Phytochemical Analysis and Mineral Content of Ethanol Extract of *Citrullus lanatus* Bark

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This study examined the phytochemical and elemental content of water melon (*Citrullus lanatus*) bark. The sample was spread in the laboratory to dry at room temperature after which it was grounded to get a coarse powder form using sterile mortar and pestle. The extracts (ethanol and aqueous) were subjected to phytochemical and elemental analysis using standard procedures. Qualitative phytochemical screening of the ethanol extract revealed the presence of tannins, alkaloids, saponins, oxalates, flavonoids, steroids, phytates and glycoside while the quantitative phytochemical screening indicated the contents of tannins to be (1.360 g/100g), alkaloid (3.965 g/100g), saponins (1.380 g/100g), oxalates (3.675 g/100g), flavonoids (1.145 g/100g), steroid (1.080 g/100g), phytates (3.160 g/100g) and glycoside (4.490 g/100g).

The elemental analysis showed that the extracts contain Ca (0.025 ± 0.002 mg/100g), K (0.065 ± 0.001 mg/100g), Fe (0.189 ± 0.007 mg/100g), Zn (0.263 ± 0.005 mg/100g), Mg (1.771 ± 0.005 mg/100g), Mn (0.670 ± 0.003 mg/100g), Na (0.033 ± 0.001 mg/100g), and Cu (0.245 ± 0.003 mg/100g) however Cr was not detected. The results of this study showed that the ethanol extract of *C. lanatus* contains bioactive compounds which might be responsible for pharmacological actions of the plant and the plant can also serve as a source of mineral elements in diet.

**Key words:** Phytochemicals, elements, composition, *Citrullus lanatus*, bark, bioactive, drugs, diet

**INTRODUCTION**

Medicinal plants are plants containing inherent active ingredient used to cure disease or relief pain (Asaolu *et al.*, 2012). Furthermore, WHO (2000) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a base for herbal product. Plants based drugs have been used worldwide in traditional medicine for the treatment of various diseases. World plant biodiversity is the largest source of herbal medicine and still about 60-80% world population rely on plant-based medicine which has been in use since the ancient ages of traditional health care system (Devaki, 2013).

Plants are a source of a large number of drugs comprising of different groups such as antispasmodics, emetics, anti-cancer, anti-microbial, anti-inflammatory, anti-malaria, anti-oxidant etc (Ncube *et al.*, 2008; Williams *et al.*, 2019). The active principle of many drugs found in plants is phytochemicals (Sadia *et al.*, 2011)). The medicinal value of these phytochemicals is because of the presence of chemical substance that produces definite physiological action on the human body (Ali *et al.*, 2012). Some of the valuable ones include: alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, and body building (Imafidon *et al.*, 2018).

Elements such as calcium play an important role in building and maintaining strong bones and teeth, large part of human blood and extracellular fluids (Victor and Chidi, 2009). Sodium has an important role in maintaining the water balance within the cells and in the function of both nerve impulse and muscles. Potassium is most commonly

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used for treating and preventing low potassium levels, treating high blood pressure, and preventing stroke. Iron is an important component of hemoglobin, the substance in red blood cells that carries oxygen from the lungs to transport it throughout the body (Abbaspour et al., 2014; Ware 2018). Nutritionists have considered the role of metals such as zinc, copper and iron in human fitness (Williams et al., 2019), and recommended that they should be considered for preparation of herbal drugs (Sadia et al., 2011). Plants have been known to relieve various diseases in India, Africa, Panama and America. Medicinal plants are the richest bio-resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, Pharmaceutical intermediates and chemical entities for synthetic drugs (Prashant, 2011).

Progress toward better understanding of plant derived has been depended on two factors which are interdependent. First is the development of strict criteria of prove that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compounds in the plant. The medicinal value of plant lies in some chemical substances that produce a definite physiological action on the human body (Imafidon et al., 2018).

The use of traditional medicine and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health have been widely observed (Banerjee et al., 2012).

