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Nutritional Evaluation, Medicinal value and Antibacterial activity of leaves of *Cucurbita maxima* D.

Okon, Okon Godwin*[1], Abraham, Nsikak Andrew[2] and Akpan, Gabriel Ukpong[3]

[1]*Department of Botany and Ecological Studies, University of Uyo, Nigeria.*
[2]*Department of Microbiology, University of Uyo, Nigeria.*
[3]*Department of Crop Science, University of Uyo, Nigeria.*
**Corresponding author: okjunior4zeeb@gmail.com*

Abstract

Proximate analysis, mineral composition, phytochemical constituents and vitamins of the leaves of Cucurbita maxima were evaluated. The leaves of C. maxima has an ash content of12.91%, fibre 11.21%, protein content of 14.21%, lipid 6.31%, carbohydrate 69.22%, moisture content of 74.41% and caloric value of 348.98 Kcal. Mineral compositions, phytochemical constituents and vitamin A and C content for the samples were investigated as well. The data indicated that the leaves of C.maxima are very good sources of mineral elements such as P, Ca, Mg, K, Fe, Cu and Zn. Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids and cardiac glycocides. The vitamin contents of the sample were also investigated, the leaves of C. maxima has a vitamin A content of 49.81mg/100g and vitamin C content of 31.42mg/100g. Also, this study revealed that C. maxima leaf extract has a significant antibacterial activity against Staphylococcus aureus, Bacillussubtilis, Pseudomonas aeruginosa and Escherichiacoli. Thus, this study has showned that leaves of Cucurbita maxima are rich sources of nutrients and can contribute significantly to the nutrient requirements and health conditions of humans.

Key Terms–Antibacterial, *Cucurbita maxima*, mineral, nutritional, phytochemical screening,proximate andvitamins.

1. Introduction

Vegetables are edible plants or parts of plants which can be eaten cooked or raw without any serious processing. They nourish the body and also serve as good sources of vitamins and minerals to the body. Vegetables contain essential food substances which include carbohydrates, proteins, minerals, oil and vitamins.These vegetables are important commodities for poor households because their prices are relatively affordable compared with other food items (Okon and James, 2014).The plant under investigation *Cucurbita maxima*, commonly known as Ndise by the Ibibio tribe in Nigeria, belongs to family Cucurbitaceae.It is most important in the cooler parts of southern Africa and the Sahel region, less important in more humid West and East Africa, where *Cucurbita moschata* is more common (Ngwerume and Grubben, 2004). *Cucurbita maxima* are rarely found growing in the wild in Nigeria. It is cultivated in northern Nigeria for the fruits. In southern Nigeria, in a largely unimproved form, it is cultivated for both the leaves and fruits (Okoli, 1984).*Cucurbita maxima* varieties are used for preparation of many dishes.*Cucurbita maxima* is widely used as a vegetable inNigeria, baked, boiled, or stewed. Young shoots and flowers of *Cucurbita maxima* are used as green vegetable. Also its vegetable is an excellent source of vitamin B (Okon, 2014).They can be cultivated on almost any well-drained soil with a neutral or slightly acid reaction (pH 5.5–6.8). They can be grown from seeds; *C. maxima*will grow on reasonably fertile soil, but do best on soils rich in organic matter (Ngwerume and Grubben, 2004). This research investigates the leaves of *Cucurbita maxima*(Ndise) commonly found in Akwa Ibom, Port Harcourt and Cross River states in South-South Nigeria. In this study, the proximate composition and the mineral content of the leavesof *C. maxima* were determined. Also the vitamins, phytochemical constituents and its antibacterial activity were determined.

2. Materials and Methods

2.1 Sources of collection of Samples

The fresh vegetables (*Cucurbita maxima*) used in this research was collected from local farms and markets in Akwa Ibom, Port Harcourt and Cross River State and were identified by a plant taxonomist in the Department of Botany, University of Uyo, Nigeria.

2.2 Samples Preparation for Analysis

The fresh leaves of *Cucurbita maxima* was air dried for one week and reduce to a coarse powder form; about 700g was macerated with cold ethanolic extraction using 1000 ml of

70% ethanol and shaken intermittently for 72 hours. It was filtered and the filtrate was concentrated (dried) *in-vacuo* at 40^0C in a water bath. The extract was weighed and stored in 100 ml beaker and covered with foil paper and stored in the refrigerator at 4^0C for the analysis.

