Original Article

Microbial Growth Inhibitory Activities of Extracts from the Peels, Juice Vesicles, and Seeds of *Citrus paradisi*

Bee-Hui Lim, Hong-Wai Tham

Biopharmaceutical Research Unit, Biology Research Laboratory, Faculty of Pharmacy, SEGi University, Petaling Jaya, Selangor, Malaysia.

Abstract

Introduction: *Citrus paradisi* is abundant in insoluble pectin fibre, rich in vitamin C and phytochemicals. With the increasing resistance of common clinically-important pathogens (especially bacteria) to antibiotics today, it brings the focus to antimicrobial properties of plant extracts containing bioactive phytochemical compounds which can potentially kill or inhibit the growth of microorganisms. **Objective:** This study aims to measure the antimicrobial effects of methanolic extracts of *C. paradisi* peels, juice vesicles, and seeds. **Materials and methods:** These samples were procured fresh and processed prior to antimicrobial assays, including disk-diffusion assays and minimum inhibitory concentration tests. In addition, independent *t*-tests and 1-way ANOVA were integrated to study the significance of microbial growth inhibition. **Results:** *B. subtilis* was found possessing the highest sensitivity towards all extracts, especially to juice vesicles and seed extracts. Other microorganisms (eg. *Staphylococcus aureus* and *Escherichia coli*) exhibited a moderate level of tolerance or sensitivity towards different extracts. **Conclusion:** To our knowledge, this is the first study reporting the antimicrobial effects of *C. paradisi* juice vesicles. We have also highlighted the needs in identifying and investigating the phytochemical(s) which are effective in inhibiting microbial growth.

Corresponding author:

Dr.Hong-Wai Tham

thamhongwai@outlook.my

Introduction

In view of the increasing concern regarding antibiotic-resistant bacterial strains worldwide, plants have been serving as a good source of medicinal agents[1], including antimicrobial agents. *Citrus paradisi*, conventionally known as grapefruit, belongs to the family of Rutaceae. It is

generally believed to have originated from a natural cross-hybridisation between *Citrus grandis* (pomelo) and *Citrus sinensis* (orange). *C. paradisi* usually presents a yellow outer peel, with their pulps in either red or pink which indicate the presence of lycopene[2].

There are various health benefits of *C. paradisi*. A rich amount of soluble fibre - pectin has been found in the pulps. Several studies have confirmed the medicinal value of pectin in preventing prostate and colon cancer[3,4]. On the other hand, a membranous content found inside the citrus fruit's endocarp - juice vesicle is filled with lipids which improve the scent or aroma of citrus fruits[5]. On the other hand, extracts from the seeds of *C. paradisi* have been shown to carry antimicrobial properties[6,7]. Despite these discoveries, the antimicrobial properties of extracts from *C. paradisi* peels, fruits (especially juice vesicles), and seeds against human pathogens remained elusive.

Despite its health benefits, the juice of *C. paradisi* has exhibited juice-drug interaction as well. These properties have affected drug bioavailability in the recipients. In 2019, it was reported that the juice of *C. paradisi* inhibited the intestinal CYP3A4 enzyme thus causing the augmentation or decrement of drug bioavailability[8]. Another organic compound that may interfere with the bioactive compound of *C. paradisi* juice is multidrug resistance protein 1 (MDR1), which can be found in the human intestinal epithelium acting as drug efflux pumps[9]. Also, the juice of *C. paradisi* inhibits the organic anion transporting polypeptides (OATPs), which serve as drug transporters across the cell membrane of kidney and liver cells[10]. In addition, several drugs which exhibit juice-drug interaction with *C. paradisi* were also reported earlier[11–13].

In order to avoid juice-drug interaction while applying antimicrobial properties of *C. paradisi* in clinical / non-clinical settings, further investigation on antimicrobial properties of Citrus fruits is needed. Numerous studies are available for antimicrobial properties of *Citrus sinensis* (oranges) and *Citrus limon* (lemons).

