

Chemical Composition and Physicochemical Analysis of Matured Stems of *Opuntia dillenii* Grown in Nigeria

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Abstract This study investigated the chemical composition and physicochemical properties of matured stems of *Opuntia dillenii* grown in Nigeria in accordance with standard procedures. The proximate analysis revealed moisture content (11.20%), crude protein (11.60%), carbohydrate content (64%) while the ash (3.33%), fat (4.42%) and crude fibre content (4.40%) were low. The phytochemical screening revealed that saponin ($118.08 \pm 0.57 \mu\text{g/ml}$) was the most concentrated while oxalate and phytate had low concentrations. The physicochemical assessment of the matured stem oil demonstrated high peroxide value, acid value and low iodine value. The fatty acid analysis revealed high concentrations of myristic acid (41.66%) followed by linoleic acid (13.95%), heptadecanoic acid (11.32%) and stearic acid (9.25%). Unsaturated fatty acids such as oleic acid and linolenic acid were not detected. The study demonstrated that the matured stems of *Opuntia dillenii* contained nutrients that are nutritionally beneficial to human health and substantial amount of saponins that could be employed in the confectionaries and cosmetic sectors, however the fatty acid analysis demonstrated that these matured stems may not be rich in unsaturated fatty acids while the matured stem oil may be susceptible to auto oxidation and possess a low shelf life.

Keywords *Opuntia dillenii*, Phytochemicals, Fatty Acids, Physicochemical, Proximate

1. Introduction

Medicinal plants have experienced enormous patronage and have been exploited in recent times. However, only few events have validated their therapeutic potentials (Sharifi *et al.*, 2014). Therapeutic plants are gaining prominence in developing countries owing to their effectiveness, safety and lesser side effects. In Nigeria, many indigenous plants are used as spices, food or

medicine where they exhibit a wide range of biological and pharmacological activities. These plants have exhibited enormous potentials in the development of modern drugs and promotion of effective health care systems in developed and developing countries, and advancement in clinical research depicts the value of herbal medicine in the prevention and treatment of diseases. Plant chemicals derived from proteins, carbohydrates and fats are components of the human body composition and chemistry (Andzouana and Mombouli, 2012), phytochemicals present in these plants, coupled with their elemental composition have been attributed to be responsible for the medicinal quality of these plants. The beneficial role of therapeutic plants in disease prevention and curbing has been attributed to the antioxidant properties of their constituents (Monica *et al.*, 2015). *Opuntia* (genus) plants are part of the *Cactaceae* family that is extensively distributed in arid and semi-arid regions all over the world (Butera *et al.*, 2002). *Opuntia ficus indica* is the largest genus and the commonest species cultivated for commercial processing among the members of this family (*Cactaceae*) in semi-arid areas of the world (Coria-Cayupan *et al.*, 2011. Moussa Ayoub *et al.*, 2011). Cactus pear cladodes are considered vegetables that originated from tropical America (Amadi *et al.*, 2017). Nopal cactus has been used as herbal remedy for various health problems in different countries, it has been adopted in health, nutrition and cosmetics in various forms such as jam, juice, oil from the seeds and tea (El-Mostafa *et al.*, 2014). The cladodes and fruits are often consumed both fresh and processed in Latin America, whereas only the fresh fruits are more widespread on European and North-American markets (Butera *et al.*, 2002). Though Prickly pear plant is abundant in many locations in Nigeria, there is little or no information regarding its nutritional value in Nigeria, resulting to its underutilization as food and fodder for both human and livestock consumption. Hence, it is important to source for alternative feeds for livestock in order to supplement scarce feeds and improve human nutrition. It was on this

foregoing that this study was carried out to evaluate the chemical and physicochemical properties of matured stems of *Opuntia dillenii* grown in Nigeria.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh matured Cactus stems (*Opuntia dillenii*) were gathered from Umuanuma Nguru in Aboh Mbaize Local Government Area of Imo State. The plant was identified and authenticated at the herbarium unit of the Department of Plant Science and Biotechnology, Imo State University Owerri. The stems were air dried for 22 days and milled into a powder that was stored under dry conditions before analysis.

