PHYTOCHEMICAL SCREENING AND NUTRITIONAL PROFILE OF *Citrullus lanatus* SEEDS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

I. J. Opara[1]*, M. E Onubia[2], E. O. Onunze[3], C. O. Oko-Udu[4] and I. Aondoyima[1]

[1]Department of Chemical Sciences, Federal University Wukari, PMB 1020 Taraba State, Nigeria
[2]Department of Pure and Applied Physics, Federal University Wukari, P.M.B 1020 Taraba State, Nigeria
[3]Department of Chemistry, Taraba State University, Nigeria
[4]Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka
*Corresponding author: j.opara@fuwukari.edu.ng, ifyuniquegreat@yahoo.com

Received: February 03, 2018 **Accepted:** March, 2018

Abstract: In this study the seeds of *Citrullus lanatus* were analyzed for their phytochemical compositions. The phytochemical screening result indicates that the seeds contain alkaloids, flavonoids, tannins, glycosides, steroids, terpenoids and saponins. The quantitative analysis for total alkaloids, saponins and flavonoids contents showed that *Citrullus lanatus* contains 35.613% alkaloids, 4.216% flavonoids and 1.820% saponins. The proximate composition results showed that the mean nutritional content of the *Citrullus lanatus* seeds contain (4.20±0.01) moisture (2.70±0.02) ash, (7.20±0.01) crude fibre, (32.08±0.03) crude protein, (30.30±0.01) crude lipid, and (23.52±0.04) carbohydrates contents. The proximate analysis also shows that the seeds of *Citrullus lanatus* havehigh fibre, proteins and lipids contents. The ash content also indicates that the seeds of *Citrullus lanatus* contain some minerals. Thus, *Citrullus lanatus* seeds can be used as food supplements.

Keywords: *Citrullus lanatus*, phytochemical, proximate, seed, extract

Introduction

Plants are rich in chemical constituents that have medicinal properties which help to sustain and improve human health (Soheil *et al.,* 2013). These chemical constituents contain various phytochemical molecules which include secondary metabolites. Secondary metabolites contain the bioactive constituents of plants which are the active ingredients of many drugs. The most important of these bioactive constituents of plants are terpenes, alkaloids, flavonoids and phenolic compounds **(**Salem e*t al.,* 2016; Usunobun *et al*., 2014)

Phytochemicals naturally occur in the medicinal plant leaves, stem bark, seeds, fruits and roots that act as defense mechanism and protect the body from diseases (Rohit, 2015). They cure diseases without causing any harm to human beings these can also be considered as "man- friendly" medicines (Sahira, 2015).

A number of research works have been carried out on seeds and oils from seed of different plants and there is an indication that some plants could become promising oil sources for many purposes including nutritional and medicinal purposes.

Plants contain therapeutic components which are often subjected to phytochemical screening to ascertain the bioactive components present in them. The therapeutic value of plants lies in some phytochemical constituents present in it that may be useful for healing of human diseases (Pradeepa, 2016)

The main aim and objective of this research study is to carry out phytochemical screening and proximate analysis on the seeds of *Citrullus lanatus* to ascertain its therapeutic and nutritional value.

Materials and Methods

Sample collection

Citrullus lanatus fruits were bought from new market Wukari, in Taraba State, Nigeria and were identified and authenticated by Dr Mrs.Shingu from Crop production Department, Federal University Wukari.

Sample preparation

Citrullus lanatus fruits were cut open and the seeds removed, the seeds were washed properly under running water to remove the rind and juices stuck to the seeds, they were air dried for 2 weeks followed by oven-drying at a temperature of 40^0C before been pulverized with an electric blender and stored in airtight containers for further work.

Serial exhaustive extraction

Cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from non polar to a more polar solvent.The solvents employed in the extraction includehexane, chloroform, ethyl acetate, acetone, ethanol and water. This was to ensure that a wide polarity range of compounds was extracted. The extracts of the seeds were prepared by soaking 200 g pulverized *Citrullus lanatus* seeds in n-hexane in an airtight container for 4 (four) days with frequent agitation until soluble matter were dissolved. The resulting mixture was filtered using Whatmann No.1 filter paper and the filtrate was concentrated by evaporation using rotary evaporator. The procedure was repeated on the residues using chloroform, ethyl acetate, acetone, ethanol and water sequentially in order of increasing polarity (Ahmed *et al.,* 2017).

