

## Antimicrobial Activity of *Chromolaena odorata* Extracts against Bacterial Human Skin Infections

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Received: October 31, 2015

Accepted: November 27, 2015

Online Published: January 7, 2016

doi:10.5539/mas.v10n2p159

URL: <http://dx.doi.org/10.5539/mas.v10n2p159>

*The research is financed by National Research Council of Thailand and Suandusit University.*

### Abstract

Leaf, stem and root of *Chromolaena odorata* were extracted by maceration extraction method using water, ethanol, methanol and hexane as solvents. The average percentage yield of leaf extracts in water ( $12.16 \pm 0.13\%$ ), ethanol ( $8.42 \pm 0.115\%$ ), methanol ( $10.45 \pm 0.012\%$ ) and hexane ( $2.37 \pm 0.215\%$ ) were significantly higher compared with stem and root extracts using the same solvents. All extracts were tested for antimicrobial activity against ten bacterial strains associated with human skin infections. Leaf extracts with ethanol, methanol and hexane solvents gave the best inhibitory activity against six gram-positive bacterial strains (*Bacillus cereus* TISTR 687, *Enterococcus faecalis* TISTR 379, *Staphylococcus epidermidis* TISTR 518, *Staphylococcus aureus* TISTR 1466, *Streptococcus pyogenes* ATCC 19615 and *Propionibacterium acnes* DMST 14916) and one gram-negative bacterial strain (*Proteus vulgaris* ATCC 13315). The hexane stem extract showed greater inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853 ( $15.3 \pm 0.5$  mm), *B. cereus* TISTR 687 ( $14.6 \pm 0.8$  mm) and *Klebsiella pneumoniae* TISTR 1843 ( $14.0 \pm 1.0$  mm), while hexane root extract showed high inhibitory activity against *Enterococcus faecalis* TISTR 379 ( $14.5 \pm 0.9$  mm) and *Kleb. pneumoniae* TISTR 1843 ( $14.7 \pm 0.6$  mm). The lowest minimum inhibitory concentration (MIC) of the ethanolic leaf extract was 0.81 mg/mL against *Staph. aureus* TISTR 1466, followed by methanolic and hexane leaf extracts with equal MIC of 1.62 mg/mL against both *Staph. aureus* TISTR 1466 and *Strep. pyogenes* ATCC 19615. The antimicrobial activity of the methanolic leaf extract of *C. odorata* was correlated with the amount of both total phenolic and flavonoid compounds. The results obtained suggest that the ethanolic, methanolic and hexane leaf extracts could be developed to treat bacterial skin infections. The hexane was suitable solvent for extraction of the stem and root parts of *C. odorata*.

**Keywords:** *Chromolaena odorata*, bacterial human skin infection, antibacterial activity, MIC, MBC

### 1. Introduction

Bacterial skin infection strains commonly infect patients in hospitals, leading to high nosocomial infection rates and use of antimicrobial drugs. The nosocomial pathogens that exhibit the greatest antimicrobial resistance are *Staph. aureus* (52.2%), *E. coli* (67.6%), *Acinetobacter baumannii* (100%) and *Ps. aeruginosa* (68.2%) (Ahoyo et al., 2014). According to the U.S. Food and Drug Administration, the bacterial pathogens that most commonly cause acute bacterial skin and skin structure infections (ABSSSI) are *Strep. pyogenes* and *Staph. aureus*, including methicillin-resistant *Staph. aureus*; less common causes are *Streptococcus* spp., *Entc. faecalis* and gram-negative bacteria. Furthermore, a report by the World Health Organization (WHO) in 2014 found antibacterial resistance in *E. coli*, *Kleb. pneumoniae*, *Staph. aureus*, *Strep. pneumoniae*, nontyphoidal *Salmonella* (NTS), *Shigella* spp. and *Neisseria gonorrhoeae*.

Nowadays, hospital-acquired infections (HAIs) are a major cause of patient morbidity and mortality, and are correlated with fomites. Fomites are reservoirs of pathogens which can infect patients by direct contact or

indirectly from touching contaminated hospital environmental surfaces (Mangicaro, 2012). At a teaching hospital in northern Nigeria, pathogenic bacteria were found on 65.7% of the samples tested (e.g., stethoscopes, doorknobs), including *Staph. aureus* (21.7% frequency), *Staph. epidermis* (8.7%), *Streptococcus* spp. (8.7%), *Bacillus* spp. (13.0%), *E. coli* (26.1%), *Pseudomonas* spp. (8.7%) and *Klebsiella* spp. (13.0%) (Maryam et al., 2014). There has also been a rapid increase in the incidence of infections with drug-resistant microorganisms acquired by patients while in the hospital. Bacterial human skin infection strains are correlated with nosocomial infection, fomites and antimicrobial-resistant strains. Obtaining new antimicrobial agents from natural resources is necessary to develop alternative drugs for safe and cost-effective health care.

