

## An Assessment of the Phytochemicals and Antibacterial Activity of Seed Extract of *Citrullus Lanatus* (Watermelon)

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### ABSTRACT

Phytochemical screening and antibacterial activities of seed extract of *Citrullus lanatus* (watermelon) against one gram-positive organism, *Staphylococcus aureus* and one gram-negative organism, *Escherichia coli* was carried out. The seed extracts were screened for the presence of tannins, saponins, terpenoids, steroids, flavonoids, glycosides, alkaloids, amino acids and balsams. The agar well diffusion method was employed to determine the effect of the extracts on the growth of the test organisms. The results of the tests carried out on the seeds of *Citrullus lanatus* (watermelon) shows the phytochemical constituents of the seed extracts which showed the presence of terpenoids, tannins, balsams, flavonoids, glycosides, alkaloids and amino acids while saponins and steroids were absent. The results also showed that not all concentrations of the aqueous and ethanolic extracts of the sample had total inhibition on the growth of the tested organisms. However, the seed extracts inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at various concentrations. It was observed that for the ethanolic extract, only concentrations at 100% and 50% inhibited the growth of the test organisms and for the aqueous extract, only concentrations at 100% inhibited their growth. The results of this study show that seed extracts of *C. lanatus* have antibacterial activity and are potent just as standard antimicrobial drugs against gram positive and gram negative bacteria.

**Keywords:** Antibacterial, *Citrullus lanatus*, Phytochemical, *Staphylococcus aureus*, watermelon.

### INTRODUCTION

It has been reported that infectious diseases account for one-half of all deaths in the tropical countries. [1] As a result, people of all continents have long applied poultice and imbibed infusions of indigenous plants

dating back to prehistory for health purposes till date. [2] Plant medicine has been used in healthcare delivery in many parts of Africa and the rest of the world and it is believed that effective health cannot be achieved in Africa unless orthodox medicine is

complemented with traditional medicine. [3] 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 illnesses and nosocomial infections. [4-9] Furthermore, 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 hydroxyanisole (BHA) that are under scrutiny as suspected cancer causing agents. [10-13]

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. [14] The most important of these bioactive constituents (phytochemicals) of plants are: steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. These phytochemicals are antibiotic principles of plants. [14] They are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques. [15] They have also been reported to exhibit hemolytic and foaming, antifungal, anti-inflammatory, fungistatic and molluscidal activities. [14] At least, 80% of Africans depend on plant medicine for their health care. [2] Many commercially proven drugs used in modern medicine were initially used in the crude form in traditional/folk healing practices. Medicinal plants generally contain a number of compounds which may be potential natural antibacterial for the treatment of common bacterial infections. [16]

It is estimated that today, plant materials are present in or have provided models for 50% of western drugs. [17] Therefore there is urgent and continuous need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and

re-emerging infectious diseases. [18] Hence more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. This study focused on the phytochemical properties of the seed extracts of *Citrullus lanatus* and also, the antibacterial activity of the extracts was also tested against one gram-positive organism, *Staphylococcus aureus* and one gram-negative organism, *Escherichia coli*.

## MATERIALS AND METHODS

### Study Area

This research project was carried out in the Microbiology laboratory of University of Uyo, main campus, Akwa Ibom State of Nigeria.

### Collection of Seeds

The seeds of *Citrullus lanatus* were obtained from watermelon fruits sold by vendors at Itam market, Uyo. The seeds were then extracted, washed and subjected to drying to constant weight at room temperature (30±5°C).

### Processing of Sample

The seeds were Dehulled and pulverized to powder using clean mortar and pestle as described by. [19] The resulting powder was stored in an air-tight container at 30°C for further use.

