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Comparative Analysis of Antioxidant Vitamins, Minerals and Bioactive Compounds of Black Pepper (*Piper guineense)* Seed, Turmeric (*Curcuma longa)* and Ginger (*Zingiber officinale)*Rhizomes Spices

[1]Okoye Obiageli I., [1]Nwobi Adaobi C. [2]Chukwuka Oluebube F., [2]Agbo Chinyere E, [2]Ezeja Enechojo P.
[1]Department of Home Science and Management, University of Nigeria, Nsukka
[2]Department of Nutrition & Dietetics, University of Nigeria, Nsukka.

Corresponding Author: obiageli.okoye@unn.edu.ng

Abstract

The study compared antioxidant vitamins, minerals and bioactive compounds of three selected local spices (turmeric, African black pepper and ginger) consumed in Nigeria. Specifically, the study analysed the antioxidant vitamin (pro vitamin A, C, E), mineral (iron, copper, sodium potassium, manganese, zinc, magnesium) and phytochemical (steroid, tannin, glycoside) compositions. The samples were prepared for chemical analysis using reference standard methods. Data were analysed using mean and standard deviation. Analysis of variance was also used to test for differences. Results on antioxidant vitamins showed that pro vitamin A content of turmeric was significantly higher (322.24 µg /100g) compared to ginger (177.16µg/100g) and black pepper (75.43µg/10g). Vitamin C content of ginger was significantly higher (27.73mg/100g) compared to black pepper (20.69mg/100g) and turmeric (20.62mg/100g). Magnesium content of black pepper was significantly higher (303.61mg/100g) than that of turmeric (241.0 mg/100g) and ginger (194.73 mg/100g). Calcium concentration of black pepper was significantly higher (357.67 mg/100g) compared to turmeric (167.67mg/100g) and ginger (162.00mg/100g). The iron content of the ginger was significantly higher (0.51mg/100g) compared to black pepper (0.34mg/100g) and turmeric (0.29mg/100g). Phytochemical analysis showed that steroid concentration in black pepper was significantly higher (1.80mg/100g) compared to turmeric (0.99mg/100g) and ginger (0.35mg/100g). The value of tannin in black pepper was significantly higher (6.27mg/100g) compared to turmeric (4.67mg/100g) and ginger (3.50mg/100g).

Keywords: Antioxidant, Vitamins, Minerals, Phytochemicals, Ginger, Black pepper, Turmeric

Introduction

Spices refer to the dried part of a plant that contains volatile oils or aromatic flavours. They are food adjuncts commonly added to foods to improve their sensory properties. They can come in various forms; whole, ground, or as extract depending on the processing method. They are used in small quantities to flavor dishes and tend to add few calories to food. Spices contribute a wide range of nutrients and bioactive components to foods (De La Torre et al., 2015). These major constituents of food have some nutritional, antioxidant and therapeutic potentials. Each has a special purpose and thus meets a specific need in the body.

Antioxidants vitamins, minerals and phytochemicals are vital substances needed by the body to perform daily functions properly. These essential substances in spices are crucial in boosting the immune system and assist in the metabolic processes of the body. Numerous epidemiological, preclinical, and clinical studies have shown that spices and herbs are excellent sources of antioxidants with their high content of phenolic compounds which are therapeutically useful in the management of diseases (Okoye & Ebeledike, 2013; De La Torre et al., 2015).

Antioxidant vitamins are organic substances needed in small amounts by humans which can help protect cells from oxidative damage. These molecules can scavenge free radicals, thus would keep the balance between oxidation and anti-oxidation (Zhou et al., 2016). Dietary antioxidants such as carotenoids, vitamins A, C and E play important roles in the body's antioxidant defense, disease management, prevent or slow damage to cells caused by free radicals and are widely used to ameliorate excessive oxidative stress (Jomova & Valko, 2013; Jiang & Xiong, 2016). Research has shown that carotenoids, vitamins A, C and E can be effective antioxidants for inhibiting the development of certain diseases (Nimse & Pal, 2015).

Spices contain minerals which are inorganic substances needed in small amounts by humans for normal growth and development (De La Torre et al., 2015). Mineral elements contained in medicinal plants are very important in human nutrition in alleviating micronutrient deficiencies (Gropper, Smith & Carr, 2018). There are two kinds of minerals: macro minerals and trace minerals. Macro minerals are required in larger amount. They include calcium, phosphorus, magnesium, sodium, potassium, chloride and sulfur. Trace minerals are required in small amounts. They include iron, manganese, copper, iodine, zinc, cobalt, fluoride and selenium. The presence of mineral elements in diet is vital for the human's metabolic processes (Gropper, Smith & Carr, 2018).

Medicinal spices contain some bioactive compounds which are chemicals produced by the plants. These bioactive components are known as phytochemicals. Phytochemicals can be seen in various parts of the plants such as the rhizome, bark, leaves, stems, fruits and seed. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Andrade-Cetto et al., 2017). They can provide a range of biological activities such as anti-inflammatory, anti-mutagenic, antimicrobial, anti-aging, anti-atherosclerosis and anti-cancerous (Zhang, 2015). Phytochemical in spices such as glycoside, steroid and tannin via their antioxidant properties play key roles in disease prevention by suppressing oxidative stress-induced DNA damage (Andrade-Cetto et al., 2017).

