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Antimicrobial properties of *Citrus maxima* flavedo extracts against food pathogens and spoilage microorganisms

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ABSTRACT

This study assesses the antimicrobial potential of ethyl acetate and dichloromethane extracts obtained from pomelo, *Citrus maxima* (*C. maxima*), flavedo against various food pathogens and spoilage microorganisms. The antimicrobial activities of these extracts were evaluated using the agar disc diffusion method against gram-positive bacteria (*Bacillus cereus*), *Staphylococcus aureus* and gram-negative bacteria (*Escherichia coli*). The results indicated that both extracts demonstrated antibacterial properties against the tested microorganisms. The ethyl acetate extract exhibited significantly higher antibacterial activity against the majority of bacterial strains compared to the dichloromethane extract, particularly against *S. aureus* and *B. cereus*. However, dichloromethane extract showed a better effect on *E. coli*, with the inhibition zone ranging from 8.7 to 11.3 mm. *S. aureus* displayed the highest sensitivity to ethyl acetate and dichloromethane extracts of pomelo flavedo with inhibition zones ranging from 1.3 to 1.5 mm, respectively. In conclusion, the findings suggest that pomelo extracts have significant potential as natural antimicrobials and can be safely utilized as food preservatives. This highlights the value of pomelo as a potential source of antimicrobial compounds for food safety and preservation purposes.

Keywords: pomelo peel; *Citrus maxima*; antimicrobial activity; antibacterial; antifungal; antiviral; natural preservative

1. INTRODUCTION

The antimicrobial, antifungal, antiviral, and anti-parasitic activities of plant extracts have gained attention from both industry and the scientific research community (Soyer et al., 2020; Gomes et al., 2019). *C. maxima*, commonly known as pomelo (Figure 1), is a tropical fruit that belongs to the *Rutaceae* family. It is widely consumed and appreciated for its refreshing taste, nutritional value, and good source of vitamins, minerals, and fiber. It is also eaten fresh and is available as a food component, especially in desserts. In recent years, there has been growing interest in exploring the antimicrobial properties of *C. maxima* extracts against food pathogens and spoilage microorganisms. Pomelo peel extract has been shown to possess antimicrobial activity against various pathogens, making it a potential natural alternative for food preservation and pathogen inhibition (Hung et al., 2020; Chollakup et al., 2020; Aichayawanich and Ngaowthong, 2012). The extract exhibits antibacterial activity against food-pathogenic bacteria, including *S. aureus*, *B. cereus*, and *E. coli* (Chollakup et al., 2020). It has also been found to have antifungal properties against plant pathogenic fungi (Mshari et al., 2022). Furthermore, studies have demonstrated that the crude extract from pomelo peel retains its antimicrobial potential even after drying (Rahman et al., 2018). Moreover, emulsified pomelo peel oil, in combination with water-soluble chitosan, has been suggested as a novel natural food preservative (Chen et al., 2018). Pomelo peel extract has also been explored for its potential applications in

cosmetics and skincare (Khang et al., 2021). This study aims to evaluate the antimicrobial properties of ethyl acetate and dichloromethane extracts derived from *C. maxima* flavedo against a range of foodborne pathogens and spoilage microorganisms, including *B. cereus*, *S. aureus*, and *E. coli*.

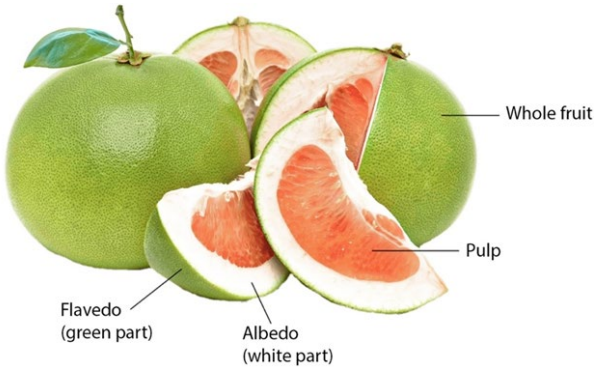


Figure 1. An illustrated picture of *C. maxima* fruit depicting pulp and peel (flavedo and albedo)

2. MATERIALS AND METHODS

2.1 Preparation of pomelo peel

Pomelo peel was utilized as the primary material. A total of 1,000 g fresh pomelo peel was collected from fruit orchards located in Tambun, Perak, Malaysia. The collected pomelo peel was thoroughly cleaned using deionized water and subsequently dried in an incubator at 50 °C for 24 h. Following drying, the pomelo peel was finely powdered using a grinding machine and stored in a sealed container in a dry environment until required for further analysis.

