https://doi.org/10.46344/JBINO.2021.v010i01.26

COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFECTS OF AQUEOUS AND METHANOLIC SEED EXTRACTS OF *Citrillus lanatus* Thunb.

[1]Sodipo O.A., [2]Yakubu J., [2]Bello F.S. and [4]Gulani I.A.

[1]Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, Borno State.
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Borno State.
[3]Department of Pure and Applied Chemistry, Faculty of Sciences, University of Maiduguri, Maiduguri, Borno State.
[4]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Borno State

Email: jamesyakubu96@gmail.com

ABSTRACT

The study aimed at evaluating the antimicrobial effects of aqueous and methanol seed extract of *Citrillus lanatus*, with the view of establishing scientific fact of the use of the plant for the treatment of microbial infections. The crude extracts of the seeds were obtained using distilled water and methanol respectively. The phytochemical and antibacterial potentials of *Citrillus lanatus* Thunb. seeds were evaluated. The seed extracts were tested for antibacterial activity using the agar well diffusion and the disc diffusion methods against clinical isolates of Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram negative bacteria *(Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeroginosa)*. The phytochemical test of the aqueous and methanolic seed extracts revealed the presence of flavonoids, cardenolides, cardiac glycosides, carbohydrates and terpenoids. The results of the disc diffusion method showed that the aqueous seed extract inhibited the growth of *K. pneumoniae* at concentrations of 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibitions of 9 mm, 12 mm and 14 mm respectively while the remaining bacteria showed resistance at all concentrations used. The methanolic seed extract inhibited the growth of *S. aureus* at concentrations of 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibitions of 7 mm, 10 mm, 15 mm and 20 mm respectively; *S. pyogenes* at concentrations of 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibitions of 9 mm, 12 mm and 15 mm respectively, while *E. coli, K. pneumoniae* and *Ps. aeroginosa* showed no sensitivity. For the agar well diffusion method, all the tested bacteria were resistant at all the concentrations used with the exception of *S. aureus* which was sensitive to the methanolic seed extract at concentrations of 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibitions of 11 mm, 13 mm, 15 mm, 17 mm and 19 mm respectively. *S. aureus* showed the highest sensitivity towards the extract among the bacteria used with the methanolic extract exhibiting a higher antibacterial activity. The result obtained in this study does not justify the traditional claim of *C. lanatus* as an antibacterial agent.

Keyword: *Citrillus lanatus*, phytochemicals, antibacterial, agar well diffusion, disc diffusion

INTRODUCTION

For centuries man has effectively used various components of plants or their extracts for the treatment of many diseases, including bacterial infections. Medicinal plants are known to contain in one or more of their organs substances that can be used for therapeutic purposes or as precursor for synthesis of useful drugs (Sofowora, 2008). Plants are considered not only as dietary supplement to living organisms but also traditionally used for treating many health problems. Over 60 % of the world human population, 80 % in developing countries depend directly on plants for their medicinal purposes (Dhillion *et al*., 2002).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect themselves, but recent research demonstrates that many phytochemicals can protect humans against diseases. Fruits and vegetables have been recognized as natural sources of various bioactive compounds (Pennington and Fisher, 2010) which could be attributed to their phytoconstituents such as flavonoids, anthocyanins, phenolic compounds, vitamins C and E, dietary fiber and carotenoids present in fruits and vegetables (Gonzalez-Aguilar *et al*., 2008). Many plants extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defence reactions of plants against infections by pathogenic microorganisms (Fawcett and Spencer, 1976).

