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The Effect of Cooking on the Goitrogenic (cyanogenic glycosides and glucosinolates) Content of Pumpkin Leaves (*Telfairia occidentalis*) and Spinach Leaves (*Spinacia olairaceae*)

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Abstract

Fresh ugwu (Telfairia occidentalis) and spinach (Spinacia olairaceae) leaves play important roles in human nutrition. Leafy vegetables including Telfairia occidentalis and Spinacia olairaceae are considered as agents of transmission of goitrogens which interfere with iodine uptake thus causing goiter. Cyanogenic glycosides are naturally occurring goitrogens found in staple foods in the tropics. Thiocyanate and isothiocyanate have been demonstrated as the goitrogenic principles of cyanogenic plants Thiocyanate ion acts as a goitrogen when present at high concentration especially when the iodine content of the diet is low. Thiocyanate inhibits the uptake of iodine by the iodide pump of the thyroid gland thus acting as a goitrogen which suppresses thyroid function leading to goiter. This study examined the effect of cooking on the goitrogenic potential of Ugwu and Spinach leaves. A total of (10g) of fresh/ andraw ugwu and spinach leaves were randomly collected and cooked. All samples were examined according to standard methods for detection of goitrogens. The cyanogenic glycosides content of rawand fresh ugwu leaves were 1. 6mg /kg - 1. 82mg/ kg while that of cooked leaves were 0. 004mg/ kg - 0. 0 l3mg/ kg. The glucosinolates content for raw and fresh Ugwu leaves were15.7mg/kg-17.28mg/kg, while that of cooked Ugwu leaves were 11.18mg/kg- 12.51mg/kg . For

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Spinach the cyanogenic glycosides content for raw and fresh were 0.24mg/kg-1.28mg/kg, while that of cooked spinach were 0.013mg/kg- 0.00Smg/kg. The glucosinolates content for raw and fresh spinach were 4.0mg/kg-2.64mg/kg while those of the cooked leaves were 2.52mg/kg-1.82mg/kg. there was a significant diference (P=0.085) in the level of reduction of glucosinolate content between raw and cooked Ugwu leaves and Spinach. There is also a significant difference (p=3.04) in the concentration of glucosinolates between Ugwu leaves and Spinach. Ugwu leaves had a higher concentration of glucosinolates than spinach in both fresh (raw) and cooked conditions respectively. These findings provide evidence that the risk of acquiring goiter from the consumption of ugwu and spinach leaves is reduced when cooked.

Keywords: Ugwu leaves, Spinach leaves, Cooking, Goitrogens, Cyanogenic glycosides, Glucosinolates

INTRODUCTION

Vegetables play a vital role in human nutritionas the most rapid and lowest source of fibers, minerals and vitamins where they are often eaten in relatively small amount as a side dish or relish with the stable food (Omale and Ugwu, 2011; Yahaya *et al*., 2014). The use of many plants for food is often limited by the composition of goitrogenic substance in them as they remain hazardous to both man and animals (Kubmarawad *et al*., 2008; Oladejo *et al*., 2018)

Goitrogens may act directly or indirectly on the thyroid gland and the referral metabolism and excretion of triiodothydomine (T3)/Thyroxin (T4) (Mondal and Chandra, 2019). The thyroid accomplishes the task of metabolic regulation through the secretion of important hormones, T4(Thyroxine) and T3(Triodothydomine) (Mullur *et al*., 2014) as well as calcitonine (Ane *et al*., 2013), which are important for calcium metabolism in the blood plasma. The level of thyroid hormone production is determined by the level of thyroid stimulating hormones (TsH) released from the pituitary gland;and by the availability of iodine and tyrosine. A number of compounds, however have the ability to prevent the synthesis of these thyroid hormones (Chudasama *et al*., 2010). Common foods rich in goitrogens include Cassava (*Manihot utilissima*) (Sadid and Joachim, 2015), Cabbage (*Brassica Oleracea* L.) (Fernado *et al*., 2012) and Pearl millet (*Pennisetum americanum*) (Eduardo *et al*., 2009; Goldiel *et al*., 2014)

