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Research Note

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Phytochemical Screening and Physiologic Functions of Metals in Seed and Peel of *Citrullus lanatus* (Watermelon)

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Abstract: The seed and peel of *Citrullus lanatus* (watermelon), a very commonly consumed fruit were examined for metals and phytochemicals. The metals were examined with atomic absorption spectrophotometer while the phytochemicals were determined through standard methods of phytochemical analysis after extraction of the *Citrullus lanatus* with ethyl acetate and water. The results of metal analysis showed that iron (Fe) was highest in concentration in both seed (29.73 mg/Kg) and peel (32. 60 mg/Kg) of the *Citrullus lanatus*, followed by manganese (Mn) in the seed (11.67 mg/Kg) and peel (14. 14 mg/Kg); zinc (Zn) in seed was 7.89 mg/Kg and peel was 9.11 mg/Kg and magnesium (Mg) in seed was 1.47 mg/Kg and peel was 1.28 mg/Kg of the *Citrullus lanatus*. However, other metal ions detected (calcium (Ca), copper, (Cu), potassium (K), nickel (Ni) and Lead (Pb) were lower than 1.0 mg/Kg in the seed or peel of the *Citrullus lanatus*. Arsenic (Ar) and selenium (Se) were undetected in both seed and peel of the *Citrullus lanatus*. Phytochemical screening of the ethyl acetate extracts of seed and peel of the *Citrullus lanatus* revealed the presence of flavonoids,

terpenoids and cardiac glycosides in both seed and peel of watermelon. Tannins was only present in the seed, while phenols, carboxylic acid, quinone and xanthoproteins were absent in the seed. All the components examined were present in the peel except tannins. Phytochemical examination of the aqueous extract of the seed and peel of the watermelon showed that only carboxylic acid was present in the seed, while in the peel, flavonoids, terpenoids, cardiac glycosides, carboxylic acid and xanthoproteins were present. The result of the analysis indicated that these supposed wastes (seed and peel) of the *Citrullus lanatus* contain vital chemical components that can serve useful purposes in health, food and feeds supplements.

Key words: phytochemicals screening, metal, seed, peel, *Citrullus lanatus*

INTRODUCTION

Fruits generally, are known to be very good source of nutrients to man and animals. They are common and easily accessible to both rural dwellers and urban settlers. Fruits are very rich in minerals, vitamins[1] and other nutritive components such as proteins and carbohydrates. Fruits are tasty and are harvested either from the wild, from agricultural farms and orchards. They serve various uses such as for food, feeds for animals, cultural uses, for the treatment of different illnesses and diseases and also as food supplements. They provide income[2] and foreign exchange. Animals need constant supply of food which contains reasonable nutritive components so that life cycle could effectively be completed in a healthy condition. Therefore the mineral requirement (nutrition) becomes an important tool in the physical and physiological wellbeing in any human or animal life [3].

It is a known fact that seed of plants and other plant parts such as the leaf, stem, bark, tubers etc possess nutritional and anti-nutritional biologically based compounds which are referred to as phytochemical compounds, which when consumed at certain concentration becomes toxic to the human body[4]. Unprocessed fruit possesses diuretic effects; this is due to the presence of minerals and some phytochemicals in them. They also possess the capacity to moderate blood and tissue pH (acidity) and also offer quick wellness to unhealthy individuals[5].*Citrullus lanatus* (Watermelon) belongs to the family *Cucurbitaceae*. It is a native of Africa and Asia, but is claimed to have originated from southern Africa. It requires warmth for cultivation and growth and so thrives better in warmer parts of the world. For proper yield, it is planted in the dry season and requires limited amount of rainfall [6]. *Citrullus lanatus* is an annual crop which creeps on the ground. The fruit of the ripe one may appear as pale yellow, light green or dark green in colour. The name of the plant was given due to large percentage (95%) of water it contains[7]. The fruit is used as a purgative in Senegal and for other remedies in diseased

conditions in Nigeria[8]. In Nigeria, *Citrullus lanatus* is majorly produced in the Northern part, but sparsely cultivated in the other parts of the country. The crop is then transported to the southern part where there is a ready market and is consumed in large quantity. When the fruit is consumed, the peels and the seeds are usually thrown away without considering if it can be of importance to man. This study examines metals and phytochemicals compositions of the seed and peel of *Citrillus lanatus* (watermelon) a common household fruit consumed in Nigeria.

