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Citrus scented natural essential oils for crystal salt deodorant

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Abstract

Alum natural mineral salt is the world's leading crystal deodorant which works in removing unpleasant body smell. Some other products attempt to block the sweat pores with harmful chemicals such as aluminum chlorohydrates or aluminum zirconium. However, crystal is hypoallergenic deodorant that is healthy, safe, and effective in inhibiting odor formation on the axillary. The light scent infused with natural essential oils might enhance the properties of the crystal deodorant and its antimicrobial activity in fighting against body odor bacteria. This study was conducted to evaluate the potential antimicrobial activity of both citrus essential oils and alum salt. The essential oils were extracted from *Citrus aurantifolia*, and *Citrus sinensis* peels by water-steam distillation and analysed using gas chromatography-mass spectrometry (GC-MS). Antibacterial assay against *Escherichia coli* was carried out on the extracted essential oils using paper disc diffusion assay. Results showed that no lead and cadmium constituents were detected, while low concentration of iron (0.993 ± 0.0059 ppm) and copper (0.134 ± 0.0078 ppm) were detected in the crystal salt (15% w/v). D-limonene, decanal and citral were major components responsible for the citrus scents aroma. The *C. aurantifolia* essential oil and 15% w/v alum salt were active against *E. coli* with inhibitory zones at 7.23 ± 0.15 mm and 11.13 ± 0.51 mm respectively. The citrus scented crystal deodorant was successfully developed and tested.

1. INTRODUCTION

Sweat itself has no smell. The unpleasant smell is actually due to sweating which enhances the bacteria's preferable environment and leaves their unwanted essence behind (Yaganza et al., 2004). Potassium alum or ammonium alum are natural mineral salts composed of molecules that are too large to be absorbed by the epidermal tissue and act as a protective layer on the skin that inhibits the growth of odor-causing bacteria, which contributes to the unpleasant smell under the armpits. These can be controlled with antimicrobials and antiperspirant salts, reducing bacterial growth and providing substantive fragrance to counter the odors. Careful selection of components with bacteriological actions may offer a dual deodorant effect – providing masking fragrance and inhibiting bacteria growth (Brockett, 1992). Citrus essential oils from peels are used in beverages, fragrance and perfume essences. The volatile aroma and distilled oils from *C. aurantifolia* peels are described as fresh, fragrant and citrusy. It is composed of aldehydes such geranial, neral and linalool. The odor-active compounds in *C. aurantifolia* essential oil also were identified as germacrene B and caryophylene oxide which contributed to sawdust-like odor notes (Chisholm et al., 2003). *C. sinensis* essential oil contains decanal, citronellal and linalool as the main aroma compound (Hognadottir and Rouseff, 2003). The test based on the nonverbal method revealed that linalool (refreshing floral-woody) and nonanal (fatty-floral) as a major component in *C. sinensis* essential oils (Gaffney et al., 1996). Other than that, limonene (fresh, light and sweet citrusy), decanal (orange-like aroma) and myrcene (sweet, balsamic-resinous) are also contribute to the aroma flavor of *C. sinensis* essential oils (Chida et al., 2006). This study aims to develop citrus scented crystal deodorant. The efficacy of the *C. aurantifolia* and *C. sinensis* as natural antibacterial agent was evaluated with the ability to inhibit the growth of *E. coli*.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Citrus aurantifolia and *Citrus sinensis* fruits were bought from Pasar Ayer Lanas, Jeli, Kelantan. samples were washed three times with tap water and one time with distilled water. The fruits were peeled and carefully separated from the flesh and seed. The peels were used for extraction of essential oils.

2.2. Essential Oil Extraction

Essential oils from both citrus peels were extracted using water-steam distillation. 117.24 gram and 67.89 gram of *C. aurantifolia* and *C. sinensis* peels were each added with 200 mL distilled water in a 500 mL round bottom flask. The distillation process was carried out for 6-7 hours at a constant temperature of 80-90 °C to ensure the hot steam water bubble did not pass through the vaporized capillary tube that may affect the result. The extracted essential oils (upper layer) were collected in a 500 mL separatory funnel whereas, the water (bottom layer) was withdrawn.

2.3. Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS with fused silica capillary column determined the volatile compounds in the essential oils; capillary column, 30 mm x 0.25 mm i.d., a film thickness of 0.25 µm (J&W Scientific Inc). The column temperature was programmed from 60 °C (2 min) to 240 °C (60 min) at the rate of 3 °C/min ; injection mode, split; split ratio 1:20; volume injected, helium carrier gas, 83 kPa; velocity at 2 ml/min at 60 °C; interference temperature 250 °C; source temperature 200 °C; El+ acquisition mass range of 41-300 amu.

