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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITIES OF *CITRULLUS LANATUS* SEED AGAINST SOME PATHOGENIC MICROORGANISMS

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ABSTRACT

The phytochemical and antibacterial potentials of *C. lanatus* were evaluated. This was carried out by the crude extraction of the seeds with hot water, ethanol and methanol. The extracts were used to verify the occurrence of phytochemicals. Stock cultures of test organism such as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis* and *Streptococcus pyogenes* were used to test the antibacterial effects of the extracts using the agar well diffusion method. The extracts showed presence of antibacterial activities which were compared to antibacterial activity of a commercial antibiotic (Ciprofloxacin) against the test organisms. At 62.5mg, ethanol extract showed a week inhibitory effect against *Proteus mirabilis* (3mm), *Staphylococcus aureus* and *Streptococcus pyogenes* (2mm respectively). Antibacterial activity of the extract was pronounced at higher concentrations (100mg, 500mg and 250mg) for all the extracts. Hot and cold water extracts showed the presence of phenol and methanol extract showed highest bacterial activity. The phytochemical analysis showed the presence of phenol, saponnin, tannin, flavonoid, alkaloid and cyanogenic glycoside. From this research, watermelon seeds when properly extracted and purified, acts as antibiotics which can be used in treatment of infections caused by pathogenic microorganisms in relation to orthodox medicine.

KEYWORDS: *Citrus lanatus* seed, phytochemical analysis, antibacterial activity, pathogenic microorganisms.

INTRODUCTION

Citrullus lanatus (water melon) is the fruit of a plant originally from a vine of Southern Africa. It produces about 93% water; hence name "water" melon [1]. *C. lanatus* is a prostrate animal plant with several herbaceous, firm and stout stems. The leaves are herbaceous but rigid, becoming rough on both sides. The leaf stalks are somewhat having and up to 150 mm long. The tendrils are rather robust and usually divided in the upper part. They are monoecious with the flower stalk up to 4mm long and to 20mm in diameter; the fruit still is up to 50mm long [2].

C. lanatus seeds are increasingly used for their oil in semi-arid regions and also the use of the oil in the cosmetic and pharmaceutical industry is increasing. There are also prospects for use of the seeds in the improvement of infant nutrition in review of their high protein and fat content [3]. In Chinese traditional medicine, watermelon rind is extensively applied to clear away heat to eliminate toxic substances and its extracts are available in powdered form [4]. In Nigeria, watermelon rind is fermented, blended and consumed as juice. High antioxidant activities have been reported on food products in microbial fermentation [5].

One generous slice of watermelon (about $1/16^{th}$ of a melon) contains large amounts of vitamin C and Beta-carotene which may help against various forms of cancer due to their antioxidant properties. Watermelon is also high in potassium which helps regulate heart function and normalize blood pressure. It is a good source of fiber also which helps maintain bowed regularity and works to prevent colon and renal cancer [5]. Emulsion obtained from the seed water extract of watermelon is used to cure catarrhal infections, disorders of the bowel, urinary passage and fever [6]. The plant contains large amount of beta-carotene and it is a natural source of lycopene. It is also rich in citrulline, an effective precursor of L-arginine [6]. Phenolic compounds are constituents of both edible and non-edible parts of the plant. The seeds are sources of protein, tannins and minerals [7].

The antimicrobial compounds found in pants are of interest because antibiotic resistance is becoming a worldwide public health concern in terms of food borne illness and nosocomial infections [8]. The plane kingdom has proven to be the most useful in the world's pharmaceuticals [9]. The most important of these bioactive constituents of plants include

phenol, tannin, saponin, alkaloid, flavonoid, steroids, carotenoids, and cyanogenic glycosides [10]. These phytochemicals constitute the antibiotic principals of plants [9]. They are found to be distributed in plants [11]. Leaves, roots, flowers, whole plants, seeds and stems have being examined in many research projects, few reports refers to seeds as sources for pharmaceutical [12]. Chemical compounds including alkaloids, lectins and phenolic compounds such as lactones, tannins and flavonoids are present in seeds and seed coat [12], and they probably function in the protection of seeds from microbial degradation until conditions are favorable for germination [13] [10]. Many studies suggest that endogenous antioxidant or exogenous antioxidants supplied by diet can function as free radical scavengers and improve human health [14] [15] [16]. Thus consumption of a variety of plant foods including watermelon seeds may provide additional health benefits [17]. Amongst all the amino acids which the body requires, there are some known as essential amino acids which the body cannot produce *C. lanatus* seeds supply some of these acids including tryptophan and glutamic acids.

