International Journal of Chemical, Material and Environmental Research 2016, 3 (3): 62-67

Research article

IJCMER
www.ijcmer.org

INTERNATIONAL JOURNAL OF CHEMICAL, MATERIAL AND ENVIRONMENTAL RESEARCH

An International Open Free Access, Peer Reviewed Research Journal

ISSN 2410-5945
2016, Vol. 3, No. 3:
pg. 62-67

Potentials of *Citrullus lanatus* Seeds as Antioxidant and Antimicrobial Agents and a Probe of their Phytochemicals

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(Received: August 11, 2016; Accepted: September 01, 2016)

Abstract

The phytochemicals, vitamins, proximate and mineral elements composition of *Citrullus lanatus* seeds were analysed by employing standard analytical methods. Phytochemical result showed the presence of alkaloids (0.35 %), saponins (0.45 %), tannins (1.09 %), flavonoids (0.36 %) and phenols (0.50 %). Vitamins composition was ascorbic acid (2.58 %), riboflavin (0.17 %), niacin (1.94 %), β-carotenoids (5.33 %) and thiamine (0.09 %). Proximate result showed crude fibre (5.25 %), lipids (4.60 %), ash (13.73 %), moisture (1.62 %) and protein (13.85 %). The sample showed the presence of mineral elements such as Ca (3.81 %), Mg (0.72 %), K (0.46 %), P (0.49 %), Fe (6.44 %), Cu (0.03 %), Zn (0.65 %) and Na (0.18 %). The oil fraction of the extract showed appreciable antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* and *Penicillum notatum*. Zones of inhibition were compared with that of ciprofloxacin used as a standard antimicrobial agent. Using α,α-diphenyl-β-picrylhydrazyl (DPPH) method, the extract was found to possess free radical scavenging activity at minimum and maximum concentrations of 4.0 and 20.0 mg/mL, corresponding to 6.27 and 48.10% inhibitions, respectively. Ascorbic acid was used as a standard free radical scavenger and its results compared with that of the extract. The potent antimicrobial and free radical scavenging activities shown by the seed extract of *C. lanatus* could be as a result of the bountiful presence of phytochemicals in them, suggesting the potential application of these seeds as antioxidant and antimicrobial agents.

Keywords: *Citrullus lanatus*, antioxidant, antimicrobial, phytochemicals, seed extract

1. Introduction

Plant extracts have played a significant role in disease prevention and therapy. They continue to be of paramount importance in herbal medicine and some have undergone specific modifications such that they could be used as tablets and infusions. While the efficacy of therapeutic values of certain plants and their extracts has been authenticated, others are still undergoing screening for their potential use as antimicrobials, antioxidants, anti-inflammatory, antihypertensive, antiasthma and anti-diabetics among other uses. This is why *Citrullus lanatus* (Thunb.) has been selected for the study. *C. lanatus* commonly known as watermelon derived its name from the fact that it produces a fruit that is about 93% water [1]. *C. lanatus* is a herbaceous creeping plant. It belongs to the family *Cucurbitaceae*, which produces from 3 to 5 fruits weighing from 3 to 10 kg [2]. *C. lanatus* fruit is round, oval or oblong, with a light green to a very dark green skin, variously patterned or striped and red, yellow or orange flesh. The seeds are flat and smooth, varying in size and may be white tan, brown, black red, green or mottled. The fruit has a smooth exterior rind (green, yellow and sometimes white and a juicy, sweet interior flesh) [3]. *C. lanatus* is thought to have originated in Southern Africa because it is found growing wild throu-

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Published online at www.ijcmer.org

ghout the area, and reaches a maximum diversity of forms there. It has been cultivated in Africa for over 4,000 years [4]. *C. lanatus* has been used in traditional herbal medicine for the treatment of a good number of diseases. The seeds can be bruised and rubbed up with water to form an emulsion, which can be used to cure catarrhal infections, disorders of the bowels, urinary tract infections, and fever. It is also being used as worm expeller to expel tapeworms and as a natural Viagra [5, 6]. The fruit is diuretic and effective in the treatment of fever, hypertension, dropsy and renal stones [7], alcoholic poisoning and diabetes [8]. The leaves are good antimalarial, analgesic, anti-inflammatory, mosquitocidal, and antimicrobial agents and can be used in the treatment of gonorrhoea [7]. *C. lanatus* has also been used in the treatment of the sore eye, scabies, itches, ulcer, pain, constipation and malaria [7, 9]. The seeds are used for oil production at the subsistence level in several African countries, and in the Middle East. They can also relieve inflammation and give tonic effects.