In 2018, a study reported the antimicrobial activity of *C. sinensis* peel organic solvents and aqueous extracts. The results proved that the extracts of *C. sinensis* peel possessed a significant antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*[14]. Other studies investigated the antibacterial activity of *C. sinensis* peel and juice extracts. Their results showed a remarkable antibacterial activity of the extracts against *S. aureus*, *E. coli* and *P. aeruginosa*[15,16]. On the other hand, several research groups have reported the antimicrobial characteristics of *C. limon* methanolic

or ethanolic extracts against important microbial pathogens, including *E. coli*, *C. albicans* and *S. aureus*[17,18].

Compared to *C. sinensis* and *C. limon*, the antimicrobial properties of *C. paradisi* extracts remained elusive. Although data were available in 2001 and 2004, Negi and Jayaprakasha reported the antimicrobial properties of *C. paradisi* peel extracts[19], while Cvetnić and Vladimir-Knezević reported using its seed extracts otherwise[20]. Therefore, this study was designed to update the research gaps pertaining to the antimicrobial properties of *C. paradisi* peel and seed extracts under the same experimental settings. In addition, we have included an additional sample - juice vesicle's extract of *C. paradisi* in this study. To our knowledge, this is the first research study focusing on the antimicrobial effects of *C. paradisi* extracts from its juice vesicles.

Materials and Methods

Procurement of samples: *C. paradisi* fruits were purchased from local grocery stores located in Klang Valley, Malaysia.

Preparation of *C. paradisi* extracts: The peels, juice vesicles, and seeds of *C. paradisi* were dried in an oven (60℃, 72 hours). The dried samples were ground into powders using pestle and mortar. Then, these powders were mixed with methanol (99%) until the samples were fully immersed. All bottles were kept in an incubator (45℃, 72 hours), with swirling using magnetic stirrers. After 72 hours, all mixtures were filtered, with filtrates placed in bottles half immersed in a water bath (50℃) to evaporate the excess methanol. The filtrates were then transferred into an oven (60℃) until the methanol was dried completely. Next, the weight of peel, juice vesicle, and seed extracts were measured and recorded.

Reconstitution of extracts: To reconstitute the dried powders, methanol (99%) was added to dissolve the dried extracts. The mixtures were sonicated to ease the process of reconstitution. The reconstituted extracts were kept in -20℃ until used.

Antimicrobial assays: All experimental procedures were conducted under an aseptic environment. The bacterial strains used in this study were *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*. Bacterial cultures were maintained on nutrient agars. Plate cultures were transferred to nutrient broth (Oxoid, CM0001) to 0.5 McFarland standard, with 50 µL of the nutrient broth culture inoculated onto a fresh nutrient agar. Inoculum was spread evenly using a sterile L-shape glass rod.

Filter paper disks were prepared, autoclaved, and dried prior to this procedure. Sterile filter paper disks were soaked thoroughly in the prepared extracts, and air-dried in a biosafety cabinet. Dried filter paper disks were placed on the surface of inoculated nutrient agars. Then, the plates were placed in an incubator (37℃, 18 hours). After that, the diameter (in milimeter, mm) of inhibition zones were measured and recorded.

Determination of minimum inhibitory concentrations (MIC): MIC tests were carried out using serial dilution method. Since *C. paradisi* juice vesicles showed a significant microbial growth inhibitory effect, MIC was conducted only to juice vesicle extracts. First, a serial dilution of bacterial cultures were prepared in sterile 1.5 mL tubes (11 tubes ranged from 1580 mg/mL to 0 mg/mL). Bacterial cultures of *S. aureus*, *E. coli*, and *B. subtilis* were prepared to 0.5 McFarland standard, with 10 µL of each culture inoculated into all mixtures with different concentrations of *C. paradisi* juice vesicle extract. All tubes were placed in an incubator (37 ℃, 18 hours). After that, the MIC of each extract was determined by observing the turbidity of the solutions, and confirmed by inoculation of each sample onto fresh nutrient agar plates.

Statistical analyses: Statistical analyses were conducted to determine the significance of difference between the type of *C. paradisi* extract (ie. peels, juice vesicles, seeds) and their antimicrobial effect against different microorganisms (ie. *E. coli, S. aureus, B. subtilis*). Analyses were conducted using Statistical Package for the Social Sciences (SPSS®, IBM®), version 23. One-way Analysis of variance (1-way ANOVA) was measured with *P* value of <0.05 considered as significant data.