2.2. Sample Preparation

A 100g of the ground sample was weighed into a thimble and soaked in 500ml of a mixture of n-hexane and isopropyl alcohol in a ratio of 3:2 according to the stipulations of Hara and Radin(1978). The thimble was stuffed with cotton to prevent the sample particles from pouring away and left for 24hrs. The solution obtained was drained, filtered using filter paper and extracted with a soxhlet extractor. The rotary evaporator was used to concentrate the sample and solvent mixture, at a low pressure with a maximum temperature of 45°C to produce the crude extracts and the extracts were stored at 4°C before analysis.

2.3 Chemical Analysis

2.3.1 Proximate Analysis

The proximate analysis was explored according to AOAC (1990) methods. The moisture content was determined by drying in an oven at 100°C until a constant weight was obtained. The total ash was determined after weighing and converting the sample to dry ash in a muffle furnace at 550°C for incineration. The crude fat content was determined by extraction with hexane via a soxhlet apparatus. The kjeldahl method was employed for crude protein determination and the carbohydrate content was determined by calculating the difference between the sums of all the proximate compositions from 100%.

2.3.2 Phytochemical Extraction and Analysis

Extraction of biological active compounds was achieved using the method explored by Nwiloh *et al.* (2016) with some modifications. One gram of sample was weighed and transferred into a test tube and 15ml and 10ml of 50% w/v potassium hydroxide was added. The test tube and its

content were heated in a water bath at 60°C for 60mins. The reaction product contained in the test tube was transferred into a separatory funnel after the reaction time, and 20ml of ethanol, 3ml of hexane, and 10ml of hot and cold water respectively, were successfully used to wash the tube and they were all transferred to the funnel. This extract was mixed and washed with 10 ml of 10%v/v ethanol aqueous solution three times, and anhydrous sodium sulphate was used to dry the solution and the solvent was evaporated. One thousand micro liter of pyridine was utilized to solubilize the sample of which 200ml was transferred to a vial for analysis.

Phytochemical analysis of the sample was carried out using an auto system buck 530 chromatographer in gas phase equipped with an on-column automatic injector, flame ionization detector, and with Hp88 capillary column (100m x 0.25mm). Chromatographic conditions were; injector temperature 220°C, detector temperature 250°C, oven temperature to 180°C, injection volume = 1ml of the sample, hydrogen was used as a carrier gas (24 pound per square inch (PSI)). The concentration of each active component was determined based on the ratio between the area and mass of internal standard and area of the peaks of the phytochemicals identified.

2.3.3 Fatty Acid Analysis

Twenty grams of the homogenized sample was combined with 60g of anhydrous sodium sulphate in agate mortar to suck up moisture. The homogenate was positioned into an extraction cellulose thimble (33 94mm), enclosed with a whatman filter paper and slotted into a soxhlet extraction compartment of the soxhlet unit. Extraction was achieved with 200ml of n-hexane via eicosapentaenoic acid (EPA) 354°C method for 8hrs. The collected crude extract was evaporated using a rotary vacuum evaporator at 40°C, to dryness. Fatty acids methyl esters from matured stems were prepared using the protocol described by (Radwan, 1978) using 1% sulphuric acid in absolute methanol. The fatty acids methyl esters obtained were separated using Gas Chromatography HP (Hewlett Packard) 6890 GC and the following protocols were observed; Column, Hp-5 (5% diphenyl, 95% dimethyl polysiloxane), 30m, 0.32mm ID, 0.25µm film thickness, Detector, flame ionization detector, initial temp, 150°C for 2min. Injector temp.220°C, injection volume 2µl, splitless mode, Detector temperature 250°C. Nitrogen was used as the carrier gas with a flow rate of 1 ml per min.

2.4 Physicochemical Analysis

The melting point, moisture, peroxide value, iodine value, acid value and saponification value were determined using the method of AOAC, (2000). The pH was detected using a pH meter, the refractive indices analyzed at 25°C following the method of Oderinde and Ajayi, (2000) by

using a Brix refractometer, and the method of Menoyo *et al.*, (2002) was adopted for the determination of TBA-reactive substances (TBARS).