2.2 Pomelo peel extraction

To prepare the pomelo peel solution, 125 g of the powdered peel was weighed and transferred into a 500 mL bijou bottle. Different containers were utilized to acquire ethyl acetate and dichloromethane extracts. The solutions, measuring 375 mL each, were added to their respective flasks and gently mixed by agitation. The bijou bottle containing the mixture was placed in a shaker at 37 °C for 24 h. After this incubation period, the crude extract was filtered using Whatman No.1 filter paper, and the resulting filtrate was concentrated under a vacuum at 40 °C using a rotary evaporator.

The extraction technique employed in this study followed a protocol modified from Chanthaphon et al. (2008), which involved using a rotary evaporator for ethyl acetate and dichloromethane extraction. The extraction process was performed under a vacuum at 90 °C to ensure the removal of all solvents. The crude extract obtained from the pomelo peel was then sealed and stored at 4 °C until further use.

2.3 Microorganisms' growth conditions

Microbial strains, including *S. aureus*, *B. cereus*, and *E. coli*, were obtained from the culture collection of the Microbiology Laboratory, Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA. The microorganisms were grown in tryptic soy broth (TSB) and subsequently inoculated onto nutrient agar, blood agar, and MacConkey agar. All bacterial strains were cultivated in brain heart infusion broth at 37 °C for 18 h. Multiple culture media were used to allow for selective growth, differentiation of bacterial species, and tailored conditions for the bacteria.

2.4 Determination of the antimicrobial activity of pomelo peel extracts

A disc diffusion technique was employed to evaluate the antimicrobial activity of the ethyl acetate and dichloromethane extracts derived from pomelo peels. Four distinct concentrations of each extract (100%, 50%, 33%, and 25%) were prepared for analysis. Mueller Hinton agar was used in this method, and filter paper discs with a diameter of 6 mm were soaked in 15 µL of the crude extracts. These extract discs were then aseptically placed over the bacterial culture on the Mueller Hinton agar plates.

The analysis involved the use of three types of organisms, with standard antibiotics (ciprofloxacin) serving as positive controls, as specified in Table 1. Two different extraction methods, ethyl acetate and dichloromethane extracts, were used to assess antimicrobial activity. A sterile blank disc was employed as the negative control and placed in the designated position on the labelled plate. Following incubation at 37 °C for 18 to 24 h, the zone of inhibition around each disc was measured using a millimeter scale. To ensure reliable results, the experiment was conducted in triplicate.

Table 1. Inhibition zones are presented as mean±standard deviation of triplicate

Concentration of extract (%)	Zone of inhibition (mm)					
	Staphylococcus aureus		*Escherichia coli*		*Bacillus cereus*	
	Ethyl acetate	Dichloromethane	Ethyl acetate	Dichloromethane	Ethyl acetate	Dichloromethane
100	14.0±0.2	12.3±0.0	9.7±0.0	11.3±0.1	14.7±0.0	13.7±0.1
50	13.0±0.2	9.3±0.1	6.7±0.1	7.3±0.1	8.7±0.9	8.7±0.1
33	11.0±0.1	13.0±0.0	6.3±0.1	6.7±0.1	8.3±0.1	8.8±0.1
25	10.7±0.1	13.3±0.1	7.7±0.1	8.7±0.0	7.7±0.1	7.7±0.1
Positive control (ciprofloxacin)	24±0.0	24±0.0	14.3±0.1	13.7±0.1	20.3±0.1	20.0±0.0
Negative control	0.0	0.0	0.0	0.0	0.0	0.0

