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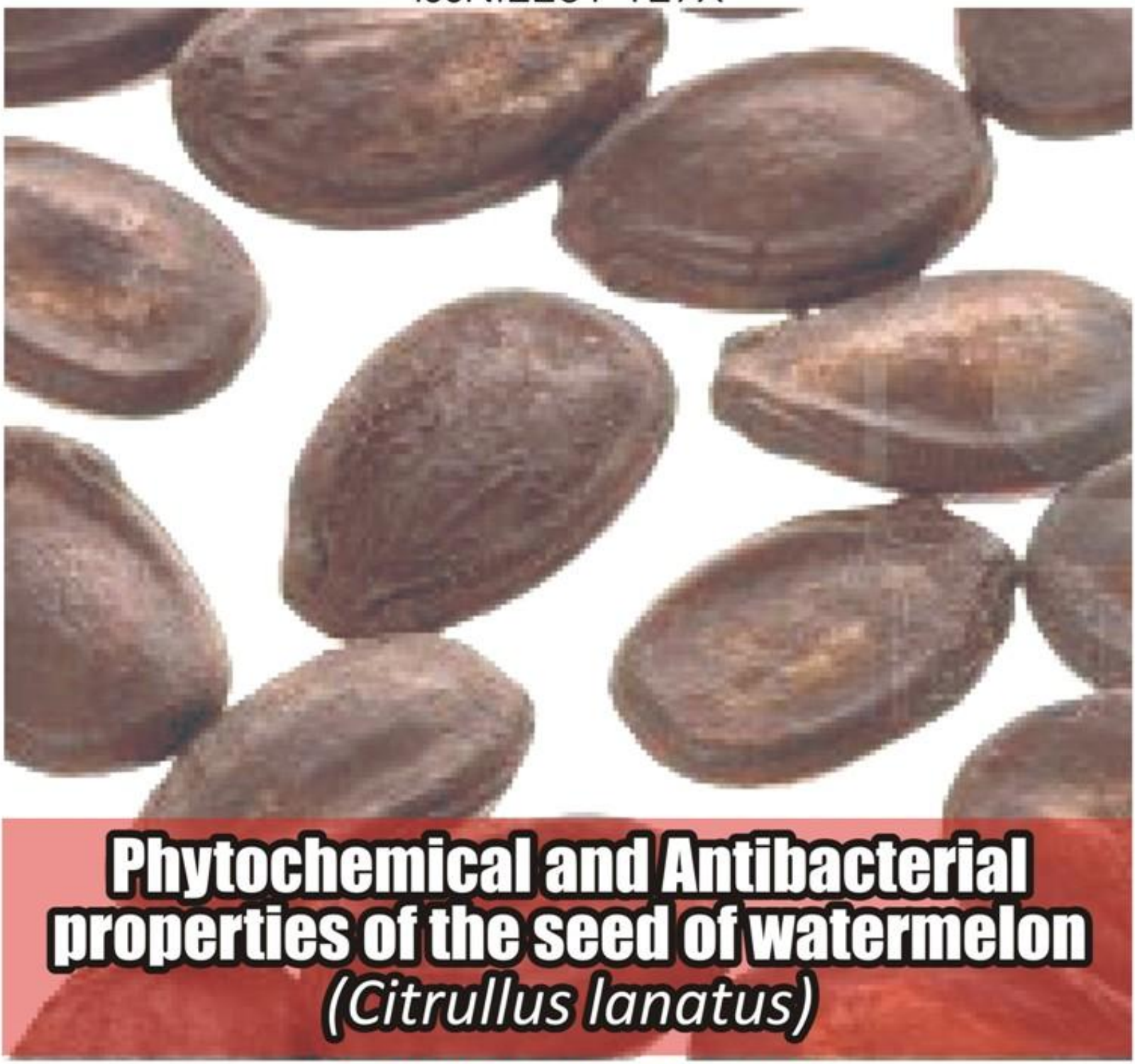

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Phytochemical and Antibacterial properties of the seed of watermelon (*Citrullus lanatus*)

