CHEMICAL COMPOSITION, NUTRITIONAL VALUES AND ANTIBACTERIAL ACTIVITIES OF WATER MELON SEED (*Citrullus lanatus)*

ABSTRACT

Plants are traditionally used for treatment of bacterial infections though they are not clinically regulated due to lack of awareness and enough data to support the reported therapeutic claims. The aim of this study was to investigate chemical composition, nutritional evaluation and antibacterial activities of water melon seeds and ginger. The qualitative analysis of watermelon indicated that alkanoids were moderately present, tannin, saponins, flavonoids, and phenols were all present. The qualitative analysis for water melon indicated 3.080mg/g for alkanoids, 0.304mg/g for phenols,0.117mg/g for tannis,0.200mg/g for saponins and 2.675mg/g for flavonoids. The vitamin composition of watermelon seeds indicated 0.030mg/100g for vitaminB_1, 0.00055 vitaminB_2, 0.6405 mg/100g for vitaminB_3, 0.0235mg /100g vitaminB_6 and 0.001 for vitaminB_{12}.The bioactivities of extracts were tested, individually and as blends, against *proteus ,Bacillus cereus ,Staphylococcus aureus, Escherichia coli ,Necropsobacter rosorum ,Tsukamurella hongkongensis, Lactobacillus* sp*, Staphylococcus petrasii, Neisseria sicca, Dietzi amaris ,Pseudomonas oryzyhabitans, Klebsiella pneumoniae, Advenella incenata, Neiserria subflava, Serriatia marcescens.*

Keywords: Plant, Vitamin, Qualitative, Quantitative, Extracts

INTRODUCTION

Many plant extracts have been shown to possess antimicrobial properties active against microorganisms *in vitro*. The scientific name of Ginger which is *Zingiber officinale* was given by the English botanist, William Roscoe (1753-1831) in an 1807 publication. As the rhizome of *Zingiber officinale* Rosc., a perennial herb, of family Zingiberaceae, probably native to Southeastern Asia, it is produced everywhere and picked and dug in Autumn and Winter. The fresh rhizome, green ginger, is used in cooking. The peeled rhizomes may be preserved by boiling in syrup. Elsewhere, slices of ginger are eaten between dishes or courses to clear the palate. Ginger is used medically to treat flatulence and colic. Their colour varies from dark yellow through light brown to pale buff (Braide *et al.*, 2012). In China, unlike the West, traditional medicine, which is herbalism, never fell out of favour. There, herbal medicines are fine and sophisticated while others use the whole ginger root (Tiwari, 2011). The Watermelon is a large annual plant with long, weak, trailing or climbing stems which are five-angled (five-sided) and up to 3 m (10 ft) long. The Water Melon is a flowering plant thought to have originated in Southern Africa, where it is found growing wild. It reaches maximum genetic diversity there, with sweet, bland and bitter forms. In the 19th century, Alphonse de Candolle considered the watermelon to be indigenous to tropical Africa (Keter and Mutiso, 2012). *Citrullus colocynthis* is often considered to be a wild ancestor of the watermelon and is now found native in north and West Africa. However, it has been suggested on the basis of chloroplast DNA investigations that the cultivated and wild watermelon diverged independently from a common ancestor, possibly *C. ecirrhosus* from Namibia. Evidence of its cultivation in the Nile Valley has been found from the second millennium BC onward. Watermelon seeds have been found at Twelfth Dynasty sites and in the tomb of Pharaoh Tutankhamun (Ahmed *et al.*, 2001).

Watermelon seeds are easily available during summer season. Though it is not an oilseed but many researchers have reported that *C. vulgaris* seed kernels contain about 52.6% oil, so these are good source of energy (628k.cal) (Gopalan *et al.*, 1971).

