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# Antibacterial Activities of Watermelon (*Citrullus lanatus*) Seed Extracts against Some Selected Bacteria

Abdulwahab Musa[1], F. A. Abadam[2], Yakaka Mohammed Alkali[2], Mohammed Buba[3]
[1] *Department of Food Science and Technology, School of Applied Sciences and Technology, Ramat Polytechnic, Maiduguri, Borno State, Nigeria*
[2] *Department of Food Science and Technology, Faculty of Applied Sciences and Technology, Ramat Polytechnic, Maiduguri, Borno State, Nigeria*
[3] *Department of Microbiology, Faculty of Sciences, Yobe State University, Damaturu, Yobe State, Nigeria*

***Abstract-*** ***Plants are reservoirs of different kinds of phytochemicals used for treatment of infections and other diseases. This study was aimed at evaluating the antibacterial activities of Citrullus lanatus (watermelon) seeds against some selected bacteria. Ethanol was used to obtain the extracts and they were analyzed for the presence of carbohydrates, alkaloids, glycosides, tannins and saponins. Out of the phytochemicals screened, only saponins were not detected in both aqueous and ethanolic extract. Staphylococcus aureus, Escherichia coli, bacillus spp. and Proteus spp. were selected for antibiotic susceptibility testing. Antibacterial activity revealed that S. aureus, E. coli, and proteus spp. were most Susceptible to the extracts at concentrations ranging from 50mg/ml to 25mg/ml. Proteus spp. was observed to be resistant especially to the ethanolic extract. Largest zone of inhibition (25mm) was produced against Staphylococcus aureus using aqueous extract at 50mg/ml. Higher concentrations were generally effective than the lower concentration whereas, ciprofloxacin (control) exert better activity than either of the extracts. The findings of this study showed that seeds of C. lanatus contained bioactive compounds with potent antibacterial activity and thus could be used as herbal preparations just as the other parts of the plant.***

***Index Terms-*** ***antibacterial, phytochemical, proximate analysis, solvent, Watermelon***

## I. INTRODUCTION

Emerging and re-emerging infectious diseases are one of the most important challenges confronting health care systems in many developing countries in the 21st century. An estimated of over 50% of all deaths was attributed to infectious diseases worldwide (Godwin et al., 2015). This is more or less connected to poor health care facilities in developing countries and most importantly, defying treatment by microorganisms to antibiotics that were hitherto efficacious. These have led to renewed interest in search for compounds with novel structures that could play role in effective health care delivery and curb the menace of resistance (Wangensteen et al., 2004). Hence, many studies have shown that plant-derived compounds are promising. The use of plants part for medication has a very long history (Schulz et al., 2001), different communities in the world have use plant in their folk medicine and the trend have been maintained up to the 21st century. In 2001 alone, an investigation revealed that about 122 compounds used in modern medicine were isolated and identified from ethno-medicinal plants (Rahman, 2014). In most of the developing countries, treatment using herbs have been a common practice and it was thought that, effective health care cannot be achieved in such countries unless orthodox medicine is complemented with traditional medicine (Elujoba et al., 2005).

*Citrullus lanatus* commonly called watermelon is a popular fruit in many parts of the world and it is notable for its high water content and attractive look. The fruit comes in various shapes, sizes and rind pattern (Wehner, 2008). Although the seed of watermelon is often discarded as waste; it contains various amounts of carbohydrate, phenol, flavonoids, protein, fibre, phosphorus and iron (Varghese et al., 2013). Proximate analysis of the seed as reported by Oyeleke et al. (2012) revealed very high fat content (47.9%) followed by protein (27.4%) and carbohydrate (9.9%). Traditionally, the seed of *C. lanatus* is said to be medicinal because it can relieve inflammation/ irritation; causes increased passing of urine and gives tonic effects (Okunrobo et al., 2012; Varghese et al., 2013).

