

## COMPARATIVE EVALUATION OF ANTIMICROBIAL PROPERTIES OF CITRUS VARIETIES AVAILABLE IN MALAYSIA MARKET

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### ABSTRACT

Citrus has been cultivated in many tropical and subtropical countries due to its culinary and medicinal properties. Its antimicrobial properties are well known. There are few varieties of citrus available in Malaysian market i.e. *Citrus aurantifolia*, *Citrus reticulata*, *Citrus microcarpa*, *Citrus limon* and *Citrus sinensis*. The present study compares the antibacterial properties of methanol extract of five varieties of citrus peels by disc diffusion method against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. There is no inhibition were observed for all the citrus peel extracts at 5 and 10 mg/ml. The methanol extract of *Citrus microcarpa*, *Citrus reticulata* and *Citrus sinensis* at 20 mg/ml showed better inhibition than compare to *Citrus aurantifolia* and *Citrus limon* against *Staphylococcus aureus* and *Escherichia coli*. However, the standards at lower concentrations showed higher inhibition than compare to all the extracts against entire organism. These findings can form the basis for further studies to isolate their active constituents to perform its biological activities.

**Keywords:** Citrus peels, Antimicrobial, Disc-diffusion method

### INTRODUCTION

Citrus family is large and varied. There are several types of citrus plants and fruits are available all over the world. These plants are widely used in both medicinal and cooking purposes. In Malaysia *Citrus aurantifolia*, *Citrus reticulata*, *Citrus microcarpa*, *Citrus limon* and *Citrus sinensis* were available in the market. They are different from colour, size, appearance and taste.

Citrus fruits reported for enormous number of biological activities such as anti-cancer, anti-diarrheal, antibacterial, antifungal, antiviral insecticidal and antioxidant. Some oils have been used in cancer treatment [1].

Medicinal plants represent a rich source of antimicrobial agents. There is also an urgent need to search for new antimicrobial compounds with novel mechanisms of action because there has been an alarming increase in the incidence of new infections diseases, as well as the development of resistance to the antibiotics in current clinical trials [2].

All the citrus varieties are well known for antimicrobial properties [3]. However, so far there is no comparative study has been reported in these varieties available in Malaysia for its antimicrobial properties. Hence, in the present study, we aimed to carry out a comparative investigation of its antimicrobial properties of different varieties of citrus using disc-diffusion method.

### MATERIALS AND METHODS

#### Collection and authentication of Citrus fruits (Fig.1)

The raw citrus fruits, *Citrus reticulata* (limau mandarin), *Citrus microcarpa* (limau kasturi), *Citrus aurantifolia* (limau nipis), *Citrus sinensis* (limaumadu) and *Citrus limon* (lemon) were collected from local market, Ipoh District, Perak, Malaysia and authenticated by botanist.

#### Extraction of dried peels of citrus fruits

The peels of the citrus fruits were removed from the fruits and washed thoroughly in distilled water to remove the contaminants; it was chopped into small pieces and dried under shade, coarsely powdered and separately subjected to extraction by maceration in

methanol (10 g of plant powder and 50 ml of methanol) at room temperature with occasional shaking for seven days. The macerate was filtered and the filtrate was dried at low temperature (40-50 °C) under vacuum. The extracts were stored in air-tight containers in a refrigerator at 4 °C until further use.

#### Antimicrobial screening

##### Test microorganism

A panel of four common pathogenic microorganisms were used in the study, which includes two gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

##### Disc-diffusion method

A suspension of the tested microorganisms was uniformly swabbed on agar. Sterile blank discs were individually impregnated with different concentration of extracts (5,10 and 20 mg/ml) and placed onto the inoculated agar plates. The plates were inverted and incubated at 37 °C for 24 h. The antimicrobial activity was measured by measuring diameter of the resulting zone of inhibition against the tested organisms. The test for positive control and negative control were performed in duplicates [2].

### RESULTS

The nature of methanol extract of citrus varieties and percentage yields were mentioned in Table 1. The results of antibacterial activity of methanol extract of citrus varieties were presented in Table 2. The antibacterial activity was tested by using three different concentrations includes 5, 10 and 20 mg/ml. There is no inhibition were observed for negative control and all the citrus peel extracts at 5 and 10 mg/ml. The concentration at 20 mg/ml showed lower to moderate inhibition against *Staphylococcus aureus* and *Escherichia coli*, but also failed to show inhibition against *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. The methanol extract of *Citrus microcarpa*, *Citrus reticulata* and *Citrus sinensis* at 20 mg/ml showed better inhibition than compare to *Citrus aurantifolia* and *Citrus limon* against *Staphylococcus aureus* and *Escherichia coli*. However, the standards at low concentrations showed higher inhibition than compare to all the extracts against entire organism.

Table 1: Yields and nature of methanol extract of citrus varieties

Plant Source	Quantity used for methanol extraction		Nature of the extracts	Yield (%)
	Powder (g)	Solvent (ml)		
<i>Citrus aurantifolia</i>	10	50	Light green residue	12.84 %
<i>Citrus limon</i>	10	50	Light yellow residue	17.26 %
<i>Citrus microcarpa</i>	10	50	Dark green residue	14.19 %
<i>Citrus reticulata</i>	10	50	Dark orange residue	21.56 %
<i>Citrus sinensis</i>	10	50	Yellowish green residue	18.38 %

Table 2: Antimicrobial activity of methanol extract of citrus varieties

S. No.	Organism used	Concentration in mg/ml															Control	Standard
		<i>Citrus aurantifolia</i>			<i>Citrus limon</i>			<i>Citrus microcarpa</i>			<i>Citrus reticulata</i>			<i>Citrus sinensis</i>				
		5	10	20	5	10	20	5	10	20	5	10	20	5	10	20		
Gram positive bacteria																		
1	<i>Streptococcus pyogenes</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	Bacitracin (+++)
2	<i>Staphylococcus aureus</i>	--	--	+	--	--	+	--	--	++	--	--	++	--	--	++	--	Penicillin (+++)
Gram negative bacteria																		
3	<i>Escherichia coli</i>	--	--	+	--	--	+	--	--	++	--	--	++	--	--	++	--	Ceftriaxone (+++)
4	<i>Pseudomonas aeruginosa</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	Ciprofloxacin (+++)

+++higher inhibition, ++moderate inhibition, +lower inhibition and --no inhibition



*Citrus reticulata*



*Citrus aurantifolia*



*Citrus limon*



*Citrus microcarpa*



*Citrus sinensis*

Fig. 1: Fruits of citrus varieties

## DISCUSSION

The results indicate that the methanol extract of citrus varieties showed lower to moderate antibacterial activity towards *Staphylococcus aureus* and *Escherichia coli*. At the same time there is no inhibition against *Streptococcus pyogenes* and *Pseudomonas aeruginosa*.

The results of this study reflect that the citrus peel extracts showed better antifungal activity than antibacterial activity [1,4]. It also closes agreement with previous report that the ethyl acetate extract of citrus peel has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with gradual increase in activity with increase in concentration indicating that the activity was concentration dependent [1,4].

There is no considerable difference has been observed in the citrus varieties peel extracts against the tested organisms. In conclusion, these findings can form the basis for further studies to isolate their active constituents to perform its biological activities.

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