*Chromolaena odorata* (L.) R. M. King and H. Robinson is a weed that is widely found in tropical Africa, North America, and South and Southeast Asia. Common names are Siam weed, Christmas bush, and common floss flower (Chakraborty, Rambhade, & Patil, 2011; Queensland Department of Agriculture, Fisheries and Forestry, 2013). Previous studies have reported that this plant exhibits biological activities: antimicrobial activity (Suksamrarn et al., 2004; Pisutthanan et al., 2005; Vital & Rivera, 2009; Naidoo, Cooposamy, & Naidoo, 2011; Sukanya, Sudisha, Prakash, & Fathima, 2011; Mondal, Bhargava, Shivapuri, & Kar, 2012; Atindehou et al., 2013; Eze, Oruche, Onuora, & Eze, 2013; Kigigha & Zige, 2013; Stanley, Ifeanyi, Nwakaego, & Esther, 2014); antibiofilm activity (Yahya, Ibrahim, Zawawi, & Hamid, 2014); antihepatotoxicity (Alisi, Onyeze, Ojiako, & Osuagwu, 2014; Asomugha, Okafor, Ijeh, Orisakwe, & Asomugha 2014); antimalarial activity (Pisutthanan et al., 2005); anthelmintic activity (Vital & Rivera, 2009); antiviral activity (Pisutthanan et al., 2005); phytopathogenic activity (Sukanya et al., 2009); antiprotozoal activity (Vital & Rivera, 2009); wound healing (Anyasor, Aina, Olushola, & Aniyikaye, 2011); and antioxidant activity (Akinmoladun, Ibukun, & Dan Ologe, 2007). Chemical compounds produced by this plant play a role in inhibiting the growth of pathogenic microorganisms: leaf extract contains coumarins, tannins, steroids, saponins, terpenoids, terpenes, flavonoids and cardiac glycosides (Phan et al., 2001; Akinmoladun et al., 2007; Vital & Rivera, 2009; Anyasor et al., 2011; Lavanya & Brahmprakash, 2011); flower extract contains flavonoids (isosakuranetin, persicogenin, 5,6,7,4'-tetramethoxyflavanone and 4'-hydroxy-5,6,7-trimethoxyflavanone) and flavones (acacetin and luteolin) (Suksamrarn et al., 2004); root extract contains triterpenes, poriferasterol, octadecane, butyrospermol acetate, bis(2-ethylhexyl) phthalate, chrysophanol and physcion (Amatya & Tuladhar, 2005); while stem extract has not been studied. The factors affecting the biological activity of medicinal plants are the plant part, season, climate, growth phase, extraction solvent, extraction procedure, and plant material to solvent ratio (Maji et al., 2010; Kothari, Gupta, & Naraniwal, 2012). Many researchers have reported that effective antimicrobial compounds have been obtained from the plant leaf, but there are few reports on other plant parts.

Previous studies on the antibacterial activity of this plant extract have been limited to clinical diarrheal strains such as *B. cereus*, *E. coli*, *Kleb. oxytoca*, *Salmonella enterica*, *Salmonella* Typhimurium, *Shigella sonnei* and *Vibrio cholera*, and skin infections with bacteria such as *Staph. aureus* and *Staph. epidermidis* (Vital & Rivera, 2009; Maji et al., 2010; Naidoo et al., 2011; Atindehou et al., 2013; Eze et al., 2013). A few reports have investigated the effect of *C. odorata* extract on bacterial human skin infection strains.

In this study, we investigated the antibacterial activity of *C. odorata* extracts against bacterial human skin infection strains. Various parts of *C. odorata*, i.e. leaf, stem and root, were extracted with water, ethanol, methanol and hexane. Antibiotics extracted from medicinal plants are an alternative method to reduce the antibiotic resistance of bacterial pathogens.

## 2. Method

### 2.1 Plant Extraction

Leaf, stem and root parts were collected from *C. odorata* plants in Amnat Charoen province, Thailand. Each plant part was washed, dried in sunlight, and then placed in a hot-air oven with temperature controlled at 60 °C. Dried plant parts were then collected, blended and weighed before extraction. A 3×4 factorial design was employed on the *C. odorata* extracts, giving an output of 12 experiments. Twenty g of dried plant part powder was soaked in a 500 mL conical flask containing 200 mL of water, 95% ethanol, methanol or hexane solvents, and shaken on a vertical shaker at 150 rpm for 24 h. The extract solutions were filtered through Whatman filter paper No. 1. The filtrates were concentrated by evaporation (R-200 rotary evaporator; Büchi, Flawil, Switzerland) and frozen in a freeze dryer (CoolSafe; Labogene, Lyngø, Denmark). Crude extract powder was stored at 4 °C until used. The percentage yield of crude extract powder was calculated by the following equation:

$$\text{Percentage yield} = \frac{\text{Extract weight}}{\text{Dried plant weight}} \times 100 \quad (1)$$

## 2.2 Determination of Total Phenols and Total Flavonoids by HPLC-DAD Method

The content of total phenols and flavonoids was analyzed by high-performance liquid chromatography (HPLC) (LC-20AD; Shimadzu, Kyoto, Japan) using a UV-vis diode array detector (DAD) recording at 280 and 360 nm (SPD-M20A, variable wavelength; Shimadzu) (Peñarrieta, Alvarado, Bergenstahl & Akesson, 2007). The conditions of phytochemical analysis were set as follows: mobile phase (methanol: 1% acetic acid), retention time (20 min), and flow rate (0.8% mL/min). The analytical column used in this study was an Intersil<sup>®</sup> ODS-3, size 4.6×150 mm (GL Sciences, Tokyo, Japan). Gallic acid and Trolox were used as standards for phenolic contents, and rutin and quercetin for flavonoid contents.

## 2.3 Pathogenic Bacteria

The pathogenic bacteria used to test the antimicrobial activity of the plant extracts were six strains of gram-positive bacteria (*B. cereus* TISTR 687, *Entc. faecalis* TISTR 379, *Staph. epidermidis* TISTR 518, *Staph. aureus* TISTR 1466, *Strep. pyogenes* ATCC 19615 and *Prop.acnes* DMST 14916) and five strains of gram-negative bacteria (*Entb. aerogenes* ATCC 13048, *E. coli* TISTR 780, *Kleb. pneumoniae* TISTR 1843, *Pr. vulgaris* ATCC 13315 and *Ps. aeruginosa* ATCC 27853). All tested bacteria were grown on trypticase soy broth (TSB) (Himedia, Mumbai, India) and incubated at  $35 \pm 2$  °C for 18–24 h before use, except *Entc. faecalis* TISTR 379 was incubated under anaerobic conditions.

