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

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ABSTRACT

The World Health Organization (WHO) defines traditional medicine as the sum total of the knowledge, skills, and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Plants are traditionally used for treatment of bacterial infections. The aim of this study was to investigate chemical composition, nutritional evaluation and antibacterial activities of watermelon seeds. The qualitative phytochemical analysis of watermelon indicated that alkaloids were moderately present, tannins, saponins, flavonoids, and phenols were all present. The quantitative analysis of watermelon indicated 3.080mg/g for alkaloids, 0.304mg/g for phenols, 0.117mg/g for tannins, 0.200mg/g for saponins and 2.675mg/g for flavonoids. The vitamin composition of watermelon seeds indicated 0.03mg/100g for vitaminB_1, 0.01 vitaminB_2, 0.64 mg/100g for vitaminB_3, 0.24mg /100g vitaminB_6 and 0.01 for vitaminB_{12}.The bioactivities of extract were tested, against *Proteus mirabilis, 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* and *Serriatia marcescens.* 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.

Keywords: Seeds, watermelon, antimicrobial activity, extract.

INTRODUCTION

Many plant extracts have been shown to possess antimicrobial properties active against microorganisms *in vitro*. 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 Watermelon 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 a good source of energy (628k.cal) (Gopalan *et al.,* 1971).

MATERIALS AND METHODS

Sample collection

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

Preparation of Extract

The extraction of Watermelon was achieved using a rotary evaporator (RE -52 A Union Laboratories, England), was carried out separately using methanol as solvent. The Watermelon was also washed and cut into slices to remove the seeds and it was also air dried for 30 days. Watermelon 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.

Proximate analysis of the plant extracts

Moisture content

A clean and well labeled dish was weighed and oven dried (W_1), enough sample of the plant parts were added into the dish and weighed (W_2), the dish was transferred and with the content to the thermo setting oven at about 105^{o}C for about 24 hours. The dish was moved from oven to desiccators and was allowed to cool for about one hour and weighed. Step 4 was repeated to get constant W_3.

$$\% \text{ Moisture} = \frac{\text{Loss in weight}}{\text{Weight of sample before drying}} \times 100$$

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

$$\% \text{ Total solid or \% dry matter} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

W_1= weight of empty dish

W_2= weight of dish + sample

W_3= weight of dish + dried sample

Ash content

Silica dish or crucible was placed in muffle furnace for about 15 minutes at 350°C, it was removed and cooled in a desiccator for about one hour, the crucible was weighed as (W1), enough sample was added into the crucible (0.5 - 2g) and content was weighed as (W_2), then the crucible was placed inside the muffle furnace and slowly increase the temperature from 200^{o}C – 450^{o}C to avoid incomplete ashing. The sample was ashed until it become whitish in colour. Later it was removed and cooled to moisten with few drops of distilled water and dried on water bath which was returned to the furnace. The furnaces were removed to desiccator and allow cooling to room temperature. The crucible and the content were reweighed as (W_3).

Calculation:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

% organic matter = 100 - % Ash.

Protein content

There are 3 stages involved in the process

Digestion

Digestion converts nitrogen in food to ammonia.

About lg of the sample was weighed into kjedahl flasks in duplicate, L – Tyrosine were weighed into another kjedahl flask as blank for standard. Copper sulfate and potassium sulphate mixed in ratio 10:150 was added as catalyst to the samples. ($CuS0_4$ – $5H_20$ and K_2S0_4). Little anti bombing granules was added to the mixtures to prevent bombing of the flask by the acid and

10mls of concentrated H_2SO_4 acid was added to digest the samples and the digestion flasks were placed into the digester at 400^0c for some time until the samples were clear.

Distillation

NaOH converts Ammonium sulphate into Ammonia gas

$(NH_3)_2SO_4 + 2NaOH \text{-------} > 2NH_3 + 2H_2O + Na_2SO_4$

The low PH of boric acid converts Ammonia gas to Ammonium ion and boric to borate ion.

$NH_3 + H_3BO_3 \text{----------} > NH_4 + H_2BO_3^-$

Fifteen mls of Boric acid (H_3Bo_3) was placed in a 200ml conical flask to serve as the receiving flask, to trap the ammonia vapor from the digest and 2 drops of mixed indicator (methylene + methyl red) was added to the boric acid. Ten mls of digested sample was added to 10ml of distil water into a digestion flask. The conical flask and the kjeldahi flask was placed into the Nitrogen/protein determinator, NaOH was added to the kjeldahi flask till color changed. The sample was steamed and distilled into the conical flask.