Watermelon seeds are a source of proteins. B vitamins, minerals, (such as magnesium, iron, potassium, phosphorus, sodium, zinc, manganese and copper) and fat among others (Vandermark, 2011; Collins et al., 2007). The antimicrobial compounds found in plants are of interest because antibiotics resistance is becoming a worldwide public health concern especially in terms of food borne illness and nosocomial infections (Anderson et al., 2001; Hsuch et al., 2005; Lin et al., 2005). Naturally occurring antimicrobials are being sought as replacements for synthetic preservation such as parabens (ethyl, methyl, butyl and propyl parabens), butyrate hydroxytolene (BHT) and butyrate 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 diseases and they provide an important source of all the worlds pharmaceuticals (Ajayi et al., 2011). The most important of these bioactive constituents (phytochemicals) of plants are the steroids, terpenoids, and carotenoids, flavonoids, alkaloids, tannins and glycosides. These phytochemicals are antibiotics 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 forming activity, antifungal, anti-inflammatory, fungi static and molluscidal (Ajayi et al., 2011). This research was carried out to examine the antibacterial activity of watermelon seeds extract against some selected microorganisms: *Bacillus* sp., *Staphylococcus* sp., *Escherichia coli*, and *Proteus* sp. and to determine the most effective extraction method between cold and warm water extraction.

## II. MATERIALS AND METHODS

### *A. Study area*

The study was conducted within the University of Maiduguri. Apparatus and reagent used were obtained from the department of microbiology, University of Maiduguri.

### *B. Collection and preparation of samples*

Twenty-five (25) watermelon fruits were purchased from fresh Gamboru Kasuwa market Maiduguri (11°50′N, 13°09′E). The fruits were washed and cut open to obtain the seeds. The seeds obtained were washed and air-dried for one week, then pulverized using mortar and pestle under aseptic conditions and ground to powder using a blender (Waring commercial blender, Model No. HGB2WTS3). Powdered Seed material were then weighed and kept in air-tight containers until further use.

### *C. Preparation of extracts*

The extraction was performed by cold maceration method as described by Tiwari et al. (2011) using water and ethanol as solvents. In the aqueous extraction, 50g of the Seeds powder was weighed and soaked in 400ml distilled water in a conical flask. The mixture was kept at room temperature for 24 hours and then filtered into a clean beaker. The filtrate was concentrated to dryness by evaporation at 45 °C in an oven. Similarly for the ethanolic extracts, 50g of the seed powder was weighed and soaked in 400ml of ethanol in a conical flask. It was subjected to treatment similar to that of the aqueous extracts.

### *D. Phytochemical analysis*

Qualitative phytochemical analysis of the various extracts was carried out according to standard procedure (Trease and Evans, 2001; Evans et al., 2002) to ascertain the qualitative composition of the seed. Phytochemicals analysis include alkaloids, phenols/ tannins, saponins, steroids, flavonoids, reducing sugars and lipids.

### *E. Preparation of test specimens*

Clinical isolates of *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp., and *Bacillus* sp. were obtained from the University of Maiduguri Teaching Hospital, Maiduguri, Borno State. The test organisms were sub-cultured and incubated at 37°C for 24 h on nutrient agar slant medium and were stored 0-4°C till use. They were then further sub-cultured in nutrient broth at 37°C for 24 h.

### *F. In vitro antibacterial activity (agar well diffusion method)*

Antibacterial activity of watermelon seed extracts was carried out using the agar well diffusion method as reported by Hassan et al. (2011), with some modifications. Mueller Hinton agar (LAB039, LabM Limited, UK) was prepared according to the manufacturer's direction. From each standard bacterial stock suspension [*Staphylococcus* sp, ($1.5 \times 10^5$ CFU/ml); *E. coli* ($2.0 \times 10^5$ CFU/ml); *P. aeruginosa* ($3.4 \times 10^5$ CFU/ml)], 1 ml of the broth culture of the respective bacteria was mixed thoroughly with 20 ml of sterile molten Mueller Hinton agar (45-50°C) before pouring into sterile petri dishes and left to solidify. Furthermore, a sterile cork-borer of diameter 6.0 mm was used to bore wells in each agar plate and 50 µl of each extract was introduced into the different wells. The plates were allowed to stand for 6 h after which they were incubated for 18 to 24 h at 37°C. After incubation, the zone of inhibition for each extract and the control was measured using a meter rule and recorded.

### *G. Screening of test organisms for viability*

Stock cultures of *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were collected from the Microbiology unit of the University of Maiduguri, Borno State, Nigeria. The isolates were screened to confirm their identities and viability prior to use (Cheesbrough, 2000; Beishir, 2001).The bacteria were sub cultured on Nutrient agar and stored on slant before use.

### *H. 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 160°C for 1h (Cheesbrough, 2000; Harrigan and McCance, 2005).