2.3Proximate Analysis

The recommended methods of the Association of Official Analytical Chemists (AOAC, 2003) was used for the determination of moisture content, crude protein, crude fat, carbohydrate, crude fibre and ash.

2.4 Mineral Analysis

Wet digestion

For wet digestion of sample, 2 ml of the plant samples was taken in digesting glass tube. 12 ml of hydrochloric acid was added to the plant samples. The mixture was kept overnight at room temperature. 4.0 ml perchloric acid (PCA) was added to these mixtures and was kept in the fumes block for digestion. The temperature was increased gradually, starting from 50ºC and increasing up to 150ºC. The digestion was completed in about 70 - 85 minutes as indicated by the appearance of white fumes. The mixture was left to cool and the contents of the tubes were transferred to one hundred millilitres (100ml) volumetric flasks and the volumes of the contents were made to one hundred millilitres (100ml) with distilled water. The wet digested solution was transferred to plastic bottles and labeled accurately. The digest was stored and used for mineral determinations (Aregheore and Hunter, 1999; Khan, Hussain, Ashraf, Valeem, and Javed, 2005). Mineral contents: calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), Iron (Fe), Copper (Cu) and Zinc (Zn) of plant samples were determined by atomic absorption spectrophotometer (AAS),flame photometry and spectrophotometry according to the methods of AOAC (2003) and Khan, Hussain, Ashraf and Mc-Dowell (2006).

2.5 Determination of Vitamins

Determination of Vitamin A (Axerphthol)

5 g of powdered samples was weighed and dissolved in 100 ml of chloroform and stored in an amber coloured bottle at -10^0C. 30% of trichloracetic acid (TCA) reagent was added and titrated with methyl chloride (CH_2CH_2) and observed for colour change (end point). The vitamin A content was then calculated (Egbuonu, 2005).

Determination of Vitamin C (Ascorbic acid)

5 g of the powdered samples was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture was shaken for 30 minutes. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for 20 minutes. It was transferred into 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipetted into volumetric flask and 1% starch indicator was added. This was added and titrated with 20% $CuS0_4$ solution to get a dark end point (AOAC, 2003, Okwu, 2005).

2.6 Phytochemical Screening

The phytochemical screening involves the simple chemical test to detect the presence of secondary metabolites. The methods of Adeneye, Adeleke, Ajagbona and Bello (2006), Sofowora (2008) and Trease and Evans (2009) were used for phytochemical screening. The phytochemical test include: tests for saponins, tannins, flavonoids, alkaloids, cardiac glycosides and terpenes.

2.7Preparation of Disc for antibacterial activities

Sterile filter papers were perforated to form disc and treated with the plant extracts of concentration 30µl while the second group treated with 50µl of the extract. The two set were allowed to absorb the extract for one hour then dried in controlled temperature to remove excess of moisture. The disc was then used for antibacterial activity.

2.8 Test Organism Used

Representative Gram positive (*Staphylococcus aureus, Bacillus subtilis)* and Gram negative (*Pseudomonasaeruginosa* and *Escherichia coli)* test organisms used in this study were obtained from the University of Uyo Medical Center, Uyo.

2.9 Plant extract susceptibility test

The Kirby-Bauer modified disc diffusion technique as recommended by National Committee for Clinical Laboratory Standards (NCCLS) was used to determine the susceptibility of the isolates to the plant extracts. The isolates were inoculated into sterile agar broth and incubated for few hours until it became slightly turbid and the turbidity of each suspension was then matched to standard turbidity (0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial test suspension and evenly inoculated on the entire surface of a sterile dry solidified agar plate. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension before inoculation. After inoculation, the surface of the agar was dried for 5 minutes with the petri dish lid in place after which the prepared disks, which have been allowed at room

temperature for about 1 hour, were aseptically placed on each inoculated plates with sterilized forceps. Each disk was firmly pressed to ensure its constant with the agar surface. The plates were allowed for 30 minutes after applying the disks then were inverted and incubated at 37^{o} C for 24 hours (CLSI, 2007; Cheesbrough, 2006).

2.10 Statistical Analysis

Results are reported as the means of triplicate experiments, and are presented in tables using the spread sheet software Microsoft Excel.

