ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Nutrient and Anti-nutrient Contents of Selected Nigerian Cucurbits Seeds

[1]Karaye IU, [1]Aliero AA, [1]Muhammad S, and [2]Bilbis LS.

[1]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto- Nigeria
[2]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto-Nigeria.

ABSTRACT

Seeds of *Luffa aegyptiaca* (Mill.), *Citrullus lanatus* (Thunb. Matsum), *Cucurbita maxima* (Duchesne, ex-Lam.), *Cucumis metuliferus* (E. Mey. ex Naudin) and *Momordica balsamina* (L.) were evaluated for their nutritional and antinutritional compositions using standard analytical procedures. Results of proximate analysis revealed a significant difference (P<0.05) in contents of proteins, lipids and carbohydrates amongst the studied species. Crude protein content ranged from 29.49-33.50% with the highest value in *C. lanatus* while *C. maxima* had the least. Crude lipid composition ranged from 28.24 33.60% with the highest in *C. lanatus* and *M. balsamina* had the least. Crude carbohydrate content ranged from 27.88-34.46% with the highest value obtained in *C. metuliferus* while the least was found in *M. balsamina*. Crude fibre analysis revealed the highest content of 3.86% in *C. maxima* while the least value of 3.16% was found in *C. metuliferus.* Ash content was highest (4.64%) in *M. balsamina* while the least (3.89%) was obtained in the seeds of *L. aegyptiaca.* Antinutritional analysis showed a significant difference (P<0.05) in the contents of oxalate, nitrate and tannins amongst the evaluated species. High nitrate and tannin contents of 8.10 and 3.7 mg/100 g dry weights were obtained in *L. aegyptiaca* and *C. maxima* respectively. Highest cyanide compositions of 0.51 and 0.50 mg/100 g were identified in *C. metuliferus* and *C. maxima* respectively. The seed extracts showed inhibition against trypsin with the highest (46.05 and 41.57%) shown by *C. maxima* and *C. lanatus* respectively. Results in this study suggest the potent of the seeds as formidable sources of nutrients that could be exploited for wider application in food and animal feed fortification schemes after further validation.
Keywords: Cucurbits, nutritional, protease inhibition, trypsin, food fortification, validation.

Corresponding author

INTRODUCTION

One of the effective ways of achieving food security is through the exploitation of available plant materials in order to satisfy the needs of the increasing population. Knowledge of the nutritive value of local dishes, soup ingredients and local foodstuffs is necessary in order to encourage the increased cultivation and consumption of those that are highly nutritive. This will help greatly in supplementing the nutrients of the staple carbohydrate foods of the poor who cannot afford enough protein foods of animal origin. There is a need to develop other sources of concentrated plant proteins that ideally should be crops that are widely grown in tropical countries [1]. The need for diversification of protein sources is imperative especially in developing countries. Plant protein is needed in such countries and could play significant role in human nutrition [2]. Although, several novel plant protein sources have been suggested, cultural food selection amongst other factors, have minimized the use of these sources. Lack of proper knowledge, especially on their nutritive value, methods of production, preservation and full exploitation is an important deterrent towards their utilization. This explains why over 30 years, the use of concentrated proteins from seeds has increased tremendously because of greater increase in knowledge of their functional properties, processing and nutritive value [1].

Cucurbit seeds are consumed directly as a snack food in many cultures throughout the world, and the seeds are especially popular in Arabian countries after salting and roasting [3]. Large segments of the population especially in developing countries suffer from protein malnutrition and projections based on current trends indicate a widening gap between human population and protein supply [1]. This explains why today, food shortage remains a serious problem especially in Africa with about 36 million humans starving to death every year coupled with childhood malnutrition, which contribute to the global burden of disease [4]. Seeds of many cucurbits are rich in protein and could be useful in the fortification of food products [5]. Thus, research output with respect to the selected seeds could help in ameliorating food security and increases income for peasants. There is grossly inadequate research output in respect of these species despite their numerous social and economic potential [6]. Seeds of many species in the Cucurbitaceae contribute substantially towards obtaining a balanced diet and a number of them have not been investigated biochemically. There is the need for thorough biochemical evaluation of the seeds of these species with the view to unlocking their potential which could be vital in providing succour to the ever-increasing demands for food especially proteins and unsaturated fats [7].