*I. Antibacterial susceptibility testing*

Antibacterial activity of the extract was investigated using modified Kirby–Bauer disc diffusion method. The disc was prepared from Whatman No. 1 filter paper using a 6mm sized perforator. The disc were placed into petri dish and sterile in hot air oven. The disc was impregnated with appropriate extract concentration following the method of Lalitha (2014). A total of 0.5g of the concentrated extract was weighed and dissolved into appropriate volume of water to obtain a concentration of 50 mg/ml, 25mg/ml and 6.25 mg/ml, ciprofloxacin was used as control at 30mg/ml concentration. The bacteria inocula was suspended in a sterile distilled water to obtain defined bacterial cells (McFarland standard No. 5). Swab stick were soaked in the standardized inocula and pressed against the test tube walls to drained-off the excess water. The swab stick were immediately used to inoculate the bacteria on dried surfaces of nutrient agar. It was allowed to stand for 10-15 minutes. The disc were then applied using sterile forceps and pressed gently to attach to agar surfaces and were incubated at 35 °C for 20 hours. Zones of inhibition were measured using meter rule and the concentration were used to determine the minimum inhibitory and minimum bactericidal concentration as described by (Benson, 2001).

## III. RESULTS

Five-hundred-gram (500g) of fine grounded watermelon (*Citrullus lanatus*) seed extract powder were dissolved in 100ml of ethanol, it was shake thoroughly and kept at room temperature for 24 hours which gives a percentage yield of 12g. 10g of the Ethanolic extract was dissolved in 100ml of distilled water and titrated using Hexane, Chloroform and Butanol to obtain the aqueous solution.

Table 1 shows the yield potential of watermelon (*Citrullus lanatus*) seed extract which affirms the yield is the ability of the solvent to extract the bioactive compounds from the seed extract. This also determine the constituents contents of plants which is either polar or non-polar. In this study, an equal amount of 500 g watermelon (*Citrullus lanatus*) seed extract were filtered with 1000ml of Ethanol, 500ml of Hexane, Butanol, Chloroform and Aqueous extract of 32.18g, 37.73g, 45g and 28.1g respectively.

**Table 1.** Yield of watermelon (Citrullus lanatus) seed extract by solvent.

| S/N | Solvent | Yield (%) |
|---|---|---|
| 1 | Hexane | 32.18 |
| 2 | Chloroform | 37.73 |
| 3 | Butanol | 45 |
| 4 | Aqueous | 28.1 |

The results of the phytochemical screening are presented in Table 2.

**Table 2.** Phytochemical screening of watermelon (Citrullus lanatus) seed extract.

| S/N | Compound | Test | Result |
|---|---|---|---|
| 1 | Carbohydrates | Molisch test | − |
| | | Barfoed test | + |
| | | Fehling A&B solution | + |
| 2 | Saponins | Frothing test | − |
| | | Fehling A&B solution | − |
| 3 | Tannins | Ferric chloride test | + |
| | | HCl ethanoate test | − |
| | | Lead ethanoate test | − |
| 4 | Alkaloids | Dragendorff test | + |
| | | Mayer test | − |
| | | Wagner test | − |
| 5 | Glycosides | Steroids test | + |
| | | Triterpenoid test | + |
| | | Salkowski test | − |
| | | Keller–Killiani test | − |

+ = present; − = absent.

In Table 2, various extracts of watermelon seeds were tested for different phyto-constituents including carbohydrates, saponins, tannins, alkaloids and glycosides using standard procedures. The result reveals that all the constituents were present. In addition, the ethanol extracts were positive for all phytochemicals screened.

The zones of inhibition of the extracts against *Staphylococcus aureus* are presented in Table 3.

**Table 3.** Zones of inhibition of watermelon (Citrullus lanatus) seed extracts against Staphylococcus aureus.

| S/N | Solvent | Extract (mg) | Zone of inhibition (mm) |
|---|---|---|---|
| 1 | Aqueous | 100 | − |
| | | 50 | − |
| | | 25 | 14 |
| | | Control | 30 |
| 2 | Chloroform | 100 | 11 |
| | | 50 | 15 |
| | | 25 | 10 |
| | | Control | 40 |
| 3 | Hexane | 100 | 11 |
| | | 50 | 22 |
| | | 25 | 7 |
| | | Control | 40 |
| 4 | Butanol | 100 | 8 |
| | | 50 | − |

| S/N | Solvent | Extract (mg) | Zone of inhibition (mm) |
|---|---|---|---|
| | | 25 | |
| | | Control | 37 |

– = no inhibition; blank = value not reported in the source.