Titration

The distillate was titrated with an acid of known concentration e.g. 0.05MHcl

CALCULATION

$$\textbf{\% NITROGEN} = \frac{(V_2 - V_1) \times C \times 0.0140 \times V \times 100}{W \times V^1}$$

V_1 – Volume of blank, V_2 –Volume of sample, C -Concentration of acid, V -Total Volume of digested Solution (ml),W -Weight of sample, V^1 - Volume of distillation (ml)

CRUDE PROTEIN = % Nitrogen × Conversion factor.

Crude fibre

Two grams of the dried sample was weighed and placed in the flask, 200 ml of boiling sulphuric acid solution was added. The extraction was boiled with petroleum ether for 1hour with 200ml of 1.25% of sodium hydroxide solution and was filtered through a filter paper and washed with distilled water. The residue was transferred into a crucible and oven dried at 105^0C, cooled in a desicator and weighed as W_2. The crucible were placed in a muffle furnace at about 500^0C for about 30minutes and cooled inside a desiccator and weighed as W_3

$$\% \text{ of crude fiber} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where W_1 = weight of empty crucible

W_2= weight of the crucible and the feed sample

W_3= weight of the crucible and ashed sample

Crude fat

Two grams of the sample was weighed into 100ml beaker and 2ml of ethanol was added to fill the round bottom flask up to 2/3 of the mark with petroleum ether. The soxhlet extractor was fixed up with a reflux condenser and was heated with heating mantle at temperature of 40-60^{0}C for 1 hour and remove. Then it was extracted with 30ml of diethylether to remove the solvent by evaporating on a water bath and later transferred to the beaker, the residue was placed inside the oven and dried for about 1hour

Weighed the residue as fat

Calculation for fat content

$$\% \text{ of fat} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100$$

Phytochemical Analysis

Phytochemical analysis of the extracts were 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 alkaloids

Five grams of each plant extracts were mixed with 5ml of 1% (v/v) aqueous hydrochloric acid on a steam bath. One millilitre of the filtrate was treated with few drops of Draggendoff's reagent. Blue-black turbidity serves as preliminary evidence of alkaloids presence.

Test for saponins

Five grams of each plant extracts was shaken with distilled water (5 ml) in a test tube. Frothing which persists on warming was taken as preliminary evidence of the presence of saponins.

Test for tannins

Five grams of each plant extracts was added to 100 ml distilled water, stirred and filtered through Whatman No 1 filter paper. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins (Trease and Evans, 1989).

Test for flavonoids

Presence of flavonoids in the plant extracts was tested by $FeCl_3$ and lead ethanoate solutions. A green-blue or violet coloration on addition of $FeCl_3$ solution and appearance of buffcoloured precipitate on addition of lead ethanoate solution indicated the presence of flavonoids in the extract (Kokate, 1994).

Test for phenols

One millilitre of each plant extract was mixed with 4 drops of ethanol 100% (v/v) and 3 drops of 1% ferric chloride solution in test tube. Formation of green or red-brown indicates the presence of phenols.

QUANTITATIVE ANALYSIS

Determination of tannins

Fine powdered ground plant sample (0.2 g) was weighed into a 500 ml sample bottle. Then, 100 ml of 70% (v/v) aqueous acetone was added and properly covered with a stopper. The bottles were kept in water bath with shaker and shaken for 2 hours at 30 ^{0}C. Each solution was then centrifuged at 1600 rpm for 5 minutes and the sediment was stored on ice. Each solution 0.2 ml) was pipetted into test tube and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml of the stock and the solution was made up to 1 ml with distilled water. Folin-ciocateau reagent (0.5 ml) was added to both the sample and the standard tannic acid solution followed by the addition of 2.5 ml of 20% (v/v) Na_2CO_3. The solutions were then shaken vigorously and allowed to incubate for 40 minutes at room temperature (28 ± 2 ^{0}C. The absorbance was read at 725 nm against a standard tannic acid curve.

Determination of saponin concentration

Spectrophotometric method of Obadoni and Ochuko, (2000) was used. Two grams of each plant extract were weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol (100% v/v) was added. The mixture was shaken in a shaker water bath for 5 hours to ensure homogeneous mixture. The mixture was filtered through No 1 Whatman filter paper into 100

ml beaker containing 20 ml of 40% (v/v) saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was again filtered with No 1 Whatman filter paper to obtain a clean colourless solution. One millilitre of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% ferric chloride ($FeCl_3$) solution was added. The mixed solution was made up to the mark of 50 ml with distilled water. It was allowed to stand for 30 minutes for colour (light brown) development. The absorbance was read against blank at 380 nm.

