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Phytochemical composition and antioxidant activity of the indigenous watermelon seeds obtained kano state, Nigeria

Bayero AS[1], Datti Y[2], Usman LA[3], Asmau AA[4], Nura T[5], Garba AA[6], Aliyu U[7], Dikko MA[8], Zakari AH[9], Yusuf M[10]
[1-4] Department of Chemistry, Yusuf Maitama Sule University, Kano State, Nigeria
[5] Department of Chemistry, FCE (T) Bichi, Kano State, Nigeria
[6, 7] Department of Science Laboratory Technology, Kano State Polytechnic, Nigeria
[8-10] Department of Science Laboratory Technology, Federal Polytechnic Nassarawa, Nigeria

Abstract
The seeds of *Citrullus lanatus* (watermelon) happen to be the most disregarded and discarded seeds after consumption of their fruits. However, recent discoveries have shown that these seeds are by far very beneficial. This study determined the phytochemicals and the antioxidant activity of the seeds of watermelon variety available in Kano State, Nigeria. The phytochemical screening was carried out using standard procedures, while the antioxidant activity was analysed using the DPPH free radical scavenging assay. The results for the phytochemical analysis revealed the presence of alkaloids, carbohydrates, oils and fats, flavonoids, phytosterols, proteins and saponins, while tannins and terpenoids were found to be absent. The DPPH% inhibition was found to be 79.44% inhibition with trolox equivalent of 109.57 µM/g. The present findings suggest that watermelon seeds not only has nutritional value, but may also have many health and economic benefits.

Keywords: phytochemicals, radical scavenging, DPPH, *Citrallus lanatus*

Introduction
Citrallus lanatus popularly known as watermelon is a herbaceous plant belonging to the family *cucurbitaceae* (Gupta *et al.*, 2018) [14] and an important horticultural crop, mostly known for its sweet juicy fruit (Munisse *et al.*, 2011; Acar *et al.*, 2012) [20, 1]. it is a tropical fruit which can be taxonomically classified as the gourd family native to Southern Africa (Kalahari Desert), and it is a drought tolerant crop which is cultivated chiefly in tropical, semi tropical and rigid regions of the world (Reetapa *et al.*, 2017) [25]. Different varieties of watermelon are available and some of the varieties are: sugar baby, golden midget, star light, jubilee, yellow baby etc. They not only vary on their size (large or small) but also in their shape (oval, round or oblong) and colour of the flesh (red, orange and yellow) (Reetapa *et al.*, 2017) [25].

The recent increase in the prevalence of nutrients deficiency diseases especially in Nigeria (and Africa in general) can be attributed to poverty and lack of sufficient knowledge of the economical and nutritional values (contents) of many of the locally available and easily accessible edible plant crops (Achu *et al.*, 2013) [2]. Exploitation of underutilised crops can boost economic and health development, especially in developing countries where intake of sufficient quantities of nutrients is less than desirable (Wani *et al.*, 2011) [29]. Studies on watermelon seeds have shown that they contain high sulphur containing amino acids, as well as high levels of essential amino acids except lysine and. They also have high crude protein content of 23.4%, with good quantities of argenine, isoleucine, leucine, and phenylalanine which are essential amino acids as well as glutamic acid and aspartic acid (Achu *et al.*, 2013) [2].

Watermelon seeds are by far the most underutilised and discarded oil seeds. However, they are known to have economic benefits especially in countries where cultivation is on the increase where snacks milled into flour and used for sauces have been prepared from the seed (Betty et al', 2016) [6]. Watermelon seeds contain an antioxidant known as cucurbocitrin, which is extracted and used in lowering blood pressure and improvement of kidney function (Oseni and Okoye, 2013) [23]. Oil from the seeds is used in cooking and constitutes the production of cosmetics (Jensen *et al.*, 2011) [16]. Seed oils have varying important nutritional sources of oils which have the industrial and pharmaceutical ethno-medicinal use (Nzikou *et al.*, 2010) [22]. The nutritional contents of watermelon seeds are of immense quality; they are rich sources of proteins, vitamins B, minerals such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese, copper and lipid, carbohydrates, fibre and the likes. It also contains some phytochemicals including saponins, alkaloids, phenols, flavonoids and tannins (Braide, 2012) [8]. These bioactive components are responsible for the antimicrobial, antimalaria, anti-inflammatory, anticancer, anti-infection (Adesanya *et al.*, 2011) [3] and antioxidant activities (Loiy *et al.*, 2011) [19]. This paper reports the phytochemical composition and antioxidant activity of the indigenous watermelon found in Kano State, Nigeria.