## 2.4 Antibacterial Activity Assay

### 2.4.1 Determination of Inhibition Zone of Crude Extracts by Agar Well Diffusion

The inhibition zone was determined using agar well diffusion method. Twenty-eight mL of trypticase soy agar (TSA) was poured into individual Petri dishes until the media became solid. One loop of each strain taken from each working stock was incubated on a TSA plate with 5 mL of trypticase soy broth (TAB) at  $35 \pm 2$  °C for 18–24 h, except *Entc. faecalis* TISTR 379 was incubated under anaerobic conditions. Bacterial strain cultures were adjusted to obtain a final concentration of  $10^8$  CFU/mL using a 0.5 McFarland standard. One hundred  $\mu$ L of the optimum cell concentration was then swabbed on a TSA plate by three-way method. Wells (7 mm diameter) were cut into the agar plates using a sterile cork borer (No. 3). About 100  $\mu$ L of extract from each plant part was poured into individual wells. Stock solutions of the extracts were prepared at a final concentration of 100 mg/mL. DMSO (dimethyl sulfoxide, 10%) was used as a negative control. The agar well plate was then incubated for 18–24 h at  $35 \pm 2$  °C. Antimicrobial activity was measured in terms of the diameter of the inhibition zone (mm). A value of the inhibition zone of  $\geq 10$  mm was selected to study the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

### 2.4.2 Determination of Bacterial Susceptibility to Extracts by MIC and MBC

The MIC and MBC were determined by two-fold serial dilution method using a macro-broth dilution technique modified from Schwalbe, Steele-Moore & Goodwin (2007). A stock solution (100 mg/mL) of each extract was serially two-fold diluted in a tube with 1 mL TSB to obtain concentrations ranging from 0 mg/mL to 100 mg/mL. The zero concentration (0 mg/mL) tube served as a growth control. The inoculum size was prepared by diluting cell cultures in TSB to obtain a cell concentration of  $10^8$  CFU/mL using 0.5 McFarland standard and then diluting with TSB to obtain a cell concentration of  $10^6$  CFU/mL. Then 0.1 mL of each standard inoculum was added to the diluted extracts. Cultivation was carried out at  $35 \pm 2$  °C for 18–20 h. The reduction of bacterial growth in each tube, containing various extract concentrations, was determined using a standard plate count compared with the growth control tube. MIC was defined as 80% inhibition in growth of the initial bacterial inoculum when compared with the control. MBC was defined the lowest concentration of a plant extract that killed the majority (99.9%) of the initial bacterial inoculum.

## 2.5 Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS Statistics 17.0 (SPSS, USA). The different treatments were analyzed using Duncan's multiple range test method at a confidence level of 95%.

## 3. Results

### 3.1 Extraction Yield

Twelve samples of *C. odorata* extracts were obtained from leaf, stem and root plant parts, and were used for water, ethanol, methanol and hexane extractions, as shown in Table 1. Leaf extracts gave significantly higher

percentage yields than stem and root extracts for all extraction solvents. The water and methanol solvents showed significantly higher yields compared with the other extraction solvents for the plant leaf and stem, but not the root. The lowest percentage yield was obtained from *C. odorata* root extracted with hexane solvent. Furthermore, hexane solvent gave a significantly lower percentage yield for all plant parts compared with water, ethanol and methanol solvents.

Table 1. Extraction yields of leaf, stem and root of *C. odorata* with four different solvents

Plant parts	Extraction solvents	Extractive value				Yield (%)	%CV
		Repeat 1 (g/10g)	Repeat 2 (g/10g)	Repeat 3 (g/10g)	Average (g/10g)		
Leaf	Water	1.38	1.19	1.08	1.22	12.16±0.134 <sup>a</sup> <sub>c</sub>	47.51
	Ethanol	0.10	0.83	0.70	0.84	8.42±0.115 <sup>b</sup> <sub>c</sub>	
	Methanol	1.06	1.04	1.03	1.05	10.45±0.012 <sup>a</sup> <sub>b</sub>	
	Hexane	0.21	0.24	0.27	0.24	2.37±0.21 <sup>c</sup> <sub>b</sub>	
Stem	Water	0.32	0.24	0.19	0.25	2.49±0.057 <sup>a</sup> <sub>b</sub>	58.33
	Ethanol	0.18	0.18	0.20	0.19	1.88±0.004 <sup>b</sup> <sub>b</sub>	
	Methanol	0.29	0.27	0.34	0.30	2.99±0.007 <sup>a</sup> <sub>a</sub>	
	Hexane	0.02	0.03	0.03	0.03	0.28±0.009 <sup>c</sup> <sub>a</sub>	
Root	Water	0.56	0.65	0.56	0.59	5.87±0.063 <sup>a</sup> <sub>a</sub>	58.78
	Ethanol	0.41	0.39	0.40	0.40	3.99±0.015 <sup>b</sup> <sub>a</sub>	
	Methanol	0.32	0.41	0.57	0.43	4.34±0.068 <sup>b</sup> <sub>a</sub>	
	Hexane	0.04	0.07	0.02	0.05	0.46±0.023 <sup>c</sup> <sub>a</sub>	
%CV		90.38	82.21	78.37			

Note. %CV = percent coefficient of variation.

Superscript letters within a column indicate significant ( $p < 0.05$ ) differences of means within solvents. Subscript letters within a column indicate significant ( $p < 0.05$ ) differences of means within plant parts. Statistics analyzed using Duncan's multiple range test.

