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Proximate Composition, Macro-Mineral Concentration and Phytochemical Screening of Seeds of Citrullus Lonatus

Okoroh P.N[1*]., Onuoha, S.C[2] ., Ukegbu C.Y[1] E.U. Godwin-Nwakwasi[3] ., Chukwuka O.C.[4] , and Okereke D.O[5].

[1,5]Department of Biochemistry, College of Natural and Applied Sciences, Gregory University, UturuPMB 1012, Abia State , Nigeria.

[2]Department of Biochemistry, Faculty of Science, University of Port Harcourt ,PMB 53,Port Harcourt , Nigeria.

[3]Department Chemistry, College of Natural and Applied Sciences, Gregory University,Uturu, PMB1012, Abia State, Nigeria

[4]Department of Optometry,College of Health Sciences, Gregory University, Uturu, PMB1012,Abia State, Nigeria

[5]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Gregory University, Uturu, PMB1012, Abia State, Nigeria

Abstract – The research was aimed at determining the nutritional value and accessing the nutraceutical potential of locally processed tropical seed of *Citrillus lonatus*.Proximate composition, macro-mineral concentrations and phytochemical screening of seeds of *Citrullus lonatus* were conducted on dry weight basis using standard methods. Phytochemical screening revealed that tannin had the highest value (6.45±0.006) followed by flavonoid (3.37±0.047) . Alkaloid was appreciable in value (1.35±0.006) while HCN and phytate were low in value. Moisture content was 14.77±0.013% and dry matter was 85.21±0.01%. Crude fat presented the highest value, 28.48±0.013% followed by crude protein (24.78±0.006%), total carbohydrate (18.44±0.006%), crude fiber (11.27±0.01%) and ash had the lowest value of 2.24±0.021%. The calorific value was high at 429.47±0.295 Kcal/100g sample. The concentrations of the analytes from the highest value to the lowest value in the sample was in the order potassium (457.60±0.670mg/kg), Magnesium(89.45±0.006mg/kg), Calcium (42.87±0.125mg/kg) and Sodium (38.26±0.008mg/kg) respectively .The potassium to sodium ratio was 12 (> 1) while the sodium to potassium ratio was 0.08(< 1). The results in this study showed that *Citrullus lonatus* seeds are rich in crude protein, crude fat, crude fiber, potassium, magnesium,calcium,sodium, tannin, alkaloid and flavonoid. They may therefore serve as good sources of food nutrients and nutraceuticals to be utilized in the production of food and feed supplements.

Keywords – Proximate composition, macro-mineral, Phytochemical, *Citrullus lonatus*, seeds.

I. INTRODUCTION

The biochemical connection between food, nutrition and health make the three essential for economic and social development of any nation (Atasie et al., 2009). The local resources within the reach of the people could be harnessed in order to satisfy the needs of the increasing population of the world particularly, in developing nations like Nigeria (Achu etal ., 2005).The type of food that we eat and even the way that we feed on the food is a fundamental factor that controls our mental health , physical and nutritional state (FAO, 2005). From age to age, humans have been known to survive by searching for food rich in nutrients or natural substances that can cure them; or even other biologically important substances present in their environment but yet unknown (Mc Clatchey, 2005).

Citrullus lanatus belongs to the family Cucurbitaceae, (Edwards et al., 2013). Its English name is watermelon. In the flowering plant family called *Cucurbitaceae*, watermelon is a vine that trails and scrambles. The plant grows in climates such as the tropical and temperate climates. It produces a large edible fruit (berry), having hard rind and lacking internal division. The flesh is sweet and juicy with color ranging from deep red to pink. It has numerous black seeds.The fruit of water melon is usually eaten raw. It can also be pickled. Its rind can be washed and consumed fresh or cooked. The flesh is also consumed as juice or as part of beverages(Wehner et al., 2001).