Citrillus lanatus (Fig. 1) commonly known as watermelon, is a popular fruit in many parts of the world. It belongs to the family Cucurbitaceae, a vine-like flowering plant originally from Africa. Cucurbitaceae is a family that comprises approximately 120 genera and over 900 species which are vastly distributed in tropical and subtropical regions of Asia, Australia, Africa and America (Rubatzky and Yamaguchi, 1997). The fruit comes in various shapes, sizes and rind pattern (Wehner, 2008). Water constitutes about 92 % of the total fruit weight. Watermelon is grown in the tropical and subtropical areas worldwide for its large edible fruit which is a special kind of berry with a hard rind and no internal division. Watermelon seeds are a source of protein, B vitamins, minerals (such as magnesium, zinc, iron, copper, sodium, phosphorus, manganese, potassium) and fat among others (Vandermark, 2011; Collins *et al*., 2007). A study carried out by Varghese *et al.* (2013) showed that watermelon seeds contain various amounts of carbohydrate, phenol, flavonoids, protein, fiber, phosphorous and iron. Also, proximate analysis of the seeds (Oyeleke *et al.,* 2012) revealed very high fat content (47.9 %) followed by protein (27.4 %) and carbohydrate (9.9 %). A study carried out by Adunola *et al*. (2015) revealed that the kind of solvent employed as well as the conditions of extraction influence the efficacy of the extract against specific test organisms.

Indiscriminate use of antimicrobials and fake substandard drugs has led to widespread resistance to commonly used

antibiotics. In addition, the high cost of conventional drugs has led many people to seek for alternative inexpensive sources of treatment. Thus, antimicrobial compounds found in plants are now the focal point of interest in the treatment of infectious diseases.

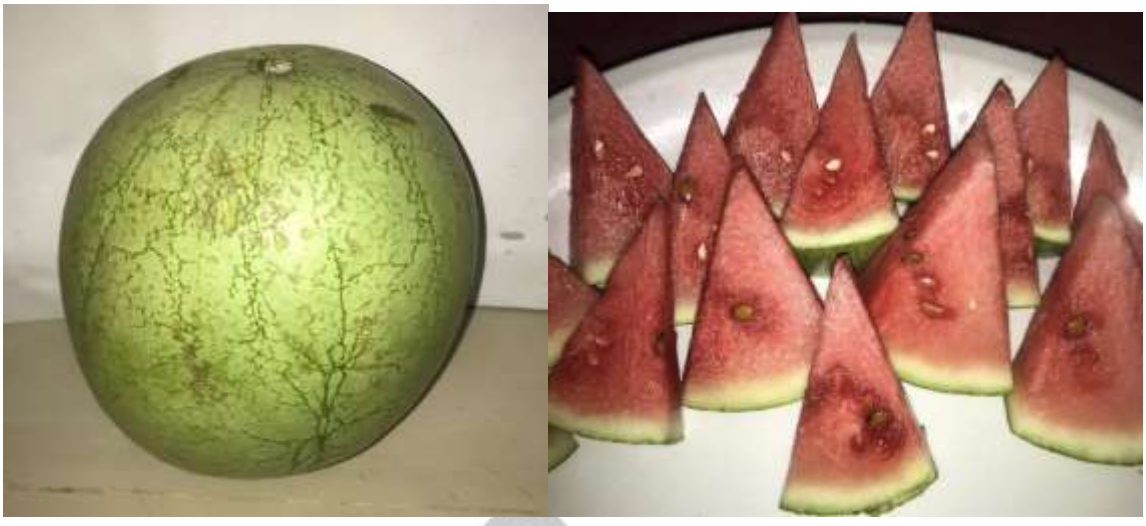

Fig. 1: *Citrillus lanatus* Thunb. Fruit (Whole and Sliced)

MATERIALS AND METHODS

Materials

The materials used include: reflux extractor, weighing balance, desiccator, test tubes, filter paper, methanol (Analar grade), measuring cylinder, mortar and pestle, stainless steel trays, dropper, sample containers, distilled water, retort stand, beaker, syringes, muslin cloth, petri dishes, nutrient agar, antibiotic discs, forceps, circular discs, Gram negative and Gram positive bacteria, cotton wool, nutrient broth, transparent ruler and a cork-borer.