### 3.2 Content of Total Phenols and Flavonoids

Evaluation of total phenolic and flavonoid contents was determined for three plant parts and four solvents, as shown in Table 2. The maximum content of total phenols was found in the ethanol leaf extract, followed by water, methanol and hexane. For the stem, water extract contained the highest total phenols. The highest flavonoid contents were found in the ethanol leaf extract, compared with other plant parts and solvents. The ethanol leaf extract also had the highest total phenolic and flavonoid contents, followed by water and methanol; hexane leaf extract only contained phenols.

### 3.3 Antibacterial Activity

Leaf extracts in ethanol, methanol and hexane exhibited strong inhibitory effects against *B. cereus* TISTR 687, *Entc. faecalis* TISTR 379, *Staph. epidermidis* TISTR 518, *Staph. aureus* TISTR 1466, *Strep. pyogenes* ATCC 19615 and *Prop. acnes* DMST 14916, with inhibition zones in the ranges of  $14.6 \pm 0.6$  to  $20.0 \pm 0.5$ ,  $14.7 \pm 1.5$  to  $17.0 \pm 1.0$ ,  $11.2 \pm 1.4$  to  $14.7 \pm 1.5$ ,  $12.0 \pm 0.5$  to  $20.3 \pm 0.6$ ,  $15.7 \pm 0.6$  to  $20.3 \pm 2.9$ , and  $9.7 \pm 0.6$  to  $14.2 \pm 0.8$  mm, respectively. In gram-negative bacteria, the ethanol, methanol and hexane leaf extracts affected *Ps. aeruginosa* ATCC 27853, with inhibition zones ranging from  $12.5 \pm 0.3$  to  $15.3 \pm 0.5$  mm. *Pr. vulgaris* ATCC 13315 was inhibited by the leaf extracts in all solvents. Methanol and hexane leaf extracts also showed antimicrobial activity against *Kleb.pneumoniae* TISTR 1843, while the water, ethanol and methanol extracts only had an effect against *E. coli* TISTR 780. None of the plant extracts were effective against *Entb. aerogenes* ATCC 13048. Hexane extract of the stem of *C. odorata* showed inhibitory activity against *B. cereus* TISTR 687, *Entc. faecalis* TISTR 379, *Staph. epidermidis* TISTR 518, *Strep. pyogenes* ATCC 19615, *Kleb. pneumoniae* TISTR 1843, *Pr. vulgaris* ATCC 13315 and *Ps. aeruginosa* ATCC 27853, while the root extract also exhibited inhibitory activity against all of the above except for *B. cereus* TISTR 687 and *Pr. vulgaris* ATCC 13315. Furthermore, the ethanolic stem and water root extracts showed inhibitory activity against *Strep. pyogenes* ATCC 19615 and *E. coli* TISTR 780, respectively (Table 3, 4 and 5 and Figures 1 and 2).

Table 2. Phytochemical analysis of leaf, stem and root of *C. odorata* extracted using different solvents

Plant parts	Solvents	Phenols			Flavonoids		
		Gallic acid (mg of GA/g of dry extract)	Trolox (mg of TL /g of dry extract)	Total phenols (mg of GA and TL /g of dry extract)	Quercetin (mg of QC/g of dry extract)	Rutin (mg of RU/g of dry extract)	Total flavonoids (mg of QC and RU/g of dry extract)
Leaf	Water	3.42	1.06	4.48	ND	2.52	2.25
	Ethanol	1.54	4.74	4.74	0.31	9.85	10.16
	Methanol	2.20	0.23	2.43	ND	2.64	2.64
	Hexane	ND	2.81	2.81	ND	ND	ND
Stem	Water	3.83	ND	3.83	ND	ND	ND
	Ethanol	1.51	0.98	2.49	ND	ND	ND
	Methanol	1.61	ND	1.61	ND	ND	ND
	Hexane	0.05	0.34	0.39	0.002	0.01	0.012
Root	Water	1.85	0.66	2.51	0.34	1.19	1.53
	Ethanol	1.55	0.10	1.65	ND	ND	ND
	Methanol	0.99	0.19	1.18	0.06	0.77	0.83
	Hexane	1.85	0.49	2.34	ND	0.04	0.04

Note. ND = Not detected.

Table 3. Antibacterial activity (zone of inhibition, mm) of *C. odorata* leaf extract

Tested microorganisms	Diameter of inhibition zone (mm)*			
	Water	Ethanol	Methanol	Hexane
<b>Gram-positive bacteria</b>				
<i>B. cereus</i> TISTR 687	NI	15.3±0.6	19.3±0.6	20.7±0.5
<i>Entc. faecalis</i> TISTR 379	NI	14.7±1.5	16.0±1.5	17.0±1.0
<i>Staph. epidermidis</i> TISTR 518	NI	13.0±1.0	12.7±2.5	14.7±1.5
<i>Staph. aureus</i> TISTR 1466	NI	16.0±0.5	20.3±0.6	12.0±0.5
<i>Strep. pyogenes</i> ATCC 19615	NI	15.7±0.6	16.0±1.7	20.3±2.9
<i>Prop. acnes</i> DMST 14916	NI	9.7±0.6	9.7±0.6	14.2±0.8
<b>Gram-negative bacteria</b>				
<i>Entb. aerogenes</i> ATCC 13048	NI	NI	NI	NI
<i>E. coli</i> TISTR 780	13.3±1.3	10.7±0.6	13.7±0.6	NI
<i>Kleb. pneumoniae</i> TISTR 1843	NI	NI	13.7±0.6	12.7±0.6
<i>Pr. vulgaris</i> ATCC 13315	10.5±0.0	11.5±0.5	13.3±0.0	15.0±0.5
<i>Ps. aeruginosa</i> ATCC 27853	NI	12.5±0.3	NI	NI

Note. NI = no inhibition; \* = included diameter of cork borer (7 mm).