Effective health cannot be achieved in Africa, unless orthodox medicine is complemented with traditional medicine. At least, 80% Africans depend on plant medicine for their healthcare [18]. Fruits and vegetables have been recognized as natural sources of various bioactive compounds [19] which could be attributed to their phyto-constituent such as flavonoids, fiber and phenolic compounds. One of such medicinal plant is *Citrullus lanatus.* Although several of its uses in traditional medicine have been documented, many of these claims are yet to be validated by scientific researchers. Therefore a review of some investigated phytochemical components and therapeutic activities of the plant are highlighted in this present study.

MATERIALS AND METHODS

Collection and Preparation of the Seeds of *C. lanatus*

C. lanatus was bought from Ariaria International Market Aba, Abia State. They were stored in a conductive atmosphere prior to analysis. The seeds were washed and dried in a SMO5E SHEL LAB oven at 30^0C for 3 days to avoid contamination. The seeds were then grinded with a warring blender and subjected to various extraction techniques.

Extraction of C. Lanatus Seed

The extraction of *C. lanatus* seed were carried out with hot water, cold water, ethanol and methanol leading to the formation of hot water, cold water, ethanol and methanol extracts respectively. About 50g of *C. lanatus* seed were added with 4 conical flasks of 25ml each

(with filter paper imbedded) then 60ml of hot water, cold water, ethanol and methanol were added respectively and allowed to settle for some time. The filtrate of the extracts was obtained by separation of the suspension in the filter paper. Ethanolic and methanolic extracts were allowed to evaporate and stored in an airtight conical flask. The hot and cold water extracts were then neatly separated and also stored.

Phytochemical Analysis

The phytochemical analysis was performed using universal laboratory techniques for qualitative determination [20] [21]. The phytochemical analyzed includes phenols, saponin, flavonoid, alkaloids, tannin and cyanogenic glycoside.

1. Phenol Analysis

2g of the sample was emerged in 20ml of methanol, extracted by filtration through filter paper. 1ml of the filtrate was testes by adding 1ml of Folin-concalteon plus 1ml of 20% $NaCO_3$, the presence of dark blue color shows the presence of phenol.

2. Saponin Analysis

About 20ml of water was added to 10.25g of the specimen in 100ml beaker and boiled gently on a hot water bath for 2 minutes. The mixture was filtered hot and allowed to cool and the filtrate used for frothing test.

Frothing Test

About 5ml of the filtrate was diluted with 20ml of water and shaken vigorously. A stable froth (foam) upon standing indicates the presence of saponins.

3. Flavonoid Analysis

10ml of ethylacetate was added to about 10g of the sample and heated in a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for ammonium test.

Ammonium Test

About 5ml of filtrate was shaken with1ml of solute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonical layer indicates the presences of flavonoids.

4. Tannin Analysis

About 5g of the specimen was boiled with 40ml of water, filtered and used for the ferric chloride test.

Ferric Chloride Test: About 3ml of the filtrate was added to few drops of ferric chloride solution. A greenish black precipitate indicates the presence of tannin.

5. Cyanogenic Glycoside Analysis

Fehling's Test: About 5ml of mixture of equal parts of Fehling's solution I and II were added to about 3ml of the filtrate and boiled for 5minutes. A more dense brick red precipitate indicates the presence of glycoside.

