EVALUATION OF THE PHYTOCHEMICAL CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *CITRULLUS LANATUS* (THUNB) SEED METHANOL EXTRACT

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ABSTRACT

The objective of the present work is to evaluate the phytochemical content, in vitro antioxidant potential and antimicrobial activity of *Citrullus lanatus* seed methanol extract. The methanol seed extract was obtained by cold maceration method. Preliminary phytochemical screening was carried out using standard procedures. *In-vitro* anti-oxidant activity of the *Citrullus lanatus* seed extract was estimated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assay. Phytochemical screening showed the presence of reducing sugars, cardiac glycoside, steroidal ring, tannins, flavonoids and saponins while alkaloids and anthraquinones were absent. For DPPH assay, the percentage inhibition range from 0.31 – 9% for *Citrullus lanatus* seed extract 95.13 – 96.54% for ascorbic acid and 59.77 – 95.85 for alpha tocopherol. FRAP assay exhibited 110 µMFe$^{2+/}$g dry material for ascorbic acid and 23.5 µMFe$^{2+/}$g dry plant for *Citrullus lanatus* seed extract. The antimicrobial assay carried out by agar well diffusion method exhibited a potent activity against selected organisms, with the minimum inhibitory concentration for Gram positive bacteria range from 4.32-19.57 mg/ml, while for Gram negative bacteria range from 7.46-14.45 mg/ml and *Candida albicans* is 9.93 mg/ml. *Klebsiella pneumonia* was resistant to the seed extract. The results of this study shows that the methanol extract of *Citrullus lanatus* seed can be used as an easily accessible source of natural antimicrobial agents. However the results revealed that the extract does not possess potent antioxidant activity compared to ascorbic acid and alpha-tocopherol. This may be attributed to environmental factors such as temperature, UV light, solar radiation, soil nutrient and soil water availability which could have impact on the plant constituents.

Keywords: Antimicrobial, antioxidant, *Citrullus lanatus* seed, phytochemical screening.
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INTRODUCTION

Watermelon (*Citrullus lanatus*) belongs to the family Curcubitaceae [1]. The origin of watermelon is in tropical Africa [2]. It is a creeping annual plant with large and rounded or oblong fruit [3]. Emulsion obtained from the seed water extract of watermelon is used to cure catarrhal infections, disorders of the bowel, urinary passage and fever [4]. The seed of watermelon contains various amounts of carbohydrates, phenolics, flavonoids, protein, fibre, phosphorus and iron [5]. Proximate analysis of the seed revealed very high fat content (47.9%), followed by protein (27.4%) and carbohydrate (9.9%) [6], traditionally seed of C*itrullus lanatus* is said to be medicinal because it can relieve inflammation/irritation caused by increased passing of urine and gives tonic effects [5, 7]. Antioxidant effect of methanol extract of *Citrullus lanatus* seed reveals that the IC_{50} values for the antioxidant activity were 28.77µg/ml and 123.8µg/ml for DPPH and lipid peroxidation assay respectively [8]. In addition , the antioxidant activities , total phenolics and flavonoid levels of fermented and unfermented watermelon rind (outer layer) have been investigated [9]. 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 [10]. The seeds of *Citrullus lanatus* were reported to have analgesic and anti-inflammatory properties [11], anti-ulcerative activity [12]. The laxative activity of the fruits was evaluated by Swapnil, [13], antioxidant activity by Naresh, [14], hepatoprotective activity by Madhavi *et al*, [15], and anti-hyperlipidemic activity by Aruna *et al*, [16].

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 aureginosa*, *Bacillus subtilis* and *Proteus vulgaris*) and fungi (*Aspergillus nigar* and *Candida albican*) had been studied [17]. 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. It showed antibacterial activity against *S. aureus*: 36mm, *B. subtilis*: 38mm, *E.coli*: 37mm, *P. valgaris*: 23mm, *P. aerguinosa*: 19mm. Furthermore, literature study reveals the uses of *Citrullus lanatus* fruit and leaves with biological activities such as antioxidants and antimicrobial [17], but the seeds are not in generally given importance. Hence the present study focuses on evaluating the antioxidant and antimicrobial activity of methanol extract of *Citrullus lanatus* seed along with its qualitative phytochemical analysis.

MATERIALS AND METHODS

Chemicals

DPPH (2, 2-diphenyl, 1-picryl hydrazyl), Ascorbic acid, Vitamin E and TPTZ (2,4,6-tripyridyl-s-triazine) were obtained from Sigma Aldrich, USA. All other reagents and chemicals were of analytical grade, obtained from BDH chemicals and Sigma Aldrich Nigeria.

