Comparative Study of the Antibacterial Efficacy of Laboratory Produced Organic Cream from *Citrullus lanatus* Seed Oil and the Seed Oil against Some Clinical Bacteria Isolates

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Abstract

A comparative study of the antibacterial efficacy of organic cream produced in the laboratory from Citrullus lanatus seed oil and the seed oil was carried out against some clinical bacteria pathogens (S aureus, S pyogenes and Ps aeruginosa) from skin. The produced organic cream and the oil from the seed of Citrullus lanatus indicates high level of antibacterial efficacies with all the concentrations (20%, 40%, 60% and 80%) used against the test organisms. The highest antibacterial efficacy of 17.0, 16.0 and 14.0 mm was indicated against S aureus, S pyogenes and Ps aeruginosa at the highest concentration used for the laboratory produced organic cream and 20.0mm against S aureus and 22.0 mm against both S pyogenes and Ps aeruginosa at the highest concentration used for the Citrullus lanatus seed oil. The MIC and MBC study of the organic cream produced in the laboratory from Citrullus lanatus seed oil and the seed oil indicates high efficacy of the test substances against the test organisms. The phytochemical studies of the the seed oil also revealed the presence of different chemical constituents. These study have provided clue to exploit Citrullus lanatus to formulated varieties of organic products.

Key words: *comparative, study, antibacterial, efficacy, Citrullus lanatus, seed oil, produce, organic cream*

INTRODUCTION

Watermelon (*Citrullus lanatus*) belongs to the family Curcubitaceae. The origin of watermelon is in tropical Africa. It is a creeping annual plant with large and rounded or oblong fruit. Emulsion obtained from the seed water extract of watermelon is used to cure catarrhal infections, disorders of the bowel, urinary passage and fever (Erickson, *et al.*, 2015; Reamake, 2011). The seed of watermelon contains various amounts of carbohydrates, phenolic, flavonoids, protein, fibre, phosphorus and iron. Proximate analysis of the seed revealed very high fat content (47.9%),

followed by protein (27.4%) and carbohydrate (9.9%), traditionally seed of *Citrullus lanatus* is said to be medicinal because it can relieve inflammation/irritation caused by increased passing of urine and gives tonic effects (Varghese, *at al.*, 2013; Oyeleke, *et al.*, 2008; Okunrobo, *et al.*, 2012). Antioxidant effect of methanol extract of *Citrullus lanatus* seed reveals that the IC50 values for the antioxidant activity were 28.77µg/ml and 123.8µg/ml for DPPH and lipid peroxidation assay respectively. In addition, the antioxidant activities, total phenolic and flavonoid levels of fermented and unfermented watermelon rind (outer layer) have been investigated. The ripe fruits are edible and largely used for making confectionary, the fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as tonic to the brain (Sathya and Shoba, 2014; Erukainure, *et al.*, 2011; Rahman, *et al.*, 2018).

Most of the information available on the antibacterial activity of *C. lanatus* seed have made it clear that cold maceration is popularly used for extraction of *C. lanatus* plant parts (Omigie and Agoreyo,2014) and in few cases Soxhlet extraction. This has brought about the need to compare extraction methods in order to determine which method will yield the highest antibacterial effect on the organisms.

The seeds of *Citrullus lanatus* were reported to have analgesic and anti-inflammatory properties, anti-ulcerative activity. The antimicrobial activity of chloroform, hexane and ethanol extracts of leaves, stem, fruits and seeds from *Citrullus lanatus* against bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis a*nd *Proteus vulgaris*) and fungi (*Aspergillus nigar* and *Candida albican*) had been studied (Madhavi, *et al.*, 2012; Alok, *et al.*, 2012; Swapuil, 2011). The antimicrobial activity was tested using cup-plate diffusion method and disc diffusion method. Analysis of the data revealed that the chloroform extract of the fruit exhibited the highest antibacterial activity.