MATERIALS AND METHODS

The *Citrullus lanatus* fruits were bought from Mile 3 market, Port-Harcourt, Rivers State. The seeds and the peels were after being thoroughly washed, carefully removed and dried in a laboratory oven at 300^0C for 24 hours. The samples were ground with xnewster blender and subjected to elemental and phytochemical analysis.

Oven dried samples of *Citrullus lanatus* of 1.0 g weight was transferred into a 125 ml volumetric flask previously washed with acid and distilled water. To the samples, 4 ml of perchloric acid, 25 ml concentrated HNO_3 and 2 ml of concentrated H_2SO_4 were added under a fume chamber and gently heated until dense white fumes appeared. The mixture was further heated with the introduction of 1-2 ml HNO_3 and 50 ml of de-ionized water until the solution became clear. The solution was filtered with Whatman No.1 filter paper into sample bottles and made up to 25 ml mark. The digests were then set aside for metal analysis. Flame solar atomic absorption spectrophotometer was used to determine the concentrations of the metals. The *Citrullus lanatus* samples were weighed into two different extractors (15.87 g of seed) and (peels 50.04 g of peel). 250 ml of the solvent (ethyl acetate) was measured into a round bottom flask and coupled with extractor to the flask and placed on a heating mantle regulated at a temperature of 220^0C and allowed to steam for 30 - 45 minutes, after which coloration occurred, and the extracts began to flux into the flask that contains the ethyl acetate until the coloration ended. For the aqueous extraction, (5.66 g of seed) and (18.98 g of peel) samples were weighed into each of two different conical flasks of 250 ml capacity and then, 80 ml of water was added to the flask containing the seed, while 150 ml was added to the flask containing the seed. Both samples were soaked for 48 hours (2 days). The resulting solutions from the aqueous extracts were carefully filtered with a Whatman No.1 filter paper into conical flasks.

The extracts were then subjected to the various phytochemical screening tests. For flavonoids, 1 ml of 10% NaOH was added to 4 ml of the extract plus a few drops of concentrated H_2SO_4 and was observed for colour change. For phenol, 2ml distilled water and few drops of 10% Ferric chloride ($FeCl_3$) were added to 1ml of the extracts and observed for colour change. For Terpenoids, 2 ml of Chloroform and 3 ml of $FeCl_3$ were added to 5 ml of the extracts and observed for colour change. For tannins, a little amount

of 1 % ferric chloride ($FeCl_3$) was added to the solution of the extract and 10 ml distilled water and observed for a change in colour. For Cardiac Glycosides tests also known as *Keller-Killani* test, to 5 ml of the extract, glacial acetic acid was added plus 1 drop each of $FeCl_3$ and H_2SO_4 and observed for colour change. For Carboxylic acid, few drops of sodium bicarbonate was added to 1ml of the extract and observed for colour change. A solution of potassium hydroxide (KOH) was added to 1 ml of the extract and observed for characteristics colour change for quinones. Few drops of concentrated nitric acid (HNO_3) was added to 1 ml of the extract and observed for the characteristics colour change for xanthoproteins

RESULTS AND DISCUSSION

The concentrations of the metals in the seed and peel of *C. lanatus* are given in **Table 1**. In human body, both Chemical and electrical processes occur. The proper functioning of these processes can take place when there is a balance of minerals in the system. The metals examined in this work have both negative and positive roles they perform in the body depending on their concentrations[9]. All the metallic elements observed in the *C. lanatus* (watermelon) were lower than the recommended daily dietary intake by RDA **(Table 2).**

Different authors have carried out the determination of metal ions in the seed of watermelon[9-11] and observed the presence of these metals in varying concentrations. The metals, potassium (K), sodium (Na), magnesium (Mg), calcium (Ca) and Iron (Fe) are macro elements required by the body for proper body mechanism, while copper, (Cu), zinc (Zn), selenium (Se), manganese (Mn) and nickel (Ni) are micro or trace elements required in trace quantity in the body. However, lead (Pb) and cadmium (Cd) constitute health risk to the body even at trace levels. The detectable values of the metals are presented in Table 1.