### Test Organisms

The test organisms were obtained from the Microbiology laboratory of University of Uyo Teaching Hospital (UUTH), Abak Road, Uyo, Akwa Ibom State. The test organisms are;

(i) *Staphylococcus aureus*

An inoculum of the test organism was cultured on mannitol salt agar. The growth produced yellowish colonies. The colonies were further subjected to Gram staining. The test organism was inoculated on a nutrient agar slant in a bijoux bottle and stored in a refrigerator at 4°C. [20]

(ii) *Escherichia coli*

The isolate obtained was plated on Eosin methylene blue agar by streaking from 18-24 hours. Colonies with distinctive metallic green sheen were observed which indicate a positive result for *E. coli*. The colonies were further subjected to Gram staining. The test organisms were inoculated on a nutrient agar slant in a bijoux bottle and stored in a refrigerator at 4°C. [21]

### **Media Preparation**

All the media used were prepared according to manufacturer's directions. Nutrient agar was used for resuscitation of the organisms, for their repeated sub-culturing and to determine the Minimum Bactericidal Concentration (MBC); Mueller-Hinton agar was used for sensitivity testing and the Nutrient broth was used for dilution broth technique.

### **Sterilization of Extracts**

The seed extracts were filter sterilized by membrane filtration using a 0.45µm pore-sized membrane filter and a vacuum pump.

### **Phytochemical Screening**

The seed extracts were screened for the presence of tannins, saponins, terpenoids, steroids, flavonoids, glycosides, alkaloids, amino acids and balsams according to the methods described by. [22-25]

### **Separation of Extracts into Component Fractions**

Chromatography is a technique by which a mixture or sample is separated into components. It is based on the principle that under the same conditions, the time between the injection of a component into a column and the elution of that component is constant. In this work, two types of chromatography were employed and they are Thin Layer Chromatography and Column Chromatography.

#### **Thin Layer Chromatography (TLC)**

TLC is a technique used to separate the components of a mixture using a thin stationary phase by an inert backing. For this

process, TLC plates also known as chromatoplates were used. To spot and develop the TLC plates, a developing chamber was required. The following procedures were carried out for TLC of the ethanolic extract;

-The plates were cut to the correct size and using a pencil, a straight line was drawn across the plate approximately 1 cm from the bottom.

-Using TLC pipettes, spots of the sample extracts were applied to the line, using n-hexane as control.

-The plates were then placed into the chamber as evenly as possible. The capillary action was allowed to draw the solvent up the plate until it was approximately 1 cm from the end.

-The plates were removed and a line drawn immediately with a pencil across the solvent front.

-The components shown were then circled with a pencil.

-After developing the TLC plates, they were visualized under UV lamp.

#### **Column Chromatography**

This is a technique routinely used to separate complicated mixtures of compounds. It is also used to separate mixtures of naturally occurring compounds isolated from plants and other living organisms. Performing the column chromatography involved packing a column with the stationary phase. The stationary phase was silica gel. In preparing and packing the column, the following procedure was employed;

-The glass column was washed, dried and clamped vertically onto a retort stand and the column packed using the dry pack method.

-A piece of cotton wool was then inserted and 20 g adsorbent silica gel of mesh size 120-150 mm was slowly poured into the column.

-The column was tapped gently to give a uniform packing, some 2.79 g of the crude extract was mixed with silica gel which was allowed to dry and then loaded into the glass column.

-Silica gel was again added on top of the sample layer before addition of solvent.

-The elution started with 100% hexane, then hexane 60%:ethyl acetate 40%, hexane 40%:ethyl acetate 60% and finally 100% ethyl acetate.

### Preparation of Extracts Concentration

The stock solutions of the seed extracts (aqueous and ethanolic) were prepared in screw capped bijoux bottles. One gram of the ethanolic seed extract was weighed and dissolved in 10 ml of DMSO<sub>4</sub> to obtain 100 mg/ml concentration of stock solution. Three varied extract concentrations which included 50 mg/ml, 25 mg/ml and 12.5 mg/ml, were prepared from the stock solution using two-fold (doubling) dilution. The same process above was equally employed for the concentrations of the aqueous seed extract as well as the control antibiotics.