3. Results and Discussion

Table 1. Proximate composition of matured stems of *Opuntia dillenii*

Parameters	Composition (%)	*Composition (g/100g)
Moisture Content	11.20±0.13	0.00
Ash Content	3.33±0.18	24.40
Crude Protein	11.60±0.21	6.90
Fat	4.42±0.19	4.90
Crude Fibre	4.40±0.06	36.00
Carbohydrate	64 ± 0.14	30.80

Values are means of three determinations ± standard errors of mean (SEM)
*Source: Carreira *et al.*, (2014)

Table 2. Physicochemical properties of *Opuntia dillenii* matured stem oil

Properties	Matured stem oil
Peroxide Value (Meq/KOH/kg)	15.60±1.26
Iodine Value (gI ₂ /100g)	63.33±2.40
Saponification Value (mgKOH/g)	158.66±1.47
Specific Gravity	1.19 ± 0.03
Melting Point (°C)	7.03 ± 0.31
pH	4.56 ± 0.26
Acid Value (mgKOH/g)	31.00±2.64
Refractive Index	1.00±0.03
Moisture (%)	7.00±1.10
Thiobarbituric Acid (µg/kg)	0.22±0.01

Values are means of three determinations ± standard errors of mean (SEM)

Table 3. Phytochemical analysis of matured stem of *Opuntia dillenii*

Phytochemicals	Concentration (µg/ml)
Anthocyanin	0.04 ± 0.02
Oxalate	1.07 ±0.01
Tanin	13.62 ±0.05
Rutin	12.41 ±0.26
Phenol	4.66 ±0.08
Lunamarine	34.43 ±0.35
Saponin	118.08 ±0.57
Sapogenin	11.88 ±0.09
Ribalinidine	3.75 ±0.09
Phytate	0.18 ±0.04
Kaempferol	7.90 ± 0.06
Catechin	44.90 ±0.38

Values are means of three determinations ± standard errors of mean (SEM)

Table 4. Fatty acid composition of matured stems of *Opuntia dillenii*

Name	Concentration (%)
C ₁₂ = Lauric acid	7.78±0.06
C _{14:0} = Myristic acid	41.24±0.55
C _{16:0} = Palmitic acid	5.48±0.06
C _{17:0} = Heptadecanoic acid	11.32±0.06
C _{18:0} = Stearic acid	9.25±0.03
C _{20:0} = Arachidic acid	1.21±0.06
C _{18:1} = Oleic acid	0.0000
C _{18:2} = Linoleic acid	13.95±0.02

Values are means of three determinations ± standard errors of mean (SEM)

The moisture content (11.20%) of matured stems of *Opuntia dillenii* (Table 1) was higher than 6.78% reported by (El-Safy, 2013) in cladode (stem) flours of *Opuntia ficus indica*, the sample had an ash content of (3.33%) and this value was lower than 27% and 21% reported by El Salfy (2013) and Carreira *et al* (2014) for *Opuntia ficus indica* cladodes and *Opuntia sulphurea cladodea* respectively. These variations may be due to Geographical location, soil, climatic conditions and maturation. The ash constituent of the sample is considerably within the tolerable ash constituent mean values of legumes (2.4 to 5.0%) recommended by FAO (1988). Pearson (1976) reported ash content as a guide to assess and grade foods in terms of nutritive quality. In addition, the ash content value of the sample (3.33%) was lower compared to the range of (5.43% to 5.75%) reported for some edible woody plants (Emmanuel *et al.*, 2011) and (6.14% and 5.54%) reported by (Andzouana and Mombouli, 2012) for the leaves of *Hippocratea myriantha* and *Urera trinervis* respectively. On the other hand, the (3.33%) ash content value of the sample was higher than the range of 0.38-1.9% for selected vegetables grown in Peshawar (Bangash *et al.*, 2011). The crude protein content (11.6%) is higher than (7.6%) and (6.9%) protein value reported by El-Safy, (2013) and Carreira *et al.*, (2014) but it is within the estimates for various *Opuntia* species though our findings remains the highest for other *Opuntia* species which ranges from 4% to 10% (Saenz *et al.*, 2006). The lipid content (4.42%) of the sample in this study is similar to the lipid content estimate (4.90%) reported by Carreira *et al.*, (2014) but higher than (2.20%) reported by Moreno- Alvarez *et al.*, (2006); El-Safy, (2013); and 1.4% of dry weight reported by Stintzing and Carle (2005) and Feugang *et al.*, (2006) for other *opuntia* species. The lipid content estimate of the same was very low in comparison to the 24% crude fat observed in *A. senegalensis* (Yameogo *et al.*, 2011). The low fat estimate recorded in matured stems of *Opuntia dilleni* suggests that these matured stems may be beneficial to individuals on weight reducing diets (Emebu and Anyika, 2011). However, the matured stems may not serve as good sources of vegetable oils. Matured stems of *Opuntia dillenii* had a dietary fiber of 4.4% of dry sample, and this