Table 4. Antibacterial activity (zone of inhibition, mm) of *C. odorata* stem extract

Tested microorganisms	Diameter of inhibition zone (mm)*			
	Water	Ethanol	Methanol	Hexane
<b>Gram-positive bacteria</b>				

<i>B. cereus</i> TISTR 687	NI	NI	NI	14.6±0.58
<i>Entc. faecalis</i> TISTR 379	NI	NI	NI	14.7±0.6
<i>Staph. epidermidis</i> TISTR 518	NI	NI	NI	11.5±1.8
<i>Staph. aureus</i> TISTR 1466	NI	NI	NI	NI
<i>Strep. pyogenes</i> ATCC 19615	NI	10.3±0.6	10.7±0.6	14.3±2.9
<i>Prop. acnes</i> DMST 14916	NI	NI	NI	NI
<b>Gram-negative bacteria</b>				
<i>Entb. aerogenes</i> ATCC 13048	NI	NI	NI	NI
<i>E. coli</i> TISTR 780	NI	NI	NI	NI
<i>Kleb. pneumoniae</i> TISTR 1843	NI	NI	NI	14.0±1.0
<i>Pr. vulgaris</i> ATCC 13315	NI	NI	NI	12.5±0.5
<i>Ps. aeruginosa</i> ATCC 27853	NI	NI	NI	15.3±0.5

Note. NI = no inhibition; \* = included diameter of cork borer (7 mm).

Table 5. Antibacterial activity (zone of inhibition, mm) of *C. odorata* root extract

Tested microorganisms	Diameter of inhibition zone (mm)*			
	Water	Ethanol	Methanol	Hexane
<b>Gram-positive bacteria</b>				
<i>B. cereus</i> TISTR 687	NI	NI	NI	NI
<i>Entc. faecalis</i> TISTR 379	NI	NI	NI	14.5±0.9
<i>Staph. epidermidis</i> TISTR 518	NI	NI	NI	11.2±1.4
<i>Staph. aureus</i> TISTR 1466	NI	NI	NI	NI
<i>Strep. pyogenes</i> ATCC 19615	NI	NI	NI	11.7±0.6
<i>Prop. acnes</i> DMST 14916	NI	NI	NI	NI
<b>Gram-negative bacteria</b>				
<i>Entb. aerogenes</i> ATCC 13048	NI	NI	NI	NI
<i>E. coli</i> TISTR 780	12.7±0.6	NI	NI	NI
<i>Kleb. pneumoniae</i> TISTR 1843	NI	NI	NI	14.7±0.6
<i>Pr. vulgaris</i> ATCC 13315	NI	NI	NI	NI
<i>Ps. aeruginosa</i> ATCC 27853	NI	NI	NI	NI

Note. NI = no inhibition; \* = included diameter of cork borer (7 mm).

The ethanolic leaf extract was found to be more active against *Staph. aureus* TISTR 1466, with a MIC of 0.81 mg/mL (MBC = 1.62 mg/mL), followed by methanolic and hexane leaf extracts, which exhibited inhibitory activity against *Staph. aureus* TISTR 1466 and *Strep. pyogenes* ATCC 19615, with an MIC equal to 1.65 mg/mL. The hexane root extract had the lowest activity, while hexane stem extract showed inhibitory activity against *B. cereus* TISTR 687 (MIC = 6.25 mg/mL) and *Pr. vulgaris* ATCC 13315 (MIC = 6.25 mg/mL). *C. odorata* extracts were most active against *Staph. aureus* TISTR 1466, followed by *Strep. pyogenes* ATCC 19615, *Staph. epidermidis* TISTR 518 and *Prop. acnes* DMST 14916; while for gram-negative bacteria, *C. odorata* extracts showed inhibitory activity against only *Pr. vulgaris* ATCC 13315 (Table 6, 7 and 8). Previous reports found that methanol leaf extract from *C. odorata* obtained in Nigeria gave a MIC value against *Staph. aureus* in a range of 0.125–2.0 mg/mL, a MIC value which closely matched our results (Eze et al., 2013; Stanley et al., 2014). However, our results indicated that the methanolic leaf extract of *C. odorata* (6.25 mg/mL) had a better MIC against *Staph. epidermidis* TISTR 518 than the methanolic leaf extract from *C. odorata* grown in Durban, South Africa (8.0 mg/mL) (Naidoo et al., 2011). Furthermore, the results obtained suggest that the extracts of *C. odorata* exhibited better antibacterial activity against gram-positive bacteria than gram-negative bacteria. The outer phospholipid membrane of gram-negative bacteria has lipopolysaccharide (LPS) components, making it impermeable to lipophilic solutes. The cell wall of gram-positive bacteria has only an outer peptidoglycan layer, which is not an effective permeability barrier (Vital & Rivera, 2009).

Table 6. Antibacterial activity (MIC and MBC values, mg/mL) of *C. odorata* leaf extract

Tested microorganisms	Water	Ethanol	Methanol	Hexane
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	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<b>Gram-positive bacteria</b>								
<i>B. cereus</i> TISTR 687	ND	ND	12.5	25.0	12.5	25	6.25	12.5
<i>Entc. faecalis</i> TISTR 379	ND	ND	12.5	25	12.5	25	12.5	25
<i>Staph. epidermidis</i> TISTR 518	ND	ND	6.25	12.5	6.25	12.5	6.25	12.5
<i>Staph. aureus</i> TISTR 1466	ND	ND	0.81	1.62	1.62	3.25	1.62	3.25
<i>Strep. pyogenes</i> ATCC 19615	ND	ND	3.25	6.25	1.62	3.25	1.62	3.25
<i>Prop. acnes</i> DMST 14916	ND	ND	12.5	25	12.5	25.0	6.25	12.5
<b>Gram-negative bacteria</b>								
<i>E. coli</i> TISTR 780	25	50	25	50	25	50	ND	ND
<i>Kleb. pneumoniae</i> TISTR 1843	ND	ND	ND	ND	25	50	12.5	25
<i>Pr. vulgaris</i> ATCC 13315	25	50	6.25	12.5	6.25	12.5	6.25	12.5
<i>Ps. aeruginosa</i> ATCC 27853	ND	ND	12.5	25.0	ND	ND	ND	ND

Note. ND = not determined.