Ethics approval:This project was conducted with approval by SEGi University Ethics Committee (Ethics number: SEGiEC/SR/FOP/10/2020-2021).

Results

C. paradisi extractions: Methanolic extracts of *C. paradisi* were measured and documented in Table 1 below. Overall, the yield from juice vesicles was higher compared to peels and seeds (Table 1).

Antimicrobial assays (disk diffusion assays): Antimicrobial properties of each extract were determined *via* disk diffusion assays. The microbial inhibitory effects of each sample were measured and presented in Figure 1.

Determination of minimum inhibitory concentrations (MIC): The presence of live microorganisms (either *E. coli, S. aureus,* or *B. subtilis*) was detected by viewing the turbidity

of each culture tube. This was complemented by observing the presence of the respective microorganisms on nutrient agars (Table 2).

The minimum inhibitory effect of *C. paradisi* juice vesicle extract was determined at between 790 - 1580 mg/mL, 395 - 790 mg/mL, and 197.50 - 395 mg/mL for *E. coli*, *S. aureus* and *B. subtilis*, respectively (Table 2).

Statistical analyses: The *P* values of One-way Analysis of Variance (1-way ANOVA) are indicated in Table 3. The significance of differences between antimicrobial effects between the parts of *C. paradisi* were measured at 0.256, 0.075, and 0.016 against *E. coli*, *S. aureus*, and *B. subtilis*, respectively.

Table 1: Concentrations of *C. paradisi* extractions.

Parts of *C. paradisi*	Concentrations of extracted sample after reconstitution (g/mL)	
	1st extraction	2nd extraction
Peels	0.3577	0.4030
Juice vesicles	1.0746	1.5801
Seeds	0.1294	0.1805

Table 2: The presence of microorganisms detected in each culture tube with serial dilution of *C. paradisi* juice vesicle extract.

Culture tube	Final concentration (mg/mL) of *C. paradisi* juice vesicle extract	Presence of microorganism		
		E. coli	*S. aureus*	*B. subtilis*
1st	1580.00	**No**	**No**	**No**
2nd	790.00	Yes	**No**	**No**

3rd	395.00	Yes	Yes	**No**
4th	197.50	Yes	Yes	Yes
5th	98.75	Yes	Yes	Yes
6th	49.38	Yes	Yes	Yes
7th	24.69	Yes	Yes	Yes
8th	12.34	Yes	Yes	Yes
9th	6.17	Yes	Yes	Yes
10th	3.08	Yes	Yes	Yes
11th	0.00	Yes	Yes	Yes

Table 3: 1-way ANOVA analyses between the types of *C. paradisi* extracts and their antimicrobial effects.

Type of microorganism	Source of variation	*P*-value
E. coli	Between Groups	0.256
S. aureus	Between Groups	0.075
B. subtilis	Between Groups	**0.016***

* Significant value(s)