Pumpkin (*Telfairiaoccidentalis*) belongs to the family cucurbitaceae. Pumpkin is cultivated in Nigeria in subsistence level with virtually no commercial importance (Aruah *et al*., 2011). In Nigeria, it is traditional vegetable crop, grown mainly for its leaves, fruit, and seed and consumed either by boiling the leaves and fruit or by roasting or baking the seeds (Aruah *et al*., 2011). The leaves, fruit, flowers and seeds are health-promoting food. The leaves are haematinic, analgesic, and also used externally for treating burns. Traditionally the pulp is used to relief intestinal inflammation or enteritis dyspepsia and stomach disorders (Sentus and Debjani 2007). Leaves extract is useful in the management of cholesterolemia, liver problems and impaired defense immune system (Oyewole and Abalaka, 2012)). *Telfairia occidentalis* has the potential to regenerate testicular damage and also increase spermatogenesis (Nwangwa *et al.,* 2007).The leaves have hypolipidemic effect which is useful in the therapy of hypercholesterolemia (Oyewole and Abalaka, 2012).Pumpkin fruit is excellent source of vitamin C and E, Lycopene and dietary fibre (Sonu and Ramana, 2013; Salman *et al*., 2018). Common name for the plant include fluted gourd, fluted pumpkin and ugwu in Igbo, Gboroko in Yoruba and Umeke in Edo. This creeping vegetable spreads low across the ground with lobed leavesand twisting tendrils (Kuku *et al*., 2014; Daramola *et al*.,

2016). The plant isdioecious, perennial and drought-tolerant, and thrives best in soil rich in organic matter and during the early part of rainy season.
Spinach (*Spinacia oleracea)* is a green leafy vegetable, low in calories and considered a good source of vitamins (ascorbic acid, riboflavin, niacin and folic acid), minerals (iron and calcium) and dietary fibres. It is a popular vegetable and consumed fresh as well as processed as raw, boiled or cooked into various delicacies (Kuku *et al*., 2014; Daramola *et al.,* 2016). Spinach is used for gastro intestinal disorder, blood generating therapy, growth stimulation in children, appetite stimulation, convalescent support and fatigue. It has been also suggested its use as an anti-cancer agent, antioxidants and cancer preventative. Spinach is also rich in iron, its use prevents diseases like osteoporosis, anaemia. Spinach may also reduce age-related eye-sight from macular degeneration and cataracts (Mahmood, 2014)..

Cyanogenic glucosides are phytoanticipins which occurs in at least 2500 plants species of which a number of species are used as food in many areas of the world (Zagrobelny *et al.,* 2011; Dar *et al*., 2016). Glucosinolates (formally called thioglucosides) commonly referred to as goitrogen are a group of more than 120 chemical compounds synthesized by thousands of plants species (Johnson *et al*., 2016). Thiocyanate inhibits the uptake of iodine by the iodide pump of the thyroid gland thus acting as a goitrogen which suppresses thyroid function leading to goiter. These chemicals can suppress thyroid function in different ways such as induction of antibodies that cross-react with the thyroid; while others interfere with thyroid peroxidase (TPO), the enzyme responsible for adding iodine during production of thyroid hormones (Chebet, 2014).

When the diet is overly rich in goitrogens, the thyroid gland swells to trap as much iodine as possible forming a goiter or a lump in the neck. Studies have shown that thiocyanate binds to the same regulatory site as iodine but with a slightly lower affinity, and in higher concentrations thiocyanate also inhibits thyroid hormone synthesis by interfering with thyroid peroxidase (TPO) (Chebet, 2014).

Goitre is a swelling on the thyroid gland. Its development is critically related to the balance between iodine and thiocynate, a goitrogen found in some African diets (Chandra, 2015). More than 5% of the world's population has goiters many of these are associated with diverse disorders and constitute a major public health problem (Endalamaw *et al*., 2019). About 300 million people with goiter live in developing countries where iodine deficiency is prevalent, while about 100 million individuals with goiter live in developed countries(Sebotsa *et al*., 2003).The most common cause of goitre worldwide is iodine deficiency usually seen in countries that do not use iodized salt (Hashemipour *et al.,* 2007). Goiter increases the risk of thyroid irradiation and hypothyroidism during pregnancy and early infancy with concomitant risk of minor brain damage and irreversible impairment of the neuropsycho-intellectual development of offspring (Hashemipour *et al.,* 2007). The enlarged thyroid comprises the trachea and oesophagus leading to symptoms such as coughing, breathing and swallowing difficulties (Norman, 2011).

