

## Proximate, Selected Metals and Metabolites Profile of *Citrullus lanatus* (Watermelon) Leaves

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**Abstract:** Herbal products have gained global importance because they have limited side effects, available and affordable compared with orthodox medicine. *Citrullus lanatus* (Water Melon) leaf is popular in traditional medical remedy for respiratory tract infections and diabetes. This research is focused on the proximate, metals and metabolites assessment of a methanolic extract obtained from *Citrullus lanatus* leaves, with the aim of substantiating on its ethno medicinal claims. The results of the proximate analysis indicated high moisture content ( $82.6 \pm 0.01$  %), crude fat ( $14.21 \pm 0.02$  %), moderate fibre content ( $30.23 \pm 0.02$  %), ash ( $6.46 \pm 0.01$  %), protein ( $3.58 \pm 0.02$  %), and moderate carbohydrate content ( $37.12 \pm 0.01$  %) while metal screening showed the presence of iron ( $0.010 \pm 0.03$  %), manganese ( $0.002 \pm 0.02$  %), calcium ( $0.820 \pm 0.02$  %), phosphorus ( $0.163 \pm 0.01$  %), and potassium ( $2.11 \pm 0.02$  %) which shows that Potassium is highest, while manganese occurred in the least amount. Metabolite screening showed that the leaves contained 15 metabolites including: Stearic acid, 3-(octadecyloxy) propyl ester (17.9 %), N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic acid, benzyl ester (23.6 %), Olean-12-ene-3,15,16,21,22,28-hexol, (3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ )- (27.1 %). The presence of Olean-12-ene-3,15,16,21,22,28-hexol (3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ ) in this extract is instructive due to its anti-tumorigenic properties. Intriguingly, the proximate, metal and metabolite screening of the methanolic extract of the leaves of *Citrullus lanatus* has substantiated its ethno medicinal claims

**Keywords:** *Citrullus lanatus*, Proximate, Metals, Metabolites

### 1. INTRODUCTION

Any plant containing substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs is considered a medicinal plant. Medicinal plants or medicinal herbs have been discovered and used in *traditional medicine* practices since prehistoric times, with one of the earliest historical records dating to 5000 years ago [1-2]. Most compounds in plants are categorized into four major biochemical classes - *polyphenols*, *glycosides*, *terpenes* and *alkaloids* [3-4]. Polyphenols include flavonoids, tannic acid, and ellagitannin, some of which have been used historically as dyes and for tanning garments [5]. Many plants store chemicals in the form of inactive glycosides which can be activated by enzyme hydrolysis. Enzyme hydrolysis reaction breaks off the sugar part of the compound, making available bioactive species that could be effectively used as medications. *Citrullus lanatus*, commonly known as *Watermelon*, belongs to the family Cucurbitaceae. It is native to Africa and introduced to Asia, Europe, and the Americas [6]. *Citrullus lanatus* species are separated into seeded and seedless types with at least 1000 varieties [7]. *Citrullus lanatus* is botanically considered as a fruit but commonly classified as a vegetable and used primarily as a dessert. Fruit can be round, oval, or oblong, with a light green to very dark thick green rind, differently spotted or striped as shown in Figure 1.

Its young plant is densely woolly with yellowish-brown hairs which disappear as the plant ages. The leaves are stemmed and are alternate, large and pinnately lobed but they become stiff and rough when old. The plant has branching tendrils, deeply notched leaves, prominent veins, deep lobes, with many tendrils [8-9].



**Figure 1.** *Citrullus lanatus* leaves and fruits [10]

In folk medicine, *Citrullus lanatus* extract is used as purgative and emetic, vermifuge, demulcent and diuretic tonic [11]. Research has reaffirmed its antimicrobial, anti-plasmodial, anti-inflammatory, anti-Prostatic, Hyperplasia, antioxidant and analgesic properties [12-13]. Islam and others [14] investigated the analgesic and antioxidant potential of methanolic extract of water melon rind and ethyl acetate extract of the flesh on Swiss Albino mice. They reported that the extracts possess potential antioxidant and analgesic activities and might be used as sources of nutraceuticals or functional foods. In 2022, Islam et al investigated and reported the antioxidant and antimicrobial effects of the peel, rind, pulp, and seeds of *Citrullus lanatus* (watermelon) [15].