value was lower than 18% of dry weight, (El-Safy, 2013) and 36% of dry sample (Carreira *et al.*, 2014) for *Opuntia ficus indica* and *Opuntia sulphurea* cladodes. The observed differences could be as a result of climatic conditions and maturation. The carbohydrate content of the studied sample was 64% of dry weight, which was higher than 30% and 45% of dry weight, reported by El-Safy (2013) and Carreira *et al.*, (2014) respectively. However, this value was in accordance with 64-71/100g of dry tissue (Stintzing and Carle 2005, Feugang *et al.*, 2006). Furthermore, the carbohydrate content of the studied sample was significantly higher than the estimates of 15.40% found in *A. hybridus*, the 30.38% in *G. Africana* and 30.40% in *C. pepo* (Iheanacho and Udeubani, 2009). The high carbohydrate content found in matured stems of *Opuntia dillenii* possibly implies that these matured stems can be considered as a potential source of energy (Andzouana and Mombouli, 2012).

The matured stem oil of the studied plant (Table 2) had a peroxide value of 15meq/kg and peroxide values greater than 10meq/kg is highly susceptible to auto-oxidation as a consequence moisture or presence of trace elements and this was demonstrated by Adebisi and Olagunju, (2011). The peroxide value of oils has been described as the most usual lipid oxidation indicator (Aremu *et al.*, 2015). Consequently, this outcome suggests that the matured stem oil possesses a lower shelf life. The iodine values in the studied sample was lower than that of *Citrullus colocynthis* (Igwenyi, 2014), peanut oil and palm oil (Birnin-Yauri and Garba, 2011). The iodine value was below 100gI₂/100g and this implies that it is a non-drying oil as regards to industrial importance and also, based on the classification of Aremu *et al.*, (2006). The saponification value of the studied sample was similar to those of *Telfaria occidentalis* oil (Nwabanne, 2012) and *Anacardium occidentale* oil (Aremu and Akinwumi, 2014) but lower than the values for *Jathropha curcas* seed oil (Elizabeth *et al.*, 2012) and Sesame seed oil (Mohammed and Hamza, 2008). Low saponification values points to the predominance of long chain fatty acids as suggested by Akintayo and Bayer, (2002). The acid value is a possible indicator of the quality of fatty acids in oil which could in turn resolve the shelf life and stability of particular oil and the pH and acid values are used to evaluate the amount of free fatty acids present in oils. The acid value found in *Opuntia dillenii* matured stem oil was higher than the acid value for groundnut oil reported by Ayoola and Adeyeye, (2010), and based on the submissions of Aremu *et al.* (2006), the matured stem oil of *Opuntia dillenii* may not be appropriate for cooking. The refractive index of the matured stem oil was below 1.4677-1.4707 (CODEX-STAN, 1999) for refined virgin oils. The lower the moisture content of a food sample, the lesser the vulnerability to microbial spoilage and thus the better the storage capacity. Lukaszewicz *et al.*, (2004) observed that the presence of thiobarbituric acid in an oil indicates that oxidation has already occurred and can be

quantified from the amounts present and in addition, these values may be used for sensory testing in order to identify rancidity in foods as suggested by these authors (Fernández *et al.*, 1997; Rhee and Myers, 2003; Campo *et al.*, 2006).