Phytochemical screening

The preliminary phytochemical analysis of the extracts were carried out to ascertain the secondary metabolites present in *Citrullus lanatus* seeds using standard described Sofowora (1990), Trease and Evans (1989), Harborne (1973).

Test for alkaloids

0.5 ml of the extract was measured into a 100 mL conical flask containing 2 ml of 5% H_2SO4 in ethanol. The mixture was heated to boiling in a water bath and was allowed to cool and then tested for the presence of alkaloids

Mayer's test:

2ml of filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test

2 mL of the filtrates were treated with Wagner s reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids

Test for flavonoids

Lead acetate test

2 mL of extracts were treated with 4 mL of 10% lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.

Test of terpenoids

Salkowski's test

1 g of each extract was mixed with 2 mL of chloroform, and 2 mL of concentrated H_2SO_4 was carefully added to form a layer. An appearance of a reddish brown color interface indicated the presence of terpenoids.

Test of phenols

Ferric chloride test

10 mg extract is dissolved in 2 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

Test of glycosides

To 2 ml of extract with dilute HCl and 2 ml Sodium nitropruside in pyridine and sodium hydroxide solution were added. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Test of tannins

2 mL of extract was mixed with 2 mL of water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates that the presence of tannins.

Test of saponins

Foam test

About 0.5 mg of the extract was shaken with 5 mL of distilled water in a test tube and warmed.The formation of stable foam was taken as an indication of the presence of saponins

Quantitative determination of some detected phytochemical constituents

Estimation of alkaloids

Determination of alkaloids was done by using Harborne (1973) method. 5 g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of flavonoids

10 g of plant sample was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through a Whatmann No.42 filter paper into a pre weighed 250 mL beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah *et al.*, 2009*).*

Estimation of total saponins

The method used was that of Obdoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 mL of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separation funnel and 20 mL of diethyl ether was added and shaked vigorously. The aqueous layer was recovered while the ether layer was discarded.

The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated.

Proximate analysis

The pulverized seeds were taken for proximate analysis. The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre contents were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition.

Results and Discussion

Table 1 and 2 show the results of preliminary phytochemical analysis and quantitative determination of some phytochemicals constituents of the seeds of *Citrullus lanatus*. It revealed the presence of six phytochemicals in the screened sample.

Table 1: Result of phytochemical screening of *Citrullus lanatus* seeds

	H. extract	C. extract	E. ace extract	Ace. extract	E. extract	Aqu. extract
Alkaloids	+	-	+	+	-	-
Flavonoids	+	-	+	+	+	+
Tannins	+	-	-	+	+	-
Glycosides	-	+	+	+	+	+
Steriods	+	+	+	+	-	-
Phenols	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	-
Saponins	+	+	+	+	-	+

+ = Present; – = Absent; **H** = Hexane; **C** = Chloroform; **E** = Ethyl acetate; **Ace** = Acetone; **E.** Ethanol; **Aqu.** = Aqueous

Table 2: Quantitative determination of some phytochemicals constituents

Phytochemical constituents	*Citrullus lanatus* (%)
Alkaloids	35.613
Flavonoids	4.216
Saponins	1.820

Alkaloids were detected in ethyl acetate, acetone and hexane extracts but absent only in chloroform, ethanol and aqueous extracts. The concentration of alkaloids present in the *Citrullus lanatus* seed is (35.61%) which iswithin the range (33.795 ± 0.035) reported by Damilola *et al.* (2015) for *Citrullus lanatus* seeds. Alkaloids are known to have muscle relaxant property and can be utilized for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004).