Muhammad *et al.* [16] reported that *Citrullus lanatus* leaf extract treatment could effectively decrease complications associated with diabetes. Wapa and others [17] affirmed the use of *Citrullus lanatus* leaf extract in the treatment of respiratory tract infections caused by bacteria. Akintunde and Thomas [18] reviewed studies published on the medicinal importance of different parts of *Citrullus lanatus*. Their report showed that the melanin found in the crude chloroform, hexane, and ethanol leaf extracts of *Citrullus lanatus* is a good antibacterial agent due to the presence of tannins, saponins, flavonoids, cyanogenic glycosides found in the extract. There is a plethora of research done on various parts of *Citrullus lanatus*.

However, the present study was carried out in order to assess the proximate, metal, and metabolite contents of *Citrullus lanatus* leaves freshly harvested from a farmstead in Angalabiri community, Bayelsa State.

## 2. MATERIALS AND METHODS

### 2.1. Materials

All chemicals used in this study were of analytical grade (BDH, Labtech chemicals and Kermel.) and they were used without further purification.

### 2.2. Methods

Samples of *Citrullus lanatus* (watermelon) leaves were collected from a farm in Angalabiri, Sagbama Local Government Area of Bayelsa state, Nigeria. Samples were properly identified at the Biological Sciences Department of the University of Africa, Toru-Orua. Samples were air-dried for twenty-one day, pulverized electronically and stored in a desiccator for further use.

#### 2.2.1. Proximate Analysis

Standard procedures as described by the Association of Official Analytical Chemists [19] were used in the determination of moisture, Fat, Crude fibre, Ash, Crude Protein, and Carbohydrates.

*Determination of Moisture Content*

A sample of 5 g was weighed into a pre-weighed Petri dish  $W_1$ . The weight of the Petri dish and the sample was taken and recorded as  $W_2$ . The Petri-dish was placed into a preset oven of 105 °C for 3 hrs. to reduce the moisture content. The Petri dish was taken out and placed inside a desiccator to cool for 30 minutes and the weight of the sample was recorded as  $W_3$ .

*Determination of Fat Content*

Approximately, of 5 g of the sample was weighed into a pre-weighed filter paper, weighed, oven dried, and tied with thread. The filter paper containing the sample was placed in the receiver of a Soxhlet apparatus. A 500 ml round bottom flask filled with 3/4 with n-hexane of boiling point 60 °C was fitted to the Soxhlet apparatus with a reflux condenser and placed in an electro mantle heater. Extraction began as the solvent refluxed several times and continued for 4 hours until the condenser detached. The filter paper containing the defatted sample was removed and dried to a constant weight in an oven at 50 °C. The difference in weight before and after extraction was recorded as the value of the extracted crude fat.

*Determination of Crude Fibre Content*

A defatted sample of 2 g was weighed and transferred into a 500 ml conical flask. 200 ml of 1.25 %  $H_2SO_4$  was added and the mixture boiled for 30 minutes using cooling fingers to maintain a constant temperature. The mixture was filtered using a butcher funnel rinsed with hot distilled water. The filtrate was transferred into a conical flask containing 200 ml 1.25 % NaOH and boiled for another 30 minutes and stirred with a magnetic stirrer. The sample solution was filtered and initially washed with hot distilled water and later with 1 % HCl, respectively. The washing was repeated twice with ethanol and twice with petroleum ether in order to remove any remaining fat. The residue was transferred into a clean dried crucible, oven dried, cooled in dessicators and weighed ( $W_2$ ). The crucible was placed in the muffle furnace at 450 °C for 2 hrs., cooled in dessicators and reweighed ( $W_3$ ).

*Determination of Ash Content*

About 2 g of the sample was weighed into a clean pre-weighed crucible. The crucible with the sample was placed in a muffle furnace at 500 °C for 3 hrs. The crucible with the ash was cooled in a desiccator and weighed.

