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Original Research Article

Nutritional and antinutritional composition of *Synsepalum dulcificum* seeds

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Abstract

Purpose: This study was designed to explore the nutritional (proximate, mineral, vitamin) and antinutritional compositions of *Synsepalumdulcificum* seeds.
Method: proximate, mineral, vitamin and antinutritional compositions were evaluated in*Synsepalum dulcificum* seeds using standard procedures.
Results: Proximate composition of the seeds showed moisture content (44.30 ± 0.26%), ash (1.44 ± 0.14%), crude fat (1.33 ± 0.58%), crude fibre (1.30 ± 0.26%), protein (12.32 ± 0.10%) and carbohydrates (39.31 ± 0.57%). Mineral analysis of the seeds showed the presence of some elements such as potassium, sodium, manganese, calcium, phosphorus, and iron. Result of the vitamin analysis showed that the seeds contain vitamin C (4.84 ± 0.95mg/g), vitamin A (3.02 ± 0.47 mg/g) and vitamin E (0.47± 0.01mg/g). The antinutritional parameters analyzed were oxalate (8.42 ± 1.63%), phytate (4.57 ± 0.48%) and hemagglutinin (3.74 ± 0.01%).
Conclusion: The results of this study indicated that *S. dulcificum* seeds have appreciable nutritional contents (such as protein, minerals and vitamins) that are of immense health benefits and low antinutrient content. Further studies aimed at the utilization of this seed in human and livestock nutrition is warranted.

Keywords: *Synsepalum dulcificum*; proximate; mineral; vitamin; antinutrients

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INTRODUCTION

The nutritional composition of foods is of paramount importance to the anthropometric and micronutrient status of individuals [1]. Plants serve as important constituents of human diet supplying the body with carbohydrates, protein, vitamin, mineral salts, lipids and water [2]. The plant parts that are used are root, stem, leaves, flower, seed and fruit. In most developing countries of the world, numerous kinds of palatable wild plants are harnessed as main sources of nutrients to reduce the effect of food scarcity [3]. Seeds of plants are indispensable sources of minerals, proteins and vitamins, which are of immense nutritive values to the human body. However, some seeds contain factors (antinutrients) that, at high levels, need to be removed to improve their nutritional quality [4]. *Synsepalumdulcificum,* previously called *Richardelladulcificum* and also referred to as miracle or magic fruit, is an evergreen plant found majorly in West Africa. Generally, it produces

small orange-like fruits of about an inch long with a splendid red colour. It has a large edible seed which is surrounded by a very thin layer of berry flesh with a swoon cherry-like flavor. It is notable for its taste-altering capacity; when eaten, the fruit makes sour food becomes sweet and tasty. The fruit also contains a glycoprotein particle with some trailing starch chain otherwise known as miraculin [5]. At the point when the fruit is eaten, the miraculin content ties to the taste buds of the tongue, making sour or bitter substance taste sweet. While the precise mechanism of this change in taste is obscure, it is accepted by various authors that the attachment of the miraculin to sweetness receptors makes them become receptive to acids, rather than sugar and other sweet things [6].

The miracle fruit pulp is utilized to improve the taste of palm wine in tropical West Africa. There have been a few endeavors to make a commercial-scale sweetner from *S. dulcificum* for diabetic patients [5]. The fruits have additionally been utilized to lessen some undesirable taste in the mouth of cancer patients on chemical treatment (Chemotherapy). Previous accounts of this plant are primarily on dietary and medicinal aspects of the fruits and leaves [7]. He et al [8] indicated that the fruit has substantial antioxidant effects.Again, the seeds are normally disposedwhen the fleshy part of the fruit is eaten raw. Consequently, the objective of this study was intended to investigate the nutritional (proximate, mineral and vitamin) and anti-nutritional composition of seeds of *S. dulcificum* to supplement the previously existing knowledge on the pulp.

EXPERIMENTAL

Preparation of *S. dulcificum* seed flour

Ripe fruits of *S. dulcificum* (miracle fruit) were collected from Uke in Anambra State, Nigeria. The plant material was identified by Mr. Alfred Ozioko, the botanist at Bioresource and Development Conservative Programme (BDCP), Nsukka, Nigeria. Fruit was cleaned and washed: the seed removed from the fruit. The seeds were dried at room temperature, pulverized into coarse powder using mechanical grinder and stored in air tight plastic container for analysis.