The global salt iodization program has resulted in the reduction in goiter prevalence. The persistence of goiter in some areas with adequate iodine prophylaxis suggests the existence of other goitrogenic factors (Zimmermann *et al*., 2000). This study determined the effect of processing and cooking on goitrogens (cyanogenic glucosides and glucosinolates) contents of ugwu leaf and spinach.

MATERIALS AND METHODS

Study site

This study was carried out in the Microbiology Laboratory Federal Polytechnic Idah, Kogi State Nigeria. Idah is a town in Kogi state North central Nigeria. It lies on a sandstone cliff on the eastern bank of Niger River in the middle belt region of Nigeria. The town is the traditional capital or the head quarter of Igala Kingdom, whose traditional ruler is the Attah of Igala. It is also a smaller Local Government Area with a population of 79, 815 at 2006 census. Its population is primarily Igala. It is located on latitude 7.1138^{o}E and Longitude 6^{o}E. The temperature of warmest period is 32.0^{o}C and coldest is 20.0^{o}C.

The main occupation of the people is farming and business of all kind. The town is rich in agriculture and the major food supplier of Kogi state with one of the largest market where all the Igala people both those from Ibaji and neighboring towns like Aganebode in Edo state come to buy and sell their goods. It has commercial routes on the river Niger linking Lokoja, the Kogi State capital to the northern part of the country and Onitsha in Anambra state to the southern part of the country and Aganebode in Edo state across the river Niger to the west.

Sample collection

Telfairia occidentalis and *Spinacia oleracea* samples were purchased randomly from Egah market in Idah, Idah L.G.A., Kogi State. Sampling was done between June and July, 2019. This is the period that corresponds with pumpkin and spinach leaves availability in ldah, thus providing enough samples for the study. The leaves were packaged in the polythene bag and transported to chemistry laboratory in the Department of Science Laboratory Technology. Federal Polytechnic Idah, Kogi State.

Preparation of Samples

The fresh samples of Ugwu and spinach leaves were separated from the stem using a knife and washed in a bowl using tap water and rinsed with little distilled water to remove the sand and other impurities. Four bowls were labeled A B C and D. were A stand for raw and B for cooked sample of Ugwu leaves and C for raw and D for raw and cooked spinach respectively. Twenty gram (20g) of the washed ugwu leaves was measured each and poured into bowl A and B. The same process was repeated for spinach leaves into bowl C and D. the wet samples of Ugwu leaves and spinach leaves in bowl A and C representing raw were squeezed to tear them apart using hand fixed with hand gloves. Then the wet samples of Ugwu leaves and spinach leaves in bowl B and D representing cooked were also squeezed to tear them apart, using hand fixed with hand gloves and steamed by microwaving to make the leaves to become soft for 5 minutes in oven with the addition of water.

Sample Processing and analysis

The leaves were boiled and cooked for 30 minutes to disintegrate the cells in order for the hydrolysis products to occur. Thereafter 1 0g each of the raw leaves were hydrolysed by the enzyme glucosidase. Hydrocyanic acid was used to detect cyanogenic glucosides quantitatively according to the method reported by Brinker and Seigler (1989). Briefly, Sodium picrate paper was prepared by dipping filter paper into an aqueous solution of 0.5% picric acid and 5% Na_2CO_3, then allowing the paper to dry. The paper was stored dry but moistened just before use. The detection limit of picrate paper with plant samples is 0.001-0-002% HCN by weight, which corresponds to about 0.01-0-02% prunasin.

Finally 10g each of the cooked leave were hydrolysed by the enzyme myrosinase. Lead acetate followed by hydrogen sulfide were used to detect glucosinolates quantitatively using UV-visible spectrophotometer by the method reported by Chandra *etal*. (2015).

Statistical analysis

R- software was used for data analysis using the Two-way ANOVA to detect the variation in the concentration of the goitrogens.

RESULTS

From the table, in raw and fresh Ugwu leaves the cyanogenic glucosides were 1.6mg/kg and 1.82mg/kg while the glucosinolates were 15.7mg/kg and 17.28mg/kg. In cooked Ugwu leaves the cyanogenic glucosides were 0.004mg/kg and glucosinolates were 0.013mg/kg and 11.8mg/kg and 12.51mg/kg respectively (table).