Watermelon is grown in sandy loam soil rich in organic matter with good drainage and pH range of 6.5-7.5(Kumar et al .,2013) .The plant has a life span of one year. It has long, weak and climbing stem which are 5-sided and about 3m long.The leaves are large, course, hairy pinnately-lobed and alternate. Watermelon is a fruit commonly consumed in many countries of the world. It contains more than 91% of water and about 7% of carbohydrates. It is a rich in organic compounds such as lycopene and citrulline Its rind contains more amount of citrulline than the flesh. It is rich in essential micronutrients, macro-elements,vitamins and phytochemicals .Due to the high level of lycopene and potassium in watermelon, it can prevent stroke and cradiovascular diseases(Le et al., 2005).Lycopene can also block inflammatory processes and works as an antioxidant to free radicals (Edwards et al., 2003). It is rich in vitamin B6, Manganese and ascorbic acid .Watermelon fruit is rich in vitamin A and these nutrients are good for immunity and vision. As a result of the high water content of the fruit, it aids digestion and rehydration. . Citrulline found in water melon seeds and rind is used in nitric oxide system in humans and has antioxidant and vasodilation roles (Rimando et al., 2005).The black water melon seeds are quite healthy and edible. They are rich in iron, zinc, protein and fiber . The seed has high arginine content showing that it has a lot of medicinal benefits (El-Adaway and Taha, 2001). They are rich in protein and essential fatty acids and there are prospects for the use of the seeds in the improvement of infant nutrition (Maynard, 2001) It is rich in antioxidants which reduces oxidative stress (Khaki et al.,2013). It is rich in phytochemicals such as flavonoids which has been reported to have positive effect on pancreative bête-cells in terms of proliferation and secretion of insulin (Mahesh and Menon, 2004).

Today, scientific research communities have shifted base to nutritional and phytochemical research on whole natural foods. This is because outside the primary nutrients it is estimated (Liu, 2004) that >5000 individual phytochemicals have been identified in fruits, vegetables , seeds and grains but a large percentage still remain unknown and need to be identified before we carefully understand the health benefits of phytochemicals such as alkaloids, flavonoids, tannins, carotenoids etc (Adekunle and Adekunle,2009). There is urgent need to find new products based on resources of biological origin and market them as they could be useful in making of food supplements, drugs and animal feeds.The study on the nutritional and therapeutic values of naturally processed tropical seeds is very vital because people will be encouraged via scientific information from the research to consume greater quantity of foods rich in seeds and nuts prepared in different forms which will eventually provide them with a better balance of nutrients to enhance health status. Recently, researchers all over the globe have indicated more interest to evaluate locally processed nuts (wild and domesticated) for their nutritional, phytochemical as well as other features for the well-being of human society. The research was aimed at determining the nutritional value and accessing the nutraceutical potential of locally processed tropical seed of *Citrillus lonatus*

II. Materials And Methods

Sample Collection and processing :

The seeds studied were those of *C. lanatus* . The seeds were extracted from the fruits of C. lanatus purchased from local farmers at Eleme, Port Harcourt , Rivers State on the 28th of July, 2020. The seeds were selected from the pulp, washed with clean water, exposed and allowed to dry under the sun. . The whole seed (unpeeled) were ground into paste and stored in labelled air tight containers for analysis on dry weight basis.

Analysis of Sample: The methods of the Association of Official Analytical Chemists (2006) were used to determine the moisture content, ash, crude protein , crude fibre and crude fat . For moisture, two grams [2g] of each sample was accurately weighed in a chemical balance and placed in a crucible with lid and dried in an oven (Plus II Sanyo Gallenkamp PLC England) set at 105ºC for 3hr, until a constant weight was obtained after cooling. The loss in weight was expressed as a percentage of the initial weight. For ash, 2g of each sample were weighed accurately into a porcelain crucible and ignited in a muffle furnace (Model LMF4 from

Carbolite, Bamford, Sheffield, England) at 550^{o}C until a light grey ash was obtained after 7 hours. To determine crude fat , 9g of the ground sample were transferred into a thimble and dried for 3 hrs at 100^{o}C. The extraction thimble containing the dried samples was in turn inserted into the extraction chamber. The extraction and its contents were fixed to a pre-weighed empty round bottomed flask which was in turn placed over an electro- thermal heater. Then 300ml of anhydrous diethyl ether was added and the condenser was fixed. The sample was then extracted for 6hrs, after which the solvent was distilled off, leaving the lipid in the flask to cool in a desiccator.

