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Antimicrobial Activities of Pomelo (Citrus maxima) Seed and Pulp Ethanolic Extract

Muhamad Sahlan[1,2, a)],Vina Damayanti[1], Dewi Tristantini[1], Heri Hermansyah[1,2]Anondho Wijanarko[1], and Yuko Olivia[1]

[1]*Chemical Engineering Department, Universitas Indonesia, Kampus UI Depok 16424, Indonesia*
[2]*Research Center for Biomedical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus UI Depok 16424, Indonesia*

[a)] Corresponding author : sahlan@che.ui.ac.id

Abstract. Grapefruit (Citrus paradisi) seed extract is generally used as naturopathic medications, supplements, antiseptic and disinfecting agents and also as preservatives in food and cosmetics products. In vitro studies have demonstrated that grapefruit seed extract has anti bacterial properties against a range of gram-positive and gram-negative organisms. Indonesian grapefruit, known as pomelo (C. maxima), has similar characteristics, contents and is under the same genus (Citrus) as grapefruit; however it has not been completely utilized as a preservative. In this work we analyze the antimicrobial activities of ethanolic extract of Indonesian pomelo (C. maxima) seeds and pulp compared to the grapefruit (C. paradisi) seeds and pulp ethanolic extract. Ethanolic extracts of pomelo and grapefruit seeds and pulp are investigated for activities against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Candida albicans. The level of antimicrobial effects is established using agar diffusion method. Both of the ethanolic do not show any antimicrobial activities against C. albicans. The ethanolic extract of pomelo seeds and pulp used in this research give positive results with growth inhibition effect on B. subtilis, S. aureus and E. coli. The zones of inhibition ranges from 22 – 30 mm in diameter, which is higher to grapefruit seeds and pulp ethanolic extract (17 – 25 mm). Ethanolic extract of pomelo seeds and pulp has an antimicrobial effect, which makes it a natural preparation for use as an alternative preservative for food and cosmetic.**Keywords**: Antimicrobial, Pomelo, ethanolic extract.

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INTRODUCTION

Over the last decade, the awareness and concern by consumers over several ingredients used in cosmetic products has increased. One of the ingredients of particular concern is preservatives. Preservatives are definitely crucial in consumables and topical, including cosmetics, because they inhibit the growth of bacteria, molds and fungus present in the air, in the water and on the skin. Preservatives prolong shelf life of the cosmetics as well. Cosmetics contaminated with bacteria and yeasts could cause irritations or infections, which can provoke various illnesses and potentially deadly diseases.

Based on their resources, preservatives are categorized into two; synthetic and natural preservative. Most cosmetics industry use synthetic preservative; by far the most used preservatives are the parabens, as much as 39% [1]. Parabens has been used as the preservative in cosmetic, food, and drug (even water supply) since 1920's, and since then have been among the most researched and studied compounds throughout the decades[2]. Several studies said, parabens were discovered to have a weak estrogenic effect, and they were also present in some breast cancer tumors[2]. Aside from the breast cancer, infertility on men, allergies, digestive disorders, and respiratory disorders are also listed as the negative effects due to the long term exposure and excessive use of parabens [3].

As a result, currently numerous cosmetic manufacturers are trying to discover alternate preservatives instead of parabens. Many cosmetic manufacturers are studying the use of natural preservatives like crude extracts, essential

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oils and vitamins to substitute parabens. This is based on the belief that natural products are basically less dangerous and can be obtained at a lower cost [4]. A number of crude extracts, essential oils and vitamins, including grapefruit (Citrus paradisi) (CP) seed extract, tea tree oil, and vitamin E, is an example of some natural ingredients that can be used to ward off bacteria.

One of the examples of natural preservative is grapefruit seeds extract which is a derivative of the pulp and seeds of grapefruit. CP seed extract products are generally used as naturopathic medications, supplements, antiseptic and disinfecting agents and also as preservatives in the food and cosmetics industry. Previous in vitro studies have demonstrated the antimicrobial properties of grapefruit seed extract against a range of gram-positive and gram-negative organisms [5]. Indonesian grapefruit, known as pomelo (C. maxima) (CM), has similar characteristics, contents and is under the same genus (Citrus) as grapefruit; however it has not been completely utilized as preservative. This condition makes CM a potential raw material for this research. Therefore, the CM seeds and pulp extract can be studied and used as an alternative preservative for cosmetics. However, some scientific studies stated that the commercially-made CP seeds extract are not fully natural and contains artificial agents such as benzethonium chloride, triclosan and methyl parabens [6]. Researchers have found that products not containing any preservatives and several self-made preparations failed to show antimicrobial efficacy and concluded that antimicrobial activity being attributed to CP seeds extract is merely due to the synthetic preservative agents it contains [7].