Raw and fresh spinach leaves had 0.024mg/kg and 1.28mg/kg cyanogenic glucosides while glucosinolates were 4.0mg/kg and 2.62mg/kg respectively (Table).In cooked spinach leaves, the cyanogenic glucosides were 0.013mg/kg and 0.005mg/kg while the glucosinolates were 2.52mg/kg and 1.82mg/kg respectively (table).

Table of Cyanogenic glycosides and Glucosinolates contents of raw fresh and cooked ugwu and spinach leaves

Plants	Cyanogenic glycosides (mg/kg wet weight)		Glucosinolate (mg/kg wet weight)	
	Raw	Cooked	Raw	Cooked
Ugwu leaves	1.71	0.008	16.49	12.16
Spinach leaves	0.76	0.009	3.32	2.17

Two –way ANOVA. P<0.05; Values are means of standard deviation of two observations

DISCUSSION

The significant proportion of human diet is composed of cruciferous vegetables that include Ugwu leaves and Spinach leaves. The goitrogenic contents Viz: Cyanogenic glucosides and glucosinolates of selected plant food were measured in both raw and cooked conditions. Goitrogenic /anti-thyroid principles in pumpkin and spinach leaves are glucosinolate and cyanogenic glucosides contents (Kavitha and Ramadas, 2013). In the present study Cyanogenic glucosides and glucosinolates were measured in uncooked/fresh and cooked conditions for Ugwu and spinach leaves, as these food stocks are generally consumed after cooking (Hemmige *et al*., 2017).

A relative variation in the content of these goitrogenic principles were found between raw/ fresh and their cooked counterparts. There was a significant reduction in cyanogenic glucosides and glucosinolates content remarkably after cooking. From the table, data showed that there was a significant diference (P=0.085) in the level of reduction of glucosinolate content between raw and cooked Ugwu leaves and Spinach. In a similar experiment with spinach, Kavitha and Ramadas (2013) observed reduction in nutritional values due to processing. There is also a significant difference (p=3.04) in the concentration of glucosinolates between Ugwu leaves and Spinach. Ugwu leaves had a higher concentration of glucosinolates than spinach in both fresh (raw) and cooked conditions respectively. In a similar study by Essack *et al*. (2017), variation was observed in the content of cyanogenic glucosides and glucosinolates between fresh and cooked South African vegetables.

In the present study, from the table, data showed that cyanogenic glucosides content was found to be higher ($p<0.05$)in raw and fresh ugwu leaves than spinach, but there was no significant difference ($p=0.501$)in the concentrations after cooking. These plant foods are generally consumed in the world mostly after cooking. There was marked reduction in the concentration of cyanogenic glycosides after cooking, thus cooking affects thecyanogenic glycosides content of the studied plants. There was also a significant difference ($p<0.255$) in the concentration of cyanogenic glycosides between the raw and cooked Ugwu leaves and spinach This observation corroborates that of Essack *et al*. (2017) in a similar study where variation was observed in the content of cyanogenic glycosides and glucosinolates between fresh and cooked South African vegetables. The plants have β-glucosidase which hydrolyzes cyanogenic glucosides to yield 2-hydroxynitrile (cyanohydrin) and hydrogen cyanide and then toaldehyde and ketone (Frisch*et al*., 2015). Cyanogenic glucosides also decompose quickly when placed in boiling water, thus traditional methods of cooking like boiling removed cyanogenic glucosides to certain extent even then some amount of residual glucosides remain on it (Frisch*et al*., 2015).