Proximate Composition of *S. dulcificum* seed flour

Percentage concentrations of protein, fat, crude fibre, moisture and ash were determined for *S.dulcificum* using the AOAC method [9].

Crude protein

Digestion: A sample of the seed (0.1 g) was weighed in a Kjeldhal flask with 2.0 g catalyst (sodium sulphate/copper sulphate). Concentrated H_2SO_4 (20 ml) was poured into the flask and the contents gently heated. The heating was increased until the contents of the flask were completely digested giving a clear solution [9]

Distillation: The content of the flask was washed with 220 ml distilled water into a distillation flask and cooled under ice blocks. Boric acid (100 ml) of 4% was poured into the flask and 3 drops of screened methyl red was added [9]

Back titration: Cooled 40% NaOH (50 ml) was added and the distillate was titrated against 0.5 N Na_2SO_4 solution [9] (eg. 1).

$$\% \text{ nitrogen} = \frac{T \times N \times Df \times MWN}{Weight\ of\ Sample} \times 100 \text{ .. (1)}$$

Crude fat

A washed, dried and cooled quick-fit flask was weighed. Fresh sample of pulp were weighed into extraction thimble and placed into the quick-fit soxhlet apparatus with a solvent flask containing 250ml of diethyl ether connected to a condenser. The set-up was heated for 16hrs for complete extraction. The extract was evaporated at 70°c to remove any remaining solvent present [9]. The apparatus was reweighed and percentage fat calculated (eq. 2).

$$\% \text{ crude fat} = \frac{Weight\ of\ Oil}{Weight\ of\ Sample} \times 100 \text{ ....(2)}$$

Moisture content

Freshly collected samples of seed (2 g) were weighed and dried in the oven at 110°c to a constant weight [9]. The dishes and samples were cooled and reweighed and percentage moisture content calculated using equation 3:

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_1} \times 100 \text{ ..... (3)}$$

Where W_1 = Weight of sample, W_2 = Initial weight of sample and dish and W_3 = Final weight of dry sample

Ash content

Samples of seed (2g each) previously weighed into porcelain dishes were put and reweighed.

The crucible and samples were placed in a muffle furnace at 600 °C for 3 hr. The ashes and crucible were cooled in a desiccator and reweighed and percentage ash content calculated using equation 4:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \quad \text{.....(4)}$$

where W_1 = Weight of crucible, W_2 = Weight of crucible and sample, and W_3 = Weight of crucible and ash

Fibre content

Sample of the seed (2g) was weighed into 500ml beakers containing pre-heated diluted H_2S0_4 about (40ml). The content was boiled for 30 minutes and filtered. The residue was washed three times with hot water, then 150ml of pre-heated KOH and drops of antifoam agent (loctanol) were added to the sample in the beaker and heated to boiling [9]. The mixture was boiled slowly for 30minutes more, filtered and washed three times with hot water. Acetone was then used in washing it three times in cold extraction unit and the content dried at 130°C for 1 hr.

Content was then turned to ash at 500°C and the ash weighed and percentage fibre calculated (eq 5).

$$\% \text{ Crude Fibre} = \frac{Weight\ of\ Fibre}{Weight\ of\ Sample} \times 100 \quad \text{.... (5)}$$

Carbohydrate or Nitrogen Free Extract (NFE)

NFE was determined by subtracting the sum of the other fraction from 100 as follows:

100 – (% moisture+ % crudeprotein + % crude fat + crude fibre + % ash) = % NFE.

Determination of Antinutrient Composition of *S. dulcificum* seed flour

Oxalates, phytates and hemaglutannin were determined by methods described by the Association of Official and Analytical chemists [10].

Oxalates

A sample (5.0) was extracted 3 times by warming (40-50 °C) and stirring with magnetic stirrer for 1 hour in 20ml of 0.3N HCL. The combined extract was diluted to 100ml with water and used for total oxalate estimation [10].