2.4 Salt Crystallization Process

500 mL of concentrated potassium aluminum sulphate solution was prepared in a beaker and left for 3 days at room temperature (27 °C) until the crystal seed formed at the bottom of the beaker. The seed crystals were collected on filter paper by filtration and tied to thread and left hanging in the beaker containing concentrated salt solution. The seed crystal was left for another 3 - 4 weeks at room temperature (27 °C). The growth of crystal seeds in the beaker was observed from time to time and the mass were recorded.

2.5 Heavy Metal Analysis on Crystal Deodorant

This method was used to determine heavy metal constituents; cobalt, copper, iron, lead, nickel and zinc in particulate matter in the concentrated salt and may be applicable to other elements. Standard solution in 6N HCl was prepared and 35 mL of 30% w/v salt concentrated was filtered using a 2.5 µm and 0.45 µm filter papers. The filtrate was placed in a 50 mL tube and added 15 mL of the standard solution to dissolve. The sample was then analysed for copper, iron, lead, and cadmium using Perkin Elmer PinAAcle 900 Flame Atomic Absorbance Spectrometry.

2.6 Citrus Scented Crystal Salt Deodorant Preparation

The crystal deodorant was prepared according to the formulation as in Table 1. Triethylene glycol was heated to 70 °C and added with essential oils. The mixture was left to cool for 30 minutes before adding ethanol to lift the aroma of the essential oils, giving a fresher smell and enhanced cooling effects when applied to skin. Triethylene glycol was used as the vehicle and easy to get due to low cost and a suitable solvent for the active ingredient and fragrance. Besides can give reasonable skin feel and was compatible with anything its stick to. Water was added as solvent for the glycol. The quantity of water to glycol will affect stick clarity, hardness, and raw material cost of the finished product. The crystal salt was then immersed in the prepared formulation and left for 30 minutes.

Table 1: Crystal deodorant formulation (Hazell S., 1999)

Ingredient	% w/v
Triethylene glycol	60.0
Water	21.0
Ethanol	18.0
Essential oil	1.0

2.7 Antibacterial Assay

The antibacterial activities of citrus essential oils and 15% w/v alum salt were tested against *Escherichia coli* using paper disc diffusion assay. Bacterial cultures were freshly prepared by inoculating a single colony of test organism in nutrient broth (NB) at 37 °C for 24 hours. 0.2 mL of the bacterial inoculums was spread evenly on nutrient agar (NA) medium. 15 µL of 0.012 mg/mL *C. aurantifolia* and 0.0081 mg/mL *C. sinensis* essential oils, were pipetted onto a sterile 6 mm paper disc. Whereas, for alum, paper discs were dried in a laminar air-flow prior to transfer onto the NA containing bacterial inoculums. Trimethoprim (50 µg/mL) was used as positive control, whereas sterile distilled water and alum salt were used as negative controls, respectively. Plates were incubated at 37 °C for 24 hours. Assays were carried out in triplicate. Presence or absence of inhibition zone was measured using a ruler and recorded during the incubation period.

3. RESULTS AND DISCUSSION

3.1 Yields and Chemical Constituents of *C. aurantifolia* and *C. sinensis* Essential Oils

The yellowish-green layer and light-yellow layer indicated the presence of *C. aurantifolia* and *C. sinensis* essential oils extracted using the water-steam distillation method (Figure 1) yielded 1.02% and 5.00% respectively (Table 2). Citrus essential oils yield differ depending on the species and reported yield of 1% to 3% (Fisher et al., 2007). Harvesting period may also influenced the yield and quality of essential oils (Kelen, 2008). The degree of freshness, genetics of the plant, climate, geography, the drying period, extraction method and the organ of the plant

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used are considered among the factors that may have a direct impact on the essential oils obtained.