It is noteworthy that *C. lanatus* is primarily harvested for juice and juice concentrates as an excellent source of vitamin C and vitamin A [10]. The fruits are widely eaten without much attention to the seeds which are discarded either as cheap animal feed or simply thrown away irrespective of its herbal therapeutic uses as mentioned above. That is to say, the seeds are underutilized. This, of course, informed our interest to investigate the potentials of *C. lanatus* seed extract as an antioxidant and antimicrobial agents as well as to probe its phytochemical constituents which are hereby reported.

2. Materials and Methods

2.1. Sample Collection and Preparation

C. lanatus fruits were bought at Ariaria International Market Aba, Abia State, Nigeria. They were taken to the Taxonomy Unit of the Department of Forestry, Michael Okpara University of Agriculture, Umudike, for identification. The fruits were washed with clean tap water and sliced open with the aid of a sharp and clean kitchen knife. The seeds were taken out, washed and then dried in a laboratory oven at 60^{o}C for 48 h. The seeds were ground with a warring blender to obtain the powdered sample.

2.2. Extraction of Oil

In a typical extraction of oil from the seeds of *C. lanatus*, 200 g of the powdered sample was introduced into a conical flask and 500 mL of methanol was added followed by continuous stirring which lasted for about 5 h. The flask was covered with filter paper and cotton fastened with a paper tape to make it airtight. Stirring continued the next day for another 5 h. The mixture was separated using a Whatman No. 1 filter paper. The process was repeated on the residue to ensure exhaustive extraction. The filtrate was pooled together and the solvent was eventually evaporated to get the oil.

2.3. Determination of Plant Chemicals

Alkaloids and phenols were determined according to the method of Harborne [11] while tannin was determined using the method of Van-Burden and Robinson [12]. Saponin was determined using the method of Obadoni and Ochuko [13]. Flavonoids were determined according to the method of Boham and Kocipia [14]. Ascorbic acid was determined using the method of the Association of Vitamin Chemists described by Kirk and Sawyer [15]. The B-complex vitamins (thiamin, riboflavin, and niacin) were determined according to SKALAR Analyzers method of Baraket *et al.* [16] while carotenoid was determined according to the method described by James [17]. The macro and micro elements comprising potassium, sodium, magnesium, calcium, phosphorus, nitrogen, iron, copper and zinc were determined according to the method of Shahidi *et al.* [18]. Protein, crude fibre, lipids, ash, moisture, carbohydrate and gross food energy contents were determined by the method described by James [17].

2.4. Antioxidant Activity Determination

The free radical scavenging activity of the oil fraction of the sample extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) method described by Man-zocco *et al*. [19]. 1.0 g of DPPH, a stable radical was dissolved in 100 mL of methanol. 3.0 mL of different concentrations of the test sample were added to 3.0 mL of a 0.004 % methanol solution of DPPH and incubated for 30 min at room temperature. The decrease in absorbance of the solution brought about by the test samples was measured at 517 nm using a spectrophotometer. Ascorbic acid, which is a known antioxidant, was used as a reference standard. The radical scavenging activity was calculated as the percentage inhibition of DPPH discoloration using Equation 1.

$$\%\ Inhibition\ of\ DPPH\ radical\ = \left[\frac{\left(A_{blank} - A_{sample}\right)}{A_{blank}}\right] \times 100 \qquad 1$$

where A_{blank} is the absorbance of the control reaction solution (containing all reagents except the test sample) and A_{sample} is the absorbance of the test sample.

