMSO 20% or control and 5 µL inoculums bacteria were put into holes inside plate (96-well plate). After inoculums were ready for concentration 10^{-2} CFU/ml, the *S. epidermidis* was incubated in the media for 48 hours at 37^{o} C. Extract concentration did not show bacterial growth (clear vision) identified by minimum inhibition concentration (MIC). 100 µL from media did not show bacterial growth, and then inoculated in100 µL new media. Concentration did not show bacterial growth after the second inoculation identified as minimum bactericidal concentration (MBC). DMSO was used as negative control.

***Escherichia coli* (*E.coli*) and *Bacillus subtilis* (*B.subtilis*) test**

Microbes of *Escherichia coli* and *Bacillus subtilis* were scratched on the surface of the nutrient agar media in different Petri dish. 15 µL dropped into upper of paper disk with 6 mm diameter, and then dried. Paper disk were put into Petri dish containing nutrient agar media. Inoculated for 24 hours at 37^{o}C and then Inhibition were calculated. Rifampicin 1000 ppm was used as antibiotic control.

RESULT AND DISCUSSION

Sample Preparation

The aims for cutting and drying for more 3 days of *curcuma spp* was able to reduce the water concentration to become ≤ 10%, this is to reduce and inhibit fungal activity and to open the oil gland as much as possible. If the plants were put in their original form, it would make the diffusion process of essential oil to run very slow (Ketaren, 1987).

1 2 3

Fig 1 *Curcuma spp* TG, TI, TL

Essential oil isolation

This research used water distillation because there are some benefits from using this method: essential oil products don't directly mixture with the air and it reduces loses of essential oil during processing. The principle of distillation method is direct contact to boiling water (hydro distillation). Hydro distillation makes hydro diffusion where the gland breaks and essential oil will be free and taken by the vapor, after which, it will be cooled by the condenser. After cooling down, the essential oil is produced. The oil separates with water. The color of essential oil can be seen on figure 2 below.

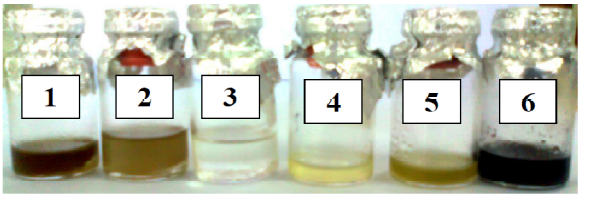


Fig 2 Essential oil extract by water distillation method, 1).TG, 2).TI, 3).TK,4).TL,5).TM,6).TP

After essential oil was obtained, the calculation of every yield of *curcuma spp* is shown in table 1.

Table 1 Yields of *Curcuma spp* Essential Oil

No	*Curcuma spp*	Yields (%)	Color
1	TG	1,00	Light Brown
2	TI	0,47	Light Green
3	TK	1,33	No color
4	TL	0,47	Light Green
5	TM	1,00	Light Yellow
6	TP	0,47	Dark purple

The highest yields of essential oil extract were TK with 1.33%. The lower yields of essential oil extract were TI, TL, and TP with 0.47%. All essential oil were determined by Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS gave results that every *Curcuma spp* contained a few compounds (TG=74 compounds, TI= 66 compounds, TK= 65 compounds, TL=37 compounds, TM=59 compounds, and TP=76 compounds). Analysis was based from Similarity Index (SI) value, base peak, and trend of mass spectra pieces. All values were compared with Libraries Database Wiley 275.L. Table 2 shows SI value >70% for every compounds.

Table 2 Composition of the essential oils of *Curcuma* species (in percentage)

No	Compounds	Curcuma spp					
		TG	TI	TK	TL	TM	TP
1	α-Pinene	-	-	-	-	3.02	-
2	Camphene	3.52	-	4.83	-	-	2.90
3	1,8-Cineole	11.80	20.28	14.71	1.19	-	4.59
4	Camphor	14.37	6.50	16.36	19.43	-	7.52
5	Borneol L	3.08	1.72	-	2.12	-	1.94
6	Cis-Ocimene	-	-	3.41	-	-	-
7	trans-β-ocimene	-	-	22.65	-	5.09	-
8	1-β-Pinene	-	1.03	-	-	48.8	3.61
9	2-β-Pinene	-	-	-	-	24.04	-
10	Limonene	-	-	-	-	1.56	-
11	Methanoazulene	-	-	-	7.05	-	-
12	Mycrene	-	-	-	-	-	2.29
13	α-terpinol	1.05	-	1.57	-	-	-
14	Caryophylene oxide	1.34	-	-	-	-	-
15	Methylxanthotoxin	15.30	-	-	-	-	-
16	Critonilide	4.23	-	-	-	-	-
17	Linalool L	-	-	2.04	-	-	-
18	Geraniol	-	-	10.46	-	-	-