The mean value of manganese in the seed was 11.67±1.21 mg/Kg and that of the peel was 14.14±1.50 mg/Kg. Manganese is very important to man with regard to proper mental health and oxygen transfer from lungs to the body cells. In biological systems it acts as an activator, co-factor or co-enzyme in a number of reactions related to carbohydrate, fat and protein metabolism[12,13]. Manganese bound enzymes are required in the detoxification of superoxide free radicals scavenging for oxygen and as an anti-oxidant. Deficiency of manganese leads to growth retardation and abnormalities in skeletal muscle. However, excessive intake leads to bioaccumulation especially when there is iron deficiency which may lead to a condition known as *manganism*, a neurodegenerative disorder that causes dopaminergic neuronal death and symptoms similar to Parkinson's disease[14,15].

The observed value of potassium in the seed was 0.25±0.00 mg/Kg and that of the peels was 0.24±0.02 mg/Kg. Potassium is an intracellular ion or electrolyte which regulates the heartbeat and muscle functions and is also involved in neuro-transmission. Potassium is a very important ion in maintenance of fluid and electrolyte balance in both humans and animals[16]. Excess or overdose of potassium in the body leads to potassium toxicity in the body known as hyperkalemia which can be fatal if untreated, causing irregular heartbeat, paralysis of the lungs, and cardiac arrest. Potassium deficiency is known as hypokalemia which is caused by water loss due to severe vomiting or diarrhea. It also leads to muscle weakness and cramping.

Sodium values for seed and peel were 0.05±0.01 mg/Kg and 0.046±0.00 mg/Kg respectively. Sodium regulates the amount of water and blood in the body and maintains muscle and nerve function[12]. Sodium is the major extracellular fluid in the body and a constituent of blood, plasma, and lymph fluid. It helps in the maintenance of electrolytes balance between the intracellular and extracellular environments[16]. Excess intake of sodium results in dehydration or hypernatremia (too little water in the body). This situation is associated with body weakness, lethargy, and in severe cases seizures or coma. Deficiency of sodium results in hyponatremia which is mostly caused by severe diarrhea or vomiting. Symptoms of hyponatremia may include headache, confusion, fatigue, hallucinations and muscle spasms. The concentrations of zinc in the seed and peel of the watermelon were 7.89±1.23 and 7.11±1.45 mg/Kg respectively. The importance of zinc lies in its antioxidant properties in the prevention of aging in man and also shortens the healing time of injury[17]. It possesses immunodulatory properties thereby increasing human immune system efficiency[18]. It is used in the treatment of diarrhea in children[19]. It boosts health of hair, and enhances the sense of taste and smell[12]. It functions in protein and carbohydrate metabolism, transfer vitamin A from liver to other parts of the body and the production DNA and RNA[20]. However, its deficiency is associated with depression[21].

The mean values of magnesium were 1.47±0.10 mg/Kg in the seed and 1.29 mg/Kg in the peel. Magnesium is very essential in many biochemical reactions required in the body. It is involved in the synthesis DNA and RNA and helps in the maintenance of normal nerve and muscle function, boosts the immune system, stabilizes the rate of heart and blood sugar, and promotes the formation of bones and teeth. The rate of utilization of magnesium is very high and so cases of high level (hypermagnesemia) are relatively rare. Toxicity of magnesium is mostly due to kidney failure or excessive intake of supplements [22] Calcium concentrations in the seed and peel were 0.57±0.01 and 0.49±0.0 mg/Kg respectively. Adequate calcium intake strengthens bones and therefore improves risk of bone fractures, osteoporosis, and diabetes in man. Furthermore, calcium in human body (bones, teeth and serum) plays a number of life important roles[23]. Calcium helps in the

differentiation and proliferation of bone and intestinal cells. However, deficiency of calcium impairs the above mentioned functions, and if allowed to continue for a long time, can lead to chronic disease[24].