*Determination of Crude Protein Content*

The crude protein was determined in 3 stages;

a) Digestion Stage:

This stage involves the digestion of the sample. 15 g of the sample was digested with 10 mL  $H_2SO_4$  in a micro-Kjeldahl digestion flask. Approximately, 0.5 g selenium was added as catalyst and the mixture was heated on an electro-thermal heater until a clear solution was obtained. The flask was allowed to cool and the digest was diluted with distilled water in a 100 ml standard flask. The sample was transferred to the Kjeldahl distillation unit.

b) Distillation Stage

10 ml of 40 % NaOH solution was added to the digest to release ammonia. 3 drops of mixed indicator were added to the receiving flask containing 10 ml of 2 % boric acid solution to give a pink colour solution. The sample was distilled until about 50 ml of the distillate was collected in the receiving flask. A colour change from red wine to green was observed indicating the presence of ammonia.

c) Titration Stage

The resulting solution was titrated with 0.1 M HCl solution until a colour change was observed (green to red wine) which indicates the end point.

*Determination of Carbohydrate Content*

Carbohydrate content was determined by difference. The summation of protein, crude fat, moisture content, ash, and crude fibre was subtracted from 100.

### 2.2.2. Metal Analysis

Standard procedures as described by the Association of Official Analytical Chemists [19] were used to determine Manganese (Mn), Iron (Fe), Calcium (Ca), Phosphorus (P), and Potassium (K).

Standard solutions were prepared separately for Manganese, Iron, Calcium, and Phosphorus (P), and values determined with an atomic absorption spectrophotometer. Measured values were plotted against the strength of solution. Thereafter, values of the various digests were measured by Atomic Absorption Spectrophotometry and the strength traced on the respective standard curves to give the corresponding values which provided the original values of the element present in the digest.

#### Determination of K using Flame Photometer

Standard solution of potassium was prepared using KCl. The standard solution was measured with the flame photometer and the value obtained was plotted against the strength of the solution. The value of the digest was measured by the flame photometer and the strength traced on the standard curve to give the corresponding value which gave the original value of the element present in the digest.

### 2.2.3. Metabolites

The chemical composition of the methanol extracts of *Citrullus lanatus* leaves were identified using GC (Agilent 6890) connected to a mass spectrophotometer (5973 MSD) fitted with Restek capillary column with a length of 30 m, 0.53 mm inner diameter and 0.12  $\mu\text{m}$  film thickness operating in electron impact mode at 60 eV. Helium (88.799 %) was used as a carrier gas at a constant flow of 1 ml /minute. 0.5 ml of sample solution was injection in the splitless mode, with a split ratio of 10:2. Injector temperature was 200  $^{\circ}\text{C}$  and the ion source temperature was 230  $^{\circ}\text{C}$ . Mass spectra were recorded at 60 eV with an interval of 0.5 seconds and fragments from 50 to 650 da. Total running time of the Gas Chromatography-mass spectrometer (GC-MS) was 45 minutes and 20 seconds.

## 3. RESULTS

The results of, proximate and mineral analyses on *Citrullus lanatus* (Watermelon) leaves are shown in Tables 1 and 2. The Gas chromatography Mass spectroscopy (GC-MS) results are presented in Table 3, while the chromatogram is shown in Figure 2.

**Table1.** Proximate Analyses of *Citrullus lanatus* leaves

Proximate Principles	Mean $\pm$ S.D
Moisture	82.6 $\pm$ 0.01
Fat	14.21 $\pm$ 0.02
Fiber	30.23 $\pm$ 0.02
Ash	6.46 $\pm$ 0.01
Protein	3.58 $\pm$ 0.02
Carbohydrate	37.12 $\pm$ 0.01