The presence of bioactive compound is related to the antimicrobial activity of medicinal plant to protect their self from against bacteria, fungi and viral infections[8]. The phytochemical constituents, i.e. flavonoids, citric acid and tannins from the plants contain anti microbial agents. The agents can be isolated by distillation, solvent extraction or cold expression methods[4, 9]. From tangerine fruits in their seeds, peels and pulp contain citric acid, coumarins, flavonoids and flavonones which have antibacterial, anti-inflammatory and insecticidal properties[10]. Hence, the research on antimicrobial activity of CM seeds and pulp ethanolic extract needs to be done. This work contributes to the identification of the antimicrobial effects of the self-made ethanolic extract of CM seeds and pulp.

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MATERIAL AND METHODS

The research procedures consist of three steps, i.e. the extraction process, evaporation and freeze drying, flavonoid content testing and antimicrobial activity testing. Each step is explained thoroughly below:

Ethanolic Extract Formation

The ethanolic extract of *Citrus maxima* and *Citrus paradise* was prepared from commercially available pomelo and grapefruit. The oven dried (150 - 200°C) powdered plant material (juiceless pulp and seeds, in quantitative ratio 4:1) was blended until the size was minimally reduced. Lastly, the sample was wrapped with the filter paper and was extracted with 70% ethanol in a Soxhlet apparatus for 6 hours. After cooling, the solvent was removed using distillation and was chemically analyzed. The remaining extract was then freeze-dried.

Flavonoid Content Testing

Determination of the flavonoid fraction in the ethanolic extract of both pomelo (*Citrus maxima*) and grapefruit (*Citrus paradisi*) sample was performed using colorimetric aluminum chloride method [**Invalid source specified.**12, 13, 14, 15]. Briefly, 0.5 mL solution of each plant extracts in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a Quant PHARO 300 Spectrophotometer located in the Bioprocess Laboratory, Department of Chemical Engineering, Universitas Indonesia. Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations of 12.5 to 100 mg ml-1 in methanol.

Antimicrobial Activity Testing

The bacteria used in this research were obtained from *Lembaga Ilmu Pengetahuan Indonesia* (LIPI) located in Cibinong, Bogor. The bacteria consists of two Gram-positive (*Bacillus subtilis* PCI 219, Ba. Su. and *Staphylococcus aureus* FDA 209P, St. Au.), and one Gram-negative (*Escherichia coli* NIHJ, Esc. Co.) bacteria strains, as well as one yeast strain (*Candida albicans* QC, Ca. Al). The microorganisms used in this research are the standard microorganisms that are usually used in the antimicrobial activity testing. The antimicrobial activity testing was based on the agar diffusion method. The agar diffusion method was performed according to the Shinji Miyadoh method [16]. The testing microorganism was swabbed on nutrient agar for bacteria and on Sabouraud dextrose agar for yeast. The blank antibiotic discs were then placed on the agar. Twenty – five to fifty microliters of test solutions were applied. The same volume of distilled water and broad spectrum antibiotic was also tested as negative and positive control, respectively. The agar plates were then incubated for a day. All experiments were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition zone diameter (mm) produced. The diameters of the clear growth inhibition zones around the cylinder were measured and noted.

RESULT AND DISCUSSION

The CM and CP were prepared to be extracted by ethanol as the solvent. Both of the sample formation were done in triplicate and the final result was combined as one sample for the flavonoid content testing and antimicrobial content testing and then freeze-dried. The total weight of dried pomelo (*Citrus maxima*) seeds and pulp (with a ratio of 1:4) was 48.58 grams. It was then extracted with 300 ml of 70% ethanol under 80°C for 6 hours. After that the ethanol was evaporated by distillation resulting in 32 mL of condensed extract of pomelo (*Citrus maxima*) seeds and pulp. The weight after freeze-drying is 8.9735 grams.

The total weight of dried grapefruit (*Citrus paradisi*) seeds and pulp (with a ratio of 1:4) was 19.99 grams then it was extracted with 300 ml of 70% ethanol under 80°C for 6 hours. After that the ethanol was evaporated by distillation resulting in 41.95 mL of condensed extract of grapefruit (*Citrus paradisi*) seeds and pulp. The weight after freeze-drying is 11.1506 grams.