Determination of the amount of alkaloids

Five grams plant extract was weighed into a 250 ml beaker and 200 ml of 10% (v/v) acetic acid in 200 ml of 100% (v/v) ethanol was added and allowed to stand for 4 minutes. It was filtered through Whatman's No. 1 filter paper and the extract was concentrated on a water bath (50^oC) for 4 hours to one quarter of the original volume. A solute ammonium hydroxide (10 ml) was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute (2 M) ammonium hydroxide and then filtered. The residue was then considered as alkaloid which was dried and weighed (Harbone and Williams, 2000). The formula written below was used to calculate concentration of alkaloid in percentage

$$\text{Alkaloid (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

Screening of Test Organisms

Bacterial isolates 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. The extracts were dispensed into the wells of the agar seeded with bacterial isolates at different concentrations. 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 Watermelon Seeds

The proximate composition of watermelon seeds were carried out (Table 1). There was relatively high moisture content of the sample, but watermelon 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 15.49%. The ash content for watermelon seed was 64.60%. The fat content of watermelon seed is 11% and lastly, the crude fibre content was 8.50% in watermelon seeds.

Table 1: Proximate composition of Watermelon seeds

Parameters	**Watermelon seeds (%)**
Moisture	9.45 ± 0.01^b
Ash	6.46 ± 0.10^c
Protein	15.49 ± 0.09^c
Crude fibre	8.50 ± 0.002^a
Fat	1.10 ± 0.001^a
Carbohydrate	59.03 ± 1.20^d

(mean±SD; n=3)

Phytochemical Composition of Watermelon Seeds

Phytochemical composition was carried out on watermelon seeds to check for the presence of phytochemical constituents which includes alkaloids, phenols, saponins, flavonoids, tannin

(Table 2 and table 3) and the result on the qualitative composition of watermelon 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**s**, 0.30mg/g in saponins and NP (not present) in flavonoids. The quantitative analysis for watermelon seeds indicates 3.080mg/g of alkaloids, 0.304mg/g of phenols, 0.117mg/g of tannin**s**, 0.200mg/g of saponins and 2.675mg/g of flavonoids.

Table 2: Phytochemical Composition of Watermelon Seeds

Parameters (mg/g)	**Watermelon seeds**
Alkaloids	++
Phenols	+
Tannins	+
Saponins	+
Flavonoids	+

KEYS: ++: Moderately present, +: Present, -: Absent

Table 3: Quantitative Composition of Watermelon Seeds

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

(mean±SD; n=3)

Vitamin composition

The vitamin composition of watermelon seeds indicated 0.03mg/100g for vitamin B1, 0.01 for vitamin B2, 0.64mg/100g for vitamin B3, 0.24mg/100g for vitamin B6 and 0.01 for vitamin B12.

Table 4: Vitamin composition in watermelon seeds

Sample	**Vitamin B_1 (mg/100g)**	**Vitamin B_2 (mg/100g)**	**Vitamin B_3 (mg/100g)**	**Vitamin B_6 (mg/100g)**	**Vitamin B_{12} (mg/100g)**
Watermelon Seeds	0.030±0.01[a]	0.01±0.0003[a]	0.644±0.02[c]	0.24±0.01[b]	0.01±0.0001[a]

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 organisms according to certain concentration while some did not show zones of inhibition i.e. the results showed different sensitivity of the extract on different organisms.

Table 5: Antibacterial effect of methanol extract of watermelon 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 mirabilis*	0	0	0	0

DISCUSSIONS

In the present study, results showed that watermelon seeds contained 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 (Mollenbeck *et al.*, 1997; Mahdi *et al.*, 2010; Okorondu *et al.*, 2010). The presence of saponins in this study was in contrast to the work reported by Ahmed *et al.*, 2001 and Palmer *et al.*, 2009, but corresponds to that of Borchardt *et al.*, 2008 and Ajaib *et al.*, 2010. 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 extract showed that not all the organisms tested were susceptible. The methanol extract of watermelon seeds produced only an effect on *Neisseria subflava*; comparatively, different researchers have reported contradictory observations in this regards (Braide *et al.*, 2010); Saxena *et al.*, 2013 and Rahman, 2014 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.

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 pneumoniae*, methicillin resistant *Staphylococcus aureus*.

However previous records showed that even new families of synthetic antimicrobial agent**s** 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 active 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 *Citrullus lanatus*. Therefore, the extract of watermelon seeds could be a good source of antimicrobial agents and thus, should be harnessed. Different governmental or private sectors that are interested in agro industry area recommend participating in this profitable area. If this should be done, it would have significant positive effect on the diversification of the Gross Domestic Product (GDP) of Nigeria as well as poverty and unemployment reduction. Researchers should investigate further on the production of watermelon in our country and the benefits obtained from the product have to be analyzed 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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