**Table2.** Metal analysis of *Citrullus lanatus* leaves per 100 g

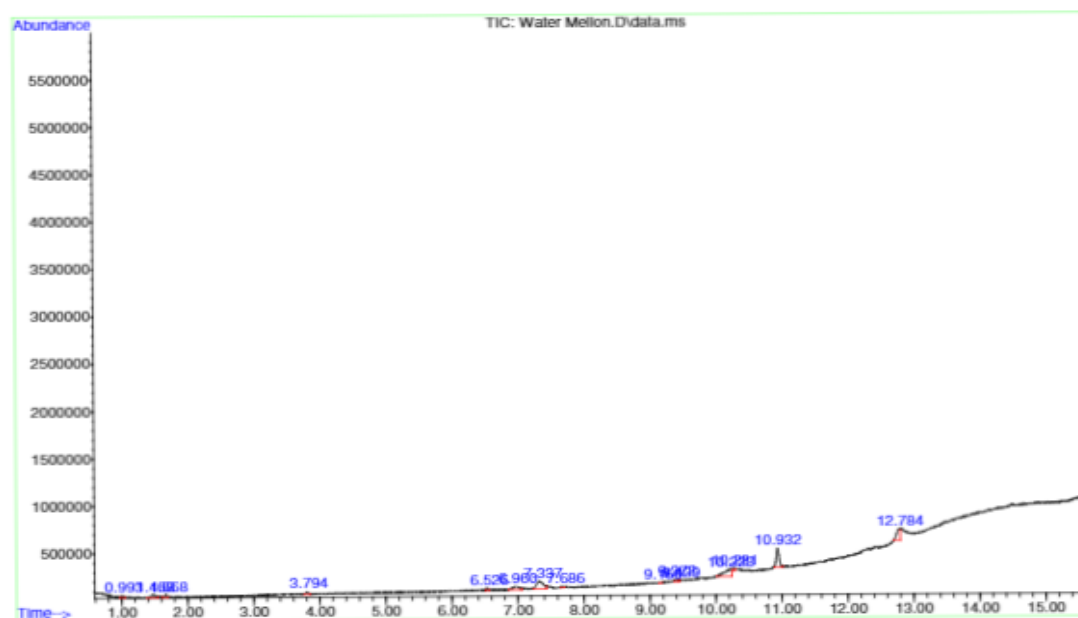
Parameters (%)	Mean $\pm$ S.D
Iron (Fe)	0.010 $\pm$ 0.03
Manganese (Mn)	0.002 $\pm$ 0.02
Calcium (Ca)	0.820 $\pm$ 0.02
Phosphorus (P)	0.163 $\pm$ 0.01
Potassium (K)	2.11 $\pm$ 0.02

**Table3.** GC-MS analysis of metabolites in *Citrullus lanatus* (Watermelon) leaves powder

S/N	Retention Time	Name(s) of compounds	Molecular formular	Molecular weight	Peak height (%)
1.	0.993	Stearic acid, 3-(octadecyloxy)propyl ester	C <sub>39</sub> H <sub>78</sub> O <sub>3</sub>	594	17.9
2.	1.468	Pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-(1,2-ethanediyl acetal)	C <sub>25</sub> H <sub>34</sub> O <sub>7</sub>	446	15.5
3.	1.668	N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic acid, benzyl ester	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	394	23.6

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4.	3.794	2-Myristinoyl pantetheine	C <sub>25</sub> H <sub>44</sub> N <sub>2</sub> O <sub>5</sub> S	484	51.9
5.	6.526	1,2-Propanediol, 3-(octadecyloxy)-, diacetate	C <sub>25</sub> H <sub>48</sub> O <sub>5</sub>	428	12.0
6.	6.960	7AH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahyd	C <sub>30</sub> H <sub>44</sub> O <sub>11</sub>	580	12.4
7.	7.337	7-Epi-cis-sesquisabinene hydrate	C <sub>15</sub> H <sub>26</sub> O	222	14.6
8.	7.686	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	C <sub>20</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	355	15.3
9.	9.160	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	C <sub>27</sub> H <sub>40</sub> O <sub>4</sub>	428	54.4
10.	9.372	Olean-12-ene-3,15,16,21,22,28-hexol, (3β,15α,16α,21β,22α)-	C <sub>30</sub> H <sub>50</sub> O <sub>6</sub>	506	27.1
11.	9.429	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	194	11.7
12.	10.223	Cyclobutane, 1,3-bis[2-(2-isopropyl-3,3-dimethyloxiran-2-yl)ethenyl]-2,4-diacetyl	C <sub>26</sub> H <sub>40</sub> O <sub>4</sub>	416	13.8
13.	10.281	Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-1	C <sub>36</sub> H <sub>58</sub> O <sub>6</sub>	586	12.3
14.	10.932	Gingerol	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>	294	9.89
15.	12.784	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	C <sub>16</sub> H <sub>50</sub> O <sub>7</sub> Si <sub>8</sub>	578	10.7



**Figure2.** Chromatogram of methanolic extract of *Citrullus lanatus* leaves

## 4. DISCUSSION

Results of the analyses of *Citrullus lanatus* leaves are discussed as follows:

### 4.1. Proximate Screening

Table 1 shows the results of the proximate content of methanolic extract of *Citrullus lanatus* leaves. Moisture content was, 82.6 %. This value was significantly higher compared to that obtained in the seeds of *Citrullus lanatus*, 10.4 % and 6.4 % [20-21]. Moisture content influences the physical properties of a substance including weight. The result indicates that the leaves of *Citrullus lanatus* have shorter shelf life with more vulnerability to microbial attacks [22-24] Crude fat content of the leaves of *Citrullus lanatus* was 14.21 %. Lower values, 0.13 % and 0.21 % were reported in the rind and pulp of *Citrullus lanatus* [25].