3. RESULTS AND DISCUSSION

Our study investigated the effectiveness of ethyl acetate and dichloromethane extractions at different concentrations (100%, 50%, 33%, and 25%) against various bacterial strains, including *S. aureus*, *E. coli*, and *B. cereus*. Ethyl acetate extraction and dichloromethane extraction are two commonly used methods for extracting bioactive compounds from *C. maxima*. These methods involve using organic solvents to extract the desired compounds from the plant material. Several studies have compared the efficacy of these two extraction methods and their effects on the bioactivity of the extracts.

Notably, the ethyl acetate extract demonstrated superior inhibitory activity, compared to the dichloromethane extract at all tested concentrations, particularly against *S. aureus* and *B. cereus*, which had exhibiting zone of inhibition values of 14.0±0.2 mm and 14.7±0.0 mm, respectively (Figure 2 and Figure 3). Several studies have examined the antimicrobial activity of *C. maxima* flavedo extracts against various food pathogens and spoilage microorganisms. Kumar et al. (2021) found significant antimicrobial activity against *S. aureus*, while minimal activity was observed against *E. coli*. In another study, Sapkota et al. (2022) reported substantial antimicrobial activity against pathogenic microbes like *Klebsiella aerogenes*, *Klebisella pneumoniae*, *Bacillus subtilis*, and *S. aureus*. In addition to their antimicrobial activity, *C. maxima* extracts have demonstrated antibiofilm properties. Barrion et al. (2014) reported that the phytochemical constituents of *C. maxima* fruit extracts exhibited antibacterial activity against *E. coli* and *Salmonella typhimurium*. Moreover, polyphenol compounds found in pomelo peels (*C. maxima*) have been shown to have antibiofilm and antiquorum-sensing effects on multidrug-resistant *Pseudomonas aeruginosa* (Sadeva et al., 2022).

The antimicrobial activity of *C. maxima* flavedo extracts can be attributed to the presence of bioactive compounds such as polyphenols. These compounds have been reported to possess antimicrobial properties and can inhibit the growth of various microorganisms (Kumar et al., 2021). The exact mechanisms of action are not fully understood. However, it is believed that the bioactive compounds in *C. maxima* extract disrupt the integrity of the microbial cell membrane, leading to cell death (Górniak et al., 2018). Additionally, the extracts may interfere with essential microbial processes, such as deoxyribonucleic acid (DNA) replication and protein synthesis, further contributing to their antimicrobial activity (Górniak et al., 2018).

The antimicrobial properties of *C. maxima* flavedo extracts make them promising candidates for use in food preservation. The extracts have been shown to exhibit higher antimicrobial activity than commercial preservatives against spoilage microorganisms and foodborne pathogens such as *Salmonella spp.*, *Listeria monocytogenes*, and *B. cereus* (Ricci et al., 2021). Incorporating *C. maxima* extracts into food products or using them as natural preservatives can help extend the shelf-life of various categories of foodstuff and improve their safety (Ricci et al., 2021).

Furthermore, using *C. maxima* extracts in combination with other antimicrobial agents, such as nisin, has been proposed as a novel approach to enhance antimicrobial efficacy and broaden the spectrum of activity (Roshanak et al., 2020). This combination could potentially prevent contamination, spoilage, and infestation of food and beverage products (Roshanak et al., 2020).

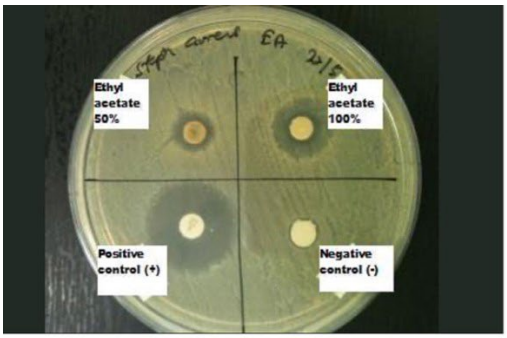
Karo et al. (2020) studied the antibacterial activity of flavonoid-rich fractions of *C. maxima* peel extract. The study used ethyl acetate as a solvent to extract bioactive compounds. The results showed that the ethyl acetate fraction exhibited significant antibacterial activity against tested bacteria. These results suggest that ethyl acetate extraction may extract bioactive compounds with antimicrobial properties more effectively.