Watermelon belongs to the family of cucurbitaceous, scientifically classified as *C. lanatus* (Donald, 2003). It is cultivated extensively for its pleasant-tasting fruit. Watermelon is of the twelve species of the family of cucurbitaceous cultivated by man. It is the major vegetable crop in the United States and other parts of the world and account for 6.8% of the world area devoted to vegetable crop production (Ali et al., 2012; Imafidon et al., 2018). Watermelon requires a long, warm growing season and grows best under temperature of 21 to 39°C. The plant also tolerates high humidity. The optimum pH range for watermelon is 6.0 - 6.5 although the plant will tolerate soil with as low as 5 (Imafidon et al., 2018; Braide et al., 2012). The vine, an annual plant is coarse and hairy and bears divided, oval leaves on short stalks and round, light yellow with individual flowers. The rounded, oblong, berrylike fruit grows to a very large size. The thick rinds are green and the watery parts are usually red in colour and contain many dark flat seeds (Prashant et al., 2011). Fruits occupy a part of daily diet of the rich and rarely the poor and there are many parts of a fruit that are not considered edible, and so are usually thrown away. One such fruit is water melon which is taken by many but the bark is thrown away and generally not included in regular diet. The juice or pulp of water melon is considered as the edible portion but bark is discarded as major solid wastes (Ali et al., 2012; Braide et al., 2012; Imafidon et al., 2018).

Effective health cannot be achieved in Africa unless modern medicine is complemented with traditional medicine. At least 80% of Africans depends on traditional medicine for their health care. Fruits and vegetables have been recognized as natural source of various bioactive compounds which could be attributed to their phyto-constituents such as flavonoids, fiber and phenolic compounds. Plants derived drugs are more inexpensive compare to synthetic drugs (Samy and Ignacimuthu, 2000; Boakge et al., 2015).

Micronutrient deficiencies are a major public health problem in many developing countries (Adebawale et al., 2013; Williams et al., 2018). According to WHO (2000), one third population of the world is affected by iron deficiency, causing varied degree of impairment in cognitive performance, lowered work capacity, lower immunity to infection, pregnancy and complication. The quality and quantity of phytochemical and elemental constituent of a given plant may vary with location due to variation in soil and other environmental conditions. In this area, there is no coherent documented information on the nutrition composition of watermelon bark although the plants are available.

The objective of the study is to investigate the phytochemical and elemental composition of watermelon bark in the study area. The findings will serve as a guide when recommending it as a source of drugs and food to the local community.

**MATERIALS AND METHODS**

**Sample Collection and Identification**

The water melon (*C. lanatus*) bark was obtained from Mubi market of Adamawa State of Nigeria the plant was identified and authenticated by a taxonomist in the Department of biological Science, Adamawa State University Mubi.

**Preparation of Sample**

The water melon (*C. lanatus*) was washed, cut open; flesh and seeds were removed and the bark was air dried in Chemistry laboratory 2, Science Complex of the Faculty of Science, Adamawa State University, Mubi. The sample was spread in the laboratory to dry at room temperature after which it was pulverized to get a coarse powder form using sterile mortar and pestle. The powder was stored in an air tight container and was used for successive analysis (Kubmarawa et al., 2009).

**Extraction**

The 2 kg of the powered samples used for the phytochemical and elemental analysis was extracted with ethanol (60 – 80°C) following the procedure described by AOAC (2000).
Phytochemical analysis

The ethanol or aqueous extract of (C. lanatus) bark was tested for the presence of bioactive compounds.

Test for tannins

The extract (200 mg) was mixed with 2 mL of 2% solution of FeCl₃. Blue-green color was observed.

Test for flavonoids (alkaline reagent test)

The extract (200 mg) was mixed with 2 mL of 2% solution of NaOH. An intense yellow colour formed which turned colorless on addition of few drops of diluted acid was observed.

Test for saponins

The extract (200 mg) was mixed with 5 mL of distilled water in a test tube and was shaken vigorously for 10 minutes and 1 mL HCl 2M was added, stable foam was formed which persisted for 15 minutes was observed.

Test for glycosides (Salkowski’s test)

The extract (200 mg) was mixed with 2 mL of chloroform. Then 2 mL of concentrated H₂SO₄ was added carefully and shaken gently. A reddish-brown color was observed.

Test for alkaloids

The extract (200 mg) was mixed with 10 mL of methanol. To 2 mL of the filtrate was added 1% HCl and then steamed. To 1 mL of the filtrate 6 drops of Wagner reagent was added Brownish-red precipitate was observed.

Test for steroids

To 2 mL of acetic anhydride 0.5 g of the sample was added followed by an addition of 2 mL H₂SO₄. The color changed from violet to blue-green (Nwankwo and Ukaegbu-Obi, 2014).