Table 7. Antibacterial activity (MIC and MBC values, mg/mL) of *C. odorata* stem extract

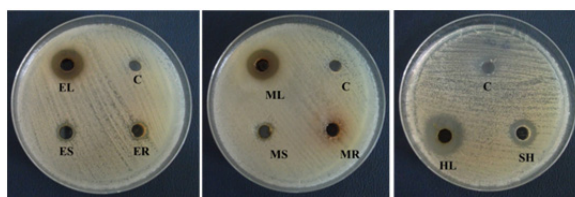
Tested microorganisms	Ethanol		Methanol		Hexane	
	MIC	MBC	MIC	MBC	MIC	MBC
<b>Gram-positive bacteria</b>						
<i>B. cereus</i> TISTR 687	ND	ND	ND	ND	6.25	12.5
<i>Entc. faecalis</i> TISTR 379	ND	ND	ND	ND	12.5	25
<i>Staph. epidermidis</i> TISTR 518	ND	ND	ND	ND	25	50
<i>Strep. pyogenes</i> ATCC 19615	25	50	25	50	25	50
<b>Gram-negative bacteria</b>						
<i>Kleb. pneumoniae</i> TISTR 1843	ND	ND	ND	ND	12.5	25
<i>Pr. vulgaris</i> ATCC 13315	ND	ND	ND	ND	6.25	12.5
<i>Ps. aeruginosa</i> ATCC 27853	ND	ND	ND	ND	25.0	50.0

Note. ND = not determined.

Table 8. Antibacterial activity (MIC and MBC values, mg/mL) of *C. odorata* root extract

Tested microorganisms	Water		Hexane	
	MIC	MBC	MIC	MBC
<b>Gram-positive bacteria</b>				
<i>Entc. faecalis</i> TISTR 379	ND	ND	12.5	25
<i>Staph. epidermidis</i> TISTR 518	ND	ND	25	50
<i>Strep. pyogenes</i> ATCC 19615	ND	ND	25	50
<b>Gram-negative bacteria</b>				
<i>E. coli</i> TISTR 780	25	50	ND	ND
<i>Kleb. pneumoniae</i> TISTR 1843	ND	ND	12.5	25

Note. ND = not determined.



*Bacillus cereus* TISTR 687

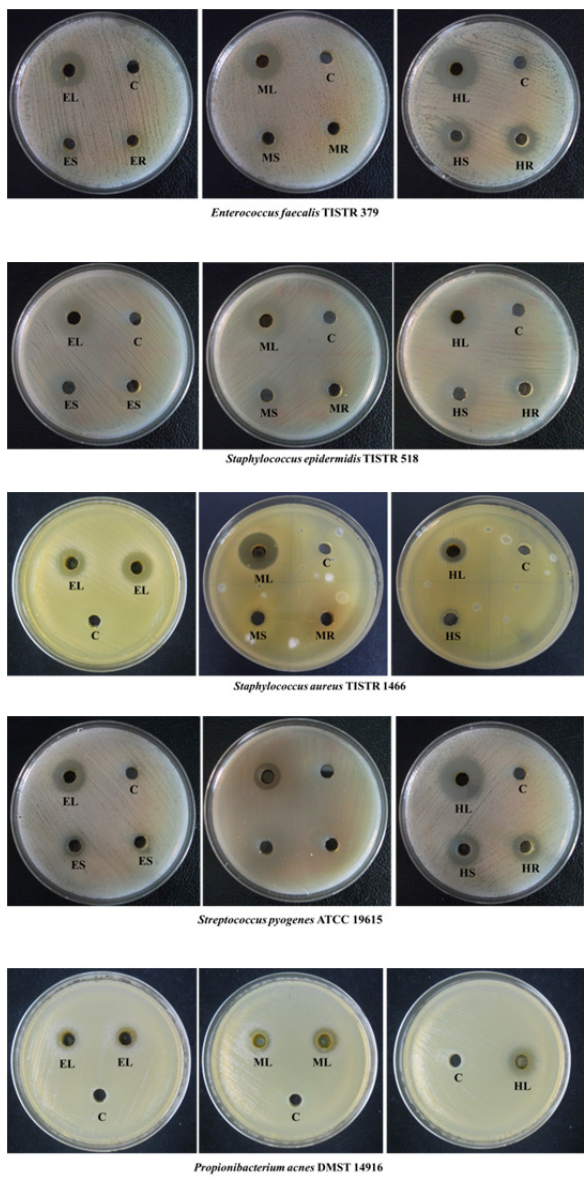
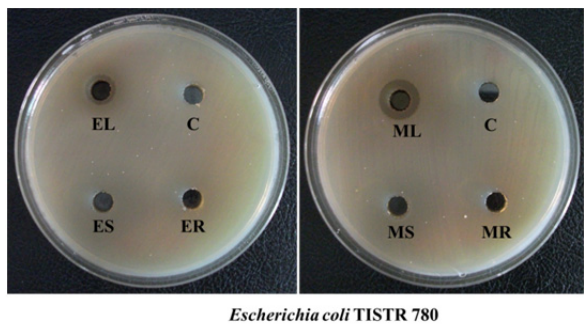