Another study by Leporini et al. (2021) evaluated the antioxidant activity of various citrus species extracts, including *C. maxima*, using different solvents. The study found that ethyl acetate extracts of *C. maxima* leaves exhibited high 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, indicating strong antioxidant potential. This suggests that ethyl acetate extraction may be more efficient in extracting antioxidant compounds from *C. maxima*.

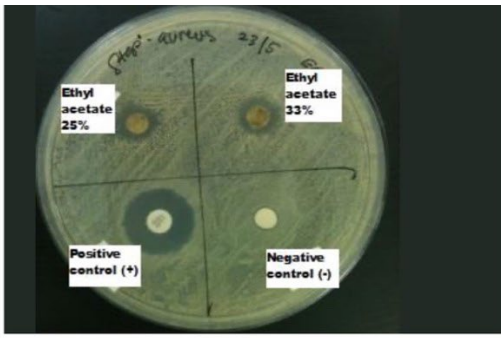
Remarkably, several studies (Jamil et al., 2021; Palacios et al., 2019) highlight that ethyl acetate extracts consistently exhibit the highest antimicrobial activity, compared to other solvent extracts. This heightened activity can be attributed to the presence of secondary metabolites such as alkaloids, phenols, and flavonoids, which are effectively extracted by these solvents (Abdu and Adamu, 2020). These compounds may disrupt the bacterial cell membrane, inhibit enzymes, or interfere with metabolic pathways.

Interestingly, in contrast to the aforementioned findings, the dichloromethane extract displayed superior efficacy compared to the ethyl acetate extract, particularly when tested against *E. coli*, resulting in a notable inhibition zone of 8.7±0.0 to 11.3±0.1 mm. However, a more rigorous statistical analysis is necessary to support this claim (Figure 4). These results indicate that the effectiveness of the extraction methods may vary depending on the specific bacterial strain, emphasizing the importance of considering the target microorganism when selecting the extraction solvent.

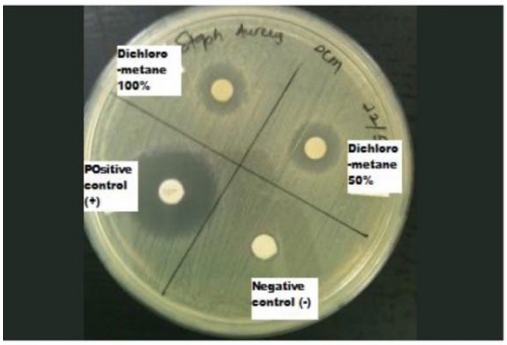
C. maxima flavedo extracts have demonstrated significant antimicrobial activity against various food pathogens and spoilage microorganisms. The bioactive compounds present in these extracts, such as polyphenols, contribute to their antimicrobial properties. The extracts have the potential to be used as natural preservatives in the food industry to extend the shelf-life of food products and improve their safety. To delve deeper into the mechanism behind the observed antimicrobial effects, further investigation is necessary to isolate and identify the specific compounds responsible for the activity (Joseph and Prakasia, 2020). Isolating active fractions and identifying the bioactive compounds through fractionation of the extracts could aid in pinpointing the most potent components (Hassan et al., 2023). These fractionation studies could help shed light on the underlying mechanisms and potentially lead to the development of targeted antimicrobial agents derived from the extracts.