The results of spectrometric determination showed that the crude ethanolic extract of grapefruit (*Citrus paradisi*) seeds and pulp absorbance is 0.353 with concentration of 48.71 mg/L. The absorbance for the pomelo (*Citrus maxima*) seeds and pulp is outside the absorbance range, which is 1.292. The pomelo (*Citrus maxima*) sample was then diluted twice to fit into the absorbance range. The diluted pomelo (*Citrus maxima*) sample absorbance is 0.585. In order to make it more general the absorbance is then converted into concentration in mg/kg unit; the concentration for grapefruit (*Citrus paradisi*) and pomelo (*Citrus maxima*) are 483.562 and 1602.740 mg/kg respectively. The thorough result of the flavonoid content testing is shown in Table 1.

TABLE 1. Analysis Result of Flavonoid Content Testing

Sample	Mass (g)	Absorbance	Concentration of Flavonoid (mg/kg)
Grapefruit	1	0.353	483.562
Pomelo	1	0.585	1602.740

Therefore, it can be stated that the pomelo (*Citrus maxima*) has flavonoid content and it is higher than the grapefruit (*Citrus paradisi*). In other words, it may be suspected that pomelo (*Citrus maxima*) shows higher antimicrobial activity than grapefruit (*Citrus paradisi*) because of its high flavonoid contents [10].

The ethanolic extract of pomelo (*Citrus maxima*) seeds and pulp and grapefruit (*Citrus paradisi*) seeds and pulp as acomparison was screened for antimicrobial activity against 3 bacterial strains and 1 yeast strain by the agar diffusion method, and the data can be seen in Table 2. In this research the sample being investigated were the comparison between ethanolic extract of pomelo (*Citrus maxima*) seeds and pulp and ethanolic extract of grapefruit (*Citrus paradisi*) seeds and pulp. Distilled water as the negative control did not show any zones of inhibition. The broad-spectrum antibiotic as the positive control showed 47 mm zone of inhibition. The investigated pomelo (*Citrus maxima*) seeds and pulp ethanolic extract was active against all Gram-positive bacteria and Gram-negative bacteria; however it exerted no inhibiting effect on the growth of the tested yeast. The grapefruit (*Citrus paradisi*) seeds and

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pulp ethanolic extract was also active against all Gram-positive bacteria and Gram-negative bacteria; however it exerted no inhibiting effect on the growth of the tested.

TABLE 2. Inhibition of Bacterial and Yeast Growth

Microorganisms	**Inhibition zone* (mm)**	
	Pomelo	Grapefruit
Gram positive bacteria		
Ba. Su.	24	18.7
St. Au.	30	25
Gram negative bacteria		
Esc. Co.	22	17
Yeast		
Ca. Al	0	0

*Diameter of zone of inhibition in mm (Data represented as mean of three reading)

The ethanolic extract of pomelo (*Citrus maxima*) seeds and pulp exhibited the largest zones of inhibition for *Staphylococcus aureus* (30 mm). The ethanolic extract of pomelo (*Citrus maxima*) seeds and pulp showed inhibition zone of 22 – 24 mm against *Escherichia coli* and *Bacillus subtilis*. The ethanolic extract of grapefruit (*Citrus paradisi*) seeds and pulp showed inhibition zones of 25 mm for *Staphylococcus aureus*, 18.7 mm for *Bacillus subtilis* and 17 mm for *Escherichia coli*. It is most likely that this beneficial antibacterial effect has been attributed to the antimicrobial activity of citrus flavonoids, such as naringenin and hesperidine as stated by Dembinski[17]. The presence of flavonoids in this research was confirmed spectrometrically. Both of the pomelo (*Citrus maxima*) and grapefruit (*Citrus paradisi*) seeds and pulp ethanolic extracts showed no activity against *Candida albicans*. The result is not in line with the research of Al-Ani (2011)[18] and Cvetnic and Knezevic (2004)[19] that also did an antimicrobial activity testing on *Candida albicans* but showed inhibition zones around 11 – 12 mm. In this research the agar used for yeast strain is Sabouroud dextrose agar whereas in Al-Ani's research Muller-Hinton agar was used. The method of extraction in this research is different with Al-Ani (2011) and Cvetnic and Knezevic (2004) whereas the extraction method will influence the chemical composition, and thus, can have repercussion regarding the biological properties [20]. Other problems influencing the analysis results are normalization of methods and laboratory conditions. The techniques used (extraction, distillation, dissolution and dispersion, culture medium) for testing biological activity of solutions at the definitive stage of laboratory screening are desirable, to provide a common basis for the comparison of results obtained in various parts of the world in different organisms tested under similar laboratory conditions.