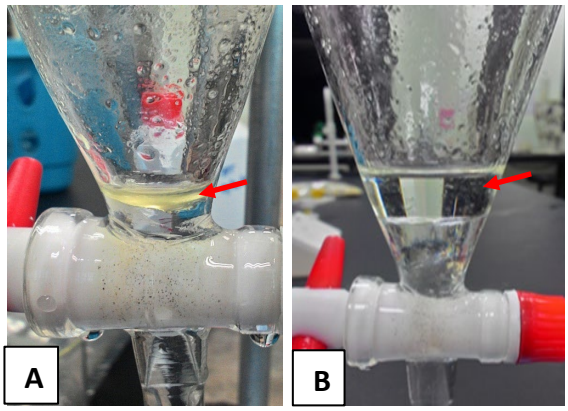

Figure 1: *C. aurantifolia* (A) and *C. sinensis* (B) peels extracted by water-steam distillation followed by separation using liquid-liquid partition. Red arrows show the extracted essential oil layers.

Table 2: The yields of essential oils extracted from *C. aurantifolia* and *C. sinensis* peels.

Sample	Weight (g)	Extracted essential oil (mL)	Percentage (%)
C. aurantifolia	117.24	1.2	1.02
C. sinensis	67.89	3.4	5.00

The chemical constituents of essential oils determine the subsequent effect on antimicrobial potential. GC-MS analysis on *C. aurantifolia* essential oils resulted in identification of tetracosane (72.14%) at higher concentrations, followed by fatty acid (56.36%). D-limonene, a colorless liquid aliphatic hydrocarbon classified as a cyclic monoterpene was also detected at 17.45% (Table 3). However, in *C. sinensis* essential oils, most of the substances were terpenes; D-Limonene (28.77%), geraniol (14.41%), citral (8.4%), carene (11.75%), linalool (43.93%), terpinene (15.47%) and citronellal (10.98%). The acyclic compound, geraniol, was identified only in the *C. sinensis* essential oils. Thus, it suggested that these compounds present in orange (Gancel et al., 2005). Previous research in citrus essential oils showed that mainly monoterpenes (97%) were identified. Whereas other compounds, such as esters, aldehydes and alcohols, are represented at low concentrations ranging from 1.80% to 2.20% (Moufida et al., 2003; Belletti et al., 2004; Rehman et al., 2007).

The difference in the chemical composition of the essential oils might due to the geographical regions and on one or combination of three factors: genetic, age and the environment of the plant (Cardile et al., 2009). The maturity of the fruit also affects the chemical composition of the essential oils obtained. No terpenes were present in essential oils of immature fruit whereas, for matured fruits, concentrations of aldehydes and aliphatic oxygenated sesquiterpenes are slightly higher (Minh Tu et al., 2002). The key factors contributing to the fresh orange flavor from essential oils were mainly from oxygenated groups, which are esters and aldehydes. Aldehydes such citronellal that present also contributed to the citrusy, fatty, floral and green aroma notes, while the terpinene impart the citrus-like and green notes. Geranial, the isomeric monoterpene aldehydes is one of the major constituent in *C. sinensis* essential oil and imparts the pleasant smell of the orange flavor. Not many compounds were detected from *C. aurantifolia* essential oil, but the presence of tetradecanal contributed to the aroma characteristic of the lime (Lan Phi et al., 2006). The presence of oleic acid in both essential oils indicates that the fatty acid occurs naturally in various vegetable fats and oils with odorless, colorless oil.

Table 3: Chemical composition (%) of *C. aurantifolia* and *C. sinensis* essential oils from fresh peel parts.

Compound	Percentage(%)	
	C. aurantifolia EO	*C. sinensis* EO
D-Limonene	17.45	28.77
n-Hexadecanoic acid	1.89	ND
Oleic acid	56.36	16.87
1,3,12-Nonadecatriene-5,14-diol	5.7	ND
Tetracosane	72.14	3.8
Acetic acid	ND	19.85
Naphthalene	ND	17.12
Pentacosane	4.45	3.87
Geraniol	ND	14.41
Citral	ND	8.4
Carene	ND	11.75
Decanal	ND	16.75
1,6-Octadien-3-ol	ND	43.93
Terpinene	ND	15.47
Citronellal	ND	10.98

Note: ND indicates not detected and EO indicates essential oil.

3.2 Antibacterial Activity of Essential Oils and Alum Salt

The antibacterial activity of *C. aurantifolia* and *C. sinensis* essential oils and alum salt were assessed according to the average diameter of the zone of inhibition against *E. coli*. The *C. sinensis* essential oil did not show any antibacterial activity against *E. coli* compared to *C. aurantifolia* essential oil and alum salt (Table 4).