### Antibacterial activity testing

The agar well diffusion method as described by [26,27] was employed to determine the effect of the extracts on the growth of the test organisms. Sterile Mueller Hinton agar was used. Working aseptically, the agar plates were uniformly seeded by flooding with 1 ml of suspension of the test organism and then left undisturbed for 15 minutes. Using a sterile 6 mm cork borer, two wells were made on each plate which were carefully labeled according to the concentrations and extracts, the wells were sufficiently spaced out and kept at least 15 mm from the edge of the plate and 25 mm from well to well to prevent overlapping of zones. Into the wells, 2 drops of the same concentration and same extract were introduced using a sterile Pasteur pipette. This was done for all the concentrations of

the extracts as well as the fraction and in duplicates after which the inoculated plates were left undisturbed for about 30 minutes and then incubated at 37 °C for 18 hours. The extracts used were aqueous and ethanolic extracts of water melon, Ampicillin was used as control for *Staphylococcus aureus* and Chloramphenicol was used as control for *Escherichia coli*. With the aid of a transparent metre rule, the zone diameters of inhibition of growth were measured and recorded to the nearest millimetre.

### Statistical Analysis

A simple T-test of hypothesis was conducted to find out whether there were variations in the activity of the seed extracts at (P> 0.05).

## RESULTS

The results of the tests carried out on the seeds of *Citrullus lanatus* (watermelon), are outlined in Tables 1-5 below. Table 1 shows the phytochemical constituents of the seed extracts which showed the presence of terpenoids, tannins, balsams, flavonoids, glycosides, alkaloids and amino acids while saponins and steroids were absent. Table 2 shows the result of the column chromatography obtained after fractionation. Different concentrations of the two solvents (n-hexane and ethyl acetate) were used. A total of four eluents were obtained and then exposed to the atmosphere for few days after which three eluents were observed to have evaporated completely leaving one which was used for further study. Table 3 shows the results of the inhibitory activity of the aqueous and ethanolic extracts of *C. lanatus* as well as that of the fraction obtained from the column chromatography. Table 4 shows the results of the Minimum Inhibitory Concentration (MIC) of the seed extracts and fraction. Table 5 shows the results of the

Minimum Bactericidal Concentration (MBC) of the seed extracts and fraction.

The ethanolic extract with an initial yield of 50 g yielded 5.6 g after evaporation with a brownish coloration and a gummy texture and also the aqueous extract with an initial weight of 50 g yielded 6.0 g with a brownish coloration and a gummy texture. Therefore, the extract yield of the two solvents, ethanolic and aqueous, were not significantly different ( $P > 0.05$ ).

For the Thin Layer Chromatography (TLC), the line on which the spots were made before elution started was taken as the solute front while the line drawn at the end was determined as the solvent front. The ratio of the solute front to the solvent front was used to determine the Retention factor ( $R_f$ ) values. 100% n-hexane yielded Plate 1 with  $R_f$  value of 0.27; 60% n-hexane : 40%

ethyl acetate yielded Plate 2 with  $R_f$  value of 0.23; 60% ethyl acetate : 40% n-hexane yielded Plate 3 with  $R_f$  value of 0.29 and 100% ethyl acetate yielded Plate 4 with  $R_f$  value of 0.30.

**Table 1: Phytochemical constituents of the extracts**

Ingredients	Ethanol	Aqueous
Terpenoids	+	-
Tannins	+	-
Saponins	-	-
Glycosides	+	+
Balsams	+	-
Flavonoids	+	-
Steroids	-	-
Alkaloids	+	+
Amino acids	+	-

Keys: + = Present, - = Absent

**Table 2: Column Chromatography**

Extract used	Concentration used	Fraction obtained
Ethanolic extract	100% H	Fraction 1
	60% H : 40% E	Fraction 2
	60% E : 40% H	Fraction 3
	100% E	Fraction 4

Keys: H= n-hexane, E= ethyl acetate

**Table 3: Antibacterial activity of the aqueous and ethanolic seed extracts of *C. lanatus* against the test isolates.**

Isolates	Average zone diameter of inhibition (mm)	Concentration (%)			
		Solvent	100	50	25
<i>S. aureus</i>	CA	16.5±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	CE	21.0±0.50	17.0±0.50	0.0±0.00	0.0±0.00
	AMP	40.0±0.50	30.5±0.50	28.0±0.50	24.5±0.50
<i>E. coli</i>	CA	13.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	CE	20.0±0.50	18.0±0.50	0.0±0.00	0.0±0.00
	CH	60.0±0.50	41.5±0.50	40.0±0.50	35.0±0.50