Source, Collection and Identification of Plant Materials

The seeds of *Citrillus lanatus* were obtained from fresh retailed watermelon fruit from Custom Market, Maiduguri, Borno State, Nigeria. The plant was identified and authenticated by Prof. S.S. Sanusi, a Plant Taxonomist in the Department of Biological Sciences, Faculty of Science, University of Maiduguri, Nigeria. A voucher specimen was prepared (UM/FPH/03/001/006) and deposited in the Faculty of Pharmacy Herbarium.

Preparation of the Plant Extract

The seeds of *C. lanatus* were dried under shade for seven days. The seeds were finely ground into powder using a wooden mortar and pestle. The powdered seeds were stored in a closed container and stored in a desiccator prior to extraction, and the solvents used for the extraction were distilled water and methanol (Analar grade).

Aqueous Extraction

The method used for the extraction was reflux. 250 g of the powdered watermelon seeds was introduced into a 5 L conical flask of the reflux extractor, 2 L distilled water was added and it was refluxed for 2

h. The extracted sample was then decanted, filtered using Whatman filter paper (No. 11) and dried on a water bath. It was then stored in a desiccator.

Extraction

The method used for the extraction was also reflux. 280 g of the powdered watermelon seed was introduced into a 5 L conical flask of the reflux extractor, 2 L of methanol was added and it was refluxed for 2 h. The extracted sample was decanted, filtered using Whatmann filter paper (No. 11) and dried on a water bath. It was then stored in a desiccator.

Phytochemical Screening

A little quantity of each of the extracts were subjected to phytochemical tests to determine the presence of the following: alkaloids, flavonoids, saponins, anthraquinones, terpenoids, phlobatannins, soluble starch, cardiac glycosides and carbohydrates as described by Vishnoi (1979); Evans (2009); Sofowora (2008); Brian and Turner (1975); Markham (1982); Silver *et al*. (1998).

Antibacterial Studies of the Aqueous and Methanolic Seed Extracts of *Citrillus lanatus*

Test Bacteria

For the antibacterial activity study, a total of five bacteria were used; three Gram negative and two Gram positive bacteria. The Gram negative bacteria used include: *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeroginosa,* while the Gram positive bacteria used were: *Staphylococcus aureus* and *Streptococcus pyogenes*. These organisms were obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Sterilization of the Materials

The media were sterilized in a portable autoclave at 121°C for 15 minutes while the pipettes and other glass wares were sterilized by dry heat in a hot air oven at 160°C for 1 hour. The cork-borer was sterilized by autoclaving and then using open flame subsequently during the boring process. The circular discs were sterilized in the petri dish using hot air oven at 160°C for 1 hour.

Antibacterial Sensitivity Tests

Two methods were used for the antibacterial sensitivity test, these are: agar well diffusion and the disc diffusion methods.

Agar Well Diffusion Method

The agar well diffusion method by Kudi *et al*. (1999); Ogundipe *et al*. (2000) as reported by Isa *et al*. (2014) was used to determine the growth inhibition of the test organisms by the plant extracts. The tests were carried out using different concentrations; 100, 200, 300, 400 and 500 mg/ml which were prepared by dissolving the aqueous extract in water while the methanolic extract was dissolved in methanol. Nutrient agar was prepared according to the manufacturer's specification; 25 ml each was poured into sterile petri-dishes and were allowed to dry and solidify.

Using a sterile cork-borer of 9 mm diameter, three holes per plate were made in the solidified agar and were inoculated with 0.5 ml suspension of the bacteria. Thereafter, the wells were filled with the prepared concentrations of the extract solutions using sterile syringes. These were done in triplicates and the plates were

incubated at 37°C for 24 h and the relative susceptibility of each bacterium to the extract as indicated by clear zones of growth inhibition (zone of inhibition) around the wells were measured with a transparent meter rule in millimeters and recorded. The standard antimicrobial discs used were Ciprofloxacin (5 µg), Gentamycin (10 µg), Erythromycin (15 µg) [Oxoid Ltd., Basingstoke, Hampshine England].