For crude fibre, 2g of the ground sample [in triplicate] were transferred into the crucible and dried for 3hrs at 100^{0}C. The dried samples were defatted by extraction with 10ml of petroleum ether (60- 80^{0}C), and air dried. The defatted sample was transferred into 100ml conical flask into which 20ml of 1.25% H_2SO_4 was added and mixed properly. An aliquot (190ml) of boiling H_2SO_4 was added into the flask and mixed properly such that a cream was produced. This flask was fitted to a reflux condenser and heated before its content was rapidly poured into shallow layer of hot water contained in a hot Buchner funnel prepared with a wet 12.5cm filter paper. Filtration was done by suction and the rate of suction adjusted such that filtration was complete within 10mins. The residue was washed free of acid with hot distilled water and quantitatively transferred into 100ml volumetric flask using 200ml of boiling 1.25% NaOH solution. Refluxing was done for 30mins and the filtrate was allowed to cool for a minute and filtered under suction. The residue was washed with several portions of boiling distilled water followed by 1% HCl, finally with boiling distilled water until an acid free residue was obtained. The residue was quantitatively and carefully transferred [using a clean spatula] into a weighed crucible, which was dried in an oven at 105^{o}C for 1 hr. Then, it was cooled in a desiccator and weighed. The dry residue was then placed in a furnace at 630^{0}C for 3hrs. The crude fibre content was calculated as well as the percentage crude fiber. The weight of ash was subtracted from that of the residue to obtain the weight of fibre. Crude protein (%Nitrogen x 6.25 0 was obtained by the method of AOAC(2006) using 2.0gportionsof the sample. The total carbohydrate content of the samples was determined by the difference method. The sum of the percentages of protein, fat, fibre and moisture was determined and the value subtracted from 100% to get the value for total carbohydrate (Onyeike and Acheru, 2002). The energy values were estimated by the Atwater factors of 4, 9 and 4 respectively, taking the sum of the products and expressing the results in kilocalories per 100g sample (Onyeike and Ehirim, 2001) . The minerals magnesium , calcium , potassium, zinc and iron were determined by atomic absorption spectrophotometry as described by AOAC (2006). Tannins , alkaloids, flavonoids, phytates and hydrocyanic acid were determined according to the method of AOAC (2006) . Saponins was determined by the method of (Obadoni and Ochuko,2001)

Data Analysis : Data were statistically analyzed by a one-way analysis of variance (ANOVA) using SPSS/PC + package. Differences between means were compared by Duncan’s (21) Multiple Range Test . Significance was accepted at a p-value of less than 0.05 ($p < 0.05$).

III. RESULTS AND DISCUSSION

Table 1: Proximate Composition (%) and Calorific value of seeds of *Citrullus lonatus*

Constituent	Percentage composition(%D.W)
Moisture	14.77±0.013
Dry matter	85.21±0.010
Ash	2.24±0.021
Crude protein	24.78±0.006
Crude fiber	11.27±0.010
Crude fat	28.48±0.013
Total carbohydrate	18.44±0.006
Calorific value(Kcal/100g sample)	429.47±0.295

Values are means ± standard deviations of triplicate determinations where %D.W.means percentage dry weight

Table 2: Macro-element composition (mg/kg) of seeds of *Citrullus lonatus*

Analyte	Composition(mg/kg D.W.)
Sodium	38.26±0.008
Potassium	457.60±0.670
Magnesium	89.45±0.006
Calcium	42.87±0.125

Values are means ± standard deviations of triplicate determinations where %D.W.means percentage dry weight

Table 3: Sodium : potassium and Potassium:sodium ratio

Sodium:potassium ratio(Na/K)	0.08
Potassium :Sodium ratio (K /Na)	11.96

Table 4: Quantitative phytochemical composition (mg/kg) of the seeds of *Citrullus lonatus*

Constituent	%Composition (mg/kg, D.W.)
Alkaloid	1.35±0.006
Flavonoid	3.37±0.047
Tannin	6.45±0.006
HCN	1.31±0.008
Phytate	0.77±0.008

Values are means ± standard deviations of triplicate determinations where %D.W.means percentage dry weight