For oxalate estimation, 5.0 ml of extract was made alkaline with 1.0 ml of 5 N ammonium hydroxide. This was made acid to phenolphthalein by drop wise addition of glacial acetic acid. A volume, 1.0 ml of 5% $CaCl_2$ solution was then added and the mixture allowed to stand for 3 hr, after which it was centrifuged at 3000 rpm for 15 min. The supernatants were discarded and the precipitate washed 3 times with hot water with thorough mixing and centrifuging each time. Then to the test tube, 2.0 ml of 3 N H_2SO_4 was added and the precipitate dissolved by warming in a water bath at 80^0C. The content of the tube was then titrated with freshly prepared 0.01 N K_2MnO_4. Titration was carried out at room temperature until the first pink colour appears throughout the solution, and then allowed to stand until the solution is colourless. The solution was then warmed to 80^0C and titration continued until a pink colour persisted for at least 30 sec.

Phytate

A quantity, 2 g of the sample was weighed into 250 ml conical flask. This was followed by the addition of 100 ml of 2 % concentrated hydrochloric acid which was used to soak each sample in the conical flask for 3hours [10]. This was filtered through a double layer of hardened filter papers [10]. The filtrate (50 ml)was placed in 250 ml marked beaker and 107ml of distilled water was added in each case to give proper acidity. Ammonium thiocyanate solution was added into the solution (10 ml of 0.3%) as indicator. This was titrated with standard ferric chloride solution (which contained 0.00195g iron per ml) to an end point of slightly brownish-yellow coloration which persisted for 5 minutes. The percentage phyate was calculated.

Vitamin Analysis of *S. dulcificum* seed flour

Vitamins A, C and E were estimated using methods described by Ojiakor and Akubugwo [11]

Vitamin A

To 10g aliquot of the seed was added 50ml of acetone:petroleum ether (1:1). After two hours, the mixture was filtered and the volume of the filtrate measured. Exactly 50 ml of 50% NaCl was added to wash the filtrate. It was shaken and transferred into a separating funnel. The lower layer was removed and the supernatant collected, the supernatant was washed with equal volume of 10% K_2CO_3 and separated. The upper layer was washed with 20 ml distilled water, separated carefully and its absorbance was read at 390 nm using 1:1 acetone/ petroleum ether as blank.

Vitamin C

To a 10g quantity of the seed was added 80ml ethanol and 20ml of distilled water; it was covered and shaken in an orbital shaker for 2hours. Then, it was filtered and the volume of the filtrate measured. The filtrate (5 ml) was measured into a conical flask, 50ml of distilled water and 2.5ml of 1M H_2SO_4 were added. 1ml of 10% starch indicator was added and titrated with 0.05M iodine solution till a blue-black colour appeared.

To get the concentration in mg/g;

$$\frac{T.V. \times 0.00886 \times Vol\ of\ Extract}{Volume\ of\ Curvette\ (5ml) \times Weight\ of\ Sample} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$

where T.V = Titre value

Vitamin E

The seed (1g) was weighed and 5ml acetone was added and allowed to stand for 10minutes. 2ml of distilled water and 5ml of petroleum ether were added to the filtrate (oily layer). 5ml of the oily layer was collected and its absorbance was read at 450 nm [11]. A standard curve was prepared using Vitamin E standard treated same as sample.

Mineral content of *S. dulcificum* seed flour

The mineral contents namely potassium, calcium, magnesium, phosphorus and iron were estimated by the use of atomic absorption spectrophotometer.

Digestion method

A 0.2 g amount of the sample was weighed out. Perchloric acid (5ml) was added and 10ml of HCl was also added. The mixture was put in the oven at 150^{o}Cfor 45minutes after which it was transferred into a 250ml conical flask and made up to mark. Atomic absorption spectrophotometer (AAS) was set at different absorbance depending on the mineral to be analysed [9].

Statistical Analysis

Results were presented as mean ± standard deviation of all parameters determined.One way analysis of variance (ANOVA) and Fisher's least significant difference (F-LSD) were used to separate the means.

RESULTS

Proximate composition of *Synsepalum dulcificum* seed flour

The result of the proximate analysis showed that *S. dulcificum* seeds have high moisture (44%) and carbohydrate (39.61%) contents. The seed is also rich in protein (12.31%) but contain low amounts of crude fat, crude fibre and ash (Table 1).