2.5. Antimicrobial Screening

The *in vitro* antimicrobial activity of the sample extract was carried out for 24 h culture of three selected each of bacteria and fungi. The test organisms were obtained from stock cultures at the Central Laboratory services Unit of National Root Crops Research Institute, Umudike, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilized in an autoclave at 121^{o}C for 15 min. They were however used within 48 h of production. The sensitivity of each test microorganism to the compound was determined using the Disc Diffusion Technique [20, 21]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the inoculum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs were carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 h in an incubator at 37^{o}C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimeter rule.

2.6. Statistical Analysis

Data were replicated three times and statistically analysed and expressed as mean ± SD.

3. Results and Discussions

The phytochemical constituents of *C. lanatus* seeds are shown in Table 1. The seed showed the presence of alkaloids, saponins, tannins, flavonoids and phenols in appreciable amounts. Flavonoids have been reported to possess anti-hypertensive effects and substantial anti-carcinogenic and antimutagenic activities due to their anti-oxidant and anti-inflammatory properties [22]. Alkaloids and tannins have been reported to possess antimicrobial activity [22, 23]. Saponins have antioxidant, anti-inflammatory, anti-apoptosis and immunostimulant properties [24]. The vitamins composition of *C. lanatus* seeds is shown in Table 2. The result showed that the seeds are good source of vitamins such as ascorbic acid, riboflavin, niacin, β-carotenoid and thiamine. β-carotenoid was the highest vitamin determined followed by ascorbic. β-carotenoid has the ability to quench singlet oxygen and thus function as antioxidants. Carotenoids may modulate processes related to mutagenesis, cell differentiation, and proliferation, independent of their role as antioxidants or precursors of vitamin A [25]. Ascorbic acid is used in wound healing. Ascorbic acid prevents scurvy which is symptomized by skin hemorrhages, bleeding gums, fragile bones and death [26].

Table 1. Phytochemical Composition of *C. lanatus* Seeds

Phytochemicals	Composition (%)
Alkaloids	0.35 ± 0.01
Saponins	0.45 ± 0.03
Tannins	1.09 ± 0.02
Flavonoids	0.36 ± 0.06
Phenols	0.50 ± 0.06

Table 2. Vitamins Composition of *C. lanatus* Seeds

Vitamins	Composition (%)
Ascorbic acid	2.58 ± 0.40
Riboflavin	0.17 ± 0.03
Niacin	1.94 ± 0.06
β-Carotenoid	5.33 ± 0.60
Thiamine	0.09 ± 0.01

Proximate analysis showed the presence of significant amounts of protein, crude fibre, lipids and ash contents (Table 3). Protein is important in growth and repair of worn-out tissues. Consumption of foods high in dietary fibre has been associ-

ated with the lower risk of several gastrointestinal diseases [25]. Fibre was fairly present in the seeds of *C. lanatus* suggesting the seed will play a role in the management of gastrointestinal diseases. The seeds also are a promising source of mineral elements such as calcium, magnesium, potassium, phosphorus, iron, copper, zinc and sodium (Table 4). Iron was the mineral in highest amount followed by calcium. Iron is a component of haemoglobin, the oxygen-carrying pigment of the red blood cells which helps in blood-building in the body. The deficiency of iron causes anaemia [25, 27]. Calcium is quantitatively the largest mineral in the body and in the ionic form regulates transport across the cell wall. It is also involved in blood clotting [27]. The seed extract of *C. lanatus* is indeed rich in phytochemicals, vitamins, and mineral elements. Phytochemicals in plant-based foods afford a myriad of pharmacological and therapeutic benefits.