Figure 1. Inhibitory activity of some *C. odorata* extracts against gram-positive bacteria C, 10% DMSO; EL, Ethanol leaf; ES, ethanol stem; ER, ethanol root; ML, Methanol leaf; MS, Methanol stem; MR, Methanol root; HL, Hexane leaf; HS, Hexane stem; HR, Hexane root





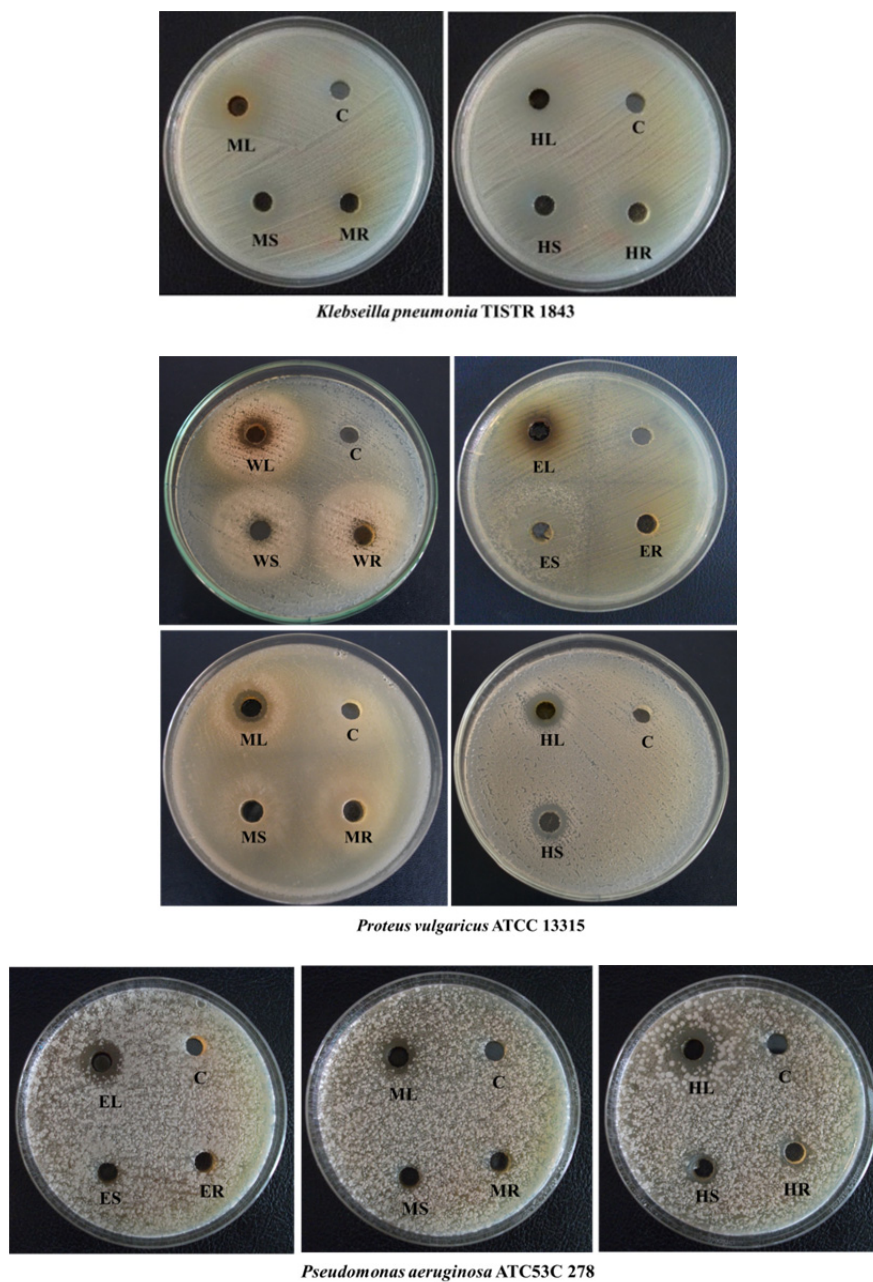


Figure 2. Inhibitory activity of some *C. odorata* extracts against gram-negative bacteria C, 10% DMSO; EL, Ethanol leaf; ES, ethanol stem; ER, ethanol root; ML, Methanol leaf; MS, Methanol stem; MR, Methanol root; HL, Hexane leaf; HS, Hexane stem; HR, Hexane root

#### 4. Discussion

##### 4.1 Effect of Solvents and Plant Parts on Yields and Compounds of *C. Odorata* Extracts

Our results indicated that water and methanol showed the highest percentage yield, followed by ethanol and hexane, when using a 1:10 (w/v) ratio of dried plant material and solvent. The effects on antibacterial activity were associated with the plant part and extraction solvent. The leaf was absorbed by polar organic solvents more than the other plant parts. These results were similar to the report of Stanley et al. (2014) but different from Mondal et al. (2012). The hexane extract of *C. odorata* gave the lowest yield which similar to the reported of Kusuma et al. (2014). Hexane is a low-polarity solvent, which can dissolve and separate the non-polar secondary compounds from the plants such as tannins, terpenes and quinines (Lavanya & Brahmprakash, 2011; Delahaye et al., 2009). The extraction efficiency from plants is related to solvent type, plant material to solvent ratio, time of extraction, and particle sizes of the plant (Tiwari, Kumar, M. Kaur, G. Kaur, & H. Kaur, 2011). The rate of

extraction can be increased by increasing the surface area and using a sample to solvent ratio of 1:10 (w/v) (Tiwari et al., 2011).

Total phenolic contents were found in almost all previous reports on *C. odorata* leaf extracts. Interestingly, in several studies no flavonoid contents were discovered during phytochemical screening (Anyasor et al., 2011; Danlami et al., 2013). However, Mondal et al. (2012) reported on the flavonoid contents found in *C. odorata* leaf extract but not the total phenolic contents. In the present study, only ethanol leaf extract showed higher flavonoid content (rutin) than total phenol contents.