The concentrations of iron in the seed and peel of the watermelon were 29.73±2.51 and 32.60±2.33 mg/Kg respectively. Due to the different metabolic processes in the human body which involves iron, it is considered as one of the most essential element. The different processes include oxygen transport through the blood to other parts of the body, DNA synthesis and electron transport[25]. Iron is a constituent of haemoglobin in animals and myoglobin in plants. It is obtained through consumption of meat, poultry, and fish, cereals, pulses, legumes, fruits, and vegetables[26]. Iron deficiency results in metabolism disorder and causes many diseases in humans which manifest in different clinical symptoms. A well-known disease of iron deficiency is anaemia and neurodegeneration [25].

Arsenic and selenium were undetected in the seeds and peels of watermelon analyzed. Naturally arsenic occurs in form of metalloids or chemical compounds. It is toxic to the humans when consumed and causes different pathological malignancies conditions in cutaneous and visceral cells[27]. Source of arsenic contamination lies in the use of arsenic based pesticides in agricultural settings and as feed additives for poultry and swine. The manure from the sources when applied to crops results in plant contamination with arsenic[28]. Selenium naturally occurs in sedimentary rocks and shales. Irrigation of agricultural lands especially from saline drainage water redistributes the metal in soil which is being taken up by plants and Selenium is an essential component of the enzyme glutathione peroxidase[29]. When it is combined with the tocopherols (vitamin E) it enhances the protection of cellular tissues and membranes against oxidative damage[30]. Selenium deficient humans are most likely susceptible to cancer and cardiovascular diseases[31].

The values of nickel obtained in this work were 0.014±0.00 mg/Kg in the seed and 0.028±0.00 mg/Kg in the peel. These values were lower than the RDA recommended daily intake value (Table 2). At high concentration, nickel inhibits mitosis in plant and reduces plant growth as well as reduction in fruit yield and quality[32-34]. The adverse effect of nickel lies in the fact that it is toxic to the blood, breaks down the immune system, toxic to neurons, genes, reproductive system, the veins, kidney, liver and also carcinogenic. Its toxicity is due to the fact that it generates reactive oxygen species which leads to the formation of free radicals in both humans and animals that result in DNA modification and imbalance[35]. The mean values of copper in the watermelon were 0.48±0.00 and 0.42±0.00 mg/Kg in the seed and peel respectively. These values were lower than the required value recommended by RDA (Table 2). These values were however higher than those obtained for different fruits and leafy vegetables sold in

Lagos, Nigeria markets[36], but were either higher or lower than those obtained from leaves and crops found around dumpsites in Lafia metropolis, Nigeria[37]. Copper is a micronutrient involved in bio-catalysis when in combination with iron and zinc. The bio-catalytic activity of copper helps in the maintenance of a healthy body by preventing of anaemia[38].

The values of lead (Pb) in the seed and peel were 0.0016±0.00 and 0.0034±0.00 mg/Kg respectively. These values were lower than the recommended daily intake of 0.1 mg/Kg by RDA (Table 2). Lead finds its way into the tissues and other parts of plant through absorption from the soil[39]. Lead is considered a very poisonous metal and its absorption has been reported to be responsible for so many neurological health effects in man which can to lead brain, liver and kidney damages[40]. Long term exposure may lead also to decrease in lifespan with health effects on both animals and man that can be carcinogenic in human[41].

Table 1: Metal concentrations (mg/Kg) in *Citrullus lanatus* Seed and Peel

Parameters	**Concentrations**	
	Seed	**Peel**
Manganese (Mn)	11.67±1.21	14.14±1.52
Potassium (K)	0.25±0.01	0.24±0.02
Sodium (Na)	0.05±0.01	0.046±0.00
Zinc (Zn)	7.89±1.23	9.11±1.45
Magnesium (Mg)	1.47±0.10	1.29±1.10
Calcium (Ca)	0.57±0.01	0.49±0.00
Iron (Fe)	29.73±2.51	32.60±2.33
Arsenic (As)	<0.001±0.00	<0.001±0.00
Nickel (Ni)	0.014±0.00	0.028±0.00
Copper (Cu)	0.48±0.00	0.42±0.00
Lead (Pb)	0.0016±0.00	0.0034±0.00
Seleniun (Se)	<0.001±0.00	<0.001±0.00