6. Alkaloid Analysis

Meyer's test: Meyer's reagent (mixture of mercuric chloride and potassium iodide dissolved in water) was added to a 5ml of the specimen's filtrate, a greenish white precipitate was formed indicating the presence of alkaloids

Test Organisms and their Screening for Viability

Stock cultures of the test organisms were collected from the Microbiology Unit of Abia State University Teaching Hospital, Aba. The test organisms are *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis* and *Streptococcus pyogenes*. The isolates were screened to confirm their identities. They were sub-cultured on nutrient agar and stored on slant before use [22].

Sensitivity Test

The antibacterial activity of the four (4) extracts of the *C. lanatus* seeds were tested using the Agar well diffusion techniques standardized inocula culture of the respective test organisms was spread evenly on the surface of nutrient agar plates. Wells of 6mm were aseptically punched on the agar using a sterile cork borer allowing at least 30mm between adjacent wells and the Petri dish. Different concentrations of the 4 different extracts (1000, 500, 125 and 62.5mg) of *C. lanatus* seeds were then introduced into the wells. Each extract was screened separately. The plates were incubated at 37^0C for 24hours [23]. Activity was determined by measuring the diameter of the zone of inhibition produced by the extracts against the test organisms. The different concentrations were used for determine the minimum inhibitory concentration using Mueller Hinton Agar.

RESULTS

Table 1 shows the phytochemical components of watermelon seed extracts. The presence of phenol, saponin, tannin, flavonoid and cyanogenic glycosides were observed. Amongst the

observed phyto-components, only cyanogenic glycoside was not present in the ethanol extracts.

Table 1: Phytochemical analysis of C. lanatus seed extracts.

Component	Cold Water Extract	Hot Water Extract	Methanol Extract	Ethanol Extract
Phenol	+	+	-	+
Saponin	-	+	+	+
Tannin	-	-	+	+
Flavonoid	+	+	+	+
Alkanoid	+	+	-	+
Cyanogenic glycoside	+	-	+	-

Key**:** + = present, - = absent

Table 2 shows the zone diameter of growth inhibition of the test organisms by methanolic extracts at different concentrations. There was no inhibitory effect observed against any of the test organisms at 62.5mg/ml concentration. At 125mg/ml, *B. cereus, P. aeruginosa* and *Proteus mirabilis* were not inhibited. There were inhibitory effects against all the test organisms at concentrations of 250-1000mg.

Table 2: Inhibitory effect of methanol extracts of *C. lanatus* seed against pathogens

	Diameter Zone Inhibition (mm)					MIC (Mg/ml)
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	30	17	9	3	0	1.25
Klebsiella pneumoniae	28	18	9	1	0	250
Escherichia coli	31	19	8	3	0	125
Pseudomonas aeruginosa	29	15	6	0	0	250
Bacillus cereus	25	14	8	0	0	250
Proteus mirabilis	20	9	3	0	0	250
Streptococcus pyogenes	24	18	8	4	0	125

The MIC value range from 125-250mg/ml. the zone diameter of growth inhibition of test organism by ethanolic extracts at different concentrations are shown in table 3. Concentrations of 250, 500, and 1000mg/ml inhibited all the organisms. Only *B. cereus* was not inhibited at 125mg/ml concentration while at 62.5mg, only *S. aureus, Proteus mirabilis* and *Streptococcus pyogenes* were inhibited. The MIC value ranged from 62.5-250 mg/ml.

Table 3: Inhibitory effect of ethanol extracts of *C. lanatus* seed against *pathogens*

	Diameter Zone Inhibition (mm)					**MIC (Mg/ml)**
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	29	19	9	5	2	6.25
Klebsiella pneumonia	29	19	8	2	0	125
Escherichia coli	30	18	8	3	0	125
Pseudomonas aeruginosa	20	16	7	2	0	125
Bacillus cereus	28	15	7	0	0	250
Proteus mirabilis	32	21	7	6	3	62.5
Streptococcus pyogenes	30	22	9	5	2	62.5

Table 4 shows the zone diameters of growth inhibition of pathogens by hot water extracts at different concentration. The MIC ranged from 125-250 mg/ml. At 125mg/ml, *S. aureus* and *B. cereus* were inhibited with diameter of growth inhibition 2mm. at 62.5mg, no test organism showed sign of inhibition.