Figures

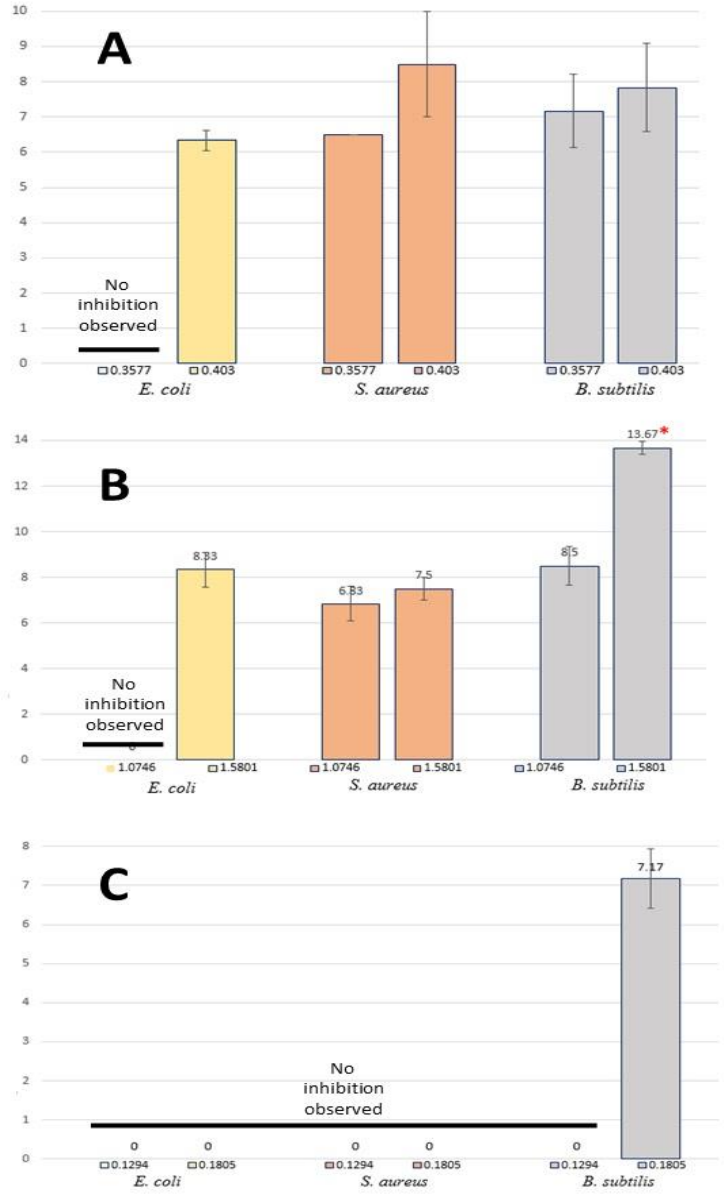

Figure 1: The average diameter of zone of inhibition (mm) of *E. coli*, *S. aureus*, and *B. subtilis* against different concentrations of extracts after reconstitution. (Y-axis) Average diameter of inhibition zones (mm); (X-axis) Different strains of microorganisms inhibited by the respective reconstituted methanolic extracts; **(A)** Peel extracts of *C. paradisi*; **(B)** Juice vesicle extracts of *C. paradisi*; **(C)** Seed extracts of *C. paradisi*. Asterisk indicates a significant value ($P = 0.0005$) found in the growth inhibition of *B. subtilis* by juice vesicle extracts.

Discussions

Despite previously reported studies, this study has highlighted several variations on antimicrobial effects of peels, juice vesicles, and seeds of *C. paradisi*. These variations can be derived from the differences in chemical constituents of polyphenols from various parts of *C. paradisi*, as reported in previous studies[21–23].

In general, *E. coli* exhibited a higher resistance against methanolic extracts of all parts of *C. paradisi*. This could be ascribed to the differences of cell wall components between Gram-positive and Gram-negative bacteria. The outer lipopolysaccharide layer and a peptidoglycan-containing periplasmic space of Gram-negative bacteria have been proven to confer additional protection to certain microorganisms[24,25]. In addition, some enzymes found in the periplasmic space of Gram-negative bacteria have been reported to prevent the entry of foreign molecules into the cytoplasm[26].

The peel methanolic extracts of *C. paradisi* were prepared at 2 different concentrations (ie. 0.3577 g/mL and 0.4030 g/mL). Other than the sample with 0.3577 g/mL against *E. coli*, both concentrations exhibited microbial inhibitory effects towards *E. coli*, *S. aureus*, and *B. subtilis* (Figure 1). For *E. coli*, the inhibitory effect increased significantly when the concentration increased from 0.3577 g/mL to 0.4030 g/mL (P = 0.0007). This result was consistent with research findings previously reported[6,27,28]. Similar trends were reported for *S. aureus* and *B. subtilis*. The findings of this study described the microbial inhibitory effects of *C. paradisi* peel extracts against *S. aureus*, and these findings are also in accordance with several previous studies[6,29]. Similarly, for *B. subtilis*, microbial inhibitory effects were also reported previously with different lengths of inhibitory zones[6,27]. Upon investigations, the differences in the zone of inhibition can be due to the variables during plant extract preparations and the status of microorganisms during inoculations. While most of the reported studies employed essential oils at high concentration, our study extracted pure methanolic extracts from fresh *C. paradisi*, which were used in sample testing without a long delay.