Table 4: Average inhibition zone (mm) of *C. aurantifolia* and *C. sinensis* essential oils and alum against *E. coli*

Sample	Inhibition zone (mm)
C. aurantifolia	7.23 $\pm$ 0.15
C. sinensis	-
Alum (15% w/v)	11.13 $\pm$ 0.51
Control (trimethoprim)	16.00 $\pm$ 0.28

Note: (-) indicates no zone of inhibition.

The *C. sinensis* essential oil is broadly used in pharmaceutical industry due to its antibacterial and antiseptic activity (Verzera et al., 2003). The different composition of essential oils varies depending on the plant's environmental conditions of the plant, even within the same species (Wang et al., 1998). This means the

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antimicrobial properties of essential oils may change as their chemical composition and the relative proportions of the various constituents are influenced by the extraction method and solvent used. These are all factors that may explain conflicting results from the different studies. Nevertheless, the lime peel oil also best inhibited the growth of gram-negative bacteria, thus lime essential oil show best as natural anti-microbe fighting agents (Mandalari et al., 2007). Based on these result, high percentage of D-limonene (28.77%) and 1,6-Octadien-3-ol (43.93%) in *C. sinensis* essential oil had no influence on *E. coli*. The compounds present in abundance may not necessarily be responsible for the antibacterial activity of the essential oils. The antimicrobial activity of some essential oils could be attributed to the minute compound(s), which exhibit antimicrobial activity (Mitiku et al., 2000).

The compounds are partly related to the antibacterial properties in their lipophilicity which causes accumulation in bacteria walls, thereby interfering with the permeability and operation of the cell membranes. Some studies also showed that to improve the D-limonene antimicrobial activity, combinations of nanoemulsion D-limonene (Zhang et al., 2014; Zahi et al., 2015) with nisin may achieve effective antimicrobial activity at low dosages. Alum salts are generally colorless and odorless crystalline solids often used as antiseptic and food preservation (Bestoon, 2012). At a concentration of 15% w/v it inhibits the growth of *E. coli* (Table 4). Alum salt may also act as antibacterial and reduce the pH below the optimum level for bacterial growth (Fitzgerald, 1988). However, alum could potentially harm in laboratory animals with low toxicity. A high level of alum solution could cause kidney damage and distraction of gum tissue with a high mortality rate due to intestinal bleeding. Thus, in this study we used a lower alum concentration to develop the crystal salt deodorant.

3.3 Crystal Salt Deodorant

Different alum concentrations were used in order to prepare the crystal salt. A lower concentration of alum produced a smaller crystal mass (Table 5). These crystal salts (Figure 2) were immersed in the formulated solution to produce crystal salt deodorant containing *C. aurantifolia* and *C. sinensis* essential oils.

Table 5: Growth of crystal salts in weeks at different salt concentration.

Crystal	Alum concentration (% w/v)	Mass of crystal (g) Week 1	Week 3	Week 5	Week 7
A	30	5.73	7.69	9.62	11.73
B	15	3.25	4.73	6.57	8.39

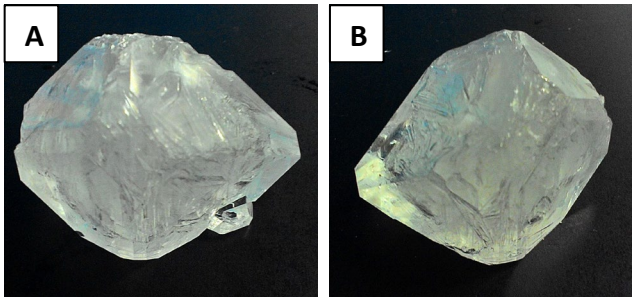

Figure 2: Salt crystals produced using 30% (A) and 15% (B) alum concentration

3.4 Heavy Metal Analysis

Heavy metal toxicity in cosmetics may result in high or low exposures due to the long term used, such as cadmium and lead, common heavy metals contaminant at any cosmetic products (Ziarati et al., 2012). Results show that no lead and cadmium were detected in the developed *C. aurantifolia* scented crystal salts (15% w/v) with low iron (0.993 ± 0.0059 ppm) and copper (0.134 ± 0.0078 ppm). Iron and copper are metallic elements and does not cause any harm to human health. However, an excessive amount of metals may cause detrimental and metabolic abnormalities to health. Thus, the low level of heavy metal content indicated that this product is safe for usage, and heavy metal toxicity may not occur.

4. CONCLUSION

In conclusion, *C. aurantifolia* essential oil and 15% w/v crystal salt are suitable for the development of citrus-scented crystal salt deodorant with antibacterial activity against *E. coli.*

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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