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Watermelon seed was evaluated for its phytochemical and antimicrobial potentials. Crude extract of the seeds was obtained using hot water, cold water, ethanol and methanol. Test organisms were screened to confirm their viability and identities using standard microbiological methods. Extracts were tested for antimicrobial activity using the standard disc diffusion assay method against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumoniae* and *Bacillus cereus*. All the seed extracts showed evidence of antibacterial properties. Hot water extract showed the highest antimicrobial activity against *Pseudomonas aeruginosa* with 14mm diameter zone of inhibition whereas ethanol and methanol extracts showed the lowest against *Escherichia coli and Klebsiella pneumoniae* with 8mm diameter zone of inhibition. Watermelon seed showed low antimicrobial activity when compared to the result of the commercial antibiotics. The analysis for phytochemical constituents was performed using generally accepted laboratory techniques for quantitative determinations. The constituents analyzed for were tannins, saponins, flavonoids, cyanogenic glycosides, oxalates and alkaloids. Alkaloid had the highest concentration of about 1.23% whereas cyanogenic glycoside had the lowest of about 0.00237%. There was a correlation between the phytochemical levels and the antimicrobial activities. The low level of phytochemicals explains the low antimicrobial activities of extracts of watermelon seeds.

Keywords: watermelon, phytochemical, antimicrobial, bacteria.

INTRODUCTION

Watermelon seeds are a source of protein, B vitamins, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others (Vandermark, 2011; Collins *et al*., 2007).

The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Anderson *et al*., 2001; Hsueh *et al.,* 2005; Lin *et al.,* 2005; Mora *et al*., 2005; Navon-Venezia *et al*., 2005 ; Vattem *et al.,* 2004). Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylated hydroxytoluene (BHT) and butylated hydroxanisole (BHA) that are under scrutiny as suspected cancer causing agents (Bergfeld *et al*., 2005; Byford *et al.,* 2002; Sun *et al*., 2003 ;Wangensteen *et al*., 2004).

The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals (Ajayi *et al*., 2011). The most important of these bioactive constituents (phytochemicals) of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. These phytochemicals are antibiotic principles of plants (Ajayi *et al*., 2011). They are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques (Hafiza *et al*., 2002). These phytochemicals have been reported to exhibit hemolytic and foaming activity, antifungal, anti-inflammatory, fungistatic, and molluscidal (Ajayi *et al*., 2011).

There has been a renewed interest in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes (Wangensteen *et al*., 2004) with the recognition that plant-derived products have great potential as sources of pharmaceuticals (Borchardt *et al*., 2008).

Although leaves, roots, flowers, whole plants, and stems were examined for useful phytochemicals in many research projects, few reports refer to seeds as sources for pharmaceuticals (Borchardt *et al*., 2008). Even though a large number of chemical compounds are present in seeds or seed coats, including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids **(**Borchardt *et al.,* 2008), these compounds probably function in the protection of seeds from microbial degradation until conditions are favorable for germination (Cai *et al.,* 2004; Komutarin *et al.,* 2004).

Many studies suggest that endogenous antioxidants, or exogenous antioxidants supplied by diet, can function as free radical scavengers and improve human health (Connor *et al*., 2002; Mojzisova and Kuchta, 2001; Oktay *et al*., 2003; Parr and Bolwell, 2000). Thus, consumption of a variety of plant foods including watermelon seeds may provide additional health benefits. Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity (Cutter, 2000 and Hao *et al*., 1998).

This paper reports on the phytochemical properties of the seed of water melon. The antibacterial potential/activity of the seed extract was also tested against five bacterial pathogens. The efficacy of the extracts was compared to some conventional antibiotics.

MATERIALS AND METHODS

Collection and preparation of the seeds of watermelon

The watermelon seeds used for this study were extracted from fresh watermelon fruits (*Citrillus lanatus*) brought from the popular Relief market, Owerri. The seeds were washed and dried in a Uniscope laboratory oven maintained at 40^0C overnight, and stored in a dry place to avoid fungal growth. The seeds were grind with a Qlink laboratory blender and subjected to various extracting agents. Crude extracts of the seed was done by the methods adopted by Cheesbrough (2000). Filtrate of the extract was obtained by separation the suspension in a glass funnel and filter paper. Ethanol and methanol were allowed to evaporate and stored in an airtight conical flask. Hot and cold water extracts were neatly separated and also stored.