Disc Diffusion Method

The agar disc diffusion method by Xu and Lee (2001); Mahasneh (2002) was used to determine the growth inhibition of the test organisms by the plant extract. Sterilized filter paper discs (6 mm in diameter) were impregnated in appropriate concentrations of the seed extract (100, 200, 300, 400 and 500 mg/ml). The discs (made from Whatman No. 1 filter paper) were allowed to absorb the extracts as described by Mahasneh (2002). The agar plates were aseptically inoculated with broth cultures of the test microorganisms using sterile pipettes and the plates allowed to dry. The discs containing the plant extract were transferred using flamed but cooled forceps onto the surface of the seeded agar plates. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The plates were then incubated for 24 h at 37°C. After 24 h, the zone of inhibition around each disc was measured and recorded in mm using a transparent meter rule.

Statistical Analysis

The statistical analyses were carried out using GraphPad Statistical package, Version 5.0 (GraphPad®, 2007). The mean and standard error of mean (SEM) as parameters were determined and the mean values of the zones of inhibition of the seed extracts were compared with that of the standard drugs.

RESULTS

Phytochemical Studies

The colour, texture and percentage yield of the extracts from the reflux extraction are presented in Table 1. The colour of the aqueous and methanolic extracts were coffee brown, the texture of both extracts was crystalline. The weights of the aqueous and methanolic extracts were 250 g and 280 g with a percentage yield of 12.68 $\%^{w}/_{w}$ and 5.64 $\%^{w}/_{w}$ respectively. The result**s** of the aqueous and methanolic extracts showed the presence of flavonoids, terpenoids, cardiac glycosides, cardenolides and carbohydrates as presented in Table 2.

Antimicrobial Susceptibility Tests of the Aqueous and Methanolic Seed Extracts of *C. lanatus*

The Agar Well Diffusion Method

The results of the *in vitro* antimicrobial susceptibility test of both the aqueous and methanolic seed extracts of *C. lanatus* using the agar well diffusion method are shown in Tables 3 and 4 respectively. The aqueous seed extract did not inhibit the growth of all the bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeroginosa*) at all concentrations (100, 200, 300, 400 and 500 mg/ml).

The methanolic seed extract inhibited the growth of *S. aureus* at concentrations of

100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibition of 11.00±0.00 mm, 13.00±0.00 mm, 15.00±0.00 mm, 17.00±0.00 mm and 19.00±0.00 mm respectively, while *S. pyogenes, E. coli, K. pneumoniae and Ps. aeroginosa* showed no sensitivity.

The Disc Diffusion Method

The results of the *in vitro* antimicrobial susceptibility test of both the aqueous and methanolic seed extracts of *C. lanatus* using the disc diffusion method are shown in Tables 5 and 6 respectively. The result showed that the aqueous seed extract did not inhibit the growth of *S. aureus, S. pyogenes, E. coli and Ps. aeroginosa* at all the concentrations (100, 200, 300, 400 and 500 mg/ml), while the growth of *K. pnemoniae* was inhibited at concentrations of 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibition of 9 mm, 12 mm, and 14 mm respectively while it showed no inhibition at concentrations of 200 mg/ml and 100 mg/ml.

The methanolic seed extract inhibited the growth of *S. aureus* at concentrations of 200 mg/ml, 300 mg/ml, 400mg/ml and 500 mg/ml with zones of inhibition of 7 mm, 10 mm, 15 mm and 20 mm respectively, while at 100 mg/ml it showed no inhibition. *S. pyogenes* also showed sensitivity at concentrations of 300 mg/ml, 400 mg/ml and 500 mg/ml with zones of inhibition of 9 mm, 12 mm and 15 mm respectively. *E. coli*, *K. pneumoniae* and *Ps. aeroginosa* showed no sensitivity to the methanolic seed extract at all concentrations (100, 200, 300, 400 and 500 mg/ml).