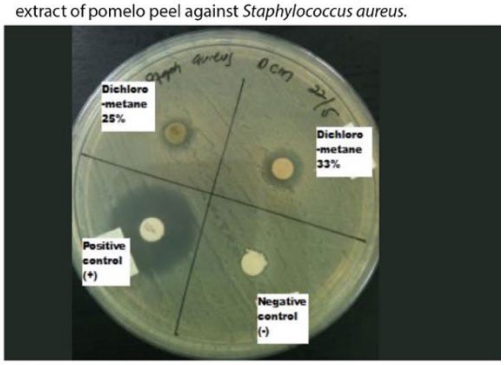
(A) Disk diffusion assay of 100% and 50% of ethyl acetate extract of pomelo peel against *Staphylococcus aureus.*



(B) Disk diffusion assay of 33% and 25% of ethyl acetate extract of pomelo peel against *Staphylococcus aureus.*

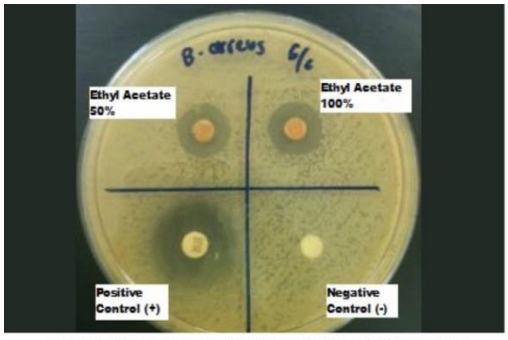


(C) Disk diffusion assay of 100% and 50% of dichlorometane extract of pomelo peel against *Staphylococcus aureus.*

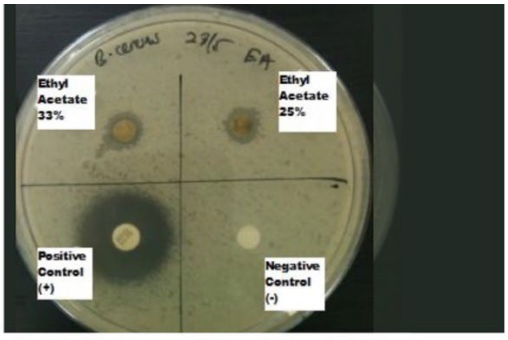


(D) Disk diffusion assay of 33% and 25% of dichlorometane extract of pomelo peel against *Staphylococcus aureus.*

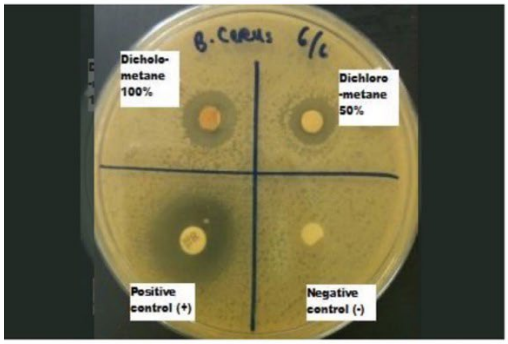
Figure 2. Antimicrobial sensitivity testing of *C. maxima* extracts against *S. aureus*
Note: Clear zone surrounding the disc reveals the presence of antimicrobial properties.



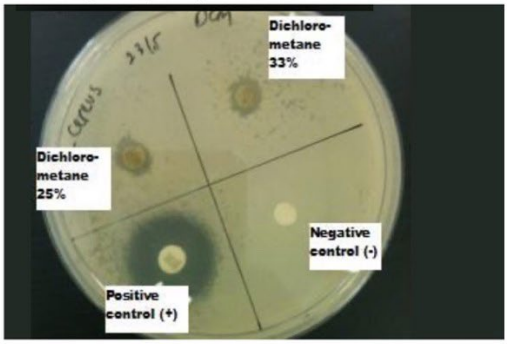
(A) Disk diffusion assay of 100% and 50% of ethyl acetate extract of pomelo peel against *Bacillus cereus.*



(B) Disk diffusion assay of 33% and 25% of ethyl acetate extract of pomelo peel against *Bacillus cereus.*



(C) Disk diffusion assay of 100% and 50% of dichlorometane extract of pomelo peel against *Bacillus cereus.*



(D) Disk diffusion assay of 33% and 25% of dichlorometane extract of pomelo peel against *Bacillus cereus.*

Figure 3. Antimicrobial sensitivity testing of *C. maxima* extracts against *B. cereus*
Note: Clear zone surrounding the disc reveals the presence of antimicrobial properties.