Table 4: Inhibitory effect of hot water extracts of *C. lanatus* seed against *pathogens*

	Diameter Zone Inhibition (mm)					**MIC (Mg/ml)**
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	27	13	7	2	0	125
Klebsiella pneumonia	25	12	6	0	0	250
Escherichia coli	29	14	7	0	0	250
Pseudomonas aeruginosa	25	12	3	0	0	125
Bacillus cereus	24	12	4	2	0	125
Proteus mirabilis	21	10	2	0	0	250
Streptococcus pyogenes	23	9	4	0	0	250

In table 5, the zone diameter of growth inhibition of the test organisms by cold water extracts at different concentrations were shown. At 62.5mg, the test organisms were not inhibited. At 125mg, *S. aureus* and *B. cereus* showed a negligible zone diameter of inhibition (1mm). The MIC value was 250mg/ml.

Table 5: Inhibitory effect of cold water extract of *C. lanatus* seed against pathogens

	Diameter Zone Inhibition (mm)					MIC (Mg/ml)
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	28	15	6	1	0	250
Klebsiella pneumonia	26	13	5	0	0	250
Escherichia coli	27	13	6	0	0	250
Pseudomonas aeruginosa	24	12	15	0	0	250
Bacillus cereus	23	11	5	1	0	250
Proteus mirabilis	20	9	3	0	0	250
Streptococcus pyogenes	20	10	5	0	0	250

Table 6 presents the comparison of the efficacy of different extracts with the standard antibiotic ciprofloxacin. The diameter zones of inhibition produced by the extracts against the test organism were comparable with that of the antibiotic. Some extracts such as methanol produces the same zone diameter (29mm) with the antibiotic against *B. cereus.*

Table 6: Sensitivity test result of the different extract and the standard antibiotics (mm)

Pathogen	C.W.E 1000mg	H.W.E 1000mg	M.E 1000mg	E.E 1000mg	CIP 1000mg
Staphylococcus aureus	28	27	30	29	34
Klebsiella pneumonia	26	25	28	29	36
Escherichia coli	27	29	31	30	38
Pseudomonas aeruginosa	24	25	29	30	32
Bacillus cereus	22	24	29	28	29
Proteus mirabilis	20	21	25	32	30
Streptococcus pyogenes	20	23	24	30	39

Key: C.W.E– Cold Water Extract H.W.E– Hot Water Extract E.E– Ethanol Extract M.E– Methanol Extract CIP – Ciprofloxacin

DISCUSSION

The phytochemical analysis showed the presence of phenol, saponin, flavonoid, alkaloid and cyanogenic glycoside. The presence of these phyto-components has been linked with the antibacterial activity of plants and plants that contain them in higher amount are considered to be superior in their antimicrobial activity [24] [25] [21].

The result of antibacterial activity of the extract against selected human pathogens indicated that the plant sample was active against a wide variety of human pathogenic bacteria. Ethanol extracts exhibited the highest inhibitory effect followed by methanol, hot water and cold water in that trend. This result agrees with the findings made by [26] where ethanol extract proved active in inhibition of the tested organisms than other extraction solvents. The low inhibition effect shown by the aqueous extracts as compared to ethanol and methanol could be due to the fact that these phyto-components are more soluble in ethanol and methanol than in water or that the hot water could have caused the denaturing of the active components.

However, most of the Gram negative organism e.g. *E. coli* showed high susceptibility than most of the Gram positive. The higher susceptibility of the Gram negative bacteria is difficult to explain in the study considering the observation of [27] that the Gram negative bacteria appear to be more resistant to antimicrobial agents than the Gram positive bacteria. This resistance has been observed to reside in the complex cell wall and cell membrane structure. More so, more antibacterial activities were observed with high concentration of the extracts than at lower concentrations. Activity even at low concentration indicates high potency of the extract against the microorganism.

CONCLUSION

These results gotten from the phytochemical analysis and antibacterial activity of the watermelon seed extracts supports the application of the extracts in ethno-medicine and will serve as a good source in pharmaceutical productions against some pathogenic microorganisms.

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