For the standard antibiotic discs used, Ciprofloxacin (5 ug) inhibited the growth of *S. aureus, S. pyogenes, E. coli, K. pneumoniae and Ps. aeroginosa* with zones of inhibition of 10 mm, 21 mm, 34 mm, 25 mm and 25 mm respectively; Gentamycin (10 ug) inhibited the growth of *S. pyogenes, E. coli, K. pneumoniae and Ps. aeroginosa* with zones of inhibition of 21 mm, 20 mm, 20 mm and 10 mm respectively, while it did not inhibit the growth of *S. aureus*. Erythromycin (15 ug) inhibited the growth of *S. pyogenes, E. coli* and *K. pneumoniae* with zones of inhibition of 20 mm, 9 mm and 11 mm respectively, while *S. aureus* and *Ps. aeroginosa* showed no sensitivity.

Table 1. The Weight, Colour, Texture and Percentage yield of the Aqueous and Methanolic Extracts of *C. lanatus* Seeds from the Reflux Extraction Process

Parameters	Aqueous Extract	Methanolic Extract
Weight of the Powder (g)	250	280
Weight of the Extract (g)	31.69	15.79
Colour	Coffee brown	Coffee brown
Texture	Crystalline	Crystalline
Percentage yield (%) $^{w}/_{w}$	12.68	5.64

Table 2. Phytochemistry of the Aqueous and Methanolic Extracts of *C. lanatus* Seeds

SN	Plant Constituents/Test	RESULTS (Aqueous and Methanolic extracts)	Observation
1.	Carbohydrates		
	i. General test (Molisch's test)	+	Dull violet
	ii. Test for Monosaccharide (Barfoed's test)	-	No colour change
	iii. Test for Free reducing sugar (fehling's test)	+	Red precipitate
	iv. Test for Combined reducing sugar	+	Reddish-brown precipitate
	v. Test for Ketoses	+	Red colour
2.	Test for Alkaloids		
	i. Dragendorff's reagent	-	No colour change
	ii. Mayer's reagent	-	No colour change
3.	Test for Flavonoids		
	i. Shinoda's test	+	Pink colour
	ii. Ferric chloride test	-	No colour change
	iii. Lead Ethanoate test	-	No colour change
	iv. Sodium Hydroxide test	+	Colourless
4.	Test for Saponins		
	i. Frothing test	-	No foam formed
5.	Test for Anthraquinones		
	i. Free Anthraquinone test (Borntrager's test)	-	No red colour formed
	ii. Combined Anthraquinones (Borntrager's test)	-	No red colour formed
6.	Test for Terpenoids	+	Violet colour
7.	Test for Tannins		
	i. Ferric chloride test	-	No green colour formed
	ii. Lead acetate test	-	No white precipitate formed
8.	Test for Phlobatannins	-	No colour change
9.	Test for Cardiac glycosides		
	i. Salkowaski's test	+	Bluish-green colour
	ii. Liebermann-Burchard's test	+	Reddish brown colour at interphase
10.	Test for Soluble starch	-	No colour change
11.	Test for Cardenolides		
	i. Keller-killiani's test	+	Brown ring at the interphase

Key

-= Absent + = Present

Table 3 Antibacterial Activity of the Aqueous Seed Extract of *C. lanatus* using the Agar Well Diffusion Method

Organisms Used	Concentrations (mg/ml) and diameter of zones of inhibition (mm), ±SEM					Standard Drugs		
	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml	Cipro	Genta	Erythro
S. aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.00	0.00±0.00	0.00±0.00
S. pyogenes	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	21.00±0.00	21.00±0.00	20.00±0.00
E. coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	34.00±0.00	20.00±0.00	9.00±0.00
K. pneumoniae	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.00	20.00±0.00	11.00±0.00
P. aeruginosa	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.00	10.00±0.00	0.00±0.00

Key: Cipro = Ciprofloxacin
Genta= Gentamycin
Erythro = Erythromycin
SEM= Standard error of mean

Table 4. Antibacterial Activity of the Methanolic Seed Extract of *C. lanatus* using the Agar Well Diffusion Method