Collection of plant material

Watermelon (*Citrullus lanatus*) fruits were purchased from Mile 12 market, Ketu, Lagos state, Nigeria. The fruits were washed and cut open to obtain the seeds. The whole plant material was subsequently taken to the Department of Botany, University of Lagos where it was properly identified by Mr O.O Oyebanji and a voucher specimen (a reference sample) was deposited with the voucher number LUH 7522. The seeds obtained were washed and shade-dried at room temperature and coarsely-powdered using a blender. Powdered seed material were then weighed and kept in air-tight containers until further usage.

Preparation of seed extract

The powdered seed material was subjected to exhaustive solvent extraction with methanol using cold maceration method for 72 h. The crude extract obtained are filtered using Whattman filter paper and evaporated to dryness using a rotary evaporator at 40°C then stored at 0-4°C in air-tight containers for further use.

Phytochemical screening

Qualitative phytochemical screening of the methanol extract was carried out according to standard procedures [18, 19] to ascertain the qualitative composition of the seed. Phytochemicals screened include reducing sugars, alkaloids, glycosides, cardiac glycosides, anthraquinones, steroids, tannins, flavonoids.

Antioxidant screening

Two methods were employed for the determination of the *in-vitro* antioxidant activities of the methanol extracts of *Citrullus lanatus* seed.

DPPH radical scavenging activity

The ability of the seed extract to scavenge 2, 2-diphenyl-1-picryhydrazyl (DPPH) free radicals was assessed by the standard method [20]. The stock solution of the extract was prepared in methanol to achieve the concentration of 100 µg/ml. Dilutions were made to obtain concentrations of 20, 40, 60, 80 and 100 µg/ml. Diluted solutions (2 ml each) were mixed with 0.5 ml of 1mM DPPH methanol solution in test tubes. The mixture was shaken and allowed to stand for 15 min at room temperature and the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm using UV-Visible Spectrophotometer. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as control. Ascorbic acid and alpha-tocopherol were used as standards. The experiments were carried out in triplicate. Percentage inhibition was calculated using the following equation.

DPPH Scavenging effect (%) = $(A_0 – A_1 / A_0) \times 100$

Where, A_0 = Absorbance of blank sample

A_1 = Absorbance of test sample or standard.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Ferric reducing antioxidant power (FRAP*)*

A modified method of Benzie and Strain, [21] was adopted for the ferric reducing antioxidant power (FRAP) assay. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (III)-TPTZ has an intense blue colour which can be read at 593nm. Freshly prepared FRAP solution containing 25ml of 300 mM acetate buffer, 2.5ml of 10 mM 2,4,6- tripyridyl-5-triazine (TPTZ) in 40 mM Hcl and 2.5ml of 20mM $Fecl_3.6H_2O$ was mixed with 1ml of extract and the absorbance read at 593 nm. The calibration curve was prepared with $FeSO_4.7H_2O$ and was linear between concentration 50 and 500µM. Results obtained were expressed in µM Fe^{2+}/ g of dry plant material and compared with that of ascorbic acid.

Antimicrobial activity

Test organisms: The organisms used comprise four Gram-negative organisms (*Eschericha coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *and Salmonella paratyphi*), two Gram-positive organisms *Staphyloccocus aureus*, *Enterococcus faecalis* and a fungus (*Candida albicans*). The test organisms were obtained from the research laboratory of Medical Microbiology and Parasitology of the College of Medicine, University of Lagos, Nigeria.

Control organisms: Control strains of *Escherichacoli* ATCC 35218, *Enterococcus faecalis* ATCC 29212 and *Staphyloccocusaureus* ATCC 25923 (Remel Ltd, UK) were used and tested along with the organisms.

Standardisation of inoculum: The test organisms were subcultured onto fresh plates of MuellerHinton agar (Oxoid, UK) for 24 h and Saboraud dextrose agar for 5 - 7 days at 37˚C for bacteria and fungi, respectively. Colonies from these plates were suspended in Mueller-Hinton broth (Oxoid, UK) and Saboraud broth (Oxoid, UK) to a turbidity matching 0.5 McFarland standard (10^8 cfu/ml). The media used for antimicrobial assays were Mueller-Hinton agar (Oxoid, UK) for bacteria and Saboraud agar (Oxoid, UK) for fungi. All were incubated appropriately as specified for each organism [22].

Agar well diffusion: Labeled media plates were uniformly seeded with the different test microorganisms, by means of a sterile swab rolled in the suspension and streaked on the plate's surfaces. Wells of 10 mm in diameter and 2 cm apart were punched on the culture media with the sterile cork borer [23]. The stock solution was prepared with methanol to give 768mg/ml and serial dilutions were made using

methanol as solvent to yield the following concentrations 384, 192, 96, 48, 24 12, 6 mg/ml respectively. 100 µl of each extract concentration was transferred into each well. Ciprofloxacin antibiotic USP (0.005%) and neat solvents (methanol) were dropped into each well at a volume of 100 µL. Each plate was kept in the refrigerator at 4^{o}C for 1 h to allow for diffusion of extract, before incubating at 37^{o}C for 18-24 hrs. Zone of inhibition around the wells was measured in millimeters and used as positive bioactivity [23].

Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentrations able to inhibit any visible bacterial growth on the culture plates. The diameter of the zone of inhibition around the well, measured in millimeter, is used as positive bioactivity. This was determined graphically, by plotting zone diameter (in mm) against the log concentration. The straight line obtained is extrapolated to a point equivalent to the diameter of the cup. The antilog of the corresponding concentration was taken as the MIC [24].

RESULTS

The phytochemical screening of the methanol extract of the watermelon seeds revealed the presence of reducing sugars, cardiac glycoside, steroidal ring, tannins, flavonoids and saponins while alkaloids and anthraquinones were absent as shown in (Table 1).

The DPPH assay showed percentage inhibition range from 0.31-9% for *Citrullus lanatus seed* extract, 95.13-96.54% for ascorbic acid and 59.77-95.85 for alpha tocopherol (Figure 1). The Ferric Reducing Antioxidant Power assay (FRAP) of the extract was 23.5 µM Fe^{2+}/g while the positive control ascorbic acid had a value of 110 µMFe^{2+}/g.

The minimum inhibitory concentration (MIC) for Gram positive Bacteria ranges from 4.32- 19.57 mg/ml, for Gram negative bacteria ranges from 7.46-14.45mg/ml and 9.93 mg/ml for *Candida albicans*. *Klebsiella pneumonia* was resistant to the *Citrullus lanatus* seed extract (Table 2).

DISCUSSION

A lot of research is being carried out worldwide directed toward finding natural antioxidants and antimicrobial agents of plant origin. The powdered *Citrullus lanatus* seeds was extracted with methanol, which is an amphiphilic solvent suitable for the extraction of lipophilic and polar compounds from plant material. In this study, cold maceration was used; the advantage of using this method is to yield more substances in the extract because all of the thermolabile and thermostable components will be preserved. The antioxidant and antimicrobial activity of the methanol extract of *Citrullus lanatus* seeds were reported along with the screening of phytochemical constituents. This result indicates that the seeds contain an appreciable amount of secondary metabolites which includes reducing sugars, cardiac glycoside, steroidal ring, tannins, flavonoids and saponins except for alkaloids and anthraquinones. The absence of alkaloids aligns with the work of Rahman *et al* [25].

Table 1: Qualitative phytochemical screening of *Citrullus lanatus* seed extract

Phytochemicals	Methanol extract
Alkaloids	+ ve
Anthraquinones	- ve
Cardiac glycosides	+ ve
Steroids	+ ve
Tannins	+ ve
Flavonoids	+ ve
Saponins	+ ve

+ve = present -ve = absent

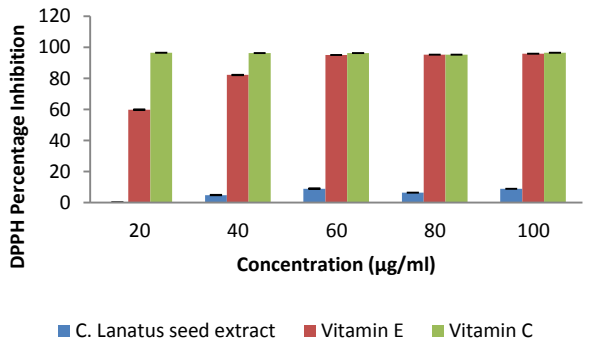

Figure 1: Percentage inhibition of DPPH against concentration of *Citrullus lanatus* seed extracts
SEM: standard error of mean. *n* = 3/group

The secondary metabolites from the seeds may be directly responsible for different activity such as the antioxidant and antimicrobial. Chemical constituents of plants are desirable because such information will be valuable for synthesis of complex chemical substances and to screen for the biological activity [25]. Phytochemicals especially flavonoids present in the extract have received increasing attention because of their biological activities; they constitute a major group of compounds that act as antioxidants [26]. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive.