Table 1: Proximate composition of *S. dulcificum* seed flour

Proximate parameters	Composition in flour (%)
Moisture	44.00 ± 0.26
Crude fat	1.33 ± 0.58
Ash	1.44 ± 0.14
Crude fibre	1.30 ± 0.26
Protein	12.32 ± 0.10
Carbohydrates	39.61 ± 0.57

The data are mean ± standard deviation of triplicate results

Mineral and vitamins contents of *Synsepalum dulcificum* seed flour

As presented in Table 2, *S. dulcificum* seed has relatively high amount of potassium (6.63 ± 1.12 mg/100g), vitamins A (4.84 ± 0.95 mg/g) and C (4.84 ± 0.95 mg/g).

Table 2: Levels of some minerals and vitamins in *S. dulcificum* seed flour

Variable	Concentration
Sodium (Na)	0.18 ± 0.02%
Potassium (K)	6.63 ± 1.12 mg/100g
Calcium (Ca)	0.62 ± 0.05%
Magnesium (Mg)	0.24 ± 0.03%
Iron (Fe)	1.00 ± 0.10 mg/100g
Phosphorous (P)	0.16 ± 0.03 mg/100g
Vitamin A	3.02 ± 0.47 mg/g
Vitamin C	4.84 ± 0.95 mg/g
Vitamin E	0.47 ± 0.01 mg/g

The data are mean ± standard deviation of triplicate results

Composition of antinutritive factors in *Synsepalum dulcificum* seed flour

The presence of antinutritive factors such as oxalate (8.42 ± 1.63 mg/100g), phytate (4.57 ± 0.48 mg/100g) and hemagglutinin (3.74 ± 0.01 mg/100g) were detected in *S. dulcificum* seeds flour

DISCUSSION

In this study, the seed of *S. dulcificum* has been found to have high content of moisture (44.3%) and protein (12.32%) but comparatively low content of fat (1.33%) and fibres (1.3%). In addition, it contains sodium, potassium, calcium, magnesium, iron, phosphorus and vitamins.

The moisture content of food can affect the shelf-life and susceptibility to microbial damage; hence any food with high moisture content usually has lower shelf-life [7]. High content of protein present in *S. dulcificum* seed suggests that the seed is a good source of protein which should be exploited for commercial purpose. It is noteworthy that plant foods which provide more than 12 % of their calorific value from proteins have been shown to be good sources of proteins [12]. The calories present in each gram of carbohydrates, proteins, and fat is 4, 4 and 9, respectively [13]. This will translate to a contribution of at least 5.4.% of the calories of fat to the calories present in the seeds. It is pertinent to note that diets providing 1-2 % of its caloric energy as fat is said to be sufficient to human beings as excess fat can cause cardiovascular disorders such as atherosclerosis as well as cancer and aging [13]. Other than the energy that can be derived from the carbohydrate of the seeds, the carbohydrate also prevents protein usage as energy, hence, conserving proteins for other metabolic uses in the system [14]. Furthermore, fibre-enriching foods extend the inner walls of the human colon, thereby enabling easier waste passage and preventing constipation. The relatively small ash content in the *S. dulcificum* seeds shows that the mineral contents in the seed analyzed is also relatively small.