Organisms Used	Concentrations (mg/ml) and diameter of zones of inhibition (mm), ±SEM					Standard Drugs		
	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml	Cipro	Genta	Erythro
S. aureus	11.00±0.00	13.00±0.00	15.00±0.00	17.00±0.00	19.00±0.00	10.00±0.00	0.00±0.00	0.00±0.00
S. pyogenes	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	21.00±0.00	21.00±0.00	20.00±0.00
E. coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	34.00±0.00	20.00±0.00	9.00±0.00
K. pneumonia	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.00	20.00±0.00	11.00±0.00
P. aeruginosa	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.00	10.00±0.00	0.00±0.00

Key: Cipro= Ciprofloxacin
Genta= Gentamycin
Erythro = Erythromycin
SEM= Standard error of mean

Table 5. Antibacterial Activity of the Aqueous Seed Extract of *C. lanatus* using the Disc Diffusion Method

Organisms Used	Concentrations (mg/ml) and diameter of zones of inhibition (mm)					Standard Drugs		
	100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	500 mg/ml	Cipro	Genta	Erythro
S. aureus	0	0	0	0	0	10	0	0
S. pyogenes	0	0	0	0	0	21	21	20
E. coli	0	0	0	0	0	34	20	9
K. pneumonia	0	0	9	12	14	25	20	11

P. aeruginosa	0	0	0	0	0	25	10	0

Key: Cipro= Ciprofloxacin
Genta= Gentamycin
Erythro = Erythromycin

Table 6. Antibacterial Activity of the Methanolic Seed Extract of *C. lanatus* using the Disc Diffusion Method

Organisms Used	**Concentrations (mg/ml) and diameter of zones of inhibition (mm)**					**Standard Drugs**		
	100 mg/ml	**200 mg/ml**	**300 mg/ml**	**400 mg/ml**	**500 mg/ml**	**Cipro**	**Genta**	**Erythro**
S. aureus	0	0	10	15	20	10	0	0
S. pyogenes	0	0	9	12	15	21	21	20
E. coli	0	0	0	0	0	34	20	9
K. pneumoniae	0	0	9	0	0	25	20	11
P. aeroginosa	0	0	0	0	0	25	10	0

Key: Cipro= Ciprofloxacin
Genta= Gentamycin
Erythro = Erythromycin

DISCUSSION

Various plant extracts have been demonstrated to possess antibacterial activity against microbial pathogens (Mahesh and Satish, 2008). The antimicrobial activity observed could be due to the various phytochemicals present. In this study, the composition and antibacterial properties of the aqueous and methanolic seed extracts of *C. lanatus* were examined. The extracts showed the presence of flavonoids, terpenoids, cardiac glycosides, cardenolides and carbohydrate. These phytochemicals have been shown to possess antimicrobial activity (Compean and Ynalvez, 2014). Plants containing toxic glycosides as reported by Aboaba *et al*. (2006) can get hydrolysed to release phenolics which are toxic to microbial pathogens. Flavonoids have been found to show *in vitro* antimicrobial activity against a wide range of bacteria. This ability is attributed to their ability to complex with extracellular and soluble protein as well as with the bacterial cell wall (Cowan, 1999). Terpenoids are terpenes to which additional elements such as oxygen have been added (Cowan, 1999). Terpenoids have been found to possess antimicrobial activity (Amara *et al.*, 1998; Mendoza *et al.*, 1997). The mechanism of action of terpenoids is not fully understood, but it is been stipulated to involve membrane disruption by the lipophilic compounds (Mendoza *et al.*, 1997). Therefore, the sensitivity shown by the bacteria in this study could probably be attributed to the presence of the above phytochemicals.