The major constraint to proper utilisation of plant-sourced nutrients is the presence of antinutritional components that may render the nutrients from such sources as bio-unavailable [8]. Plants produce a variety of proteins (peptides) that are involved in the defence against pathogens and invading organisms, including ribosome-inactivating proteins, lectins, protease inhibitors and antifungal peptides (proteins). Specially, the protease inhibitors inhibit aspartic, serine and cysteine proteinases [9]. Protease inhibitors inhibit the function of proteases; many naturally occurring protease inhibitors are proteins [10]. The objectives of the study were to evaluate the nutritional and antinutritional compositions of selected cucubits seed as well their protease inhibitory effect against trypsin.

MATERIALS AND METHODS

Samples Collection and Preparation

Seeds of five cucurbits namely *Luffa aegyptiaca* (Mill.), *Citrullus lanatus* (Thunb. Matsum and Nakai), *Cucurbita maxima* (Duchesne, ex Lam), *Cucumis metuliferus* (E.Mey. ex Naudin) and *Momordica balsamina* (Linn.) were obtained at different locations in Sokoto. The plant materials were authenticated at the Department of Biological Sciences Herbarium, Usmanu Danfodiyo University, Sokoto where voucher specimens were deposited. Seeds were removed from the fruits by cutting the individual fruit longitudinally and scrapping out the seeds using a clean knife and seeds were screened to remove bad ones. The seeds were dried to a constant weight in an oven at 70^0C, ground using mechanical blender, placed in airtight containers and stored in desiccators for further analyses. The plant materials were authenticated by a plant taxonomist at the Herbarium, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. Voucher numbers were issued while the identified samples were deposited in the herbarium. Analyses were carried out in the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto Main Campus. The location lies between 13^0 01′N, Latitude and 5^0 15′E Longitude. Climate of Sokoto is tropical continental dominated by two opposing air masses, tropical maritime and tropical continental. Daily maximum and minimum temperature between February and November are in the ranges of 38-40^0C and 21-23^0C respectively. Temperature is lowest during the harmattan months of December and February between 20^0C and 23^0C. Relative humidity ranges from 17-80%.

Experimental

All determinations were carried out in triplicate. Proximate compositions were carried out following the standard methods of [11]. Estimation of crude protein involved the determination of total nitrogen by kjeldahl procedure. The amount of crude protein was obtained by multiplying the nitrogen content by 6.25. This factor was based on assumption that all feed proteins contain 16% nitrogen and that all the nitrogen in the tissue is present as protein. Nitrogen Free Extractive (N.F.E.) referred to as soluble carbohydrate is not determined directly but obtained as the difference between crude protein and the sum of ash, protein, fat and crude fibre. N.F.E. = 100 - (% ash + % crude fibre + % crude fat + %crude protein).

Tannin content was determined according to the method of [12] that involved the reaction of tannin with ferric ammonium citrate at pH 8 bringing about a characteristic reaction in which a dark coloured complex was formed measured spectrophotometrically at 250 nm. Content of phytate was evaluated using the method described by [13]. Phytin-phosphorus was determined and phytate content calculated by multiplying the value of phytin-phosphorus by 3.55. Oxalate composition was determined according to the method adopted by [14], in which the oxalate was precipitated as calcium oxalate; the concentration is determined by filtration with potassium permanganate, which gives a faint pink colour at end-point. Cyanide content was determined according to the method used by [15] which involved the extraction of cyanide with water and its quantitative measurement by the alkaline picrate reagent. Amount of cyanide was then extrapolated from cyanide standard calibration curve while nitrate content was evaluated using the method described by [16]. Evaluation of trypsin inhibitors was carried out using N-benzoyl- L-arginine ethyl ester (BAEE) as substrate. The data obtained was analyzed using one-way analysis of variance (ANOVA) test with SPSS version 10.0 and results were reported as means ± Standard error of triplicate values. The differences between means were determined using the least significant difference (LSD) as described [17].

RESULTS AND DISCUSSION

Table 1 presents the result of proximate analysis of the studied species with a significant difference ($p < 0.05$) in contents of crude protein, carbohydrate and lipid. Crude protein of 33.48% was found in *C. maxima* while *M. balsamina* and *C. lanatus* had 30.86% and 29.49% respectively. The highest lipid content of 33.56 % was observed in *M. balsamina* while *C. lanatus* and *C. metuliferus* had 32.97% and 28.24% respectively. The crude carbohydrate content of 34.46% was found in *C. lanatus* while *C. metuliferus* had 27.88%. The crude fibre and available energy contents did not differ significantly ($p < 0.05$) amongst the studied seeds. Ash content ranged from 3.89% to 4.64%, the highest value of 4.654% was identified in the seeds of *L. aegyptiaca*, and *M. balsamina* had 3.89 %. The five species evaluated contain appreciable amount of energy that ranged from 509.96-to 538.24 K/cal and *M. balsamina* had the highest content of 538.96 K/cal while *C. lanatus* had 509.96k/cal.