3.0 Results and Discussion

The results of the proximate analysis are shown on Table 1. The leaves of *C. maxima* has an ash content of 12.91%, fibre 11.21%, protein content of 14.21%, lipid 6.31%, carbohydrate 69.22%, moisture content of 74.41% and caloric value of 348.98 Kcal. This results show that leaves of *C. maxima* has a high content of carbohydrate and protein. Lipid content and fibre are relatively low but within the range of edible vegetables when compared with other edible traditional vegetables. Okon and James (2014) reported similar observations when comparing the proximate composition of *C. maxima* to other traditional vegetables in Akwa Ibom State.

Table 1: Proximate analysis of *Cucurbita maxima*

Parameters	Leaves
Ash (%)	12.91
Fibre (%)	11.21
Protein (%)	14.21
Lipid (%)	6.31
Carbohydrate (%)	69.22
Moisture Content (%)	74.41
Caloric Value (Kcal)	348.98

The mean ± standard deviation (SD) of triplicate analysis

The mineral compositions of the leavesof *Cucurbita maxima* are presented in Table 2. The results show that *C. maxima* are rich in minerals.The result here shows that calcium, potassium, phosphorus and magnesium are higher in *C. maxima* than other minerals. Also, the iron, copper and zinc content in these vegetable are in minute quantities.

Table 2: Mineral composition of *Cucurbita maxima*

Minerals (mg/kg)	Leaves
P	849.62
Ca	2340.40
Mg	671.21
K	4214.00
Fe	1040.00
Cu	610.00
Zn	590.00

The mean ± standard deviation (SD) of triplicate analysis

Table 3 shows the vitamin constituents of *Cucurbita maxima*. The results indicate that *C. maxima* are a rich source of vitamin A and C.When consumed in diet, vitamin A helps to maintain good sight and prevents diseases of the eye. Vitamin C, on the other hand, has anti-infective properties, promotes wound healing and strong immune system (Nwaogu and Ujowundu, 2010). Vitamins are essential nutrients that promote growth, development, reproduction, digestion, disease reduction, and overall health and life maintenance (Ujowundu *et al.*, 2013).

Table 3: Vitamin A and C composition of *Cucurbita maxima*

Vitamins (mg/100g)	Leaves
A	49.81
C	31.42

The mean ± standard deviation (SD) of triplicate analysis

Table 4 shows the results of the phytochemical analysis present in leaves of *Cucurbita maxima*. The result revealed the presence of alkaloids, tannins, flavonoids and cardiac glycocides. While saponins and terpenes are absent. Similar observation was recorded by Okon (2014). In humans and most animals, alkaloids and flavonoids have been observed to possess antidiuretic, antispasmodic, anti-inflammatory and analgesic effects (Owoyele, 2002).Tannins are polyphenols which have shown appreciable antimicrobial actions (Enechi and Odonwodo, 2003).

Table 4: phytochemical constituents of leave extracts of *C. maxima*

Phytochemical	leaves
Alkaloids	++
Saponins	-
Tannin	+++
Flavonoids	++
Terpenes	-
Cardiac glycocides	+++

As shown in Table5, *Cucurbita maxima* leaf extract has an antibacterial activity against some potential human pathogens such as *Staphylococcus aureus*, *Bacillussubtilis*, *Pseudomonas aeruginosa* and *Escherichiacoli*.The potency of the extract was also seen to increase as the concentration of the extract increased. Of the entire test organisms, *S. aureus and E. coli* were seen to be most sensitive to the extract with a diameter of inhibition zone of 20 mm while *B. subtilis* was the most resistant with inhibition zone of 14 mm. similar observations was made by Villasenor*et al.* (1995) who reported minimal inhibition activity of *C. maxima* leaf extract on *B. subtilis* and *E. coli.*

Table 5: Antimicrobial activity of *Cucurbita maxima*

Plant Extract	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
30µl	20 mm	14 mm	18 mm	20 mm
50µl	28 mm	18 mm	22 mm	24 mm

4.0 Conclusion

This study revealed that the leaves of *Cucurbita maxima* contain considerable amounts of proximates and mineral nutrients which are necessary for growth and maintenance of the body. It can be used as alternative source of food and vitamins and should be incorporated into our diet.*C. maxima* leaf extract has a significant antibacterial activity against *Staphylococcus aureus*, *Bacillussubtilis*, *Pseudomonas aeruginosa* and *Escherichiacoli*. This work explains the rationale of using the plant leafs in treating various infections.

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