Screening of test organisms for viability

Stock cultures of *Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Staphyloccocus aureu*s and *Pseudomonas aeruginosa* were collected from the Microbiology unit of the Federal University of Technology Medical Center, Owerri, Imo State, Nigeria. The isolates were screened to confirm their identities and viability prior to use (Cheesbrough, 2000; Beishir, 1987).The bacteria were sub cultured on Nutrient agar and stored on slant before use.

Preparation of paper discs

Small circular high potency discs (6.25mm) in diameter made from Whatman No.1 grade filter paper with the aid of a mechanical perforator. Discs were sterilized in a glass Petri dish using the hot air oven at a temperature maintained at 160ºC for 1h (Cheesbrough, 2000; Harrigan and McCance, 1990).

Phytochemical analysis

The analyses for phytochemical constituents were performed using generally accepted laboratory techniques for quantitative determinations (AOAC, 1984). The constituents analyzed for were tannins, saponins, flavonoids, cyanogenic glycosides, oxalates and alkaloids.

Sensitivity test

Four extracts of the watermelon seed, including hot water, cold water, ethanol and methanol were tested for antibacterial activity using a disc diffusion assay method (Cruickshank *et al*., 1975; Carter and Chengappa, 1991; Cheesbrough, 2000) against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumoniae* and *Bacillus cereus* .

The diameter of the zone of inhibition near the respective discs was measured to the nearest millimeter. To compare the efficacy of the seed extracts to commercially available antibiotics, the test organisms were subjected to the routine laboratory susceptibility test against ten standard antibiotics such as Ciproflox, Erythromycin, Lincocin, Gentamycin, Ampiclox, Rifampin, Floxapen, Streptomycin, Norfloxacin, Chloramphenicol.

RESULTS

The colonial and cell morphologies of the bacteria pure cultures obtained from a research and diagnostic laboratory is shown in Table 1. The identities of the pure cultures were further confirmed with few biochemical tests as shown in Table 2. The features of the test organisms were compared with those in standard manual (Buchanan and Gibbon, 1974; Sneath *et al*., 1986; Carter and Chengappa, 1991).

Table 3 shows the result of the phytochemical analysis of watermelon seeds. The study indicated that saponins,

Table 1: Colonial and cell morphology of bacteria isolated from wounds

Colonial characteristics	Grams morphology	Capsule	Spore	Motility	Flagellum	Probable identity
Smooth circular and golden yellow colonies	Gram positive oval cells in clusters	-	-	-	-	*Staphylococcus* sp
Smooth and shiny colonies with green pigments	Gram negative small short single rods	-	-	+	+	*Pseudomonas* sp
Smooth moist shiny colonies	Gram negative small short single rods	-	-	+	+	*Escherichia coli*
Moist and mucoid raised creamy colonies	Gram negative large rods in short chains	+	-	-	-	*Klebsiella* sp
dull and dry serrated cream colonies	Gram positive beaded short swollen rods	-	+	+	+	*Bacillus* sp

Table 2: Biochemical characteristics of bacteria isolated from wounds

Sugar Fermentation															Identity of isolate
Cat	Oxi	Coag	In	MR	VP	Cit	Urease	NO_3	H_2S	Glu	Suc	Lac	Mn	Xyl	
+	-	+	-	+	-	-	-	+	+	+	+	+	+	-	*Staphylococcus aureus*
+	+	+	-	+	-	+	+	+	+	+	-	-	+	+	*Pseudomonas aeruginosa*
+	-	-	+	+	-	-	-	+	-	+	-	+	+	+	*Escherichia coli*
+	-	-	-	-	+	+	$+^s$	-	-	+	+	+	+	+	*Klebsiella pneumoniae*
+	-	-	-	-	+	+	-	+	+	+	-	$+^s$	-	-	*Bacillus cereus*

Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, methyl red; VP, voges proskaeur; Cit, citrate; NO_3, nitrate reduction; H_2S, hydrogen sulphide; Glu, glucose; Suc, sucrose; Lac, lactose; Mn, mannitol; Xyl, xylose; s, slow reaction

Table 3: Phytochemical analysis of watermelon seed

Constituents	(%)
Saponin	0.720
Alkaloid	1.23
Cyanogenic Glycoside	0.00237
Flavonoid	0.97
Oxalate	0.0275
Tannin	0.035

flavonoids, cyanogenic glycosides, alkaloids, tannins and oxalate were present in watermelon seeds in varying quantities. Alkaloid had the highest concentration of about 1.23% whereas cyanogenic glycoside had the lowest of about 0.00237%.