The research result showed clear differences between the antimicrobial activity of the pomelo (*Citrus maxima*) seeds and pulp ethanolic extract and the grapefruit (*Citrus paradisi*) seeds and pulp ethanolicextract (Figure 1). The inhibition zones for the *Bacillus subtilis* in the investigated pomelo (*Citrus maxima*) seeds and pulp ethanolic extract is 24 mm, which is higher than [19]grapefruit seeds extract sample which is 14 mm. The antimicrobial activity of pomelo (*Citrus maxima*) seeds and pulp ethanolic extract against *Staphylococcus aureus*is is moderately higher than the grapefruit (*Citrus paradisi*) seeds and pulp ethanolic extract and it is three times the inhibition zones from Al-Ani's research and Cvetnic and Knezevic's research; which is 10 mm. The inhibiting zones of pomelo (*Citrus maxima*) seeds and pulp ethanolic extract against *Escherichia coli* showed moderately higher value than the

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grapefruit (*Citrus paradisi*) seeds and pulp ethanolic extract. Both tested ethanolic extracts showed no activity in the yeast strain whose cause is probably due to an unfitted method of testing. The pomelo (*Citrus maxima*) seeds and pulp ethanolic extract were found to be superior to the Grapefruit (*Citrus paradisi*) seeds and pulp ethanolic extract in general;it showed higher inhibition zones in all Gram-positive and Gram-negative bacteria strains.

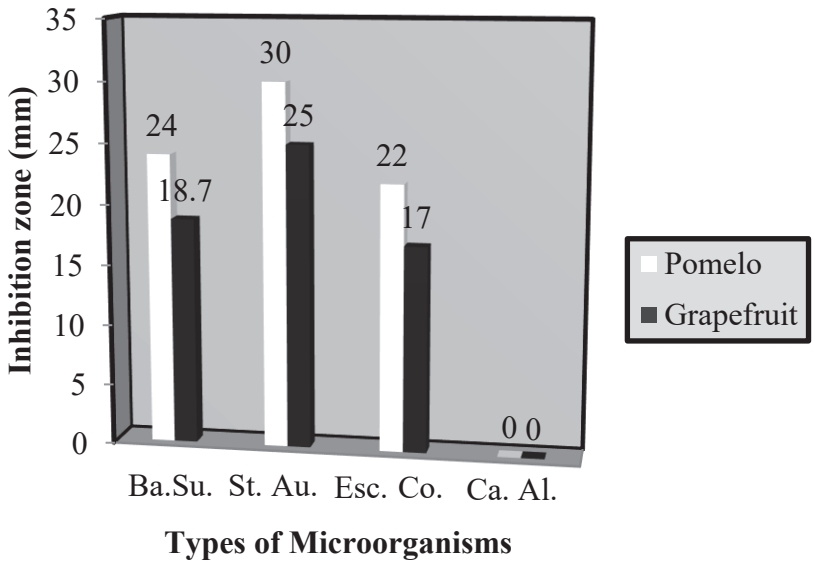

FIGURE 1. Comparisons between the Effect of Pomelo and Grapefruit Seeds and Pulp Extract on Different Types of Microorganisms

CONCLUSION

The formation of the ethanolic extract has been successfully done from pomelo (Citrus maxima) and grapefruit (Citrus paradisi) seeds and pulp. Antimicrobial activity testing against Bacillus subtilis, Staphylococcus aureus and Escherichia coli showed that the pomelo (Citrus maxima) extract exerts more antimicrobials efficacy as compared to the grapefruit (Citrus paradisi) extract formulated from this research;it showed higher inhibition zones in all Gram-positive and Gram-negative bacteria strains. While for Candida albicans it shows no activity, it may be because of the different method of antimicrobial activity testing being used. This leads to the conclusion that pomelo (Citrus maxima) seeds and pulp ethanolic extract has an antimicrobial activity and can be used as natural preservatives for cosmetic that are environmentally friendly and safe for the body.

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