SCIENTIFIC HYPOTHESIS OF THIS RESEARCH

- Can water melon seeds contain reasonable proximate components
- Can water melon seeds contain some amount of Alkaloids and Flavonoids
- Can water melon seeds using methanol solvent for extraction has an antibacterial effect on some bacterial isolates.

MATERIALS AND METHODS

Sample collection

Water Melon (*Citrullus lanatus*) seeds used for this study were obtained from fresh retailed fruit sellers from Oja-Oba Market, Ado Ekiti, Ekiti State.

Preparation of Extract

The extraction of Water Melon was achieved using a rotary evaporator (RE -52 A Union Laboratories, England), was carried out separately using methanol as solvent. The Water Melon was also washed and cut into slices to remove the seeds and it was also air dried for 30 days. Water Melon seeds were ground to fine powder separately, with the use of an electric blender (Thomas Wiley machine, model 5 USA). The sample was weighed 100g and soaked in 400ml of methanol separately in a conical flask. The mixture in the flasks was kept at room temperature for 24 hours with intermittent agitation and the sample was filtered into a beaker. The filtrate of the sample was concentrated to dryness by evaporation at 45^0C in a water bath for 3days.

Phytochemical Analysis

Phytochemical analysis of the extracts was carried out qualitatively using accepted laboratory techniques as described by (Nagalingam *et al.*, 2012).Tests on the presence of alkaloid, flavonoids, glycosides, saponins and tannin were conducted accordingly.

Qualitative analysis

Test for Saponins

The test was carried with froth test method.1 gram of each sample were weighed into a separate conical flask, which contained 10ml of distilled water and was boiled for 5 minutes. The two mixtures were filtered and 2.5 ml of the filtrate were added to 10ml of sterile distilled water in a test tube. The test tube was subjected to shaken vigorously for about 30 seconds and was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins (Nagalingam *et al*., 2012).

Test for Flavanoids

Five millilitre of dilute ammonia solution were added to a portion of the aqueous filtrates of the sample followed by addition of concentrated H_2SO_4. Formation of yellow colour observed in each sample indicated the presence of flavonoids (Nagalingam *et al.,* 2012).

Test for Tannins

The sample was mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of Tannins.

Test for Alkaloids

Two drops of Mayers's reagent were added along the side of test tubes containing few ml of both samples. Appearance of white creamy precipitate indicates the presence of alkaloids.

Test for Phenol

The test was carried out using Ferric chloride test to check for the presence of phenolic compounds. 50(mg) of each samples were dissolved in 5ml of distilled water. Few drops of neutral 5% Ferric chloride solution was added both mixtures. Presence of phenolic compound indicates a dark green colour (Nagalingam et *et al*., 2012).

Screening of Test Organisms

Bacterial isolates obtained which were obtained from Sputum, Air and patients with peridonatal disease and preserved on Nutrient agar (Sigma- Aldrich product) slant in the microbiology laboratory of the Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria. The isolates include *Esherichia coli, Staphylococcus aureus, proteus mirabilis, Bacillus cereus, Necropsobacter rosorum, Tsukamurella hongkongensis, Lactobacillus* sp, *Staphylococcus petrasii, Neisseria sicca, Dietzia maris, Pseudomonas oryzyhabitans, Advenella incenata, Neiserria subflava* and *Serriatia marcescens.* The stock cultures were inoculated into freshly prepared nutrient agar to test for their viability. They were stored at controlled temperature for subsequent antibacterial testing. Further subculturing was carried out until pure cultures of the isolates were obtained.

Antibacterial screening

The Antibacterial susceptibility test was performed by agar-well diffusion method using Mueller-Hinton agar (Sigma- Aldrich product). The test was performed under sterile condition. The Mueller-Hinton agar was inoculated separately with a suspension of each test bacterial strain culture evenly spread with the use of a sterile swab sticks on the entire surface of each sterile surface of sterile Petri-dishes. The agar was carefully punched using a cork-borer of 5mm in diameter and the extracts were dispensed into the wells at different concentrations in the wells of the agar seeded with bacterial isolates. The positive antibacterial activities were established by the presence of measurable zones of inhibition after 24 hours of incubation.