Our results indicated that the water, ethanol and methanol leaf extracts of *C. odorata* possessed antibacterial activity, as they exhibited the highest total phenol and flavonoid contents (Table 4). Both ethanol and methanol solvents could extract antimicrobial compounds (flavonoids, flavones, polyphenols, terpenes and tannins) from the leaf of *C. odorata* (Akinmoladun et al., 2007; Tiwari et al., 2011; Mondal et al., 2012; Vijayaraghavan, Ali, & Maruthi, 2013; Stanley et al., 2014). Normally, the polarity of a solvent plays a key role in increasing phenolic solubility (Naczka & Shahidi, 2006). The polar secondary compounds from medical plants contain alkaloids, flavonoid and some phenols (Delahaye et al., 2009). In many plants, the types of flavonoids and phenols that show antimicrobial activity are chrysin, quercetin and rutin, and catechols, epicatechin and cinnamic acid (Tiwari et al., 2011). The antimicrobial compounds of *C. odorata* (flavonoids) are effective at inhibiting the growth of pathogens by binding to the bacterial cell wall, leading to inhibition of cell wall biosynthesis (Anyasor et al., 2011; Lavanya & BrahmaPrakash, 2011). In terms of antibacterial activity, the results indicated that the organic solvents ethanol, methanol and hexane were the most suitable extraction solvents. Highly polar organic solvents such as ethanol and methanol are typically used for extraction of bioactive compounds from plants.

#### 4.2 Effect of *C. Odorata* Extracts on Antibacterial Activity

Our results were similar to previous findings on the effect of *C. odorata* extracts against pathogens. Leaf extracts demonstrated the best antimicrobial activity, followed by stem and root extracts. The best extraction solvent for antibacterial activity was methanol, followed by ethanol and hexane. Most of the antibacterial activities found in the aqueous, ethanol and methanol leaf extracts were against gram-positive bacteria (*B. subtilis*, *B. cereus*, *Staph. aureus* and *Staph. epidermidis*) and gram-negative bacteria (*E. coli*, *Kleb. pneumoniae*, *Neisseria gonorrhoeae*, *Ps. aeruginosa*, *Salmonella Typhimurium* and *Vibrio cholerae*) (Vital & Rivera, 2009; Maji et al., 2010; Naidoo et al., 2011; Mondal et al., 2012; Eze et al., 2013; Stanley et al., 2014). Furthermore, it was found that the gram-negative bacteria *Entc. aerogenes* was not inhibited by water, ethanol, methanol or hexane leaf extracts, results which are in accordance with the leaf extract from *C. odorata* in Durban, South Africa (Naidoo et al., 2011). *E. coli* and *Klebsiella pneumoniae* were inhibited by leaf extracts with acetone and benzene from *C. odorata* in India but not by water leaf extract (Maji et al., 2010). The previous report found that the hexane leaf extracts of *Callistemon viminalis* leaf showed more potent inhibition against skin pathogen (*Staph. aureus*, *Strep. pyogenes* and the enteric *B. cereus*) than intestinal pathogen (*Shigella sonnei*, *Salmonella enteritidis* and *E. coli*) (Delahaye et al., 2009). The factors affecting the quality of plant extracts are the nature of the plant material (season, topography and climate) and its origin (locations) (Tiwari et al., 2011). The type of solvent used in the extraction procedure plays an important role in the diversity of compounds in the plant extracts (Tiwari et al., 2011).

*C. odorata* stem and root extracts in hexane showed better antibacterial activity compared with other solvents. Venkata et al. (2012) reported that extracts from the root of *C. odorata*, using ethyl alcohol, methanol, chloroform, and methanol:chloroform:water in a ratio of 12:5:3 as solvents, exhibited activity against the same pathogen strains as in our results.

### 5. Conclusions

In comparing the antibacterial activities of different plant parts and extraction solvents, it was found that the most successful extraction procedure for *C. odorata* leaf was by using ethanol and methanol solvents and maceration extraction method. The ethanolic and methanolic leaf extracts gave high extraction yields and high contents of both total phenols and flavonoids. Ethanolic and methanolic leaf extracts also exhibited good antibacterial activity against the gram-positive bacteria *Staph. aureus* TISTR 1466, *Strep. pyogenes* ATCC 19615 and *Staph. epidermidis* TISTR 518 and the gram-negative bacteria *Pr. vulgaris* ATCC 13315. *C. odorata* extracts were also more active against gram-positive bacteria than gram-negative bacteria. The antimicrobial activity of *C. odorata* extracts was correlated with the amount of both total phenol and flavonoid compounds. For stem and root parts, hexane solvent was more suitable for extraction of antibacterial compounds than other solvents. This is the first report to demonstrate the antibacterial activity of ethanolic and methanolic leaf extracts of *C. odorata* against *Strep. pyogenes* ATCC 19615 and *Prop. acnes* DMST 14916, as well as the inhibitory activity of stem

extract of *C. odorata* against the bacterial skin infection (*B. cereus* TISTR 687, *Pr. vulgaris* ATCC 13315, *Entc. faecalis* TISTR 379 and *Strep. pyogenes* ATCC 19615). The results obtained suggest that the ethanolic and methanolic leaf extracts could be used as a potential natural source for drug development to treat bacterial skin infections.

### Acknowledgments

This work was part of a project financially supported by the National Research Council of Thailand and Suan Dusit University. We would like to gratefully acknowledge Dr. Thongchai Mala for approved the final manuscript. The authors would like to thanks Dr. Suchada Thophon, Dr. Piyaporn Waranusantigul and Dr. Niwat Kangwanransan for their help in providing plant materials.

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