The glucosinolate content of Ugwu and spinach leaves also reduced markedly after cooking. In a similar study of Broccolini and Kale, Baenas *et al*. (2019) reported that, cooking broccolini and kale affected glucosinolates (GLS) and isothiocyanates (ITC) concentrations, with individual GLS being directly affected by the cooking method. Heat exposure increases the degradation of GLS by myrosinase, and consequently, significantly altering the ITC and indole levels (Baenas *et al*., 2019). Glucosinolate can be gained or lost by vegetables during storage, they may be degraded or leached during processing or preserved by thermal inactivation of myrosinase (Essack *et al*. (2017)). Following tissue damage, myrosinase enzymes catalyse the decomposition of glucosinolates to a variety of volatile and non-volatile products (Raybould and Moyes, 2001). Processing methods such as chopping, boiling and cooking cause cell damage, glucosinolates hydrolysis and the formation of a variety of hydrolysis products (Frisch *et al*., 2015). It has been shown that the level of leaching into the cooking is more strongly related to the amount of cooking water used rather than cooking time or method (Essack *et al*., 2017). Processing of cruciferous vegetables in domestic food preparation or industrial processing will influence levels of glucosinolates considerably and thus affect their health protective capacity (Song *et al*., 2007). ITC exerts antioxidant, anti-inflammatory and multi-faceted anticancer activities in cells, through the in vivo inhibition of inflammation pathways and activation of detoxification enzymes, thus, the highest benefit of cruciferous foods occurs when they are consumed raw, avoiding the degradation of the enzyme myrosinase by cooking or processing. The hydrolysis of GLS to ITC and indoles is crucial for the health-promoting activities related to cruciferous consumption, and is produced after the loss of the cellular integrity because of tissue disruption, by crushing or chewing, or by the action of the gut microbiota (Baenas *et al*., 2019). The normal level of thiocyanate in blood serum recommended by WHO should not exceed 100 mg/L (Chebet, 2014). During cooking the glucosinolate–myrosinase system may be modified as a result of inactivation of plant myrosinase, loss of enzymic cofactors such as epithiospecifier protein, thermal breakdown and/or leaching of glucosinolates and their metabolites or volatilisation of metabolites. Cooking also affects the site of release of breakdown products of glucosinolates, which is the upper gastrointestinal tract, and also makes it easier for it to be hydrolysed in the colon under the action of the resident microflora (Rungapamestry *et al*., 2007). Rungapamestry *et al*. (2007) reported that feeding trials with human subjects have shown that hydrolysis of glucosinolates and absorption of isothiocyanates are greater following ingestion of raw brassica with active plant myrosinase than after consumption of the cooked plant with denatured myrosinase, and the digestive fate of glucosinolates may be further influenced by the extent of cell rupture during ingestion, gastrointestinal transit time, meal composition, individual genotype and differences in colonic microflora.

CONCLUSION

The results of this study indicated that ugwu and spinach contain goitrogensand cooking reduces significantly the amount to a safe level. The findings of the study may have important implications in food safety. Prevention of goitrogens can be achieved by washing, chopping, boiling and cooking of vegetables. More research is needed to provide effective ways of vegetable treatment that can be applied in individual household or at the community level to reduce the goitrogenic contents of consumable foods.

REFERENCES

Ane, G.M., Silvia S., Francisco, G. and Juan, C. G (2013). The incidence and prevalence of thyroid dysfunction in Europe A Meta-analysis. *Archive of Internal Medicine*. **160**: 526.

Aruah, C. B.,Uguru, M. I. and Oyiga, B. C.(2011).Nutritional Evaluation of Some Nigerian Pumpkins (*Cucurbita* spp).*Fruit, Vegetable and Cereal Science and Biotechnology.* **5**(2)**:** 64-71.

Baenas, N., Marhuenda,J., García-Viguera,C., Zafrilla, P. and Moreno,D.A. (2019). Influence of Cooking Methods on Glucosinolates andIsothiocyanates Content in Novel Cruciferous Foods. *Foods*,**8**, 257. doi:10.3390/foods8070257

Brinker, A. M. and Seigler, D.S. (1989). Methods for the Detection and Quantitative Determination of Cyanide in Plant Materials. *Phytochemical Bulletin*. 21(2): 24-31.

Chandra, A.K. (2015): Iodine, Thiocyanate and the Thyroid. *Biochem Pharmacol*. (Los Angel) **4**: 171. doi:10.4172/2167-0501.1000171

Chebet, S. J. (2014). Assessment Of The Levels Of Thiocyanate In Processed And Unprocessed Red And Brown Finger Millet (*Eleusine Coracana*) Grown In Mogotio Area, Baringo County, Kenya. Unpublishedmsc Thesis Department Of Chemistry , School Of Pure And Applied Sciences Kenyatta University, Kenya.

Chudasama R.K., Verma P.M. and Mahajan R.G.(2010). Iodine nutritional status and goiter prevalence in 6 -12 years primary school children of Saurashtra region. India. *World Journal of Pediatrics*. **6**(3): 233 -237.