Table 1: Proximate compositions evaluation from the seeds of five selected species in Cucurbitaceae (presented as % DM).

Species	Protein	Carbohydrates	Lipid	Fiber	Ash	Available energy(Kcal)
L.aegyptiaca	29.86^{c}	31.38^{b}	30.87^{c}	3.16	4.64	522.79
C. lanatus	29.49^{c}	34.46^{a}	28.24^{d}	3.28	4.37	509.96
C. maxima	33.48^{a}	28.68^{c}	30.66^{c}	3.07	3.98	524.58
C.metuliferus	30.38^{b}	27.88^{e}	32.97^{b}	3.86	4.29	529.77
M. balsamina	30.86^{b}	28.19^{d}	33.56^{a}	3.49	3.89	538.24
SE ±	0.87	0.42	0.51	NS	NS	NS

Means in a column followed by same letter are not significantly different at ($p< 0.05$).

Crude protein obtained was lower than that reported by [18] on *C. maxima* with higher crude fibre content in this study. Ranges of crude protein and fats obtained in this study were also higher than that reported by [19] on *C. lanatus* and [20] on loofah gourd seeds, but lower than the reports of [21] on *Luffa* seed flour; [22] on pumpkin and melon seeds. The amounts of protein and fat identified in the five species were higher than (13.19-26.86 % protein) and (14.48-24.62% fat) reported by [20] on seeds of *Citrullus colocynthis, Coccinia grandis, C. metuliferus* and *Cucumis prophetarum.* However, ash content obtained in the result is in close agreement with 2.00-4.46% as reported by [20]. The amounts of fats and carbohydrate contents of *C. maxima* obtained in this work are in close agreement with the reported (38.92% fats and 24.30% carbohydrates) by [23]. The obtained results on fat contents is also within the reported range on seeds of *C. lanatus* and *C. lanatus* by [24] but lower than the reported range of oil contents of 41.0-56.6% by [25] on melon seeds. Carbohydrate contents of the studied species are higher than the reported 11.48% on *C. maxima* [18].

Evaluated antinutritional components in the five seed samples are presented in Table 7. The highest phytate content (0.4 mg/100 g Dry weights) was obtained in the seeds of *L. aegyptiaca* followed by *C. maxima* (0.3 mg/100 g). The highest oxalate content (0.91 mg) was identified in *L. aegyptiaca*, followed by 0.9 mg identified in the seeds of *C. maxima, C. metuliferus* and *M. balsamina.* Cyanide is another antinutritional component identified in the five seed samples with varying concentrations. The highest cyanide content (0.51 mg/ 100 g) was identified in *C. metuliferus* followed by 0.50 mg/100 g identified in *C. maxima*. There is no significant difference ($P < 0.05$) in cyanide content among the five seed types. The highest nitrate content (8.30 mg/100 g) was identified in *C. maxima*, followed by (6.80 mg/100 g) identified in *C. metuliferus.* There is a significant difference ($P < 0.05$) between the nitrate content in *C. maxima* and *C. lanatus*. There is no significant difference ($P < 0.05$) between the nitrate content in *C. lanatus* and *C. metuliferus.* Tannin, another antinutritional component identified in the five seeds at varying concentrations. The highest tannin content (3.70 mg/100 g) was identified in *C. maxima* followed by 2.11 mg/100 g identified in *C. lanatus*, while the least value (1.10 mg/ml) was identified in *M. balsamina.* There is a significant difference ($P < 0.05$) in tannin content between the seeds of *C. maxima* and *C. lanatus.* There is no significant difference ($P < 0.05$) between the tannin compositions in *L. aegyptiaca*, *C. metuliferus* and *M. balsamina.*

The amount of phytate obtained in *C. lanatus* (0.20 mg /100 g) was lower than that reported [26]. These antinutrients could be toxic when consumed in unprocessed foods. However, at the identified low concentrations, they may not constitute major danger provided they were properly cooked before consumption. Table 3 presents the result of inhibition against trypsin by the five seeds studied. The inhibition ranged from 46.05-38.02% respectively with the highest shown by *C. maxima* while the least was exhibited by *L. aegyptiaca.*

CONCLUSION

From the results obtained in this study, the studied cucurbits could be viewed to be formidable source of valuable nutrients such as proteins, fats, carbohydrates, fibre which could be exploited in ameliorating the problem of malnutrition by incorporation in various food and feed fortification formulations, after further validation. Thus, considering the numerous potential of these species, their cultivation should be encouraged.