The *C. paradisi* juice vesicle methanolic extracts exhibited a similar inhibitory pattern against *E. coli* and *S. aureus* (Figure 1B). A significant improvement in growth inhibitory effect was observed in *E. coli* when the extracts of juice vesicles increased from 1.0746 g/mL to 1.5801 g/mL (P = 0.003). The same pattern was also observed in *B. bacillus* (P < 0.0005) (Figure 1B, marked with asterisk). To our knowledge, this is the first study reporting the microbial growth inhibitory effect of *C. paradisi* juice vesicle extracts.

Although the seed extract of *C. paradisi* was reported not to hold any antimicrobial effect against *E. coli*, *S. aureus*, and *B. subtilis*[30], our results suggest that the extracts possess microbial growth inhibitory effect against *B. subtilis* at concentration of 0.1805 g/mL (Figure 1C). Compared to a study by Sahlan *et al.*, our seed extract does not exhibit a strong inhibitory effect towards *B. subtilis*[31]. This can be explained by the different parts of *C. paradisi* used in extract preparation, whereby Sahlan *et al.* employed a mixture of *C. paradisi* seeds and pulps, while this study presented the inhibitory effect of extracts purely prepared from seeds only. Similar to juice vesicle extracts, the findings on seed extracts have highlighted the need to further investigate the phytochemical compounds which play their antimicrobial roles.

The significant increase ($P = 0.0005$) in microbial growth inhibitory effect towards *B. subtilis* by juice vesicle extracts has drawn our interest in its antimicrobial activities (Figure 1B). Therefore, its minimum inhibitory concentration (MIC) against different microorganisms was tested, with results tabulated in Table 2. Limited studies are available to deduce the MIC of *C. paradisi* juice vesicle extracts. Although some researchers reported a much lower MIC of *C. paradisi* extract towards *E. coli*, *S. aureus* and/or *B. subtilis*[20], these studies analysed phytochemical activities of *C. paradisi* seeds, pulps, or citrus juice concentrates. Our findings tabulated in Table 2 show the same inhibitory patterns as disk-diffusion assay (Figure 1B), whereby *B. subtilis* exhibited the highest sensitivity towards the juice vesicle extract of *C. paradisi*. This phenomenon indicated the presence of specific phytocompounds which can effectively suppress the growth of *B. subtilis*, which can be lethal to vertebrate animals once being introduced into the animals[32].

One-way Analysis of variance (1-way ANOVA) analyses were conducted to measure the significance of difference between the type of *C. paradisi* extract (ie. peels, juice vesicles, seeds) and their antimicrobial effect against different species of microorganisms (ie. *E. coli*, *S. aureus*, *B. subtilis*). As shown in Table 3, our results have clearly indicated that *B. subtilis* exhibited a significant difference in sensitivity towards different types of extract from *C. paradisi*. This finding has again highlighted the needs in exploring and identifying the phytochemical compounds in the seeds and juice vesicles of *C. paradisi* that exhibit the antimicrobial properties.

Conclusion

This study has reported the antimicrobial effect of *C. paradisi* extracts from its peels, juice vesicles, and seeds against both Gram-positive and Gram-negative bacteria (ie. *E. coli*, *S.*

aureus, *B. subtilis*), using common methodologies including disk-diffusion assays, minimum inhibitory effect (MIC) tests, and some integrations of statistical analyses (independent *t*-test, 1-way ANOVA). The results indicated that the well-studied Gram-positive bacteria - *B. subtilis* possessed a high sensitivity towards *C. paradisi* extracts, especially those prepared from juice vesicles and seeds. MIC tests have also highlighted the research needs in identifying the phytochemical compounds in these samples for characterisation studies. To our knowledge, this is the first study elucidating the antimicrobial effect of dried *C. paradisi* juice vesicles, instead of the while pulp or extracted fruit juice.

Acknowledgement

The authors acknowledge the research support given by Faculty of Pharmacy (SEGi University) and Research & Innovation Management Centre (RIMC, SEGi University).

References

1. Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res. 2012 Oct;3(4):200–1.

2. Dasgupta A, Klein K. Herbal and other dietary supplements that are antioxidants. In: Antioxidants in Food, Vitamins and Supplements. Elsevier; 2014. p. 295–315.