Dar, S.A., Wani, A.B., Wani, M.Y.,Hussain, S. and Majid, M.S. (2016).Plant-Insect Interactions-Cyanogenic Glucosides.*Imperial Journal of Interdisciplinary Research*. **2**(11): 1107-1118.

Daramola, O-O.O., Oyeyemi, W.A. and Onyendilefu, G. (2016): Effects of Methanol Extract of *Telfairia occidentalis* Seed on Serum Lipid Profile, Biochemical and Antioxidant Activity in Female Wistar Rats. *European Journal of Medicinal Plants.* **15**(2): 1-8.

Eduardo G., Raymond H.L., Robert D.R., Sidney H.I., Robert C.C., Jim L., Edward F.M., John H. and Ken K. (2009), Antithyroid and Goitrogenic Effects of Millet: Role of C-Glicosyl Flavones. *The Journal of Clinical Endocrinology and Metabolism*. **68**(4): 4-7

Endalamaw, Y., Kedir,H. and Alemayehu, T. (2019): Visible Goiter among Pregnant Women Attending AntenatalClinic in Public Health Facilities of Debre Markos Town, EastGojjam, North West Ethiopia**.** *Journal of Nutrition and Metabolism.* Volume **2019**, Article ID 2484523, 7 pageshttps://doi.org/10.1155/2019/2484523.

Essack, H., Odhav, B. and Mellem, J.J. (2017):Screening of traditional South African leafy vegetables for specific anti-nutritional factors before and after processing. *Food Science and Technology*. DOI: http://dx.doi.org/10.1590/1678-457X.20416

Fernando R., Pinto M.D .P. and Pathmeswaran W. (2012) Goitrogenic food and prevalence of Goitre in Sri Lanka. *International Journal of Internal Medicine*. **1**(3): 17 -20

Goldiel J.B., Atawodi S.E., lsah H.S. and Bevezi E.P. (2014) Effect of combined millet and leafyvegetables, Balance Diet on thyroid functions. *Journal of Biological Agriculture and health Care*. **4**(20): 2224-3208.

Frisch, T., Motawia, M.S., Olsen, C.E., Agerbirk, N., Møller, B.L. and Bjarnholt, N. (2015): Diversified glucosinolate metabolism: biosynthesis of hydrogen cyanide and of the hydroxynitrile glucoside alliarinoside in relation to sinigrin metabolism in *Alliaria petiolata*. *Frontiers in Plant Science*.**6**(926): 1-16.

Hashemipour, M., Masaoud, A., Ashrat, A., Mansour S. Hassan R Ali K., M Mohammad, M., Roya, K., Zhale, A., Sassan H. and Farida.,M ,(,2007) high presence of goiter in an iodine replete area. *Asia Pacific Journal of clinical Nutritional*.**16**:403-1410

Hemmige, N.N., Abbey, L. and Asiedu, S.K. (2017): An overview of nutritional and anti nutritional factors in green leafy vegetables. *Horticult Int J*. **1**(2):58–65.

Johnson, T.L., Dinkova-Kostova, A.T. and Fahey, J.W. (2016). Glucosinolates from the Brassica Vegetables and Their Health Effects. In: Caballero, B., Finglas, P. and Toldrá, F. (eds.) The *Encyclopedia of Food and Health*. **3**: 248-255.

Kavitha, V. and Ramadas, V. S. (2013): Nutritional composition of raw fresh and shade dried form of spinach leaf (*Spinach oleracea*). JPR:BioMedRx: *An International Journal*. **1**(8): 767-770.

Kubmarawa D., Andenyand I. F. H. and Magomya A. M (2008) Amino Acid and two non-convential leafy vegetables; Gesamumand Balaciteaegutiaca L. *Africa Journal of Biotechnology***7**(19): 3502-3504.

Kuku, A., Etti, U.J. and Ibironke, I.S.(2014).Processing Of Fluted Pumpkin Seeds, *Telfairia Occidentalis* (Hook F) As It Affects Growth Performance And Nutrient Metabolism In Rats. *African Journal of Food, Agriculture, Nutrition and Development*. **14**(5): 1992-2014.

Mahmood, L. (2014). The metabolic processes of folic acid and Vitamin B12 deficiency. *J Health Res Rev*. **1**:5-9.