Table 4 shows the evaluation of watermelon seeds for its antimicrobial potentials. Four extracts of the seeds, including hot water, cold water, ethanol and methanol which were tested for antimicrobial activity using a disc diffusion assay method against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumoniae* and *Bacillus cereus* . Hot water extract showed the highest antimicrobial activity against *Pseudomonas aeruginosa* with 14mm diameter zone of inhibition whereas ethanol and methanol extracts showed the lowest against *Escherichia coli and Klebsiella pneumoniae* with 8mm diameter zone of inhibition. Table 5 shows the susceptibility pattern of the test organisms to ten commercially available antibiotics.

DISCUSSION

The viability and identities of the test organisms were confirmed (Tables 1 and 2) with reference to standard laboratory manuals (Buchannan and Gibbon, 1974, Sneath *et al*., 1986; Carter and Chengappa, 1991). Six antioxidant are present in the watermelon seed analysed (Table 3). The phytochemical screening revealed the presence of saponin (0.720%), alkaloid (1.23%), cyanogenic glycoside (0.00237%), flavonoid (0.97%), oxalate (0.027%) and tannin (0.035%) in a low concentration. The antimicrobial activities of these photochemical compounds had been reported (Okorondu *et al*., 2006; Nwaoguikpe *et al*., 2008; Okorondu *et al*., 2010; Ajayi *et al*., 2011; Nwaoguikpe *et al*., 2011). Cutter (2000) and Hao *et al*. (1998) had also reported on the antimicrobial potentials of antioxidants present in plants and plant extracts. The four seed extracts showed evidence of ability to resist the growth of the test organisms. The water extracts presents better response to the antibacterial activities than the ethanol and

Table 4: Microorganisms and their zone of inhibition using watermelon seed extracts

Organism	Diameter of zone of inhibition (mm)			
	HWE	CWE	ETE	MTE
Klebsiella pneumoniae	12	10	8	8
Pseudomonas aeruginosa	14	10	10	11
Escherichia coli	10	10	9	8
Bacillus cereus	10	9	9	9
Staphylococcus aureus	11	10	9	9

HWE, hot water extract; CWE, cold water extract; ETE, ethanol extract; MTE, methanol extract

Table 5: Microorganisms and their zone of inhibition for commercial antibiotics

Organism	Diameter of zone of inhibition (mm)									
	CPX	E	LC	GN	APX	RD	FLX	S	NB	CH
Klebsiella pneumonia	-	-	-	11	-	-	-	11	-	-
Pseudomonas aeruginosa	27	17	19	17	11	13	15	13	23	21
Escherichia coli	23	19	23	19	17	15	13	13	17	13
Bacillus cereus	23	17	17	13	13	17	17	15	13	15
Staphylococcus aureus	25	15	13	19	19	13	17	27	15	17

CPX, Ciproflox (10mcg); E, Erythromycin (30mcg); LC, Lincocin (30mcg); GN, Gentamycin (10mcg); APX, Ampiclox (30mcg); RD, Rifampin (10mcg); FLX, Floxapen (30mcg); S, Streptomycin (30mcg); NB, Norfloxacin (30mcg); CH,Chloramphenicol (20mcg)

methanol extracts (Table 4). The low antimicrobial activities of methanol and ethanol extracts may be due to their ability to dissolve fat during extraction. Essien *et al*. (2009) reported that Watermelon seed contains a lot of fat (about 40%).

Klebsiella pneumonia and *Pseudomonas aeruginosa* are susceptible to all the extracts. The antimicrobial activities of the extracts were also compared with that of the commercial antibiotics (Table 4). Watermelon seed extracts exhibited very low antimicrobial activity when compared with the result of the commercial antibiotics (Tables 4 and 5). The low concentration of the phytochemicals present in the seeds may account for this poor performance. *Klebsiella pneumonia* showed high resistance to eighty percent of the commercial antibiotics. The multiple determinant factors such enzymes, capsules etc could account for this erratic behavior (Perry and Staley, 1997; Prescott *et al.*, 1999; Prescott *et al*., 2002). Others test organisms were susceptible to the antibiotics and presents varying zone of inhibitions (Table 4).

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