3. Huang Y, Wang T. Pectin oligosaccharides enhance α2,6-sialylation modification that promotes apoptosis of bladder cancer cells by targeting the hedgehog pathway. Cell Biochem Biophys [Internet]. 2021 May 26; Available from: http://dx.doi.org/10.1007/s12013-021-00996-9

4. Wikiera A, Grabacka M, Byczyński Ł, Stodolak B, Mika M. Enzymatically extracted apple pectin possesses antioxidant and antitumor activity. Molecules [Internet]. 2021 Mar 6;26(5). Available from: http://dx.doi.org/10.3390/molecules26051434

5. Simitzis PE, Deligeorgis SG. Agroindustrial by-products and animal products: A great alternative for improving food-quality characteristics and preserving human health. In: Food Quality: Balancing Health and Disease. Elsevier; 2018. p. 253–90.

6. Deng W, Liu K, Cao S, Sun J, Zhong B, Chun J. Chemical composition, antimicrobial, antioxidant, and antiproliferative properties of grapefruit essential oil prepared by molecular distillation. Molecules [Internet]. 2020 Jan 5;25(1). Available from:

http://dx.doi.org/10.3390/molecules25010217

7. Reagor L, Gusman J, McCoy L, Carino E, Heggers JP. The effectiveness of processed grapefruit-seed extract as an antibacterial agent: An *in vitro* agar assay [Internet]. Vol. 8, The Journal of Alternative and Complementary Medicine. 2002. p. 325–32. Available from: http://dx.doi.org/10.1089/10755530260128014

8. Dasgupta A. Effects of herbal supplements on clinical laboratory test results. In: Accurate Results in the Clinical Laboratory. Elsevier; 2019. p. 295–318.

9. Honda Y, Ushigome F, Koyabu N, Morimoto S, Shoyama Y, Uchiumi T, et al. Effects of grapefruit juice and orange juice components on P-glycoprotein- and MRP2-mediated drug efflux. Br J Pharmacol. 2004 Dec;143(7):856–64.

10. Bajraktari-Sylejmani G, Weiss J. Potential risk of food-drug interactions: Citrus polymethoxyflavones and flavanones as inhibitors of the organic anion transporting polypeptides (oatp) 1b1, 1b3, and 2b1. Eur J Drug Metab Pharmacokinet. 2020 Dec;45(6):809–15.

11. Srinivas, Srinivas. Review of grapefruit juice mediated drug-drug interactions – trends from a chirality perspective [Internet]. Journal of Applied Biopharmaceutics and Pharmacokinetics. 2013. Available from: http://dx.doi.org/10.14205/2309-4435.2013.01.01.8

12. Kogure N, Akiyoshi T, Imaoka A, Ohtani H. Prediction of the extent and variation of grapefruit juice-drug interactions from the pharmacokinetic profile in the absence of grapefruit juice [Internet]. Vol. 35, Biopharmaceutics & Drug Disposition. 2014. p. 373–81. Available from: http://dx.doi.org/10.1002/bdd.1904

13. Bailey DG, Dresser G, Arnold JMO. Grapefruit-medication interactions: forbidden fruit or avoidable consequences? CMAJ. 2013 Mar 5;185(4):309–16.

14. Haroen U, Budiansyah A, . N. Phytochemical Screening and *in vitro* antimicrobial effect of orange (*Citrus sinensis*) ethyl acetate extract silage [Internet]. Vol. 17, Pakistan Journal of Nutrition. 2018. p. 214–8. Available from: http://dx.doi.org/10.3923/pjn.2018.214.218

15. Hassan IA, Ekum MI, Ogunsanya AS. Antibacterial activity of sweet orange (*Citrus sinensis*) juice extract on selected bacteria [Internet]. Vol. 15, African Journal of

Microbiology Research. 2021. p. 178–82. Available from: http://dx.doi.org/10.5897/ajmr2020.9387

16. Egbuonu A, Osuji C. Proximate compositions and antibacterial activity of *Citrus sinensis* (sweet orange) peel and seed extracts [Internet]. Vol. 12, European Journal of Medicinal Plants. 2016. p. 1–7. Available from: http://dx.doi.org/10.9734/ejmp/2016/24122

17. Ali J, Das B, Saikia T. Antimicrobial activity of lemon peel (*Citrus limon*) extract. Int J Curr Pharm Res. 2017 Jul 14;9(4):79.

