**Citrus x microcarpa** bunge fruit extract as antibacterial against *staphylococcus aureus*

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**Abstract.** *Citrus x microcarpa* Bunge is a plant that is used optimally by the people of Bangka Belitung known as the 'Jeruk Kunci'. Utilization of this plant in the fruit is used as an acid enhancer in food and is made in the form of syrup. So that the jeruk kunci fruit waste is widely available and has not been utilized. Based on literature, *Citrus x microcarpa* contains flavonoids and a monoterpeneshydrocarbon. Bioactivity which belongs as antimicrobial, antibacterial and antioxidant. Therefore, jeruk kunci fruit waste has the potential to be developed as an antibacterial herbal medicine based on literature studies. *Citrus x microcarpa* Bunge extract was obtained by maceration for 3 x 24 hours using ethanol solvent. Then phytochemical testing was conducted qualitatively using several reagents and quantitative phytochemical testing was carried out using functional group analysis contained in the extract uses the Fourier Transform Infrared (FT-IR) at the wavenumber region 4000-400 cm⁻¹. Then, determination of antibacterial activity using the disk diffusion method. Based on FT-IR spectrum data analysis *Citrus x microcarpa* extract contains tannins. The results of antibacterial testing of *Citrus x microcarpa* Bunge extract obtained concentration of 20%, 40% and 60% has a relatively strong antibacterial inhibition. whereas for 80% and 100% concentrations the ability to inhibit is very strong.

**1. Introduction**

Infectious diseases caused by bacteria are still a problem both in developing countries and in developed countries. *Staphylococcus aureus* is one of the most common bacteria that cause infections in the world. This bacterium can live on human skin and is very easy to breed at an optimum temperature of 30°C. Diseases caused by *S. aureus* include respiratory infections, abscesses, skin inflammation, mastitis, impetigo, and food poisoning that cause diarrhea accompanied by nausea and vomiting [1],[2].

Antibacterial is a compound that can inhibit bacterial growth. Antibacterial can be a synthetic compound and natural compounds. Antibacterial that can be used to inhibit the growth of *Staphylococcus aureus* such as erythromycin, cloxacillin and dicloxacillin. The use of synthetic antibiotics allows for resistance. Natural compounds found in organisms are known as secondary metabolites. Secondary metabolites that act as antibacterial include alkaloids, phenols and flavonoids. The mechanism of action of secondary metabolites, among others, by damaging bacterial cell walls,
changing membrane permeability, inhibiting the work of enzymes, and disrupt the process of protein synthesis [3].

Citrus x microcarpa Bunge is widespread in the Bangka Belitung area and is known by the name of the jeruk kunci. Jeruk kunci are used as an acid enhancer in foods, and are processed into key orange syrup, so that the jeruk kunci fruit waste is abundant and has not been utilized. Research on phytochemicals and bioactivity of Citrus x microcarpa Bunge found that C. x microcarpa Bunge contains flavonoids, alkaloids, polyphenols, monoterpenes hydrocarbon such as limonene, sabinen, citronellal, linalool and hedycaryol with bioactivity as antimicrobial, antibacterial, antioxidant and antifeedant [4-8]. Flavonoid compounds also act as antibacteria. Therefore, this research will examine the antibacterial bioactivity of jeruk kunci fruit waste against Staphylococcus aureus

2. Materials and methods
2.1. Material and equipment
The materials used in this study were jeruk kunci fruit waste from industrial waste of jeruk kunci syrup Air Duren Village, dimethyl sulfoxide (DMSO), nutrient agar, nutrient broth, ethanol, paper discs, amoxicillin, Mueller-Hinton agar (MHA), aquades, alunnum foil, cotton, and label paper. Equipment used includes petri dishes, test tubes, erlenmeyers, micro pipettes, rotary evaporators, vacuum pumps, Buchner funnel, vial bottle volumetric flask and spatula.

2.2. Sample Preparation
Waste of fresh jeruk kunci fruits as much as 1 kg is cleaned by washing with running water and then dried in direct sunlight. A 450 gram dry sample was blended using a blender, to increase contact between the sample and the solvent [9].

2.3. Extraction
450 gram sample powder was macerated for 3 x 24 hours with comparison of sample with solvent 1:10. Every 1 x 24 hours, filtering and solvent replacement are carried out so that the extraction process runs optimally [10]. The filtrate obtained was evaporated using a rotary evaporator, so that a thick extract was obtained [11]. The next step, the viscous extract obtained was tested for antibacterial against Staphylococcus aureus and confirmed the presence of secondary metabolite functional groups using Fourier Transform Infrared (FT-IR) in the wave number range 4000-400 cm⁻¹.

2.4. Antibacterial testing
Antibacterial testing is carried out using the agar diffusion method. A bacterial suspension is applied to the petri dish which contains a solid nutrient agar. Next 6 mm disc paper saturated with 20%, 40%, 60%, 80%, and 100% concentration test solution is placed on a petri dish. The same work for disc paper as a positive control and negative control. Positive control in this test uses amoxillin and negative control uses DMSO solvent. Then the petri dish was incubated for 24 hours at 370C. Measurement of antibacterial activity is done by measuring clear zones formed using calipers. Furthermore, the clear zone diameters formed were classified into four categories, namely <5 mm weak, 5-10 mm moderate, 10-20 mm strong and> 20 mm very strong [12].

3. Result and Discussion
Citrus x microcarpa Bunge is widespread in the Bangka Belitung area and is known by the name of the jeruk kunci (Figure 1). Based on research that has been done. Citrus x microcarpa contains tannin compounds based on tests with FeCL3 with a greenish-black color change.
Figure 1. Fruit of *Citrus x microcarpa* Bunge

Spectrum FTIR of *Citrus x microcarpa* on Figure 2. FTIR functions to find out the functional groups contained in the synthesized product.