The phytochemical analysis of the matured stems of *Opuntia dillenii* (Table 3) revealed that catechin and kaempferol are flavonoids with the highest concentrations (44.90µg/ml and 12.41µg/ml) respectively while anthocyanin (0.04µg/ml) and rutin (0.88µg/ml) exhibited low concentrations. Catechin, a major constituent of green tea has been reported to have anti-cancer, hypotensive, and hypocholesterolemic activities (Yang *et al.*, 2004; Combra *et al.*, 2006; Butt *et al.*, 2013; Masuda *et al.*, 2014). Kaempferol have been attributed to possess antimicrobial potentials as well as antioxidant properties (Singh *et al.*, 1988; Quarenghi *et al.*, 2000; Paolillo *et al.*, 2011). The oxalate and phytate concentrations were low (1.07µg/ml and 0.18µg/ml) while tannin was (13.62µg/ml) and tannins have been attributed to possess possible anti-carcinogenic effect (Butler, 1989), antimicrobial and antioxidant properties (Lin *et al.*, 2001; Buzzini *et al.*, 2008). However, the precipitation of tannins have been observed to obstruct the absorption of protein (Shimada, 2006) thereby underscoring its potentials as an anti-nutrient. Lunamarine and ribalinidine are known alkaloids and their concentrations in the studied sample are (34.43µg/ml and 3.75) respectively, alkaloids have exhibited potent therapeutic potentials which range from anti-malarial (Cordell, 1981), analgesic, and antioxidant properties (Stary, 1998) and anti-plasmodial properties (Fozia *et al.*, 2010) among other effects. Saponins demonstrated the highest value (118.08µg/ml) and its beneficial effects includes anti-inflammatory properties (Odeyemi *et al.*, 2015), anti-cancer (Man *et al.*, 2010) and anti-platelet aggregation activity (Huang *et al.*, 1999) among other benefits. The physicochemical properties of saponins and in addition to its biological activities have led to an increase in its application in cosmetic, food and pharmaceutical industries. Therefore the high saponin content present in matured stems of *Opuntia dillenii*, qualifies them as useful candidates to be applied in the cosmetic and confectionary sectors.

Fatty acid analysis of the studied plant (Table 4) indicated the presence of eight fatty acids. Myristic acid with 41.66% composition had the highest concentration. Myristic acid coupled with linoleic acid (13.95%), heptadecanoic acid (11.32%) and stearic acid were the major fatty acids found in matured stem of *Opuntia dillenii*. Lopez-Cervantes *et al.*, (2011) and Carreira *et al.*, 2014 reported that linoleic acid, palmitic acid and oleic acids as the main constituents of *Opuntia sulphurea* cladodes and *Opuntia ficus indica* cladodes. However, the absence of oleic acids, linolenic acids and the low concentrations of linoleic acid portray possible low concentrations of these unsaturated fatty acids and this could be a consequence of growing conditions of cladodes, degree of maturation and

age of cladodes (Coskuner and Tekin, 2003; Ennouri *et al.*, 2005), these researchers observed increase in saturated fatty acid content at the end of fruit maturation of *Opuntia ficus indica* fruits. Unsaturated fatty acid improves lipid profile while excessive intake of saturated fatty acids results in obesity and elevated cholesterol levels (Brenna *et al.*, 2009). This value indicates further that the matured stems of *Opuntia dillenii* may not be a rich source of unsaturated fatty acids as a result of the absence of linolenic acid, oleic acids and low concentrations of linoleic acid.

4. Conclusions

The matured stems of *Opuntia dillenii* exhibited useful potentials as a great source of energy as a result of the high carbohydrate content while the phytochemical screening indicates that these matured stems are rich in flavonoids, saponins and alkaloids, this outcome could be of immense benefits due to the enormous therapeutic potentials accrued to these phytochemicals while the physicochemical analysis of the matured stem oil of *Opuntia dillenii* revealed that the oil may not be suitable for cooking and may be susceptible to auto oxidation and the matured stems demonstrated low concentrations of unsaturated fatty acids.

Conflict of Interest

The authors declare no conflict of interest regarding this article.

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