Materials and Methods
Sample Collection and Preparation
Ten matured watermelon fruits (five for each species) were purchased from Na'ibawa and Yankaba markets in Nasarawa and Tarauni Local Governments of Kano State,

Nigeria. The fruits were sliced open using a clean stainless steel laboratory knife and the seeds were removed and washed with distilled water then air-dried for two week. The dried seeds were then milled with a laboratory blender then packed in an air tight container and stored in a dessicator (containing silica gel) ready for further analysis.
All the chemicals used were of analytical grade.

Phytochemical Screening
The qualitative phytochemical screening of the watermelon seed extracts was carried out according to different standard procedures to ascertain the phytochemical composition of the seed.

Test for Alkaloids
Mayer' s test: Two drops of Mayer's reagent were added to 2 ml of the watermelon seed extract along the sides of test tube (Evans 1997) [10].

Test for Carbohydrates
Benedict' s test: Benedict's reagent (0.5 ml) was added to 0.5 ml of the watermelon seed filtrate. The mixture was heated on a boiling water bath for 2 minutes (Benedict, 1908) [5].

Test for Oils and Fats
Spot Test: A small quantity of extract is pressed between two filter papers (Sahira and Cathrine 2015) [26].

Test for Flavonoids
Alkaline Reagent Test: A measure of 2 ml of 2% NaOH was mixed with aqueous watermelon seed extract, a concentrated yellow color was produced which became colorless when 2 drops of the diluted acid was added (Sofowora 1993) [27].

Test for Phytosterols
Libermann-Burchard's Test: The watermelon seed extract (50 mg) was dissolved in of 2 ml acetic anhydride, and to these 2 drops of concentrated sulphuric acid were slowly added along the sides of the test tube (Finar 1986) [11].

Test for Proteins
The extract (10 mg) was dissolved in 5 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate was subjected to Biuret test for proteins. Here 2 ml of the filtrate was treated with 1 drop of 2% copper sulphate solution, and 1 ml of (95%) ethanol was added, followed by excess of potassium hydroxide pellets (Gahan 1984) [12].

Test for Saponins
The watermelon seed extract (50 mg) was diluted with distilled water and made up to 10 ml. The suspension is shaken in a graduated cylinder for 15 minutes (Kokate 1999) [17].

Test for Terpenoids
Noller's Test: The watermelon extract (2 mg) was taken in a dry test tube and was treated with a bit of tin foil and 0.5 ml of thionyl chloride, followed by gentle heating (Sourabh *et al.,* 2014) [28].

DPPH Antioxidant Activity of Watermelon Seed
Watermelon seeds flour ability to scavenge lipid-soluble 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, which resulted in the bleaching of the purple color exhibited by the stable DPPH radical was determined using the procedure of Amarowicz et al. (2008). Two ml of the watermelon seed extract was added to 1 ml of methanolic solution of DPPH. The mixture was vigorously shaken and kept in a dark chamber for 30 min before measuring the absorbance at 517 nm using UV-Vis spectrophotometer. Free radical scavenging ability was calculated as percentage of DPPH radical discolouration.

Results
The sample of the watermelon seed was tested for different phytochemical composition including alkaloids, carbohydrates, oils and fats, flavonoids, phytosterols, proteins, saponins, tannins and terpenes and the results are presented in Table 1. The result for the antioxidant activity of watermelon seed is presented in Table 2.