The result of the proximate composition of seeds of C.lotanus is shown in Table 1.The moisture content was low.The value reported in this study was lower than those reported for F.capensis leaves by Okoroh et al (2019) and Uzoekwe et al., (2015), P.ostreatus sample (SAWCS) revealed by Okoroh et al (2017) but comparable to the moisture content reported for P.ostreatus sample (AVOS) highlighted by Okoroh et al (2017) . The moisturecontent of food reflects the water activity (Olutiola et al.,(1991) and it is used tomeasurefoodstability to contamination by microorganisms (Uraih and Izuagbe, 1990). The seeds can stay a longer period and their shelf life is enhanced. For low moisture content, the solid matter will definitely be high indicating that the level of certain food nutrients will relatively increase because of drying.

The seeds had high crude fat content .Okoroh and Onuoha (2019) also reported high crude fat content in peeled and unpeeled friedseeds of Anarcadum occidentale and A.hypogaea. The value was higher than those reported by Adeyeye and Omolayo (2011) for A.hybridus and T.ocidentalis leaves. Fredrick et al , (2013), Okoroh et al (2017), and Sumaira et al, (2016) reported lower fat contents for P. citrinopilaetus, P.ostreatus samples (AVOS, WWS and SAWCS), and G.lucidium,,P.ostreatus and H.erinaceus respectively. *P. ostreatus* samples , P. *citrinopilaetus* and *G. lucidum* respectively. Seeds and nuts are natural plant foods which serve as good sources oils rich in monounsaturated and polyunsaturated fatty acids. They have been assumed as an essential part of optimal diets for the prevention of CHD by leading experts in the field (Hu and Willet, 2002).

The study revealed high protein content in the seeds of C.loatus. Adeyeye and Omolayo (2011), Aletor (1999) reported higher protein value for A.hybridus and T.ocidentalis leaves . Uzoekwe et al., (2015), Danlani et al.,(2012) , Olaniyi et al .,(2018) , Okoroh et al., (2019) and Okoroh et al., (2017) reported low protein content for F.capensis leaves, S.virosa leaves, C.cujete leaves, F.capensis leaves and P.ostratus fruiting bodies from organically supplemented samples (SAWCS, WWS and AVOS) respectively. Since theseeds of C.lonatus analyzed in thisstudy are rich in protein, they can therefore be eaten in diets as good sources of protein for humans. Proteins are building blocks in the cells and tissues for repair and growth.

The seeds of C.lonatus investigated in this study revealed high fiber value compared to the value reported by Uzoekwe et al., (2015) and Okoroh et al., (2019) for leaves of F.capensis and unpeeled fried seeds of Anarcadum occidentale and A.hypogaea (Okoroh et al .,2019) respectively and but lower than values reported by Olaniyi et al., (2018) for C.cujete leaves and higher than values reported by Okoroh et al., (2017) , Akubugwo et al., (2007) for P.ostratus and A.hybridus respectively. Dietary fiber is important in the system as it helps tolower cholesterol level andreduces the risk of heart diseases and diabetes (Ishida et al., 2000). The consumption of appreciable amount of the seeds of C.lonatus as snacks, in foods and supplements can be recommended as fiber aids digestion, softens stool,prevents constipation and enhances bowel movement (Ayoola and Adeyeye, 2010). However, consumption of too much seeds of C.lonatus implies high level of fibe and this may cause irritation of the intestine. It may also cause decrease in nutrient availability.

The study showed low ash content of the seeds of C.lonatus. The value was lower than those reported by Uzoekwe et al., (2015), Olaniyi et al., (2018), Sumaira et al., (2016) for F. capensis leaves, C.cujete leaves and P.ostreatus respectively on dry weight basis. Ash reflects mineral values of the seeds.

Total carbohydrate in this study was found to be lower than the value reported by Dike (2012) for M.lucida but the value was in consonant with that reported by Sofowora (1993) for M.poggei.

The calorific value of C.lonatus seeds was found to be high.Thiscould be due to the high protein and high crude fat content of the sample. This is in consonant with the carbohydrate value highlighted by Okoroh and Onuoha (2019) for peeled and unpeeled fried seeds of Anarcadum occidentale and A.hypogaea but lower than the value reported for A.wilkensiana and T.procumbens (Ikewuchi, 2012). The results obtained showed that seeds of C.lonatus are good sources of proteins, plant oils, fiber and energy.