BOTANICAL DESCRIPTION

Kingdom: Plantae
Order: Cucurbitales
Family: Cucurbitaceae
Genus: Citrullus
Species: C. lanatus
Botanical Name: *Citrus lanatus*
(Chomicki and Renner, 2014).

Diagram of Watermelon seeds and Oil. (Parsons and Cuthbertson 2011).

STATEMENT OF THE PROBLEM

The society in which we live today is face with the challenges of emerging diseases such as bacteria, fungi, viruses and parasites and with other disease conditions such as diabetes, hypertension, cancer and they have continually to plaque the human race. This is as a result of the interaction between humans with the environment and with the items we consume on a daily bases. The skin being the largest organ of the body, have also become the easiest portal of entry by pathogenic microorganisms to gain asses to the body to cause disease and other disease conditions. This is because the skin is directly expose to the environment and the items we use such as soaps, detergents, creams etc, most of this items are synthetic and can cause toxicity, allergy and can also be carcinogenic (Patel and Rauf, 2017). When infection develops, it becomes difficult to treat with the available synthetic antimicrobial agents. Since humans are constantly interacting with the environment. It will be helpful for humans to interact with items of plant base or from plant products (organic base), so as to eliminate or reduce the challenges of emerging diseases and different disease conditions in our communities. Watermelon (*Citrullus lanatus*) is a plant that's common and available in northern part of Nigeria and can be used for the production of different organic products that are safer and cheaper for consumption than the synthetic ones.

JUSTIFICATION OF THE STUDY

The world have been so colonize by microorganisms, which have continued to cause diseases and hospital admissions and even death and it as becomes evident that this disease and disease conditions are as a result of human interactions with the environment and also, with the items we consume on a daily bases. It therefore became necessary to make our environment safe from microbial colonization and to also search for plant base products or organic products that are more available, safer, affordable and renewable than the synthetic items that toxic, carcinogenic to the body. *Citrullus lanatus* seed oil is helpful in providing profitable uses in the production organic cream and the antimicrobial potentials against microbes from the skin origin. That's why this research work is with the focus to produce organic cream from *Citrullus lanatus* seed oil and to test and compare the efficacy of the produced organic cream with the seed oil against clinical bacteria pathogens from skin origin

METHODOLOGY

STUDY AREA

The Study area of this research work is Chemistry and Microbiology Laboratory of Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State

SAMPLE COLLECTION

The watermelon (*Citrullus lanatus*) seed used in this study was collected within Birnin Kebbi, Kebbi State (Nigeria). The sample was packed in polythene bags, and transported to the department of Science technology for processing.

SAMPLE PROCESSING

The seeds of watermelon (*Citrullus lanatus*) was collected and the seeds were allowed to dry under shade for 14 days. The seeds were then pounded into powdered form using mortar and

pestle. The fine powdered obtained was stored in dark plastic bag at room temperature for further use.

EXTRACTION PROCEDURE

Soxhlet Extraction Method as described by Akbar *et al.* (2009) with slight modification was used. The fine powdered seed were defatted in a soxhlet apparatus. The extraction was carried out by using n-hexane as the solvent. The process was continued for 6 hours. Solvent was removed by vacuum evaporation and exposed to heat in a drying oven at 50°C. The amount of oil recovered was calculated as percentage of total oil present in seed (Kashari, 2021).

MEDIA PREPARATION

All the media used in this research work were prepared in accordance to the Manufacturer's instructions as contained in the label of the container of the media.

PRODUCTION OF CREAM FROM CITRULLUS LANATUS SEED OIL

Reagents used

1. Stearic acid - 100g
   Croda wax - 75g
   Cetfyle alcohol - 50g
   Borax - 50g
   Honey - 50g
   Petroleum jelly - 50g
   Citrullus lanatus oil - 50ml
   Glycerin - 30ml
   Vitamin E - 50ml
   Colour pigment - 1tbs
   Perfume - 40m
   Sodium benzoate - 1teaspoon

PROCEDURE

Step 1

Exactly 4 liters of water was measured, boiled and borax was poured into it for it to dissolve and was properly stirred and the solution was kept until when need.