![FTIR Spectrum of Citrus x microcarpa](image)

**Figure 2.** FTIR Spectrum of *Citrus x microcarpa* Bunge

Widened absorption from 3400 to 3100 cm\(^{-1}\) indicates the vibration of the -OH (hydroxyl) group, the widening band caused by the interaction of intermolecular hydrogen bonds. This hydroxyl group can also originate from the phenol group (Ar-OH) which is strengthened by absorption at the 738 cm\(^{-1}\) wave number. The uptake of this OH OH group may also overlap with C-H aromatic stretching uptake which is usually at 3159-3050 cm\(^{-1}\). This indicates the presence of aromatic groups in the extract. The presence of aromatics is also supported by the presence of vibration C = C stretching at 1614 cm\(^{-1}\). The analysis showed that *Citrus x microcarpa* Bunge extract contained aromatic compounds in the form of phenolic compounds or polyphenols such as tannins.

Bands with wave numbers 2923 cm\(^{-1}\) indicate the presence of CH\(_3\) (methyl) groups and CH as asymmetric vibrations (methylene). The existence of this group is also supported by the absorption of wave numbers from 1448 to 1334 cm\(^{-1}\). This indicates that the *Citrus x microcarpa* extract contains methyl, methylene and metin groups.

Based on FT-IR spectrum data analysis (Figure 2. and Table 2), *Citrus x microcarpa* extract contains compounds that have Ar-OH (phenolic), -OH (hydroxyl), and C = C (aromatic) groups. This indicates that the functional group is a functional group on the structure of polyphenols such as tannins.
Table 1. Vibrational mode of *C. x microcarpa* Extract

| Wavenumber (cm⁻¹) | C. x microcarpa | C. ferruginea [3] | Vibration modes               |
|-------------------|----------------|------------------|-------------------------------|
| 3351              | 3332           | -OH stretch alcohol/phenol |
| 2923              | 2924           | C-H stretch CH₃/asymmetric CH₂ |
| 1614              | 1607           | C=C aromatic      |
| 1448              | 1435           | CH₃ bend         |
| 1334              | 1245           | C-H bend         |
| 1210              | 1020           | C-C stretch      |
| 1031              | 777            | C-O alcohol stretch |
| 738               |                | C-H aromatic bend |

Antibacterial testing of *C. x microcarpa* Bunge extract (jeruk kunci) was carried out using the disc diffusion method. The potential for antibacterial activity is known by measuring in the clear zone area around the disc paper. After testing antibacterial against gram-positive *Staphylococcus aureus* with test material in the form of ethanol extract of jeruk kunci fruits waste, DMSO (negative control) and amoxillin (positive control) solvents, the data shown in the table below.

Table 2. Inhibition zone of ethanol extract of *Citrus x microcarpa* Bunge on the growth of *Staphylococcus aureus*

| Concentration of extract | Average Inhibition Zone Diameter (mm) |
|--------------------------|---------------------------------------|
| 20%                      | 12.36                                 |
| 40%                      | 17.37                                 |
| 60%                      | 19.61                                 |
| 80%                      | 22.90                                 |
| 100%                     | 26.63                                 |

Based on the above data, ethanol extract of jeruk kunci fruit waste has the potential to inhibit the growth of *Staphylococcus aureus*. Positive control in this study using amoxillin with inhibition zone diameter of 18.45 mm and negative control using DMSO solvent did not show any inhibition of the growth of *Staphylococcus aureus* bacteria.

The antibacterial potential of ethanol extract of jeruk kunci fruits waste in inhibiting the growth of *S. aureus* began to be seen at the lowest concentration of 20% of 12, 36 mm. At extract concentration of 40% has a inhibitory power of 17.37 mm. The extract concentration of 60% formed a clear zone of 19.61 mm, and at the extract concentration of 80% and 100% formed a clear zone of 22.90 mm and 26.63 mm respectively. The growth of *S. aureus* decreased with increasing concentration of jeruk kunci fruits waste extracts. Inhibition of the growth of *S.aureus* over positive control was shown at extract concentrations of 60%, 80% and 100%. Based on these data extract concentrations of 20%, 40% and 60% have a relatively strong bacterial inhibition strength, at a concentration of 80% and 100% the inhibitory strength of bacteria is very strong. Research on pure compounds 3’, 4’, 5,6,7,8-hexamethoxyflavone isolated from *Citrus microcarpa* from Manila did not show any antibacterial activity against *S. aureus* [4].

Based on FTIR spectrum data analysis, *Citrus x microcarpa* Bunge contains tannins. The tannin / phenol hydroquinone compound as an antibacterial by inhibiting the transcriptase enzyme and the DNA topoisomerase being not formed. The mechanism of action of other tannins as antibacterials by activating cell adhesin, activating enzymes and disrupting protein transport. Disruption in the protein synthesis of bacterial cells due to the presence of tannin compounds will be fatal, leading to bacterial cell death [13].

4. Conclusion

*Citrus x microcarpa* Bunge extract contains compounds that have Ar-OH (phenolic), -OH (hydroxyl), and C = C (aromatic) groups. This indicates that the functional group is a functional group on the
structure of polyphenols such as tannins. Tannins including phenolic groups like flavonoid. The results of antibacterial testing of *Citrus x microcarpa* Bunge extract obtained concentration of 20%, 40% and 60% has a relatively strong antibacterial inhibition, whereas for 80% and 100% concentrations the ability to inhibit is very strong.

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