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Antimicrobial Effect of Grapefruit Crude Extracts on Selected Bacterial Isolates

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ABSTRACT

The antimicrobial activity of crude aqueous extracts (pulp and rind) of two varieties (white pulp and pink pulp) of grapefruit against five clinical bacterial isolates (*Escherichia coli*, *Staphylococcus aureus*, *Yersinia entercolitica*, *Salmonella* sp., *Streptococcus mutans* and *Klebsiella aerogenes*) was investigated *in vitro*. The pulp and rind were macerated mechanically and water-extracted, serially diluted to get 100, 50, 25, 12.5, 6.25, 3.125, and 1.563% concentrations, and the punch-hole method employed for the antimicrobial activity test using nutrient agar medium. Culture plates containing peptone water instead of extract served as control. Plates were incubated at 37°C for 24 hours and diameter of zone of inhibition measured. The inhibitory effect increased with concentration of extract. The pulp extracts had no effect on *Y. enterocolitica* and *S. mutans*. At 12.5% concentration, white pulp extract did not inhibit growth of *S. aureus*. Generally, diameters of zone of inhibition of each bacterium at same concentration of both white and pulp extracts were not significantly different except for *Salmonella* sp. and *K. aerogenes* ($P = 0.05$). Also, *E. coli*, *Salmonella* sp., and *K. aerogenes* were affected by the rind oil while others were not. At 6.25% concentration, pink rind but not the white, inhibited growth of *Salmonella* sp. Where applicable, there was no significant difference between minimum inhibitory concentration (MIC) of pulp and rind extracts, except for *Salmonella* sp. in which the pink variety rind had lower MIC (6.25%) as against 12.5% of pulp. Grapefruit pulp and rind extracts have antimicrobial potential that can be harnessed.

Keywords: activity, diameter of clear zone, growth inhibition, *in vitro*, pulp, rind

INTRODUCTION

There have been increased interests in the use of plants as sources of medicines due to their fewer, nonexistent or less severe side effects, and their relative abundance and therapeutic potency over orthodox medicines (Idu *et al.* 2007; Okwori *et al*. 2008). The continuous search for the medical application of plant parts informed this study.

The grapefruit, *Citrus paradisi* Macf. is a perennial plant of the family Rutaceae, closely related to orange, lime, lemon and tangerine, and believed to have originated from Jamaica (Wainwright 2004; Oreagba *et al.* 2008). The tree is deciduous with many varieties well-known all over the world. The fruit is large, round, with yellow peel and weighing approximately 250-700 g. Its pulp is very juicy and has a refreshing aromatic taste. It is a berry with an outer covering (exocarp), white mesocarp and an endocarp with 8-10 segments of juice sacs (vesicles). The fruit is eaten raw or is used as a jam, marmalade or jelly. It has an acidic taste due to the presence of the glycoside naringin; this makes it a strong antibacterial agent (Bailey *et al*. 1998). It is also a good source of vitamin C (Mahan and Escott-Stump 2000) therefore having an antioxidant property which makes it find use in healing of bites, injuries, dental caries, and enhancement of digestive and immune systems. Grapefruit interacts with some drugs such as cholesterol-reducing drugs (Bailey and Dresser 2004). In addition, the extract of pulp and seeds of grapefruit contains bioflavonoids, amino acids, samlarides, iron, magnesium, sodium, potassium and the B-group vitamins. The essential oil (extracted from the rind) is used as a fragrance component in soaps, detergents, cosmetics and perfumes. It is also extensively used in disserts, soft drinks and alcoholic beverages, as it has antidepressant, antiseptic, antitoxic, bactericidal, diuretic property.

There are documented evidences on the antimicrobial activity of plant parts (Oyedeji *et al.* 2005; Avato *et al.* 2006; Kalyoncu *et al*. 2006). Higher plants have been a source of antibiotics (Iwu *et al.* 1999; Omar *et al.* 2000).

MATERIALS AND METHODS

Preparation of materials

1. Isolation and identification of test organisms

Clinical bacterial isolates (*Escherichia coli*, *Staphylococcus aureus*, *Yersinia entercolitica*, *Salmonella* sp., *Streptococcus mutans* and *Klebsiella aerogenes*) were obtained from Lahor Public Health and Research Centre (www.lahorresearch.org) a standard research centre located in Benin City, Nigeria. Fresh sterile culture plates of the test bacteria were made from the stored cultures on McConkey Agar (Oxoid, England) at 37°C and sub-cultured for purity, and re identified using colony and microscopic characteristics (Okwori *et al.* 2007). The organisms were then transferred to Nutrient Agar (Biotec, UK) slants and stored at 4°C after incubation for 24 hrs.