Potassium is the most abundant mineral element found in *S. dulcificum* seed with a concentration of 6.6 %. High level of potassium increases the utilization of iron, and is of immense benefit to people taking diuretics to manage hypertension. The concentration of sodium in the seed is 0.18%. Potassium is requisite for the regulation of blood pressure, fluid balance and blood volume. It also functions in the proper performance of the nerves and muscles [15]. Calcium, on the other hand was found to be 0.62%. It is a vital mineral required in the body for neurologic functions and bone formation. It also helps to ease insomnia and aids blood clotting as well as normal functioning of muscles [16]. Magnesium is essential for calcium metabolism, regulation of blood pressure and insulin secretion [17]. The concentration of magnesium in the seed from this study is 0.24 mg/100g. Magnesium deficiency reduces the osteoblast bone formation, uncontrollable twisting of muscles which could lead to convulsion and prevention of cell formation [16]. The iron content of the *S. dulcificum* seed is 1.00 mg/100g. Iron plays many beneficial roles in human body. It is important in blood formation and the transport of oxygen and carbon dioxide from one tissue to another [18]. Its deficiency also results in anemia and impaired muscles metabolism. In children, iron deficiency leads to impaired learning ability and behavioral problems [19]. Phosphorus content of the *S. dulcificum* seed is 0.16 mg/100g. Phosphorous is essential as cellular components as well as blood. It is found as part of nucleic acids, adenosine triphosphate, sugar phosphate and phospholipids [15]. Phosphates may also take part in as buffers in the body and facilitate the movement of nutrients across the cell membrane [18]. Looking at the recommended dietary allowance (RDA) for minerals, calcium (100 mg/day); phosphorus (800 mg/day); magnesium (400 mg/day) and iron (8 mg/day), *s.dulcificum*seeds when comple-mented with other diets could cover RDA and contribute for improving human health [12].

The levels of antioxidant vitamins were analyzed in this study and the result shows that *S. dulcificum* seeds contain 4.84 mg/g of vitamin C, 3.02 mg/100g of vitamin A and 0.47 mg/100g of vitamin E (Table 3). Vitamin C plays an important role in wound healing especially in children and women above 18 years [14]. Furthermore, when it is inadequate could result in scurvy in children. Vitamin C facilitates collagen production, wound healing and red blood cells formation. It also boosts immune system and acts as an antioxidant that prevents oxidative damage to cellular components such as proteins, nucleic acids or lipids [20]. Vitamin A helps in maintaining good sight and normal vision [21]. Vitamin E, an antioxidant known as anti-sterility vitamin is crucial in the development and normal functioning of red blood cells and muscles [22]. Health benefits of vitamins in humans have received extensive attention recently. Many individuals especially those in underdeveloped nations where malnutrition is common face the risk of vitamin deficiencies. Such situations are managed by vitamin supplementation (usually by intake of multivitamin tablets) which has been associated with overdose of vitamins and vitaminosis [23]. In addition, there is an evidence of interactions of vitamins and other drugs [24]. Attention should be given to natural sources of vitamins which usually provide moderate amount and reduce the risk of vitamin overdose as well as deficiencies.

Antinutrients pose a major constraint in the use of plant protein sources in livestock feed or human food without adequate and effective processing.

Hurrel*et* al. [25] reported that a phytic acid intake of 4-9 mg (dry matter) reduces the absorption of iron by 4-5 folds in humans. Toxicity of oxalates for humans was set as 2.5 g/day and the consumption of diet high in these antinutrients may result in kidney disease [26]. The levels of phytate and other antinutrients in *S. dulcificum* seed are considerably low to be considered toxic. However, these antinutrients present in the seeds could be easily detoxified by soaking, boiling or frying.

CONCLUSION

From the results obtained from the study, it can be concluded that *S. dulcificum* seeds are rich in protein and some vitamins and contain low levels of antinutrients compared with some non-edible seeds. However, there is a need for further studies aimed at the utilization of these seeds in human and animal nutrition.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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REFERENCES