Antibiotics Susceptibility Testing

This test was carried out in order to determine whether or not an etiological agent is sufficiently sensitive to a particular antimicrobial agent to permit its use for treatment. The test was carried out with discs containing known concentrations of the antibiotics to be used for both Gram positive and Gram negative bacteria. The test was carried out by making an even spread of the pure isolates on prepared Meuller-Hinton agar using sterile swab sticks and aseptic placement of the particular disc meant for Gram positive and negative bacteria. The plates containing the discs were then incubated at 37^0C for 24

hours. The plates are evaluated based on the sizes of the zones of inhibition of each of the antimicrobial agents.

RESULTS

Proximate Composition of Water Melon Seeds

The proximate composition of watermelon seeds were carried out (Table 1). There was relatively high moisture content of the sample, but water melon seeds had high percentage of moisture content of 94.50% (Table 1). The amount of carbohydrate content in watermelon seeds were 62.22%. The protein content was 15.490%. The ash content for water melon seed is 64.60%. The fat content of watermelon seed is 11% and lastly, the crude fibre content was 8.50% in water melon seeds.

Proximate composition of Water melon seeds

Parameters	**Water melon seeds(%)**
Moisture	9.45±0.01
Ash	6.46±0.10
Protein	15.49±0.09
Crude fibre	8.50±0.002
Fat	1.10±0.001
Carbohydrate	59.03±1.20

(mean±SD; n=3)

Phytochemical Composition of Water Melon Seeds

Phytochemical composition was carried out on water melon seeds to check for the presence of phytochemical constituents which included alkaloids, phenols. saponins, flavonoids, tannin (Table 2 and table 3) and the result on the qualitative composition of water Melon seeds showed that alkanoids were moderately present, tannin, saponins, flavonoids, and phenols were all present. The quantitative analysis indicated 5.640mg/g for alkaloids, 0.015mg/g in phenols, NP (not present) for tannin, 0.3000mg/g in saponins and NP (not present) in flavonoids. The quantitative analysis for water melon seeds indicated

3.080mg/g of alkaloids, 0.304mg/g of phenols, 0.117mg/g of tannin, 0.200mg/g of saponins and 2.675mg/g of flavonoids.

Table 2: Phytochemical Composition of Water Melon Seeds

Parameters (mg/g)	Water melon seeds
Alkaloids	++
Phenols	+
Tannis	-
Saponins	+
Flavonoids	-

KEYS: ++: Moderately present

+: Present

-: Absent

Table 3: Quantitative Composition of Water Melon Seeds

Parameters (mg/g)	Watermelon seeds
Alkaloids	3.080±0.02
Phenols	0.304±0.001
Tannins	0.147±0.005
Saponins	0.200±0.003
Flavonoids	2.675±0.01

(mean±SD; n=3)

Vitamin composition

The vitamin composition of watermelon seeds indicated 0.03mg/100g for vitamin B1, 0.000055 for vitamin B2, 0.6405mg/100g for vitamin B3, 0.0235mg/100g for vitamin B6 and 0.001 for vitamin B12.

Table 4: Vitamin composition in water melon seeds

Sample	**Vitamin B_1 (mg/100g)**	**Vitamin B_2 (mg/100g)**	**VitaminB_3 (mg/100g)**	**VitaminB_6 (mg/100g)**	**VitaminB_{12} (mg/100g)**
Watermelon seeds	0.030 0.030=0.03	0.001 0.0001=0.000055	0.644 0.637=0.6405	0.025 0.022=0.0235	0.001 0.001=0.001

Antibacterial Effect

Antibacterial effect of methanol extract of watermelon seeds were carried out on the bacterial isolates (Table 5). The results indicated zones of inhibition on some organism according to certain concentration while some didn't show zones of inhibition i.e. the results showed different sensitivity of both extract on different organisms.