Further identification of the bacteria was done using cultural morphology, Gram- stain, motility and biochemical tests (catalase, coagulase, indole, sugar fermentation, methyl red and Voges-Proskauer) following the methods of Buchanan and Gibbons (1994). For the tests, 24-hr old cultures prepared from the stored cultures by streaking on nutrient agar and incubating at 37°C were used.

2. Extraction of grapefruit juice

Grapefruit (var. 'Duncan', white pulp; var. 'Forster', pink pulp) were washed thoroughly under running tap and rinsed in several

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Research Note

Table 1 Diameter of zone of inhibition of bacteria isolates treated with grapefruit pulp extracts.

Bacteria species	Extract	Diameter of zone of inhibition (mm) ± SD at different concentrations						
		100	50	25	12.5	6.25	3.125	1.563
Escherichia coli	W	$15 \pm 0.003^{1}a$	$11 \pm 0.001^{1}a$	$10 \pm 0.000^{1}a$	$9 \pm 0.01^{1}a$	$0^{2}a$	$0^{2}a$	$0^{2}a$
	P	$11 \pm 0.001^{1}a$	$10 \pm 0.100^{1}a$	$9.5 \pm 0.00^{1}a$	$9 \pm 0.001^{1}a$	$0^{2}a$	$0^{2}a$	$0^{2}a$
Staphylococcus aureus	W	$6 \pm 0.002^{1}a$	$4 \pm 0.000^{1}b$	$2 \pm 0.020^{1}b$	$0^{2}b$	$0^{2}a$	$0^{2}a$	$0^{2}a$
	P	$8 \pm 0.001^{1}a$	$5 \pm 0.000^{1}b$	$4 \pm 0.000^{1}b$	$2 \pm 0.00^{2}b$	$0^{3}a$	$0^{3}a$	$0^{3}a$
Yersinia entercolitica	W	$0^{1}b$	$0^{1}c$	$0^{1}c$	$0^{1}b$	$0^{1}a$	$0^{1}a$	$0^{1}a$
	P	$0^{1}b$	$0^{1}c$	$0^{1}c$	$0^{1}b$	$0^{1}a$	$0^{1}a$	$0^{1}a$
Salmonella sp.	W	$0^{1}b$	$0^{1}c$	$0^{1}c$	$0^{1}b$	$0^{1}a$	$0^{1}a$	$0^{1}a$
	P	$25 \pm 0.000^{1}c$	$20 \pm 0.000^{1}d$	$10 \pm 0.000^{2}a$	$5 \pm 0.000^{2}a$	$0^{3}a$	$0^{3}a$	$0^{3}a$
Streptococcus mutans	W	$0^{1}b$	$0^{1}c$	$0^{1}c$	$0^{1}b$	$0^{1}a$	$0^{1}a$	$0^{1}a$
	P	$0^{1}b$	$0^{1}c$	$0^{1}c$	$0^{1}b$	$0^{1}a$	$0^{1}a$	$0^{1}a$
Klebsiella aerogenes	W	$0^{1}b$	$0^{1}c$	$0^{1}c$	$0^{1}b$	$0^{1}a$	$0^{1}a$	$0^{1}a$
	P	$9 \pm 0.001^{1}a$	$7 \pm 0.001^{1}a$	$6 \pm 0.001^{1}b$	$5.2 \pm 0.002^{1}a$	$5 \pm 0.001^{1}b$	$0^{2}a$	$0^{2}a$

W = white pulp extract; P = pink pulp extract; SD = standard deviation. Values are means of three replicates from two independent experiments. Values bearing different letters in the same column are significantly different from each other, while values with different numbers in superscript along the same row are significantly different from each other, using a student's *t*-test.

changes of sterile distilled water. Each fruit was cut in four equal sections with a sharp sterile kitchen knife. The rind was peeled off with the aid of the knife and the seeds removed. Then 50 g of the cut fruit was blended aseptically in 50 ml distilled water using an electrical blender (Mixer model 830 L, Hong Kong) after which the mixture was filtered with a Whatman No. 1 filter paper and the extract collected in sterile 10 ml universal bottles.

3. Extraction of grapefruit rind oil

10 ml of hot water (40°C) was added to 10 g of rind and macerated as before. The blended mixture was collected in 10-ml test tubes and allowed to stand for a few minutes. The mixture formed two layers. The upper layer which contained the rind oil was transferred to sterile 10 ml universal bottles with the aid of a sterile pipette.

4. Serial dilution of extracts

Sterile distilled water was used as diluent for both the pulp and rind extracts. The extracts were serially diluted to give 100, 50, 25, 12.5, 6.25, 3.125, and 1.563% concentrations of white pulp extract (WPE), white rind extract (WRE), pink pulp extract (PPE) and pink rind extract (PRE). The undiluted extracts represented 100% concentration.