Saponins were detected in hexane, ethylacetate, acetone, chloroform, and aqueous extracts but absent in ethanol extract. The value obtained for saponins in *C. lanatus is* 1.82%. The presence of saponins in the seeds can be useful in treating inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness (Rita *et al.*, 2015). Also in nature, saponins appear to act as antibiotics that protect plants from microbes. In humans, saponins might fight cancer and infection (World health.net). Adelani *et al.* (2015) reported the presence of saponin in cold water extract of *Citrullus lanatus*.

Flavonoids were detected in hexane, ethylacetate, acetone, ethanol and aqueous extracts but absent in chloroform extract. The value of flavonoids found in *C. lanatus* is 4.21%. Flavonoids in plants comprise a vast array of biologically active compounds which have been used in traditional medicine for many years and have antioxidant and anti-proliferative effects especially against chronic inflammatory and allergic diseases, breast cancer and coronary artery disease (Ochwang'I *et al.*, 2016). They are also potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Okwu *et al.*, 2006).

The screening revealed the presence of tannins in hexane, acetone and ethanol extract. Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane (Njoku & Akumefula, 2007). Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism. Hence, the seeds could act as an efficient antimicrobial drug (Pradeepa *et al.,* 2016). Tannins also interfere with protein synthesis.

Terpenoids were detected in hexane, ethylacetate, acetone, chloroform, and ethanol extracts but absent in aqueous extract. Terpenoids are antifungal and antibacterial which is attributed to their membrane disruption action and inhibitory action on bacterial cell or fungus (Tawheed and Monika, 2014).

Steroids were detected in hexane, ethylacetate, acetone andchloroform but absent in ethanol and aqueous extract. Steroids in plants have been shown to exhibits analgesic properties and responsible for central nervous system activities (Ahmed and Mohammad, 2014).

Glycosides were detected in ethylacetate, acetone, chloroform, ethanol extracts and aqueous extracts but absent in hexane extract. Glycosides are beneficial in reducing inflammation, protecting against endotoxemia and may be used in cardiac treatment of congestive heart failure (Tawheed and Monika, 2014)

Proximate analysis

Table 3 shows the proximate analysis of *Citrullus lanatus*. The result revealed that the seeds of *C. lanatus* contained 4.20±0.01 moisture, 30.30±0.01 crude lipid, 32.08±0.03 crude protein 7.20±0.01crude fiber, 2.70 ±0.02 ash and 23.52±0.04 carbohydrate. *C. lanatus* seeds were found to have high crude lipid content 30.30% which is higher than that reported by (Betty *et al*., 2016) for three varieties of water melon; Charleston gray (26.83±4.24), Crimson Sweet (26.50±4.27) and Black diamond (27.83±2.63) and lower than that reported for *Cocosnucifera linn* (48.80±0.38) and *Cococynthis citrullus* (50.42±0.52). (Mabalaha *et al*., 2007) also reported oil yields of seeds ranging from 24.8-30.0% in *Citrillus lanatus* and *C. colocynth* species respectively. The percentage of fat extracted from *C. lanatus* seeds as reported by (Oyeleke *et al*. 2012) was 47.9% which is higher than the results obtained from the seeds of *C. lanatus* in this study. The high crude lipid content found in the seed is an indication that the seed could be source for good, cheap and novel source of oils that would be utilized for both domestic and industrial purposes. Crude lipids are essential due to the ability to provide the body with maximum energy (Otutu *et al.,* 2015).

Table 3: Proximate analysis of *Citrullus lanatus*

Proximate composition	*Citrullus lanatus* (%)
Moisture content	4.20±0.01
Ash content	2.70±0.02
Crude fibre	7.20±0.01
Crude protein	32.08±0.03
Crude lipid	30.30±0.01
Carbohydrate	23.52±0.04