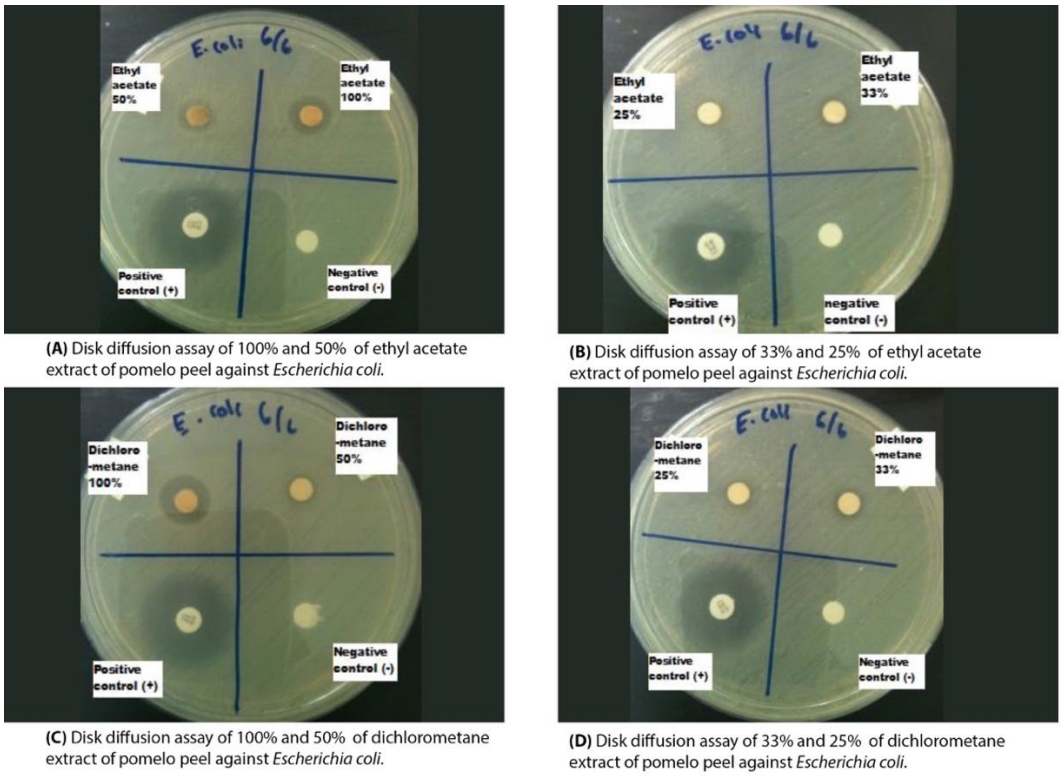


Figure 4. Antimicrobial sensitivity testing of *C. maxima* extracts against *E. coli*
Note: Clear zone surrounding the disc reveals the presence of antimicrobial properties.

4. CONCLUSION

In conclusion, the antimicrobial properties of *C. maxima* flavedo extracts against food pathogens and spoilage microorganisms have been clearly demonstrated in this study. The ethyl acetate and dichloromethane extracts exhibited significant inhibitory activity against various bacterial strains, including *S. aureus*, *E. coli*, and *B. cereus*. The superior inhibitory activity of the ethyl acetate extract, particularly against *S. aureus* and *B. cereus*, suggests its potential as a natural antimicrobial for food preservation. However, the dichloromethane extract showed notable effectiveness against *E. coli*, indicating the importance of considering the specific bacterial strain when selecting extraction solvents. These findings underscore the value of *C. maxima* flavedo as a potential source of bioactive compounds, such as polyphenols, with antimicrobial properties. However, further studies isolating and identifying the active constituents within the extracts are needed to optimize their antimicrobial efficacy. Purifying the active compounds could enable the development of more targeted antimicrobial drugs with improved potency and stability.

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