Note: <0.001 = Below Detectable Limit (BDL)

Table 2: RDA Recommended Daily Intake of Minerals

Metal	Recommended Daily Allowances (mg)	Tolerable Upper Intake Limit (mg)
Manganese (Mn)	1.8	11
Potassium (K)	4700	-
Sodium (Na)	1500	2300
Zinc (Zn)	11	40
Magnesium (Mg)	310	400
Calcium (Ca)	1000	2500
Iron (Fe)	8	45
Arsenic (As)	-	-
Nickel (Ni)	-	1.0
Copper (Cu)	900µg	10000µg
Lead (Pb)	0.10	-
Selenium (Se)	55µg	400µg

The phytochemical screening of the ethyl acetate extracts of seed of the watermelon showed the presence of flavonoids, terpenoids, tannins and cardiac glycosides and the absence of phenols, carboxylic acid, quinones and xanthoproteins. That of the peel showed the presence of flavonoids, terpenoids, cardiac glycosides, phenols, carboxylic acid, quinones and xanthoproteins and the absence of tannins (**Table 3).** The aqueous extracts of the seed only showed the presence of carboxylic acid, while that of the peel showed the presence of flavonoids, terpenoids, cardiac glycosides, carboxylic acid and xanthoproteins **(Table 4**).

Several authors have worked on the phytochemical composition of watermelon *Citrillus lanatus* (watermelon). Observed the presence of tannins, saponins, carbohydrates, alkaloids, terpenes, glycosides, flavonoids and phenolics in the seed of watermelon[42]. Observed the presence of saponins, alkaloids, cynogenic glycosides, flavonoids, oxalate and tannins in both ethanol and methanol extracts of the seed of watermelon also observed the presence of alkaloids, anthraquinones, flavonoids, gallic acid, saponins and tannins in the seed of different species of watermelon.

The different extraction solvents used in this work gave rise to different phytochemicals. This finding corroborates the finding of[43,44], when they subjected watermelon (*Citrullus lanatus*) to different solvent extractions. The presence of these phytochemicals is an indication of the health importance of the plant. These phytochemicals are known to possess antimicrobial activities[45], which is a vital component of health delivery services. These constituents are natural compounds commonly found in plants and are bioactive against microorganisms. Different stress conditions and diseases are taken care by

phytochemicals when they work in combination with other components of plants referred to as nutrients[46].

Phytochemicals also referred to as secondary metabolites are very important in pharmacological studies because they possess such bioactive capacities or activities such as antiulcer, antimicrobial, antioxidant, analgesic, aphrodisiac and many other vital and important healing and health implications[47].

Table 3: Phytochemical Screening of Ethyl Acetate Extracts of seed and peel of watermelon (*Citrulluslanatus*)

Phytochemical Component	Seed	Peel
Flavonoids	+	+
Phenols	-	+
Terpenoids	+	+
Tannins	+	-
Cardiac glycosides	+	+
Carboxylic acid	-	+
Quinone	-	+
Xanthoproteins	-	+

Key: + = present, - = absent

Table 4: Phytochemical Screening of Aqueous Extracts of Seed and Peel of Watermelon (*Citrulluslanatus*)

Phytochemical Component	Seed	Peel
Flavonoids	-	+
Phenols	-	-
Terpenoids	-	+
Tanins	-	-
Cardiac glycosides	-	+
Carboxylic acid	+	+
Quinone	-	-
Xanthoprotoeins	-	+

Key: + = present, - = absent

CONCLUSION

This study showed the presence of metal ions and phytochemicals in the seed and peel (waste components) of *Citrulluslanatus*. The concentrations of all the metal ions were all below the recommended daily intake by RDA. The phytochemical constituents present in

the seeds and peels were flavonoids, terpenoids, tannins, cardiac glycosides, phenols, carboxylic acid, quinones and xanthoproteins. Considering the concentration levels of the metal ions and the phytochemicals present in the watermelon (seed and peel), these components can be integrated into food supplements to take care of human and animal nutritional needs.

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