19	Terpehyl	-	15.56	-	-	-	-
20	Curcumol	-	12.34	-	-	-	-
21	Furan	-	-	-	-	1.18	-
22	Tetranoriabd	-	-	-	-	1.11	-
23	Curcumene	-	-	-	13.81	-	-
24	Sesquisabinene hydrate	-	-	-	2.46	-	-
25	β-elemene	-	-	-	5.46	-	1.57
26	Bicyclo	-	-	-	-	-	5.26
27	β-elemenone	-	-	-	-	-	1.28
28	Eremophilene	-	-	-	-	-	1.18
29	Methoxymethyl	2.59	-	-	8.49	-	-
30	Xanthorizol	1.05	-	1.21	-	-	-
31	Methyl Cinnamate	-	-	3.67	-	-	-
32	Valence	-	-	-	-	-	1.19
33	Germacrene	-	-	-	2.34	-	2.22
34	ɑ-terpineol	-	1.17	-	-	-	-
35	ethoxy/ethyl/trimethyl	-	-	-	-	-	2.58

Testing of Antibacterial Agent

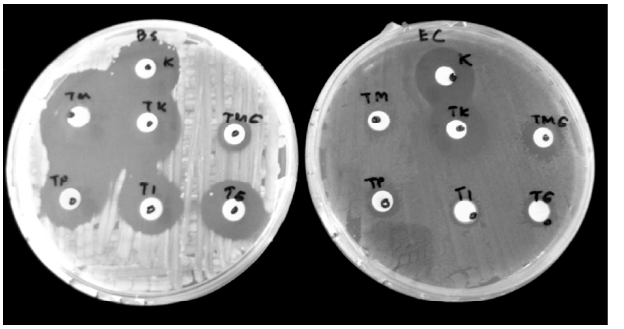
Six *Curcuma spp* used three bacteria, *Staphylococcus epidermidis*, *Escherichia coli*, and *Bacillus subtilis.* Microbe test result for *E. coli* and *B. subtilis* can be seen in Table 3.

Table 3 Test result of *E. coli* and *B.Subtilis*.

No	Sample	Inhibit Diameter (mm)	
		Escherichia coli	***Bacillus subtilis***
1	TG	9.97	17.48
2	TI	9.57	17.59
3	TK	18.11	19.42
4	TL	-	25.88
5	TM	12.29	9.91
6	TP	10.24	16.92
7	Rifampicin control (1000 ppm)	19.13	18.84

The microbe tested is shown on Figure 3. *Curcuma spp* made the nutrition media with white color to become clear zones. Clear zone is identified as inhibition power. Based on that result, all *Curcuma spp* have antibacterial activity for *E.coli* and *B. subtilis*, except TL. For inhibition of *E.coli*, TG, TI, TK, TL, TM, and TP showed the following clear zones (in mm): 9.97; 9.57; 18.11; negative; 12.29; 10.24, lower compared to the control of 19,94. For inhibition of *B.subtilis,* TG, TI, TK, TL, TM, and TP

showed the following clear zones (in mm): 17.48; 17.59; 19.42; 25.88; 9.91; 16.92; and the control was 18.84. Clear zone of TK and TL were bigger than control.



Bacillus subtilis *Escherichia coli*

Fig 3 Activity of six *Curcuma spp* as anti-bacterial agent for *Bacillus subtilis* and *Escherichia coli* (clear zones).

For inhibition of *S.epidermidis* bacteria, *Curcuma spp* (TG, TI, TK, TL, TM, and TP) had inhibition activity. The minimum inhibition concentration (MIC) of TL is the most active with the MIC determined to be 125µg/ml and TG had the minimum bactericidal concentration (MBC) which was determined to be 1000 µg/ml (Figures 4 and 5). MIC was of a lower level, where essential oil isn't able to inhibit of *S.epidermidis* bacteria anymore.