Mondal, C. and Chandra, A. K. (2019). Goitrogenic/antithyroidal potential of moringa leaves (Moringa oleifera) and spinach (Spinacia oleracea) of Indian origin on thyroid status in male albino rats. *Brazilian Journal of Pharmaceutical Sciences*. **55**:e18005

Mullur,R.,Liu,Y-Y. and Brent, G.A. (2014).Thyroid Hormone Regulation of Metabolism. Physiol Rev. **94**(2): 355–382. doi: 10.1152/physrev.00030.2013

Norman, J. (2011). Thyroid goiter treatments. Enlargement of the thyroid. Retrievedon 27/5/2011 from http://vvwvv.endocrinen1eb.co111/conditions/thvroid/thvroidooiter **3**(1): 25-30

Nwangwa E.K., Mardi J., Ebeye O.E. and Ojieh A.E. (2007), Testicular regenerative effects induced by the extracts of Telfairiaoccidentalis in rats, Caderno de peszvisa, *Series Biol*. **19**:27-35.

Oladejo, A. A., Okesola, M. A., Oyerinde, A. S., Jaiyesimi, K. F. and Kolawole, J.A.(2018). Evaluation of Goitrogenic Content of CommonVegetables in South West Nigeria. *Asian Food Science Journal*. **4**(1):1-6.

Omale J. and Ugwu C.E.(2011) Comparative Studies on the protein and mineral composition of some selected Nigerian vegetables. Africa. *Journal of Food Science*. **5**(1):22-25 .

Oyewole O. A. and Abalaka M. E. (2012). Antimicrobial Activities of *Telfairia occidentalis* (fluted pumpkins) Leaf Extract against Selected Intestinal Pathogens. *Journal of Health Science,***2**(2): 1-4 DOI: 10.5923/j.health.20120202.01

Raybould, A.F. and Moyes, C. (2001): The ecological genetics of aliphatic glucosinolates. *Heredity*.**87**(4):383-91.

Rungapamestry , V., Duncan , A. J., Fuller, Z. and Ratcliffe, B. (2007). Effect of cooking Brassica vegetables on the subsequent hydrolysis and metabolic fate of glucosinolates. *Proceedings of the Nutrition Society*, (1): 69 – 81. DOI: https://doi.org/10.1017/S0029665107005319

Sadid, L. and Joachim, M. (2015). Potential of Cassava leaves in Human Nutrition. A Review: *Trends in Food Science and Technology*. **442**(2): 33-37.

Salman, T.M., Lawal, O.R., Sulaiman, S.O., Feyitimi, A.A. and Alada, A.R.A.(2018): Effects of Te*lfairia Occidentalis* Leaf Extract on Plasma Lactate and Liver Glycogen in Rats. *Niger. J. Physiol. Sci.* **33**: 169-175

Sebotsa, M.. L. D., Dannhauser, A., Jooste, P. L. and Joubert, G. (2003). Prevalence of goitre and urinary iodine status of primary-school children in Lesotho. *Bulletin of the World Health Organization*, **81** (1): 28-34

Sentu S. and Debjani G. (2007), Effect of ripe fruit pulp extract of cucurbita pepo linn. In: *Aspirin-induced Gastria and duodenal ulcer in animals*, S.N. Pradhan Center for Neuroscience, University of Calcutta, Kalkata, India, pp.639-645.

Song, L. and Thornalley, P.J. (2007): Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. Food and Chemical Toxicology.**45**(2):216-24

Sonu, S. and Ramana R. T. V. (2013): Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. *International Food Research Journal*. **20**(5): 2309-2316

Yahaya A.I., Suleiman I., Rahmatallah A.A. and Bello U.B. (2014) Nutrient Content of selected Edible leafy vegetables. *American Journal of Applied Chemistry*. **2**(3): 42-45.

Zagrobelny, M. and Møller, B. L. (2011). Cyanogenic glucosides in the biological warfare between plants and insects: The burnet moth-birdsfoot trefoil model system. *Phytochemistry*, **72**: 1585–1592.

Zimmermann, M., Adou,P., Torresani,T., Zeder,C. and Hurrell, R. (2000). Persistence of goiter despite oral iodine supplementation ingoitrous children with iron deficiency anemia in Côte d'Ivoire1–3. *Am J Clin Nutr.***71**:88–93.