Determination of phytochemicals

The phytochemicals of the samples were estimated using Buck scientific (USA) BLC 10/11– model high performance liquid chromatography (HPLC) following the procedure adopted by Nwankwo and Ukaegbu-Obi, (2014). The prepared sample (5 g) was placed into 25 cm³ standard volumetric flask and made up to mark over diluent. The solution was refluxed, shake, centrifuged and decanted. Then filtrate was filtered using the HPLC grade filter paper and then inject in to HPLC buck scientific (USA) BLC 10/11 - model (AOAC, 2000).

Elemental analysis

The dried sample was weighed into a crucible and placed in a muffle furnace at room temperature and the temperature was raised to 550°C for three hours to complete ash. The ash was dissolved in hot 10% HNO₃, filtered and diluted to required volume in a standard flask with 0.01 M HNO₃. The elements in solution were determined using atomic absorption spectrophotometer (AAS) following the procedure adopted by AOAC (2000). The result of the elemental analysis was presented in the form of means ±SD of triplicates of determination.

RESULTS

Preliminary phytochemical screening conducted on the ethanol extract of C. lanatus revealed the presence of tannins, alkaloids, saponins, oxalates, flavonoids, steroids, phytates and glycosides (Table 1):

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+ +</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ + +</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ +</td>
</tr>
<tr>
<td>Oxalates</td>
<td>+ +</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ + +</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Phytates</td>
<td>+ + +</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

Key + = presence of constituents  
+ + = high presence of constituents  
+ + + = very high presence of constituents

The result of quantitative phytochemical analysis revealed 1.360 g/100 g, 3.965 g/100 g, 1.380 g/100 g, 3.675 g/100 g, 1.145 g/100 g, 1.080 g/100 g, 3.160 g/100 g and 4.490 g/100 g for tannins, alkaloids, saponins, oxalates, flavonoids, steroids, phytates and glycosides respectively (Table 2):

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>(g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>1.360</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>3.965</td>
</tr>
<tr>
<td>Saponins</td>
<td>1.380</td>
</tr>
<tr>
<td>Oxalates</td>
<td>3.675</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1.145</td>
</tr>
<tr>
<td>Steroids</td>
<td>1.080</td>
</tr>
<tr>
<td>Phytates</td>
<td>3.160</td>
</tr>
<tr>
<td>Glycosides</td>
<td>4.490</td>
</tr>
</tbody>
</table>

The result of elemental analysis revealed 0.025 ± 0.002 mg/100 g, 0.065 ± 0.001 mg/100 g, 0.189 ± 0.003 mg/100 g, 0.263 ± 0.005 mg/100 g, 1.770 ± 0.007 mg/100 g, 0.670 ± 0.003 mg/100 g, 0.033 ± 0.001 mg/100 g and 0.245 ± 0.003 mg/100 g for Ca, K, Fe, Zn, Mg, Mn, Na and Cu respectively while Cr was below detection level (Table 3):
DISCUSSION

The values of the quantitative phytochemical analysis ranged from 1.08 - 4.490g/100g similar value (1.12 – 3.42g/100g) was observed by Williams et al., 2019. Glycoside has the highest value followed by alkaloids, flavonoids, phytates, saponins, tannins, oxalates and steroids (Table2). This is in agreement with the report of Ali et al. (2012), who showed that alkaloids and terpenes are widely distributed in the genus “citrullus.” Phytochemicals have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxifying enzymes, stimulation of the immune system and general modulation of hormonal activities (Imafidon et al., 2018)

The presence of these metabolites indicates the great potentials of the water melon bark as a source of useful phytomedicines. As some flavonoids have anti-inflammatory effects on both acute and chronic inflammation (Kim et al., 2012; Hussain et al., 2013).

Some plants that possess alkaloids are known for decreasing blood pressure and balancing the nervous system in case of mental illness. The presence of tannins could also show that it is an astringent help in wound healing and anti-parasitic. The presence of saponins shows the class of natural products involved and can be used to enhance penetration of micro molecules such as protein through cell membrane. It also indicates the water melon bark potential activity on antimicrobial agents (Eujoba, 2012; Williams et al., 2018). However, in high quantity it is toxic. Oxalates may be responsible for its use in the treatment of cough, dysentery, inflammation and ringworm (Ouilly et al., 2017). Alkaloids are known to possess anti-malarial property, hence the bark of water melon may be a good source of anti-malaria for which it is traditionally used. Also water melon bark (Citrullus lanatus) as a genital stimulant may attribute to the presence of alkaloids (Ouilly et al., 2017). The presence of terpenes suggests its possible use as anti-tumor and antiviral agent as some terpenes are known to be cytotoxic to tumor cells. Some of the eudesmane (sesquiterpenes) has been reported to exhibit antibacterial properties possess anti-malaria property; hence the bark may be a good source of anti-malaria (Tona et al., 2001). Any plants parts containing saponins and phenols are believed to have antioxidant, anti-cancer, anti-inflammatory, anti-viral and anti-diarrheal this is the reason why water melon bark can be used as medicine (Sharma et al., 2000; Sadique et al., 1987).