18. Henderson AH, Fachrial E, Nyoman Ehrich Lister I. Antimicrobial activity of lemon (*Citrus limon*) peel extract against *Escherichia coli*. American Academic Scientific Research Journal for Engineering, Technology, and Sciences. 2018 Feb 10;39(1):268–73.

19. P. N, G. J. Antibacterial activity of grapefruit (*Citrus paradisi*) peel extracts [Internet]. Vol. 213, European Food Research and Technology. 2001. p. 484–7. Available from: http://dx.doi.org/10.1007/s002170100394

20. Cvetnić Z, Vladimir-Knezević S. Antimicrobial activity of grapefruit seed and pulp ethanolic extract. Acta Pharm. 2004 Sep;54(3):243–50.

21. Bouarab-Chibane L, Forquet V, Lantéri P, Clément Y, Léonard-Akkari L, Oulahal N, et al. Antibacterial properties of polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) models [Internet]. Vol. 10, Frontiers in Microbiology. 2019. Available from: http://dx.doi.org/10.3389/fmicb.2019.00829

22. Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol. 2012 Apr;23(2):174–81.

23. Tong C, Peng M, Tong R, Ma R, Guo K, Shi S. Use of an online extraction liquid chromatography quadrupole time-of-flight tandem mass spectrometry method for the characterization of polyphenols in *Citrus paradisi* cv. Changshanhuyu peel. J Chromatogr A. 2018 Jan 19;1533:87–93.

24. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009 May;1794(5):808.

25. Miller SI. Antibiotic resistance and regulation of the gram-negative bacterial outer

membrane barrier by host innate immune molecules. MBio [Internet]. 2016 Sep 27;7(5). Available from: http://dx.doi.org/10.1128/mBio.01541-16

26. Dik DA, Fisher JF, Mobashery S. Cell-wall recycling of the gram-negative bacteria and the nexus to antibiotic resistance. Chem Rev. 2018 Jun 27;118(12):5952–84.

27. Ayat AA, Alaa AM, Einas GM, Kowther IE, Layla FY, Marvit OW, et al. Phytochemical investigation and assessment of antimicrobial, anti-inflammatory and antioxidant activities of Sudanese *Citrus paradisi* peel extract [Internet]. Vol. 11, Journal of Pharmacognosy and Phytotherapy. 2019. p. 1–8. Available from: http://dx.doi.org/10.5897/jpp2018.0531

28. Uysal B, Sozmen F, Aktas O, Oksal BS, Kose EO. Essential oil composition and antibacterial activity of the grapefruit (*Citrus Paradisi*. L) peel essential oils obtained by solvent-free microwave extraction: comparison with hydrodistillation [Internet]. Vol. 46, International Journal of Food Science & Technology. 2011. p. 1455–61. Available from: http://dx.doi.org/10.1111/j.1365-2621.2011.02640.x

29. Özogul Y, Özogul F, Kulawik P. The antimicrobial effect of grapefruit peel essential oil and its nanoemulsion on fish spoilage bacteria and food-borne pathogens. Lebenson Wiss Technol. 2021 Jan;136(110362):110362.

30. Polat DÇ, Eryilmaz M, Akalin K, Coşkun M. Antimicrobial activity of grapefruit seed. Hacettepe University Journal of the Faculty of Pharmacy. 2018;38(1):1–3.

31. Sahlan M, Damayanti V, Tristantini D, Hermansyah H, Wijanarko A, Olivia Y. Antimicrobial activities of pomelo (*Citrus maxima*) seed and pulp ethanolic extract. In Author(s); 2018. Available from: http://aip.scitation.org/doi/abs/10.1063/1.5023949

32. Gu H-J, Sun Q-L, Luo J-C, Zhang J, Sun L. A first study of the virulence potential of a *Bacillus subtilis* isolate from deep-sea hydrothermal vent. Front Cell Infect Microbiol [Internet]. 2019 [cited 2021 Aug 5];0. Available from: http://dx.doi.org/10.3389/fcimb.2019.00183