The macro-element composition of the seeds of C.lonatus are shown in Table 2. The mineral consentrations revealed in this study showed that potassium was very high while that of sodium was appreciable in the seeds on dry weight basis. Uzoekwe et al., (2015) reported lower value of potassium and sodium for the bark of F.carpensis while Olaniyi et al ., (2018) also reported very low value of sodium for F. capensis on dry weight basis. Sodium and potassium are found in extracellular and intracellular fluids in humans and help to maintain electrolyte balance and membrane fluidity. Potassium helps to maintain acid-base balance of the hydrogen ion concentration of the body tissues. Islam et al.,(2004) reported that potassium aids with calcium, magnesium, phosphorus and iron to complete theabsorption of proteins, carbohydrates and vitamins.

The value of magnesium was found to be high in the seeds analyzed. This concentration is lower than the level of magnesium found in the leaves and bark of F.capensis reported by Uzoekwe et al.,(2015) but higher than the value reported for C.cujete leaves (Olaniyi et al., 2018). Magnesium helps in metabolism in the body. It plays great role in specificneuro-muscular activities aswell as actions in organsand systems linked with theheart and itsassociated vessels (Ryan, 1991).

The value of calcium reported in this study on dry weight basis was high. Okoroh et al.,(2019) reported a comparable value for F.capensis leaves in their study but Uzoekwe et al .,(2015) reported a higher value for F.capensis bark while Olaniyi et al., (2018) reported a lower value for C.cujete leaves. Living tissues need calcium as essential structural and functional cation.Calcium helps in the building and maintain of bone mass and its lack could lead to malformation of bones in young animals and egg shell formation. Calcium intake is essential and safer for cancer patients than certainchemotherapeutic agents that may cause osteopenia and osteoporosis(Hasan et al ., 2004).

The sodium:potassium ratio and potassium sodium ratio calculated in this study are revealed in Table 3. The sodium to potassium ratio was less than 1 and the potassium to sodium ratio was greater than 1. These values indicate that the consumption of appreciable quantity of theseeds of C.lonatus may help to improve cardiovascular health.

The result of the quantitative phytochemical analysis of theseeds of C.lonatus is depicted in Table 4. Tannin was highest was high in value followed by flavonoid and then alkaloid. HCN and phytate were also present but in low levels.The level of alkaloid reported in this study was lower than that highlighted by Nkafamiya et al (2010) for F.asperifolia and F.sycomorus respectively but the value of tannin reported in this study was higher than that reported for F.sycomorus(Nkafamiyaet al ., 2010) but the level of HCN and phytate was comparable. Phytates are known to bind minerals and vitamins in foods thereby reducing their availability for boy utilization (Olusanya, 2008). The seeds of C.lonatus have been highlighted to be rich in phytochemicals such as flavonoids which has been indicated to have positive effect on pancreatic bête-cells in terms of proliferation and secretion of insulin (Mahesh and Menon , 2004).These phytochemicals (bioactive compounds) may be responsible for their medicinal properties.

IV. Conclusion

This study shows that the seeds of C.lonatus are rich in crude protein, crude fiber fiber, crude fat and total carbohydrate,essential minerals (potassium, calcium, magnesium and sodium), bioactive compoundssuch as tannin, alkaloid and flavonoid. These results therefore suggest that the seeds of C.lonatus are good sources of these nutrients and bioactive compounds and their consumption in appreciable quantities may aid in meeting nutritional and nutraceutical needs for normal growth, development and prevention of deficiency diseases in localities where they exist.

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Competing interests

The authors hereby declare that there was no conflict of interest or financial inducement which may have negatively influenced them in writing this scholarly article

Author's contributions

P.N.O. designed the study and drafted the manuscript ,O.D.O carried out the experiments,   C.OC. collected resource materials , U.C.Y. conducted statistical analysis , S.C.O. supervised laboratory work   and edited the manuscript , E.U.G. revised and edited the manuscript . The authors read and approved the manuscript.

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