Table 5: Antibacterial effect of Methanol Extract of Water Melon Seeds on some selected Bacterial Isolates

S/N	ORGANISM	300(mg/ml)	200(mg/ml)	100(mg/ml)	Control
1	*Lactobacillus sp*	12	0	0	0
2	*Klebsiella pnuemoniae*	0	0	0	0
3	*Neisseria subflava*	0	18	0	0
4	*Necropsobacter rosorum*	0	13	14	0
5	*Pseudomonas oryzihabitans*	16	0	0	0
6	*Neisseria sicca*	0	17	0	0
7	*Tsukmurella hongkongenis*	0	0	0	0
8	*Proteus mirabilis*	0	0	0	0
9	*Advenella incenata*	0	0	0	0
10	*Staphylococcus petrasii*	0	0	0	0
11	*Staphylococcus aureus*	0	0	0	0
12	*Neisseria sicca*	0	0	0	0
13	*Proteus*	0	0	0	0

DISCUSSION

In the present study, results showed that watermelon seeds contain phytochemicals including

alkaloids, flavonoids, phenols, and tannins. The role of these phytochemicals as antimicrobial has been reported by many researchers (Mahdi *et al.*, 2010; Godwin *et al.*, 2015; Wangensteen *et al.*, 2004).

Their presence in watermelon seeds were also reported by many authors (Mahdi *et al.*, 2010; Mollenbeck *et al.*, 1997; Okorondu *et al.*, 2010). The presence of saponins in this study was in contrast to the works reported by (Ahmed *et al.*, 2001; Palmer *et al.*, 2009). But corresponds to that of Ajaib *et al.*, 2010 and Borchardt *et al.*, 2008. The disparity observed might be attributed to differences in geographical location, factors like climate, soil and propagation method. Presence of these phytochemicals in the extract was a clear indication of antimicrobial potentials of the watermelon seeds extract.

Antibacterial activity of the extracts showed that not all the organisms tested were susceptible. The methanol extract of water melon seeds which produced only an effect on *Neisseria subflava* Comparatively, different researchers have reported contradictory observations in this regards (Braide, *et al.*2010); Rahman, 2014; Saxena *et al.*, 2013); observed that water extract presents better response to the antibacterial activities than the methanol whereas (Braide *et al.*, 2012) reported the contrary. This contradiction might be a function of methodological differences and strain variability. This organism has been reported to be resistant to the watermelon seeds extract at concentrations lower than 125mg/ml (Hassan *et al.*, 2011).

CONCLUSION

Indiscriminate use of antimicrobial drugs has created very dangerous drug resistance to microbial strains; many bacterial strains have developed resistance against antibiotics, such as penicillin resistant *Streptococcus pnemoniae*, methicillin resistant *Staphylococcus aureus*.

However previous records showed that even new families of synthetic antimicrobial agent will have short life expectancy. Researchers are advised to turn their attention towards plants products, which is most

promising area in search of new biologically activity compounds with better activity against multi drug resistant strains and reduced antibiotic related side effects (Teoh *et al.,* 2012).

The activity observed is a clear indication of therapeutic property possessed by the plant just like other parts of the plant. Therefore, the extract of watermelon seeds could be a good source of antimicrobial agents and thus, should be harnessed.

RECOMMENDATIONS

- Different governmental or private sectors that are interested in agro industry area recommend participating in this profitable area. If this effort should be done, it would have significant positive effect on the diversification of the Gross Domestic Product (GDP) of Nigeria as well as the poverty and unemployment reduction. Researchers should investigate further on the production of water melon seeds in our country and the benefits obtained from the product have to analyze deeply to encourage the pharmaceutical industries and other investors locally.
- It is also important that more species of pathogenic bacteria be tested in order to ascertain the spectra of activities of the antimicrobial substances present in watermelon seeds.

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