Table 3 shows the zone of inhibition of the ethanolic extract of watermelon (*Citrullus lanatus*) against *Staphylococcus aureus*, watermelon seed have high range of antibacterial activities against gram positive bacteria. The results shows that the inhibitory effect of watermelon seed extract against the growth of *Staphylococcus aureus* with Hexane giving the highest inhibition of 22mm followed by Chloroform 15 mm and Aqueous 14mm while the lowest inhibition was observed with Butanol which has the inhibition of 8mm.

The zones of inhibition against *Bacillus subtilis* are presented in Table 4.

**Table 4.** Zones of inhibition of watermelon (Citrullus lanatus) seed extracts against Gram-positive Bacillus subtilis.

| S/N | Solvent | Extract (mg) | Zone of inhibition (mm) |
|---|---|---|---|
| 1 | Aqueous | 100 | 9 ± 0.05 |
| | | 50 | – |
| | | 25 | – |
| | | Control | 45 |
| 2 | Chloroform | 100 | 10 |
| | | 50 | – |
| | | 25 | – |
| | | Control | 40 |
| 3 | Hexane | 100 | 10 |
| | | 50 | 13 |
| | | 25 | 7 |
| | | Control | 39 |
| 4 | Butanol | 100 | 18 |
| | | 50 | |
| | | 25 | 24 |
| | | Control | 37 |

– = no inhibition; blank = value not reported in the source.

Table 4 shows the zone of inhibition of the ethanolic extract of watermelon (*Citrullus lanatus*) against *Bacillus subtilis*. According to Table 4, the inhibitory effect of watermelon seed extract against the growth of *Bacillus subtilis* with Butanol giving the highest inhibition of 24mm followed by Hexane 13mm and Aqueous 9mm while the lowest was Chloroform which has no zone of inhibition.

The zones of inhibition against *Escherichia coli* are presented in Table 5.

**Table 5.** Zones of inhibition of watermelon (Citrullus lanatus) seed extracts against Gram-negative Escherichia coli.

| S/N | Solvent | Extract (mg) | Zone of inhibition (mm) |
|---|---|---|---|
| 1 | Aqueous | 100 | 18 |
| | | 50 | – |
| | | 25 | – |
| | | Control | 40 |
| 2 | Chloroform | 100 | 5 |
| | | 50 | 2 |
| | | 25 | 23 |
| | | Control | 45 |
| 3 | Hexane | 100 | 10 |
| | | 50 | – |
| | | 25 | 8 |
| | | Control | 33 |
| 4 | Butanol | 100 | 13 |
| | | 50 | |
| | | 25 | 8 |
| | | Control | 37 |

– = no inhibition; blank = value not reported in the source.

Table 5 shows the zone of inhibition of the ethanolic extract of watermelon (*Citrullus lanatus*) against *Escherichia coli*. According to Table 5, the inhibitory effect of watermelon seed extract against the growth of *Escherichia coli* with chloroform giving the highest inhibition of 23mm followed by Aqueous 18 mm and Butanol 13mm while the lowest was Hexane which has the inhibition of 10 mm.

The zones of inhibition against *Proteus* spp. are presented in Table 6.

**Table 6.** Zones of inhibition of watermelon (Citrullus lanatus) seed extracts against Gram-negative Proteus spp.

| S/N | Solvent | Extract (mg) | Zone of inhibition (mm) |
|---|---|---|---|
| 1 | Aqueous | 100 | 9 ± 0.05 |
| | | 50 | 10 |
| | | 25 | 4 |
| | | Control | 50 |
| 2 | Chloroform | 100 | 14 |
| | | 50 | 10 |
| | | 25 | 6 |
| | | Control | 45 |
| 3 | Hexane | 100 | – |
| | | 50 | 12 |
| | | 25 | 8 |
| | | Control | 39 |
| 4 | Butanol | 100 | – |
| | | 50 | – |
| | | 25 | – |
| | | Control | 37 |

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− = no inhibition.

Table 6 shows the zone of inhibition of the ethanolic extract of watermelon (*Citrullus lanatus*) against *Proteus* spp. According to Table 6, the inhibitory effect of watermelon seed extract against the growth of *Escherichia coli* with Chloroform giving the highest inhibition of 14mm followed by Hexane and Aqueous 9 ± 0.05 mm while the lowest was Butanol which has no zones of inhibition.

The zones of inhibition produced by the antibiotic sensitivity discs are presented in Table 7.