Previous studies carried out on watermelon seeds showed the presence of alkaloids, tannins, and saponins, which have been found to possess antibacterial activity against various bacteria (Compean and

Ynalvez, 2014). However, in this study, all these phytochemicals were found to be absent. This as reported by Adunola *et al.* (2015) could be due to the kind of solvents employed as well as the conditions for extraction which can influence the efficacy of the extract against the test organisms. This was evident in a study carried out by Adunola *et al.* (2015) in which the chloroform extracts of *C. lanatus* seed also showed the absence of saponins, tannins and alkaloids, while the aqueous extract showed the absence of only alkaloids. The methanolic extract however showed the presence of all the phytochemicals tested. Another scenario for the effect of extraction process can be seen in the study carried out by Braide *et al.* (2012) using ethanolic, methanolic and aqueous extraction in the absence of heat which showed the presence of all the phytochemicals and all the test microorganisms used showed susceptibility. According to Essien *et al*. (2009) methanol has the ability of dissolving fat during extraction process and watermelon is known to contain a lot of fat (about 40 %), thus this could have resulted in the low antibacterial activity exhibited by the methanolic extract against the tested microorganisms.

Another reason for the absence of alkaloids, saponins and tannins, in this study may be due to the fact that the composition of these secondary metabolites varies from species to species, climatic conditions and physiological state of development of the endemic plants (Hussain and Deeni, 1991).

The lack of sensitivity showed by some of the bacteria used in this study may therefore be attributed to the absence of some the above phytochemicals, and the poor antibacterial activity obtained (i.e. the low zones of inhibitions) may also be due to the low concentrations of the phytochemicals that were found to be present according to Braide *et al.* (2012).

In this study, the Gram-positive bacteria showed more sensitivity than the Gram-negative bacteria to the seed extract. Gram-negative bacteria have outer phospholipid membrane carrying the structural lipopolysaccharides components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible because the peptidoglycan constituting the outer layer is not an effective permeability barrier. Therefore, the walls of Gram-negative bacteria act as a diffusional barrier making them less susceptible to antimicrobial agents than Gram-positive bacteria (Nostro *et al.,* 2000; Hodges, 2002).

K. pneumoniae was the only Gram-negative bacteria that showed sensitivity to the extract. This aligns with the report of Braide *et al.* (2012) in which *K. pneumoniae* was susceptible to the aqueous seed extract of *C. lanatus*. *S. aureus* showed the highest zone of inhibition among all the tested microorganisms. This is in concordance with the reports of Adunola *et al.* (2015); Braide *et al.* (2012); Essien *et al*. (2009) in which *S. aureus* exhibited the highest zones of inhibition among other tested microorganisms. This shows that *S.*

aureus is the most susceptible microorganism to the antibacterial effect of *C. lanatus* seed extract. The methanolic seeds extract showed better activity which is in contrast to the study of Braide *et al.* (2012). This may probably imply that more of the phytochemicals were extracted with methanol.

The *C. lanatus* seed extracts exhibited low antibacterial activity than that of the standard antibiotics used, with the exception of *S. aureus* which was resistant to gentamycin and erythromycin but showed sensitivity towards the methanolic seed extract at concentrations of 500 mg/ml, 400 mg/ml, 300 mg/ml and 200 mg/ml. This resistance to gentamycin is in accordance to the study carried out by Dowding (1977) in which clinical isolates of *S. aureus* were shown to be resistant to gentamycin. Also the resistance of *S. aureus* to erythromycin is in agreement with a study carried out by Vlkova *et al.* (2008). *Ps. aeroginosa* also showed resistance to erythromycin in accordance with a study conducted by Morita *et al*. (2013).

Conclusion

In this study, the aqueous and methanolic seed extracts of *C. lanatus* showed the presence of flavonoids, terpenoids, carbohydrates, cardenolides and cardiac glycosides. The extracts showed poor activity towards some of the tested microorganisms while some showed no sensitivity at all. The Gram-positive bacteria showed more sensitivity than the Gram-negative bacteria with the methanolic extract exhibiting more activity.

This study does not justify the potential of *C. lanatus* as an antibacterial agent but further investigation should be done as it could serve as a source of drug for some microbial infections as it showed the presence of some useful phytochemicals.

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