The protein contents of obtained in the *C. lanatus* is high and similar to what has earlier been reported by (Damilola *et al*., 2015). Proteins are essential component of the diet needed for survival of animals and humans, which function basically in nutrition by supplying adequate amounts of required amino acids. The presence of seed coat (shell) in the ground seed used for analysis accounted for the high fiber content of the seeds under study 7.20±0.01. Crude fibre in the diet consists mostly of the plant polysaccharides that cannot be digested by human dietary enzymes such as cellulose, hemicelluloses and some materials that make up the cell wall. This suggests that both seeds would provide additional dietary fibre in the diet. The ash contents of *C. lanatus* seeds 2.70 ±0.02 in the present study are higher than that of earlier report (2.4%) by (Yanty *et al.,* 2008), and lower than that reported by (Domilola *et al.,* 2015) (4.138 ± 0.015).The ash percentages of the seeds indicate the total inorganic content from where the mineral content could be obtained. Mineral elements speed up metabolic processes, improve growth and development. The moisture content result obtained for *C. lanatus* was 4.20±0.01 which is higher than those reported for kersting's groundnut (1.7 ± 0.12%) and cranberry bean (1.7 ± 0.51%) (Aremu *et al.,* 2006) but lower to those reported for *Luffa cylindrical* (5.8%) (Olaofe *et al.,* 2008). The high moisture content is an indication that both seeds may likely be susceptible to microbial attack. The carbohydrate content of the seed was 23.52±0.04. The carbohydrate content obtained from the present study is similar to earlier report (23.18%) by (Loukou *et al*., 2007). Carbohydrates are polar compounds which are readily converted into glucose as source of energy.

Differences in percentages of protein, lipid, fibre, moisture, ash and carbohydrate contents in comparison to the present results could likely be attributed to varietal and regional/soil differences.

Conclusion

The present study showed that the seed of *Citrullus lanatus* contains nutritional components and various phytochemicals like alkaloids, steroids, glycosides, saponins, flavonoids, terpenoids and tannins. These compounds naturally occur in most plant materials and have proven to have medicinal properties including anticancer, antitumor, anti-malarial, anti-diuretic, antipyretic, antimicrobial, antifungal activities among others. Thus, the seeds of *C. lanatus* have potential effect on degenerative diseases.

References

Adelani A, Tabitha A, AjibaLilian C, Dahunsi SO & Oluyori AP 2015. Antibacterial activity of watermelon (*Citrullus lanatus*) seed against selected microorganisms. *Afr. J. Biotechnol.*, 14(14): 1224-1229.

Ahmed O & Mohammad Ali., 2014. Qualitative and Quantitative Analysis of Phytochemicals of *Loranthus bengwensi* leaf. *Int. R. J. Pharm. Sci*., 05(01): 1-3.

Ahmed RN, Jumah SS, Arekemase MO, Agbabiaka TO, Adam AI & Adejoro DO 2017. Serial exhaustive extraction: Influence of solvent polarity on antibacterial activity of extracts of leaves of *Tithonia diversifolia*. *Nig. J. Pure & Appl. Sci*., 30 (1): 1-10.

AOAC 2000. Association of Official Analytical Chemists; Official method of analysis. 15thEdn, Washington DC, p. 212.

Aremu MO, Olaofe O & Akintayo ET 2006. A comparative study on the chemical and amino acid composition of some Nigerian underutilized legume flours. *Pak. J. Nutr*., 5: 34 – 38.

Betty T, Jacob KA, Faustina D & Elsa IO 2016. Watermelon seeds as food: Nutrient composition, phytochemicals and antioxidant activity. *Int. J. Food Sci. Nutr*., 5(2), 139-144.

Damilola AO, Glory OC, Garba JD, Chintua EI, Kerian CN, Martin UO & Flourence AA 2015. Evaluation of chemical compositions of *Citrulluslanatus* seed & *Cocos nucifera* stem bark. *Afr. J. Food Sci*., 6(3): 75-83.

Harborne JB 1973. Phytochemical Methods. 3rd edn. Chapman and Hall Ltd., London, pp. 135-203.

Krishnaiah D, Devi T, Bono A & Sarbatly R 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Plants Res*., 3(2): 67-72.

Loukou AL, Gnakri D, Dje Y, Kippre AV, Malice M, Baudoin JP & Zoro B 2007. Macronutrient composition of three cucurbit species cultivated for seed consumption in Cote D'lvoire. *Afr. J. Biotech*., 6(5): 529-533.