**Table 7.** Zones of inhibition (mm) of antibiotic sensitivity discs against Gram-positive and Gram-negative bacteria.

| Organism | Gram reaction | CPX | R | E | APX | AM | CH | AU | OFX |
|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | Positive | 22 | 14 | 7 | − | 12 | n.t. | n.t. | n.t. |
| *Bacillus* spp. | Positive | 11 | 8 | − | 10 | − | n.t. | n.t. | n.t. |
| *E. coli* | Negative | − | n.t. | n.t. | n.t. | − | 14 | − | 18 |
| *Proteus* spp. | Negative | 18 | n.t. | n.t. | n.t. | 11 | − | 14 | 11 |

CPX = ciprofloxacin; R = Rocephin; E = erythromycin; APX = Ampiclox; AM = amoxicillin; CH = chloramphenicol; AU = Augmentin; OFX = ofloxacin; − = no inhibition; n.t. = not tested.

Table 7 shows the zone of inhibition of sensitivity discs of the tested organisms in millimetres (mm). The Gram-positive bacteria (*Staphylococcus aureus*) was observed with high degree of sensitivity of (22mm, 14mm and 12mm) against Ciprofloxacin, Ampiclox and amoxicillin and shows very high sensitive to gram positive sensitivity discs than *Bacillus subtilis* while *Escherichia coli* showed variation in their sensitivity to the antibiotic discs with high degree of sensitivity of (14mm and 18mm) against Chloramphenicol and ofloxacin and so also *Proteus* spp. has high degree of (11mm, 18mm and 14mm) against Ciprofloxacin, Rocephin and Ampiclox respectively.

*Bacillus* spp. showed the highest resistance especially to the ethanolic extract. This organism has been reported to be resistant to seeds extract of watermelon at concentrations lower than 25mg/ml. Susceptibility of *S. aureus* and *E. coli* observed in this study is in agreement with that of Godwin et al. (2015) who made similar observations. In a recent study, susceptibility of *S. aureus* was attributed to presence of saponins. In this study the findings therefore, support the view that other phytochemicals besides saponins are active against Gram positive bacteria also have higher extract concentrations were shown to be more potent than the lower concentrations.

Nwankwo et al. (2014) have made similar observations although activity even at low concentration indicates high potency of the extract against the organism. The potency of the extract was further revealed by the results of antibacterial activity in which most of the concentrations were in close range. The ciprofloxacin (control) used however, showed more effect than the extract which may reflect variation in the concentration of the active compounds in the antibacterial.

## IV. DISCUSSION

In the present study, results showed that watermelon seeds contain phytochemicals including Carbohydrates, saponins, tannins, alkaloids and glycosides. No saponins were observed in both the aqueous and ethanolic extracts. The absence of saponins in this study was in contrast to the works reported by Hassan et al. (2011) and Braide et al. (2012) but corresponds to that of Godwin et al. (2015) and Nwankwo et al. (2014). The disparity observed might be attributed to differences in geographical location as it can affect plant's active constituents, which may be induced by many factors like climate, soil and propagation method. Presence of these phytochemicals in the extract was a clear indication of antibacterial potentials of the watermelon seeds.

Antibacterial activity of the seed extracts showed that all the organisms tested were susceptible. The ethanolic extract exhibited more potency on *S. aureus* and *Proteus* spp. in contrast to the aqueous extract which produced more effect on E.coli and comparatively, different researchers have reported contradictory observations in this regards.

In this study it was observed that ethanolic extracts presents better response to the bacterial activities than the ethanol whereas Nwankwo et al. (2014) reported the contrary. This contradiction might be a function of methodological differences.

## V. CONCLUSION

Results from this study showed the antibacterial potentials of seed extract of watermelon (*Citrullus lanatus*) due to the presence of some phytochemicals. Most of the bacteria tested were observed to be susceptible to the extract. The activity observed is a clear indication of therapeutic properties possessed by the seed of the plants. The antibacterial effects of *C. lanatus* seed extracts against the selected bacteria suggests that extracts obtained by cold maceration as well as using butanol and chloroform have potential as antibacterial agents especially against *Staphylococcus* sp. and proteus spp. Further isolation of saponins from seed and testing it against more Gram positive bacteria

will be necessary to confirm that saponins are responsible for the antibacterial activity observed. Therefore, it can be applied as a home remedy for treatment of diseases caused by *Bacillus* sp., *Klebsiella* sp., *Staphylococcus aureus* and *Escherichia coli*.

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