Table 3. Proximate Compositions of *C. lanatus* Seeds

Constituents	Composition (%)
Crude fibre	5.25 ± 0.76
Lipids	4.60 ± 0.40
Ash	13.73 ± 0.31
Moisture	1.62 ± 0.09
Protein	13.85 ± 0.91

Table 4. Mineral Composition of *C. lanatus* Seeds

Mineral Elements	Composition (%)
Calcium	3.81 ± 0.20
Magnesium	0.72 ± 0.07
Potassium	0.46 ± 0.01
Phosphorus	0.49 ± 0.01
Iron	6.44 ± 1.47
Copper	0.03 ± 0.02
Zinc	0.65 ± 0.01
Sodium	0.18 ± 0.02

The antimicrobial activity of *C. lanatus* seed extract is shown in Table 5. The extract was found to be efficacious in inhibiting the growth of *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, *A. niger* and *P. notatum*. Zones of inhibition were compared with that of ciprofloxacin used as a standard antimicrobial agent. The extract showed the highest sensitivity against *C. albicans* followed by *P. notatum* and least against *S. aureus*. Flavonoids are effective antimicrobial substances *in vitro* against a wide array of microorganisms by inhibiting the membrane-bound enzymes [23]. Tannins have been reported to possess antimicrobial activity [22, 23]. Alkaloids also are commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms [22]. The presence of sufficient quantities of alkaloids, flavonoids, and tannins in the seed extract of *C. lanatus* could be responsible for the antimicrobial activity exhibited by the extract. The ability of the extract to exhibit potent antimicrobial activity against these pathogens justifies the medicinal use of the seed extract to cure catarrhal infections, disorders of the bowels, urinary tract infections and fever [5, 6].

Table 5. Antimicrobial Activity of *C. lanatus* Seed Extract

Microorganism	Extract (mm)	Ciprofloxacin (mm)
S. aureus	7.00 ± 1.41	18.00 ± 1.6
E. coli	9.00 ± 0.50	17.00 ± 0.50
P. aeruginosa	14.00 ± 1.70	16.00 ± 1.71
C. albicans	20.00 ± 1.60	29.00 ± 1.61
A. niger	13.00 ± 1.41	21.00 ± 1.05
P. notatum	15.00 ± 0.05	19.00 ± 0.05

Table 6: Free Radical Scavenging Activity of *C. lanatus* Seed Extract

Concentration (mg/mL)	Radical Scavenging (%)	
	Extract	Ascorbic acid
4.0	6.27 ± 0.03	13.00 ± 0.99
8.0	16.31 ± 0.11	21.10 ± 0.16
12.0	32.91 ± 1.86	37.11 ± 0.91
16.0	44.01 ± 0.01	83.00 ± 0.02
20.0	48.10 ± 0.05	86.20 ± 0.13

The free radical scavenging activity of *C. lanatus* seed extract is shown in Table 6. The extract exhibited significant free radical scavenging activity at minimum and maximum concentrations of 4.0 and 20.0 mg/mL compared to the ascorbic acid used as a standard free radical scavenger. DPPH acts a stable free radical and gives a strong absorption band at 517 nm in the visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption

reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract [28]. Phenolics considered as the largest group of phytochemicals have been reported as accounting for most of the antioxidant activity of plants or plant products [29]. The phytochemical analysis of *C. lanatus* seeds showed tannins and phenols to be in highest amounts. The presence of phenols, flavonoids, and tannins in the seed extract of *C. lanatus* could be responsible for the free radical scavenging activity observed. Flavonoids and tannins are phenolic compounds which have been reported as primary free radical scavengers [30]. β-carotenoid has also been reported to possess antioxidant activity [25]. The DPPH scavenging activities of *C. lanatus* seed extract showed a good correlation with its high ascorbic acid and β-carotenoid contents which of course were the highest analytes detected as shown in Table 2. These facts suggest that the seed extract of *C. lanatus* could be employed in the management of health challenges mediated through oxidative stress and free radical activities.

4. Conclusions

The seed of *C. lanatus* which is used in traditional herbal medicine to cure catarrhal infections, disorders of the bowels, urinary tract infections and fever was analysed and found to contain appreciable quantities of phytochemicals and vitamins as well as mineral elements. The extract exhibited potent antimicrobial and free radical scavenging activity. The medicinal value of *C. lanatus* seed extract could be a function of its bioactive phytochemicals and vitamins constituents that produce definite physiological action in/on the human body.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. I. K. Ndukwe of Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, for identifying and authenticating the plant sample.

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