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Research Article

COMPARATIVE EVALUATION OF ANTIMICROBIAL PROPERTIES OF RED AND YELLOW WATERMELON SEEDS

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ABSTARCT

Watermelon fruits are originated from the West Africa and distributed all over the world including Malaysia. Its antimicrobial properties are well known. There are two varieties of watermelon fruits are available in the Malaysian market i.e. red and yellow. In the present study, a qualitative phytochemical analysis of methanol extracts of two varieties of watermelon seeds were carried out to identify the difference in their active constituents. A detailed antimicrobial study was carried out for both of the extracts and compared. The zone of inhibition produced by the crude methanolic extract of red watermelon seeds against *Streptococcus pyogenes* and *Staphylococcus aureus* were 7-20 mm and 7-10 mm, respectively in different concentrations. At the same time crude methanolic extract of red watermelon seeds do not showed zone of inhibition against *Escherichia coli* and *Pseudomonas aeruginosa*. The methanolic extract of yellow watermelon seeds do not showed zone of inhibition against all the tested organisms. In conclusion, these results showed that methanolic extract of red watermelon seeds are good candidate for further investigation against gram positive bacteria.

Keywords: *Citrullus lanatus*, Red and yellow watermelon, Antimicrobial.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. There is also an urgent need to search for a new antimicrobial compounds with novel mechanisms of action because there have 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 (Kamazeri et al., 2012).

Watermelon (*Citrullus lanatus*) belongs to the Cucurbitaceae family and is a source of multiple minerals, vitamins and proteins that are present in skin, pulp and seeds.

Beneficial effects of *C. lanatus* on cardiovascular diseases may be due to several mechanisms including antioxidant, anti-inflammatory and vasodilatory properties. *C. lanatus* is a rich source of citrulline that is responsible for the biological activities (Poduria et al., 2013).

A numeral commercial variety of watermelon exists. In Malaysia, red and yellow watermelon fruits are available in the market. The watermelon fruit, loosely considered a type of watermelon has a smooth exterior rind and a juicy, sweet, usually red, but sometimes orange, yellow, or pink interior flesh (Daniel and Maria, 2000). Water melon seeds have both nutritional and cosmetic importance, the seed contain vitamins B2, minerals, riboflavin, fat carbohydrates and protein (Lazos., 1986). Its antibacterial activities are well known against various common pathogens.

The seeds of red and yellow watermelon are different in colour and shape. Definitely there may be a different constituents and biological activities between those varieties.

However, so far there is no comparative study has been reported in watermelon varieties, though various extracts of watermelon seeds have been reported for antibacterial activity (Ajayi et al., 2011). Hence, in the present study, we aimed to carry out a comparative investigation of its antimicrobial properties of different varieties of watermelon fruits using standard methods.

MATERIALS AND METHODS

Collection and authentication of plant materials

The fruits of red and yellow watermelon (Fig.1, *Citrullus lanatus*) were collected from local market, Ipoh District, Perak, Malaysia and authenticated by botanist.

Fig. 1: Red and yellow watermelon fruits

Extraction of red and yellow watermelon seeds

The flesh was removed and the seeds of red and yellow watermelon were collected and washed thoroughly in distilled water to remove contaminants; it was dried under shade, coarsely powdered and separately subjected to extraction by maceration in methanol at room temperature with occasional shaking for seven days.

The macerate extracts were 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.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of both the extracts were carried out by using various chemical tests to identify the phytoconstituents present in it.

Tests for carbohydrates: Molisch's test, Fehling's test, Benedict's test.

Tests for alkaloids: Mayer's test, Dragendorff's test, Wagner's test.

Tests for steroids and sterols: Liebermann Burchard's and Salkowski test.

Tests for glycosides: Baljet's test, Borntrager's test and Keller-Kiliani test.

Tests for saponins: Foam test and hemolysis test.

Test for flavonoids: Shinoda test.

Tests for tannins: Lead acetate test and gelatin test.