The fiber content of the leaves was 30.23 %, indicating that *Citrullus lanatus* leaves are a richer source of fiber. The ash value in *Citrullus lanatus* leaves was 6.46 %. Lower value, 2.70 %, was reported in the seeds of *Citrullus lanatus* [26], indicating the presence of high organic matter in the leaves. Protein content was 3.58 % in the leaves. Suwaibatu and others reported a much higher value, 15.23 %, in the dried seeds of *Citrullus lanatus* [27]. The carbohydrate content of the leaves of *Citrullus lanatus* was 37.12 %. Significantly lower carbohydrate value, 7.03 % was reported in the dried seeds [27]. Intriguingly, the leaves could be a better source of energy than the seeds [28].

### 4.2. Metal Analysis

Results of the metal screening of *Citrullus lanatus* leaves are presented in Table 2.

Potassium (K) in the leaves of *Citrullus lanatus* was 2.11 %. Higher concentration, 19.00 % of potassium was reported in the seeds [27]. Iron (Fe) concentration value was 0.010 %. A significantly higher value was reported in the seeds [27]. The concentration of manganese (Mn) in the leaves of *Citrullus lanatus* was 0.002 %. Calcium value in the leaves was 0.820 %. Phosphorus content was 0.163 %. Clearly, the present study has shown that the leaves of *Citrullus lanatus* contain essential components for bone, teeth, tissues, muscle, blood, and nerve cells. Calcium is an essential trace element for healthy bones, nerves, and muscles [29]. Magnesium is a component of chlorophyll and important in calcium metabolism in bone [30]. Iron (Fe) participates in oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport [31]. Potassium triggers the heart to squeeze blood to the body [32].

### 4.3. Metabolites Analysis

Gas chromatography Mass spectrometry (GC-MS) examination of the methanol extract of *Citrullus lanatus* leaves revealed the presence of fifteen metabolites, Table 3. These include: Stearic acid, 3-(octadecyloxy)propyl ester, 17.9 %, N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic acid, benzyl ester, 23.6 %, Olean-12-ene-3,15,16,21,22,28-hexol, (3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ )-, 27.1 %. Maximum peak, 51.9 % was shown by 2-Myristinoyl pantetheine and the lowest peak, 10.7 %, was shown by Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl. Peak area percentage less than 5 % were considered insignificant, Figure 2. Olean-12-ene-3,15,16,21,22,28-hexol (3 $\beta$ ,15 $\alpha$ ,16 $\alpha$ ,21 $\beta$ ,22 $\alpha$ )- is a metabolite that exhibits anti-tumorigenic properties [33]. Interestingly, the GC-MS screening has corroborated the use of *Citrullus lanatus* leaves in traditional medicine.

## 5. CONCLUSION

Proximate result shows that the leaves contain a high amount of moisture. High moisture content means high susceptibility of the leaves to bacterial attack. A moderate amount of carbohydrate was shown, indicating that the leaves can be a potential source of energy. Metal screening revealed that the leaves contain important trace metals that are beneficial to health. Potassium was present in the highest value, while manganese occurred in the least amount. Gas chromatography Mass spectrometry (GC-MS) screening of the methanolic extract of the leaves showed 15 metabolites, implying that the leaves contain important bioactive components with different biological activities, substantiating the use of the leaves in traditional medicine.

## 6. RECOMMENDATION

Extract of *Citrullus lanatus* (watermelon) leaves is used locally to cure respiratory tract infection and diabetes. However, the present study recommends researches on the dosage prescription of the leaf extract.

## 7. DATA AVAILABILITY

Data that are generated in the findings of this study are available on request from the corresponding author (godwinj2012@gmail.com).

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