Experiment to determine the antimicrobial activity of grapefruit extracts on bacteria

1. Preparation of culture plates

With the aid of a sterile wire loop, a few colonies of fresh cultures of each test organism were suspended in 5 ml nutrient broth in a 10-ml universal bottle. Then 1ml of this suspension was diluted serially with peptone water using the two-fold serial dilution method to get a standard inoculum of 1-2 × 10^{7} cfu/ml. Nutrient agar plates (three plates for each organism) were flooded with the standard inoculum of each organism. Excess culture suspension was poured away after ensuring uniform distribution. The culture plates were dried in an incubator at 37°C for 30 min.

2. Determination of antimicrobial activity

The punch-hole method (Stokes 1975) was used. Three equidistant holes were made in each dried culture plate using a sterile 10 mm cork borer. Then 0.1 ml sterile liquefied nutrient agar was poured into the holes to seal the floor so as to prevent contamination resulting from inter-well leakage. The three holes were inoculated aseptically with 2 ml of one concentration of an extract. There were three culture plates for each concentration and tests with each organism were duplicated. Culture plates containing peptone water only instead of extract served as control. All culture plates were incubated at 37°C for 24 hrs. The plates were examined for zone of inhibition and, where present, measured with a meter rule; two measurements, vertical and horizontal were taken for each plate and the mean diameter calculated (Adeleye and Opiah 2003; Junaid *et al*. 2006).

3. Determination of minimum inhibitory concentration (MIC)

A serial dilution of extract was performed according to the broth macro-dilution technique (Akinyemi *et al*. 2005). For each extract, 5 ml of nutrient broth was pipetted into a universal bottle containing 5 ml extract. Then 5 ml of this mixture was transferred into a fresh universal bottle containing 5 ml nutrient broth after mixing thoroughly. This dilution process continued to the seventh bottle, to get serially diluted extracts of 100, 50, 25, 12.5, 6.25, 3.125, 1.563% concentrations. The eighth bottle contained nutrient broth only and served as control. Plates flooded with the organisms and dried as earlier explained were punched (eight holes per plate) and filled with 0.2 ml volume of the different concentrations of the each extract and incubated at 37°C for 24 hrs. The MIC was read as the least concentration that inhibited the growth of the test organisms.

Statistical analysis of data

Culture plates were in triplicates and experiment for each treatment was done twice. Mean values for two experiments were therefore used for all treatments. Data were analyzed using student's *t*-test and level of significance determined at $P = 0.05$.

RESULTS AND DISCUSSION

Effects of grapefruit pulp extract on bacteria

Extracts of both grapefruit varieties had varied effects on the tested bacteria. The inhibitory effect increased with concentration of extract (**Table 1**). Both WPE and PPE had no effect on *Y. enterocolitica* and *S. mutans*. Whereas WPE had no effect on *Salmonella* sp., PPE inhibited growth of *Y. enterocolitica* and *S. mutans*. At a concentration of 12.5%, WPE did not inhibit growth of *S. aureus*. Generally, diameters of zone of inhibition of each bacterium at same concentration of both extracts (WPE and PPE) were not significantly different except for *Salmonella sp* and *K. aerogenes*. Whereas WPE was active against two organisms (*E. coli* and *S. aureus*) PPE was active against four (*E. coli*, *S. aureus*, *Salmonella* sp. and *K. aerogenes*).

Effects of grapefruit rind extract on bacteria

Three of the organisms (*E. coli*, *Salmonella* sp., and *K. aerogenes*) were affected by the rind oil while the others (*S. aureus*, *Y. enterocolitica* and *S. mutans*) were not affected (**Table 2**). The diameter of clear zone increased with increase in concentration. At a concentration of 6.25% PRE inhibited growth of *Salmonella* whereas WRE had no effect on this organism.

Table 2 Diameter of zone of inhibition of bacteria isolates treated with grapefruit rind extracts.