Table 1: Qualitative Analysis of the Watermelon Seed Extract

S/NO	Phytochemical Constituents	Watermelon Seed Extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Oils and Fats	+
4.	Flavonoids	+
5.	Phytosterols	+
6.	Proteins	+
7.	Saponins	+
8.	Tannins	-
9.	Terpenoids	-

+ Present-Absent

Table 2: Antioxidant activity of the Watermelon seed Sample

Sample	% DPPH Inhibitor	Trolox Equivalent (µM/g)
Watermelon Seed	79.44%	109.57 µM/g

Discussion
The phytochemical analysis/screening of the watermelon seeds showed the presence of alkaloids, carbohydrates, oils and fats, flavonoids, phytosterols and proteins, while terpenoids and saponins were absent. This result resemble that reported by Betty *et al.,* (2016) [6]. Presence of saponin in this study is supported by finding from Kudirat and Otutu (2016) [18] (Edeoga *et al.*, 2005; Kim *et al.*, 2003). Saponins have been reported to be effective and responsible for the treatment of many conditions such as inflammation, pre-and post-menopausal symptoms (Bombardelli and Gabetta, 2001) [7], cardiovascular and hypertension (Yao *et al.*, 2005). Purified saponins have been used in the manufacture of food and drinks primarily as foaming agents and antioxidants (Takashi *et al.,* 1986). Kudirat and Otutu (2016) [18] reported the presence of alkaloids in dried watermelon seeds, and this is in agreement to this study which confirms the presence of this phytochemical in watermelon seed. Some alkaloids from plant sources are reported to of medicinal actions as analgesics, antispasmodics, anti-cholinergics and anaesthetics (Okwu, 2004), antimicrobial activity of some alkaloids have also been reported (Olaleye, 2007). The presence of flavonoids in the analysed sample of watermelon seed is in agreement

with the findings by Braide *et al.,* (2012) [8]; Nwankwo *et al.,* (2014) [21] and Kudirat and Otutu (2016) [18]. Flavonoids are a group of secondary metabolites found in some plants which have been shown to exert potent antioxidant activity against the superoxide radical (Kudirat and Otutu 2016) [18]. They have also been used as natural antioxidants in foods and pharmaceutical drugs due to their ability to scavenge reactive oxygen species (Bombardelli and Gabetta, 2001) [7]. Other phytochemical present in the analyzed watermelon seeds are carbohydrates, fats and oils, proteins and phytosterols, and this finding is supported by similar findings by Braide *et al.,* (2012) [8]; Nwankwo *et al.,* (2014) [21] and Kudirat and Otutu (2016) [18]

The absence of tannins in this reseach work is supported by similar report by Braide *et al.,* (2012) [8]; Nwankwo *et al.,* (2014) [21] and Kudirat and Otutu (2016) [18]. Tannins are known to have many biological avtivities (Arapitsas 2012) [4] but if ingested in large quantities, they tend to inhibit the absorption of minerals such as iron and calcium, and this may lead to aneamia or osteoporosis (Varadharajan *et al.,* 2012). However, the absence of terpenoids in this reseach work contradicts many researches that confirm the presence of triterpenes in watermelon seeds. Reports by Braide *et al.,* (2012) [8]; Betty *et al.,* (2016) [6]; Edori and Marcus (2016) [9] have all confirmed the presence of terpenoids in watermelon seeds.

The antioxidant activity expressed as % DPPH inhibition presented in Table 3. From the result, watermelon seed gave a percentage DPPH scavenging ability with 79.44, and this agrees with what was reported be Oseni and Okoye (2013) [23] who reported 56.93% DPPH free radical scavenging ability of watermelon seeds, as well as that reported by Gill SN. (2011) [13] and Kudirat and Otutu (2016) [18] who all reported a positive radical scavenging ability of the watermelon seeds against the DPPH free radicals. The antioxidant activity may be due to presence of phytochemicals like tannin and flavonoids all of which are known to possess high antioxidant activity as compared to other phytochemicals (Rahman *et al.,* 2013).

Conclusion

The phytochemical composition along side the antioxidant activity of watermelon seed is an indication of its (the seed's) potency/efficacy towards many health problems, thus rather than discarding the watermelon seeds as waste products they should rather be consumed, for they will serve as promoters of good health. The watermelon seed is found to possess some excellent source of secondary metabolites that provides them with an ability to be used as an indigenous ethno-medicine by traditional healers.

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