Step II

Croda wax, steric acid and Cetfyle alcohol were mixed together in a dry pot and was heated until the chemicals melt. Petroleum jelly, *Citrullus lanatus* oil, Glycerin and Vitamin E was then added to the content of the pot

Step III

The prepared borax solution in **Step 1** was mixed with the contents in the pot in **Step II** and Sodium benzoate was added the mixture was stirred together and was allowed to cool down for $1^{1/2}$ hours to obtain the organic cream from *Citrullus lanatus* oil. Perfume and colour pigment was then added to give a desired fragrant and attraction (Maslowski, 2014).

ISOLATION OF BACTERIAL

The organisms used in this research work are clinical isolates obtained from Hospital laboratory in Birnin Kebbi metropolis. The isolates obtained are *Staphylococcus aureus*, *Streptococcus*

pyogenes and *Pseudomonas aeruginosa* and was transported aseptically to microbiology laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State for microbiological confirmatory investigations and for subsequent use.

FORMULATION OF THE DIFFERENT CONCENTRATIONS FOR ANTIBACTERIAL TESTING

About 2m1 of the produced organic cream was placed in 10ml of distilled and was done for 3ml, 5ml and 7ml in other to obtain various concentrations of 20%, 40%, 60% and 80% respectively. The same procedure was repeated for the *Citrullus lanatus* seed oil.

ANTIBACTERIAL TESTING

Agar well diffusion techniques was used to determine the antimicrobial activity of the laboratory produced organic cream and the oil from the seed of *Citrullus lanatus*. The selected strains of bacteria *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* were inoculated on the surface of sterile Mueller Hington Agar plates, after making the wells. The varying concentrations of 20%, 40%, 60% and 80% of the produced organic creams and the *Citrullus lanatus* seed oil were then added to each well contain in separate plates and the plates were then incubated in an upright position at 37^0C for 24 hours. This was done in duplicate. The diameter of the zone inhibition was measured in millimeters and the results were recorded (Cheesbrough, 2000). Pure Egyptician cream was used as control.

DETERMINATION OF MIC AND MBC

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the produced organic cream and the oil from the seed of *Citrullus lanatus* against the test bacteria (*Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*) was determined according to the tube dilution method described by (Kashari, 2021).

PHOTOCHEMICAL ANALYSIS

The the oil from the seed of *Citrullus lanatus* s was screened for the presence of alkaloids, tannins, saponins, flavonoids, glycosides, anthraquinones, glycosides, terpenoids and steroids (Puerta and Cisneros, 2012).

FINDINGS AND DISCUSSIONS

The findings of this research works are presented on tables.

Table. 1: Shows Results of Antibacterial Activity of *Citrullus lanatus Produced Cream* and *Citrullus lanatus Seed Oil* against Bacteria from skin origin.

	Concentration in (mg/ml/%)/ Zone of inh in (mm)				
	20	**40**	**60**	**80**	**Control**
Organic Cream CL					
S aureus	15.0	12.0	17.0	17.0	18.0
S pyogenes	12.0	12.0	10.0	16.0	20.0
Ps aeruginosa	10.0	10.0	14.0	14.0	18.0
Seed Oil CL					
S aureus	10.0	14.0	14.0	20.0	18.0
S pyogenes	14.0	14.0	16.0	22.0	18.0

Ps aeruginosa	12.0	16.0	16.0	22.0	18.0

Key: MM = Millimetre, mg/ml/% = Milligram/m, CL= *Citrullus lanatus*

Table 2: Shows Results of MIC and MBC of *Citrullus lanatus* Seed Oil lab Produced Organic Cream and *Citrullus lanatus* Seed Oil against the test Bacteria