Mabalaha MB, Mitei YC & Yoboah SO 2007. A Comparative study of the properties of selected melon seeds oils as potential candidates for development into commercial edible vegetable oil. *J. Am. Oil Chem. Soc*., 84: 31-34.

Njoku PC & Akumefula MI 2007. Phytochemical and nutrient evaluation of spondias mombin leaves. *Pak. J. Nutr*., 6: 613-615.

Obadoni BO & Ochuko PO 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global J. Pure & Appl. Sci*., 8: 203-208.

Ochwang'I DO, Kimwele CN, Oduma JA, Gathumbi PK & Kiama SG 2016. Phytochemical screening of medicinal plants of the Kakamega Country, Kenya commonly used against *Cancer. Med Aromat Plants*, 5: 277.

Okwu D 2004. Phytochemicals and vitamin content of indigenous species of south-eastern Nigeria. *J. Sustain. Agric. Environ*., 6(1): 30-37.

Okwu DE & Josiah C 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr. J. Biotech*., 5 (4): 357-361.

Olaofe O, Okiribiti BY & Aremu MO 2008. Chemical evaluation of the nutritive value of smooth luffa (*Luffa cylindrica*) seed's kernel. *Elec. J. Env. Agricult. Food Chem*., 7(10): 3444 – 3452.

Otutu OL, Seidu KT, Muibi BO, Oladokun F & Oyalowo MR 2015. Potential food value of watermelon (*Citrullus lanatus*) seed constituents. *IJST*., 3(7): 1-10.

Oyeleke GO, Olagunju EO. & Ojo A 2012. Functional and physicochemical properties of watermelon (*Citrullus lanatus*) seed and seed-oil. *IOSR-JAC*., 2(2): 29-31.

Pradeepa M, Kalidas V & Geetha N 2016. Qualitative and quantitative phytochemical analysis and bactericidal activity of pelargonium graveolens l'her. Int J App Pharm., 8 (3): 7-11

Rita N., Baruah KK, Sarma S, Bhuyan R., Roy DC & Mithu D2015. Phytochemical Screening of Different Plants of North-Eastern Region of India.J Biosci Bioeng., 2:9-11

Rohit KB 2015. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. *J. Pharmacogn Phytochem*., 4(1): 07-09

Sahira B & Catherine L 2015 General techniques involved in phytochemical analysis. *IJARCS.*, 2(4): 25-32.

Salem ME, Atega A, Afifa O & Fouzy A 2016. Qualitative and quantities analysis of phytochemicals of various extract for *Ephedraaltissima* from Libya. *J. Med. Plants Stud.*, 4(3): 119-121.

Sofowara A 1990. "Phytochemical screening of nigerian medicinal plants". *Parts III, Lioyeria*., 41: 234-246.

Soheil ZM, Bey HG, Chim KC, Tara S & Habsah AK 2013. Biological activities and phytochemicals of *Swietenia macrophylla King. Molecules*., 18: 10465-10483.

Stray F 1998. The natural guide to medicinal herbs and plants. Tiger Books International, London, pp. 12-16.

Tawheed A & Monika T 2014. A comparative study on proximate composition, phytochemical screening, antioxidant and antimicrobial activities of *Linum usitatisimum L*. (flaxseeds). *Int. J. Curr. Microbiol. App. Sci*., 3(4): 465-481.

Trease GE & Evans WC 1989. "Pharmacognosy*"* 11th edn, Baillere Tindoll, London, pp. 45-50.

Usunobun U, Okolie NP, Anyanwu OG & Adegbegi AJ 2014.Phytochemical screening and proximate composition of *Annona muricata* leaves. *EJBPSP*., 2 (1): 18-28.

Yanty NA, Lai OM, Osman KL & Ghazali HM 2008. Physicochemical properties of cucumis Melo Var. Inodorus (*Honeydew melon*) seed and seed oil. *J. Food Lipids*., 15(1): 42-55.