Bacteria sp	Extract	Diameter of zone of inhibition (mm) ± SD at different concentrations						
		100	50	25	12.5	6.25	3.125	1.563
Escherichia coli	W	30 ± 0.00^{1}a	24 ± 0.002^{1}a	15 ± 0.00^{2}a	10 ± 0.001^{2} a	0^{3}a	0^{3}a	0^{3}a
	P	39 ± 0.00^{1}b	33 ± 0.001^{1}b	24 ± 0.00^{2}a	17 ± 0.001^{2} b	0^{3}a	0^{3}a	0^{3}a
Staphylococcus aureus	W	0^{1}c	0^{1}c	0^{1}b	0^{1}c	0^{1}a	0^{1}a	0^{1}a
	P	0^{1}c	0^{1}c	0^{1}b	0^{1}c	0^{1}a	0^{1}a	0^{1}a
Yersinia entercolitica	W	0^{1}c	0^{1}c	0^{1}b	0^{1}c	0^{1}a	0^{1}a	0^{1}a
	P	0^{1}c	0^{1}c	0^{1}b	0^{1}c	0^{1}a	0^{1}a	0^{1}a
Salmonella sp.	W	20 ± 0.001^{1}d	16 ± 0.00^{1}d	12 ± 0.00^{2}a	8 ± 0.001^{2}a	0^{3}a	0^{3}a	0^{3}a
	P	28 ± 0.002^{1}a	23 ± 0.00^{1}a	17 ± 0.00^{2}a	11 ± 0.00^{3}a	4 ± 0.00^{4}b	0^{5}a	0^{5}a
Streptococcus mutans	W	0^{1}c	0^{1}c	0^{1}b	0^{1}c	0^{1}a	0^{1}a	0^{1}a
	P	0^{1}c	0^{1}c	0^{1}b	0^{1}c	0^{1}a	0^{1}a	0^{1}a
Klebsiella aerogenes	W	18 ± 0.00^{1}d	14 ± 0.00^{2}d	11 ± 0.001^{2}a	9 ± 0.00^{2}a	7 ± 0.00^{2}c	0^{3}a	0^{3}a
	P	26 ± 0.002^{1}a	19 ± 0.001^{2}a,d	14 ± 0.001^{3}a	10 ± 0.00^{3}a	8 ± 0.00^{3}c	0^{4}a	0^{4}a

W = white pulp extract; P = pink pulp extract; SD = standard deviation. Values are means of three replicates from two independent experiments. Values bearing different letters in the same column are significantly different from each other, while values with different numbers in superscript along the same row are significantly different from each other, using a student's *t*-test.

Table 3 Minimum inhibitory concentration of aqueous extracts of white and pink pulp grapefruits against bacterial isolates at 37°C and 24 h incubation.

Bacteria sp	Minimum Inhibitory Concentration of extracts (%)			
	WPE	WRE	PPE	PRE
Escherichia coli	12.5 ± 0.000^{1}a	12.5 ± 0.000^{1}a	12.5 ± 0.000^{1}a	12.5 ± 0.000^{1}a
Staphylococcus aureus	25 ± 0.000^{1}b	NA	12.5 ± 0.001^{2}a	NA
Yersinia entercolitica	NA	NA	NA	NA
Salmonella sp.	NA	12.5 ± 0.002^{1}a	12.5 ± 0.000^{1}a	26.5 ± 0.000^{2}b
Streptococcus mutans	NA	NA	NA	NA
Klebsiella aerogenes	NA	6.25 ± 0.001^{1}b	6.25 ± 0.000^{1}b	6.25 ± 0.001^{1}c

NA = not applicable; WPE = white pulp extract; WRE = white rind extract; PPE = pink pulp extract; PRE = pink rind extract. SD = standard deviation. Values are means of three replicates from two independent experiments. Values bearing different letters in the same column are significantly different from each other, while values with different numbers in superscript along the same row are significantly different from each other, using a student's *t*-test.

Minimum inhibitory concentration

Where applicable, there was no significant difference between minimum MIC of pulp and rind extracts, except for *Salmonella* sp. in which PRE had lower MIC (6.25%) as against 12.5% of pulp (**Table 3**).

Although the activity of several plant extracts has been tested using some of the organisms in our work (Omenka and Osuoha 2000; Kareem *et al.* 2008; Timothy *et al.* 2008), information on the antimicrobial effect of grapefruit extracts is scarce. Cvetni *et al.* (2004) reported earlier that grapefruit extract was active against bacteria, with *Salmonella* sp. having the highest growth inhibition (MIC 2.06%) among the tested bacteria. In our report, WPE did not inhibit growth of *Salmonella* but PPE did. The difference in MIC might be due to variety of fruit and species of *Salmonella* used. These workers also found that grapefruit pulp contains about 3.92% polyphenols and 0.11% flavonoids; these compounds no doubt contributed to the antibacterial activity of the extract. Also, Xu *et al.* (2007) reported that grapefruit seed extract inhibited growth of food isolates of *Salmonella* sp. Lime, a close ally of grapefruit, was reported to have inhibited growth of *S. aureus*, *Streptococcus* sp., *K. aerogenes*, *E. coli* and *Salmonella* sp. (Adeleye and Opiah 2003).

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