Table 4 MIC and MKC of six *Curcuma spp* as anti *S. epidermidis* bacteria

Conc (ppm)	Material Tested													
	TG		TI		TK		TL		TM		TP		control (+)	control (-)
2000	+	+	+	+	+	+	+	+	+	+	+	+	+	-
1000	+	+	+	+	+	+	+	+	+	+	+	+	+	-
500	-	-	+	+	-	-	+	+	+	+	+	+	+	-
250	-	-	-	-	-	-	+	+	-	-	-	-	+	-
125	-	-	-	-	-	-	+	+	-	-	-	-	+	-
62,5	-	-	-	-	-	-	-	-	-	-	-	-	+	-
31,25	-	-	-	-	-	-	-	-	-	-	-	-	+	-
15,63	-	-	-	-	-	-	-	-	-	-	-	-	+	-
MIC*	1000	1000	500	500	500	1000	1000	1000	125	125	500	500	500	

The MIC is important to know antibacterial potential of essential oil from *Curcuma spp*. MIC is used to determine lower effective doses from essential oil. The lower the value of MIC, the essential oil of *Curcuma spp* has more potential to inhibit bacteria growth.

Table 5 MKC of six *Curcuma spp* as anti-bacterial agent for *S. epidermidis*

Conc (ppm)	Material Tested												
	TG		TI		TK		TL		TM		TP		control (+)
2000	+	+	-	-	-	-	-	-	-	-	-	-	+
1000	+	-	-	-	-	-	-	-	-	-	-	-	+
500	-	-	-	-	-	-	-	-	-	-	-	-	+
250	-	-	-	-	-	-	-	-	-	-	-	-	+
125	-	-	-	-	-	-	-	-	-	-	-	-	+
62,5	-	-	-	-	-	-	-	-	-	-	-	-	+
31,25	-	-	-	-	-	-	-	-	-	-	-	-	+
15,63	-	-	-	-	-	-	-	-	-	-	-	-	+
MKC*	1000	2000	-	-	-	-	-	-	-	-	-	-	-

Essential oil can inhibit or kill by interfering with cell wall, cell membrane, inhibiting enzyme process or crushing the genetic materials of bacteria. Cell wall of bacteria is built from peptidoglycan slide. Essential oil increased the osmosis level of bacteria cell wall causing lysis. Antibacterial agents on essential oils dissolve phospholipids (the primary material of cell membrane). This is because phospholipid has two parts, hydrophilic side which has phosphate group and the other side is hydrophobic which has fat. Essential oil has branch of phenol group and alcohol dissolve phospholipids. Soluble phospholipids in essential oil decreases cell permeability, cell will experience lysis and protein will be denaturized, inhibiting the protein cytoplasm and nucleate acid form. Moreover, Rupilu and Lamapaha (2008) reported, phospholipids damage causes the damage of cell membrane and finally causes leakage of important components inside bacteria like protein, nucleate acid, and nucleotide. All component will flow outside because unstable cell permeability, inhibiting bacteria live and growth, and even collapsing it.

CONCLUSION

The highest yield of essential soil of the six *Curcuma spp* was 1.33 % (w/v) on TK or *Curcuma boesenbergia pandurata* and the lower ones were TI, TL,TP on 0.47%(w/v). MIC of six *Curcuma spp* tested with *Staphylococcus epidermidis* are as follows: TK and TG 1000µg/ml, TI, TM, TP 500 µg/ml, and TL 125µg/ml. TG was the most active with MBC 1000 µg/ml. The other essential oil wasn't able to kill at concentration of 2000

µg/ml. *E.coli* activity were tested, clear zone diameter (in mm) of TG, TI, TK, TL, TM, and TP were 9.97; 9.57; 18.11(negative); 12.29; 10.24 with smaller diameters compared to clear zone of control 19.94 mm. *B. subtilis* activity was tested, clear zone (in mm) of TG, TI, TK, TL, TM, and TP were 17.48; 17.59; 19.42; 25.88; 9.91; 16.92. Clear zone of control is 18,84mm. *B. subtilis* activity TK and TL were showing clear zones which were bigger than control. Primary compounds of essential oil with GC-MS were determined as: 1,8-Cineole, Camphene, Camphor, Borneol L, 1-β-Pinene, β-elemene, Methoxymethyl, Xanthorhizol, Germacrene, 1-α-terpineol, caryophyllene oxide, 6-methylxanthotoxin, critonilide, 2-methoxymethyl-4,4-dimethyl-5-phenyldihydro pyran (table 2).

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