The present study showed the presence of a number of metals in citrullus lanatus bark. The most abundant element was Mg followed by Fe, Mn, Zn, Cu, K, Na and Ca (Table 3), as previously reported by Imafidon et al. (2018) Nutritionists have considered the role of metals such as zinc, copper and iron in human fitness (Williams et al., 2019), and recommended that they should be considered for preparation of herbal drugs (Sadia et al., 2011).

Magnesium is an important mineral element in connection with circulatory diseases such as heart disease (Eujoba, 2012). High magnesium concentration is a component of leaf chlorophyll in plants.

Fe is not a toxic metal because it serves as micronutrient. Iron is an essential element for blood production. About 70 percent of human body’s iron is found in the red blood cells called hemoglobin and in muscle cells called myoglobin. Iron is an important component of hemoglobin, the substance in red blood cells that carries oxygen from the lungs to transport it throughout the body. If the body doesn’t have enough iron, it can’t make enough healthy oxygen-carrying red blood cells. A lack of red blood cells is called iron deficiency anemia (Abbaspour et al., 2014; Ware 2018). Hence water melon bark can serve as a source of Fe supplement for the body.

Copper is an essential trace element in human body and exist as an integral part of copper proteins. Ceruloplasmin which is concerned with the release of iron from the cells into the plasma and is involved in energy metabolism (Ayoola et al., 2010). The presence of copper, zinc and manganese indicates that the water melon bark is essential for immune function (Tona et al., 2001).

Potassium is a mineral that plays many important roles in the body. Food sources of potassium include fruits (especially dried fruits), cereals, beans, milk, and vegetables. Potassium is most commonly used for treating and preventing low potassium levels, treating high blood pressure, and preventing stroke. Potassium is necessary for the function of all living cells. Potassium shift across nerve cell membranes and necessary for the normal nerve transmissions. Potassium depletion or excess can result in numerous abnormalities, including abnormal heart rhythm and various electrocardiographic abnormalities. Fresh fruits and vegetables are good dietary source of potassium (Raman, 2017). Therefore water melon bark can be a good source of K.

**Table 3: Elementary composition of ethanol extract of C. lanatus bark**

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>0.025 ± 0.002</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>0.065 ± 0.001</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.189 ± 0.003</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.263 ± 0.005</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>1.770 ± 0.007</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.670 ± 0.003</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>0.033± 0.001</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.245 ± 0.003</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>BDL</td>
</tr>
</tbody>
</table>

BDL = below detection limit

All values represent mean ± SD (Standard Deviation).
Sodium has an important role in maintaining the water balance within the cells and in the function of both nerve impulse and muscles. It also helps in the maintenance of normal acid-base balance. An adult need about 3g per day of sodium but modern dietary habits take in 5 – 20 g per day (Vashishtha et al., 2007).

Calcium plays an important role in building and maintaining strong bones and teeth, large part of human blood and extracellular fluids. Approximately 99% of the body calcium is stored in the bones and teeth (Vctor and Chidi, 2009). The studied water melon bark is essential in building up the level of calcium in the body.

Cr (though not detected in the extract) in trivalent state is an essential trace element that potentiates insulin action and those influences carbohydrate, lipid and protein metabolism (Kim et al., 2012).

CONCLUSION

Results of this study indicate that the water melon bark extract contains some major bioactive contents: tannins, alkaloids, saponins, oxalates, flavonoids, steroids, phytates and glycosides that inhibit the growth of micro-organism there by proving very effective sources of derived drugs. This may imply that the bark of water melon can be used for the treatment of dysentry, constipation, gastro internal disorder and diabetes. Therefore, the metabolites identified are believed to have antioxidant, anthelmintic, anti-bacterial, demulcent and diuretic being used in the treatment of dropsy and renal stones.

The elemental analysis has further shown an appreciable amount of minerals contained in the water melon bark. The bark can be used as source of mineral in diet and also as part of drugs in pharmaceuticals.

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