Keys: CA = *C. lanatus* Aqueous extract CE = *C. lanatus* Ethanolic extract CH = Chloramphenicol AMP = Ampicillin

**Table 4: Minimum Inhibitory Concentration**

Isolates	Extracts	Minimum inhibitory concentration (%)						
		100	50	25	12.5	6.25	3.125	1.563
<i>S. aureus</i>	CE	-	-	-	MIC	+	+	+
	CA	-	-	-	-	MIC	+	+
<i>E. coli</i>	CE	-	-	-	MIC	+	+	+
	CA	-	-	-	-	MIC	+	+

Keys: CA = *C. lanatus* Aqueous extract CE = *C. lanatus* Ethanolic extract  
- = No growth + = growth

**Table 5: Minimum Bactericidal Concentration**

Isolates	Extracts	Minimum bactericidal concentration (%)				
		100	50	25	12.5	6.25
<i>S. aureus</i>	CE	-	-	MBC	+	+
	CA	-	-	-	-	MBC
<i>E. coli</i>	CE	-	-	MBC	+	+
	CA	-	-	-	-	MBC

Keys: CA = *C. lanatus* Aqueous extract CE = *C. lanatus* Ethanolic extract  
- = No growth + = growth

## DISCUSSION

The results of the extraction showed that a total yield of 11.2 g and 12.0 g were obtained for the ethanolic and aqueous seed extracts respectively from the original weight of 50 g. The phytochemical constituents showed that the seed extracts contained bioactive elements seven of which were obtained in this study. Terpenoids, tannins, balsams, flavonoids, glycosides, alkaloids and amino acids were present while saponins and steroids were absent. The report of [28] supports these results. These phytochemical compounds have also been reported to possess antimicrobial activities. [29-32,14] Furthermore, [33] and [34] also reported the antimicrobial potentials of antioxidants present in plants and plant extracts. It is therefore not surprising that the ethanolic and aqueous extracts of *C. lanatus* used in this study indicated varying degrees of antibacterial effect [35] reported that the geographical location of a plant can affect its active constituents, which may be induced by many factors like climate, soil and propagation method. Also, time of collection of plant parts also affects its effectiveness. [36]

The results presented in Table 3 show that not all concentrations of the aqueous and ethanolic extracts of the sample had total inhibition on the growth of the tested organisms, and this may be due to the dilution effect. However, the absence of terpenoids, flavonoids and amino acids in the aqueous extract may be related to solubility of active compounds in aqueous solution. [37] The seed extracts inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at various concentrations. It was observed that for the ethanolic extract, only concentrations at 100% and 50% inhibited the growth of the test organisms and for the aqueous extract, only concentrations at 100% inhibited their growth. The effects of the control

(antibiotics) were significantly greater (Zone Diameter of Inhibition > 28 mm). The MIC and MBC results indicated that the values on *Staphylococcus aureus* and *Escherichia coli* were different (Tables 4 and 5). The seed extracts may have been bacteriostatic and bactericidal at higher concentrations. Statistical analysis showed that there was a significant difference ( $P < 0.05$ ) in the effectiveness of the aqueous and ethanolic extracts of the seed.

The results of the Thin Layer Chromatography (TLC) shows that the two solvents used (n-hexane and ethylacetate) at different concentrations yielded different plates with different Retention factor ( $R_f$ ) values. In the Column fractionation (Table 2), n-hexane and ethyl acetate were also used at different concentrations which gave rise to four different fractions with differences in colour and consistency. After evaporation process, three of the fractions dried off completely remaining one fraction which was then used for further studies. It is possible that the extracted components were volatile and were lost on evaporation.

## CONCLUSIONS

The results of this study show that seed extracts of *C. lanatus* have antibacterial activity and are potent just as standard antimicrobial drugs against gram positive and gram negative bacteria. However, further research is necessary in order to determine and identify the antibacterial compounds within the seeds and also to determine their spectrum of activities against disease-causing microorganisms.

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