1. *Sosanya M, Freelad-Graves J, Gbemileke A, Adeosun E, Shokunbi O. Proximate and mineral composition of traditional, composite foods consumed in rural areas in Bauchi state, Nigeria. Curr Dev Nutr 2020; 4(2):780*
2. *Odoh UE, Agbachi BC. Phytochemical, proximate and nutritive composition analyses of the seed of landolphiaowariensisp. Beauv. (Apocynaceae). World J Innov Res. 2020; 8(5):104-109*
3. *Aletan UI, Kwazo HA. Analysis of the proximate composition, antinutrients and mineral content of maeruacrossifolia leaves. Nig J basic Appl Sci 2019;27(1):89-96*
4. *Patricia O, Zoue L M, Doue R, Niamke S. Proximate composition and nutritive value of leafy vegetables consumed in northern Cote d'ivoire. Eur Sci J 2014;10(6):1857-1881*
5. *Jian H, Qiao F, Chen J, He N. Physicochemical characterisation of polysaccharides from the seeds and leaves of miracle fruit (Synsepalum dulcificum) and their antioxidant and α-glucosidase inhibitory activities in vitro. J Chem 2017; Article ID 8948639*
6. *Wong JM, Kern M. Miracle fruit improves sweetness of a low-calorie dessert without promoting subsequent energy compensation. Appetite 2011; 56, 163–166.*
7. *Nkwocha CC, Njoku OU, Ekwueme FN. Phytochemical, antinutrient and amino acid composition of Synsepalum dulcificum pulp. IOSR J Pharm Bio Sci 2014; 9(2): 25-29.*
8. *He Z, Tan JS, Abbasiliasi S, Lai OM, Tam YJ, Ariff AB. Phytochemicals, nutritionals and antioxidant properties of miracle fruit Synsepalum dulcificum. Ind Crops Prod 2016; 86: 87-94.*
9. *Association of Official Analytical Chemists (AOAC).Official Methods of Analysis.18th Ed., Washington, D.C; 2012*
10. *Association of Official Analytical Chemists (AOAC). Official Methods of Analysis.20th Ed., Washington, D.C.; 2016*
11. *Ojiakor OA, Akubugwo EI. An Introductory Approach to Practical Biochemistry.CRC Publishers, Owerri, Nigeria. 1997; pp. 156-161.*
12. *Ali A. Proximate and mineral composition of marchubeh (Asparagus officinalis). World Dairy Food S 2009;4:142-14913. Kris-Etherton P M, Hecker KD, Bonanone A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Pubmed 2002; 9:71-88*
13. *Whitney ES, Cataldo CB, Rolfes SR. Understanding Normal and Clinical Nutrition. 6th ED.,Wadsworth/ Thomson learning, USA.2002*
14. *AlozieYE,Udo AU, Orisa C. Proximate, antinutrient and vitamin composition of full-fat and defatted seed flour of Telfaira occidentalis. Turk J Agric 2017; 5(11): 1256-1260.*
15. *Payne WJA. An introduction to animal husbandry in the tropics.Longman Publishers, Singapore; 1990; pp. 92-110.*
16. *Galsworthy TD, Wilson PL. Osteoporosis: It steals more than bone. Am J Nurs 96; 27-32.*
17. *Onyiriuka SO, Nwadinigwe CA, Nwaji MN, Akpansi LES, Okoro UC. Principles of Organic Chemistry (1st Edition); 1997; 12: 206-209*
18. *Guthrie HA. Introductory Nutrition (7thedn.).Time Mirror Mosby College Publishers, Boston; 1989*
19. *MC Donald A, Edwards RA, Greenhulgh FD, Morgan CA. Animal Nutrition.Prentices Hall, London; 1995; pp. 101-122.*
20. *Riccioni G, Bucciarelli T, Mancini B, Corradi F, Di Ilio C, Mattei PA, D'Orazio N. Antioxidant vitamin supplementation in cardiovascular diseases. Ann Clin Lab Sci 2007; 37(1): 89-95.*
21. *Achikanu CE, Ani ON, Akpata EI. Proximate, vitamin and phytochemical composition of cucumismetuliferus seed. Int J Food Sci Nutr 2020;5(3):20-24*
22. *Onwuka GI. Food analysis and instrumentation (theory and practice). 1st edn, Napthal prints, Surulere, Lagos, Nigeria 2005, 140-160*
23. *Weiss WPA. 100-Year Review: From ascorbic acid to zinc—Mineral and vitamin nutrition of dairy cows. J. Dairy Sci 2017; 100, 10045–10060.*
24. *Péter S, Navis G, de Borst MH, von Schacky C, van Orten-Luiten ACB, Zhernakova A, Witkamp RF, Janse A, Weber P, Bakker SJL. Public health relevance of drug–nutrition interactions. Eur. J. Nutr 2017; 56, 23–36.*

25. Hurrel RF, Juillert MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate and iron absorption in humans. Am J Clin Nutr 1992; 56:573-578

26. Hassan LG, Umar KJ, Umar Z. Antinutritive factors in Tribulus terrestris (Linn) leaves and predicted calcium and zinc bioavailability. J Trop Biosci 2007; 7:33-36.