Table 2: Antinutritional compositions of the seeds of five selected species of Cucurbitaceae (mg/100 g D W).

Species	Phytate	Oxalate	Cyanide	Nitrate	Tannins
L. aegyptiaca	0.40	0.91^{a}	0.40	4.06^{c}	1.53^{c}
C. lanatus	0.20	0.50^{b}	0.30	6.80^{b}	2.11^{b}
C. maxima	0.30	0.90^{a}	0.50	8.10^{a}	3.70^{a}
C. metuliferus	0.20	0.90^{a}	0.51	6.80^{b}	1.40^{c}
M. balsamina	0.20	0.90^{a}	0.20	1.56	1.10^{C}
SE ±	NS	0.36	NS	0.63	1.56

Means in a column followed by same superscripts are not significantly different at $P < 0.05$ (n = 3).

Table 3: Change in absorbance per minute (TIU) of seed extracts using BAEE substrate.

	L. aegyptiaca	*C. lanatus*	*C. maxima*	*C. metuliferus*	*M. balsamina*
1.	3.68	5.06	3.91	6.67	5.06
2.	2.76	2.30	2.53	3.22	2.30
3.	2.07	1.84	1.38	1.84	2.07
$\overline{X}$	**2.84**	**3.07**	**2.61**	**3.91**	**2.79**

REFERENCES

[1] Abdelatief SHE. International Journal of Agriculture 2011; 1 (2): 68-75.
[2] Abitogun AS, Ashogbon AO. Ethno-botanical leaflets 2009; 14:235.
[3] Hamza MA. J King Saud University 1995; 7 (2): 179-186.
[4] Al-Khalifa AS. J Agr Food Chem 1996; 44:964-966.
[5] Murray C, Lopez A. The Lancet 1997; 349 (9): 1436-42.
[6] Abiodun O A, Adeleke RO. Pakistan J Nutr 2010; 9 (9): 905-908.
[7] Loukou AL, Gnarkri D, Dje Y, Kippre AV, Malice M, Baudoin JP and Zoro IA. African J Biotechnol 2007;6 (5): 529-533.
[8] Fokou B, Achu MB and Changeups TM. African J Nutr Agr Develop 2004; 4: 8-12.
[9] Ali M S, Zahoor MN and Qadri IM. African J Biotechnol 2010; 9 (11): 1551–1554.
[10] Jin YK, Seong CP, Indeok H, Hyeomasook C, Jae KS and Yoonkyung P. Int J Mol Sci 2009; 10 (6): 2860-2872.
[11] Rawlings D. Biochem J 2004; 378 (3).
[12] AOAC, (1990). Official Methods of Analysis. 14th edition. Association Official Analytical Chemists. Washington DC.
[13] Van-Buren JP and Robinson WB. J Agr Food Chem 1981; 17:772-777.
[14] Young SM and Greaves JE. Food Research 1940; 5:103-108.
[15] Day RA and Underwood AL. (1986). Quantitative Analysis. 5th edition. 701. Prentice Hall publication.
[16] Wang TL, Tong XY, Yan LQ, Shang JY and Liu SM. Chromatographia 2005; 62:631.
[17] IITA (1988). Selected methods for soil and plant analysis; Manual series NO. 1).
[18] International Institute for Tropical Agriculture, Ibadan, Nigeria, 55-56. Gomez KA and Gomez AA. (1984). Statistical Procedure for Agricultural Research. Second edition, 20-30.
[19] Muhammad AA. (2004). Chemical Composition and oil Characteristics of pumpkin seed kernel. Research Bulletin. By Food and Agricultural Research Centre, King Saud, University, Kingdom of Saudi Arabia. 129: 5-18.
[20] Sadou H, Sabo H, Alma MM, Saadou M and Léger C. Bull Chem Soc Ethiopia 2007; 21 (3): 323-330.
[21] Dairo FAS. (2008). Assessment of Loofa gourd seeds (Luffa cylindrica Roem.) on performance and some haematological indices of rabbit wieners. Nutrition and Digestive Physiology. 9th World Rabbit Congress, Verona- Italy, 613-618.
[22] Evangelos SL. J Food Sci 2008; 51 (5): 1382-1283.
[23] Ojiako OA and Igwe CU. J Med Food 2007; 10 (4): 735-8.
[24] Mabalaha MB, Mitei YC and Yoboah SO. J American Oil Chem Soc 2007; 84:31-34.
[25] Madaan TR and Lai BM. Plant Foods and Human Nutrition 1984; 34:81-86.
[26] Jyothi AL and Kaul P. Food Sci Technol 2011; 44: 1821-1826.