Test for triterpenoids: Tin and thionyl chloride test.

Tests for proteins and amino acids: Biuret test and Ninhydrin.

Antimicrobial screening

Test microorganisms

A panel of four common pathogenic microorganisms were used in the study, which includes gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus),* 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 (1000, 500, 250 µg/ml) and placed onto the inoculated agar plates (Kamazeri et al., 2012). The plates were inverted and incubated at 37 ºC 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 duplicate.

RESULTS AND DISCUSSION

The nature of two varieties of watermelon seed extracts and yields were mentioned in Table 1.

Table 1: Yields and nature of methanolic extract of red and yellow watermelon seeds

Plant Source	Quantity used for methanol extraction		Nature of the extracts	Yield (%)
	Powder (g)	Solvent (ml)		
Red watermelon seeds	25	200	Brownish white semisolid	3.40
Yellow watermelon seeds	25	200	Yellowish white semisolid	4.28

Phytochemical analysis (Table 2) of crude methanolic extracts of red and yellow watermelon seeds showed the presence of alkaloids, carbohydrates, proteins, glycosides, steroids, flavonoids, tannins, triterpenoids and fixed oils.

Table 2: Qualitative phytochemical analysis of Methanolic extract of Red and yellow watermelon seeds

Phytoconstituents	Methanolic extract of Red watermelon seeds	Methanolic extract of watermelon seeds
Alkaloids	+	+
Carbohydrates	+	+
Proteins	+	+
Aminoacids	-	-
Steroids and sterols	+	+
Glycosides	+	+
Flavonoids	+	+
Tannins	+	+
Triterpenoids	+	+
Fixed oils	+	+

+ Present, - Absent

Table 3: Antimicrobial activity of methanolic extract of red and yellow watermelon seeds

S. No.	Organism used	Concentration in µg/ml						Control	Standard
		Methanolic extract of Red watermelon seeds			Methanolic extract of Yellow watermelon seeds				
		1000	500	250	1000	500	250		
Gram positive bacteria									
1	*Streptococcus pyogenes*	20 mm	10 mm	7 mm	-	-	-	-	Bacitracin (4 µg/ml, 25 mm)
2	*Staphylococcus aureus*	10 mm	7 mm	7 mm	-	-	-	-	Penicillin (10 µg/ml, 17 mm)
Gram negative bacteria									
3	*Escherichia coli*	-	-	-	-	-	-	-	Cefriaxone (30 µg/ml, 26 mm)
4	*Pseudomonas aeruginosa*	-	-	-	-	-	-	-	Ciprofloxcin (5 µg/ml, 21 mm)

No inhibition

The antibacterial activity of crude methanolic extract of red and yellow watermelon seeds against *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were presented in Table 3. The zone of inhibition produced by the crude methanolic extract of red watermelon seeds against *Streptococcus pyogenes* and *Staphylococcus aureus* were 7-20 mm and 7-10 mm, respectively in different concentrations. At the same time crude methanolic extract of red watermelon seeds do not showed zone of inhibition against *Escherichia coli* and *Pseudomonas aeruginosa*.

The methanolic extract of yellow watermelon seeds do not showed zone of inhibition against all the tested organisms. However, the standards showed better activity with lower concentration when compared to both the extracts against the entire organism.

The results indicate that the methonolic extract of red watermelon seeds showed antibacterial activity toward only in gram positive (*Streptococcus pyogenes* and *Streptococcus aureus)* and not in gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The highest antibacterial activity was recorded in methonolic extract of red watermelon seeds against *Streptococcus pyogenes* (20 mm).

These findings are supported by the reported results of earlier study (Poduria et al., 2013). The results of this study reflect that potent phytochemicals are present in the methanolic extract of red watermelon seeds than yellow watermelon seeds. The crude methanolic extract of red watermelon seeds was biologically active when compared to yellow watermelon seeds. In conclusion, these results showed that methanolic extract of red watermelon seeds are good candidate for further investigation against gram positive bacteria.

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