Test Substance	**Concentration in (mg/ml/%)**	
Test Bacteria	**MIC Values**	**MBC Values**
Lab Produced Organic Cream		
Staphylococcus aureus	5.0	10.0
Streptococcus pyogenes	5.0	10.0
Pseudomonas aeruginosa	1.25	2.50
Citrus Lanatus Seed Oil		
Staphylococcus aureus	5.0	10.0
Streptococcus pyogenes	5.0	10.0
Pseudomonas aeruginosa	5.0	10.0

Key: MIC= Minimum inhibitory Concentration,
MBC = Minimum Bactericidal Concentration

Table 3: Shows the Phytochemical Compounds present in *Citrullus lanatus* Seed Oil

Phytochemicals	**Level of Presence**
Alkaloids	++
Flavonoids	+
Tannins	++
Glycosides	-
Saponins	++
Steroids	++
Terpense	+
Anthraquibone	-

Key: ++ = Highly present, + = Slightly present, - = Absent

DISCUSSION

Comparative study of the antibacterial efficacy of the organic cream produced from *Citrullus lanatus* seed oil and that of the oil was determined using agar "well" diffusion method against some selected clinical bacterial isolates (*Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*) from skin origin. The Laboratory produced organic cream indicates the following antibacterial potentials: 15.0, 12.0, 17.0, and 17.0 mm, 12.0, 12.0, 10.0 and 16.0 mm and 10.0, 10.0, 14.0, and 14.0mm with the concentration of 20, 40, 60, and 80% used against *S aureus, S pyogenes* and *Ps aeruginosa* while the *Citrullus lanatus* Oil from the seed shows activity of 10.0, 14.0, 14.0, and 20.0mm, 14.0, 14.0, 16.0 and 22.0mm and 12.0, 16.0, 16.0, and 22.0mm respectively at the concentration of 20, 40, 60, and 80% used against *S aureus, S pyogenes and Ps aeruginosa*. The pure Egyptian Cream used as control also indicates varying degree of activities against the test bacteria. 18.0, 20.0 and 18.0 mm recorded against *S aureus,*

S pyogenes and Ps aeruginosa for the laboratory produced organic cream while 18.0mm was recorded for *S aureus, S pyogenes and Ps aeruginosa* against the seed oil of *Citrullus lanatus*. This research work is in line with the findings Adelani, *et al.,* 2015, who reported *Citrullus lanatus* leaf extracts to have high antibacterial properties against both gram positive and gram negative bacteria. The minimum inhibitory concentration and minimum bactericidal concentration study of the Laboratory produced organic cream and the seed oil of *Citrullus lanatus* indicates that the test substances have both bacteriostatic and Bactericidal abilities against the test organisms (*S aureus, S pyogenes and Pseudomonas aeruginosa*). The phytochemical screening carried out on the seed oil of *Citrullus lanatus* revealed the presence of some important bioactive compounds such as alkaloids, flavonoids, tannins, Saponins, Steroids and Terpenes, glycoside while anthraquinone was absent. This is in line with the findings of Oyeleke *et al.,* (2008), who reported *Citrullus lanatus* to have wide range of phytochemical constituents, saturated and unsaturated fatty acid. The result of this research work has provided suggestion of the possibility of using oil from the seed of *Citrullus lanatus* for the production of organic cream or skin remedies that will help to inhibit or destroy pathogens that can cause skin disease as well as to reduce and replace the use of synthetic products that are carcinogenic to the body.

CONCLUSION

This research work indicates that organic creams and other products can be produce from plant base and organic products can be as effective as the synthetic products. *Citrullus lanatus* seed have provided clue on this study for the use of the oil from the seed to produce organic cream with high antibacterial potentials against the bacterial pathogens under study and the efficacies observed was as a result of the presence of major bioactive compounds present in the *Citrullus lanatus* oil.

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