DPPH is a stable free radical which on addition of antioxidant compounds, the DPPH gets decolorized and can be quantitatively measured from the changes in absorbance. DPPH is a free radical and it produces a strong absorption band at 517nm, in the visible region of the electromagnetic radiation. The colour turns from purple to yellow as the molar absorptivity of the DPPH reduces i.e. when the odd electron of DPPH becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced

DPPH-H [27]. The percent inhibition of DPPH calculated from absorbance is directly proportional to the free radical scavenging activity of test sample. The DPPH test shows that the ability of the seed extracts tested to act as a free radical scavenger is less potent when compared to ascorbic acid and alpha-tocopherol (see figure 1). This result did not align with the study of carried out in India, which showed a maximum percentage inhibition of 96.79% at 1000 µg/ml and minimum percentage inhibition of 48.66% at 1.95 µg/ml of methanol extract of *Citrullus lanatus* seed [8]. The reason(s) for this variation in result may be associated with environmental factors such as temperature, UV light, solar radiation, soil nutrient and soil water availability which could have impact on the plant constituents.

Table 2: Zones of inhibition (mm) of organisms to *Citrullus lanatus* seed extract

	Concentration (mg/ml)									
Test Organisms	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**MIC(mg/ml)**	**Cip**	**Met**
	384	**192**	**96**	**48**	**24**	**12**	**6**		**0.005%**	**99%**
					(mm)					
Gram positive bacteria										
Staphylococcus aureus ATCC 25923	15	13	11	10	10	10	10	15.13	25	10
Staphylococcus aureus	15	14	13	14	12	11	10	4.32	27	10
Enterococcus faecalis ATCC 29212	20	16	13	12	10	10	11	19.59	19	10
Enterococcus faecalis	11	10	10	10	10	10	10	18.62	18	10
Gram negative bacteria										
Salmonella Paratyphi	13	12	11	10	12	10	10	7.46	24	10
Klebsiella pneumonia	N	N	N	N	N	N	N	N	26	12
Escherichia coli ATCC 35218	15	14	14	13	11	10	10	7.73	26	12
Escherichia coli	14	13	11	11	10	10	10	12.73	11	10
Pseudomonas aeruginosa	12	10	11	10	10	10	10	14.45	19	10
Fungus										
Candida albicans	25	20	18	15	12	10	10	9.93	0	12

Met = Methanol; Cip = Ciprofloxacin; N= non-active; D1-D7= Extract concentration

For example, in experiments on effect of altitudinal variation study in genetically uniform plants, significant variation in phenolic contents was found. Furthermore the results were confirmed by antioxidant activity which confirmed that higher antioxidant activity is found in extract of plants at higher altitude compare to plants at lower altitude [28, 29].

FRAP (Ferric reducing antioxidant power) is one of the most rapid test and very useful for routine analysis. The antioxidant activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent. The reducing ability of the extract (23.5 µM Fe^{2+}/g) shows that it is less active when compared to ascorbic acid (110 µM$Fe^{2+/}$g). The decreased reducing power in the

extract indicated that some components in the extract were not donating electron that could react with the free radicals to convert them into more stable products to terminate radical chain reaction.

The extract had potent activity against *Staphylococcus aureus,Staphylococcus aureus* ATCC 25923, *Salmonella paratyphi, , Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis, , Escherichia coli* ATCC 35218, *Escherichia coli,Pseudomonas aeruginosa* and *Candida albicans* from 48 -384 mg/ml based on the zone of inhibition except for *Klebsiella pneumonia* which was non susceptible to the extracts. The extra coating on the Gram negative organism did not prevent the penetration of the seed extract to inhibit the bacteria; hence the susceptibility of the test organisms. Ciprofloxacin was more potent as an antibacterial agent when compared to the diluted extracts. Saponins which were among the seed extract phytoconstituents have been reported to have antibacterial effect against Gram positive bacteria but not against Gram negative organisms [30]. The antibacterial activity of *Citrullus lanatus* seed against the *Staphylococcus aureus* in the present study may be ascribed to the presence of flavonoids, tannins and saponins and this was corroborated by the report of Soetan *et al* [30]. Thus the study confirmed a strong antimicrobial activity of *Citrullus lanatus* seed due to the presence of saponins and other phytochemical present in the extracts.

CONCLUSION

The *Citrullus lanatus* seed methanol extract showed a less potent antioxidant activity when compared to ascorbic acid using DPPH scavenging and Ferric reducing antioxidant power assays but exhibited a potent antimicrobial activity against selected organisms. The MIC for Gram positive bacteria ranges from 4.32-19.57 mg/ml, while for Gram negative bacteria range from 7.46-14.45mg/ml and 9.93 mg/ml for *Candida albicans*. *Klebsiella pneumonia* was resistant to the *Citrullus lanatus* seed extract. In addition, the extract was found to contain phyochemicals which include reducing sugars, cardiac glycoside, steroidal ring, tannins, flavonoids and saponins while alkaloids and anthraquinones were absent. The results of this study show that the methanol extract of *Citrullus lanatus* seed can be used as an easily accessible source of natural antimicrobial agents.

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