Nutrition therapy is a key in preventing diseases, managing existing diseases, controlling or reducing the rate of development of various diseases and complications. Unhealthy foods can increase the risk of developing many nutrition related chronic diseases such as diabetes mellitus, cancer, stroke and other cardiovascular diseases (Andrade-Cetto et al., 2017). Poor nutrition may have significant number of dangerous effects on health. Poor diet and sedentary lifestyle contribute to oxidative stress and excess free radical production (Nimse & Pal, 2015). Oxidative stress can play a crucial role in both diseases progression and other diet-related diseases.

Turmeric is a flowering plant of the ginger family known as *Zingiberaceae*. The rhizomes or underground stems have been used from antiquity as a spice, condiment, a textile dye, medicine and stimulant. Turmeric is a plant native to tropical South Asia. African black pepper is a flowering plant in the piper family known as *Piperaceae*. It is normally grown in tropical regions of central Western Africa. It is cultivated for its fruits, which is usually dried and used as a spice and seasoning. Ginger is an underground rhizome belonging to the family *Zingiberaceae.* It is one of the most widely consumed spices worldwide. Ginger is a plant native to China, South East Asia, West Africa and the Caribbean. Studies have reported that these functional spices are rich in biological properties such as anti-hyperglycemic, anti-inflammatory, antiviral, antibacterial, cleaning, anti-cancer, antioxidant, antiseptic, radioprotective, and cardioprotective properties of ginger, turmeric and black pepper in vitro and in vivo (Panahi et al., 2012; Okoye & Ebeledike, 2013; Zhu, Chen & Song, 2018). They are considered to have beneficial effects in the body beyond basic nutritional requirements (Lobo et al., 2010).

Turmeric is a polyphenolic compound with diverse pharmacologic effects including anti-inflammatory, antioxidant, antiproliferative, hypoglycemic properties and antiangiogenic, activities (Panahi et al., 2015). Ginger is an important herb which exhibits many medicinal and nutritional values (Zhu et al., 2018). *Piper. guineense* seed has antioxidant, anti-inflammatory, anti-flatulent, anti-bacterial, anti-virus, astringent, carminative and bioavaliability properties (Okoye & Ebeledike, 2013).

Plant sources contain various nutrients with antioxidant benefits which are considered to be safer than synthetic antioxidants in commercial food additives or oral orthodox drugs. These synthetic antioxidants may contain high amounts of preservatives, deficient in nutrients and may be toxic to health. They may have a number of serious adverse effects on health and can cause metabolic alterations and other degenerative conditions. Nasri and Shirzad (2013) reported that synthetic antioxidants have side effects and can be toxic to human. The search for medicinal spices rich in nutrients with antioxidant benefits has continued to be an important area of research and of public interest in Nigeria.

Epidemiological studies have reported that many herbs and spices are clinically effective and relatively less toxic than the synthetic drugs in the treatment and management of chronic health problems (Nasri & Shirzad, 2013; Kaur & Arora, 2015). Clinical studies on these spices are limited. Therefore, identifying plant sources with various nutrients and antioxidant benefits can be a very good alternative to preventing, treating and managing health problems. Several Studies supported the view that *Curcuma longa, Piper guineense and Zingiber officinale* are rich in antioxidant vitamins, minerals and phytochemicals and can be used to manage and prevent diseases (Morvaridzadeh et al., 2020; Verma et al., 2018; Hong et al., 2020). Based on these, these spices were evaluated in order to establish enough evidence for the above claims.

Objectives of the study

The general objective of the study was to analyse the antioxidant vitamin, mineral and bioactive compounds of turmeric rhizome, African black pepper seed, and ginger rhizome (flour) spices. Specifically, the study determined:

1. antioxidant vitamin composition (pro- vitamin A, vitamin C and E) of the samples.
2. mineral contents (iron, calcium, magnesium, potassium, copper phosphorus, sodium, manganese, zinc) of the samples.
3. phytochemical constituents (steroid, tannin, glycoside) of the samples.

Materials and Methods

Design of the Study: The study adopted pure experimental research design.

Procurement of Materials: *Curcuma longa* rhizome (tumeric), *Piper guineense* seed (African black pepper) and *Zingiber officnale* rhizome (ginger) were used for the study. They were procured from Ogige local market Nsukka, Enugu State, Nigeria. They were identified in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Procedure for preparation of samples for chemical analysis: Three kilogrammes each of the samples: *Curcuma longa* rhizome (turmeric), *Zingiber officanale* rhizomes (ginger) and *Piper guineense* seed were sorted to remove debris and defects. The rhizomes and seeds were carefully washed with distilled water to remove dirt and sand, then allowed to drain in a plastic sieve. The rhizomes were peeled and cut into small sizes. They were dried in an oven at a temperature of 40 °C for 48 hours. The seeds were oven- dried at 40°C for 4 hours The dried rhizomes were polished to remove rough surface by handpicking. Ginger, turmeric and black pepper were ground into flour using a high speed electric blender (Soyona Japan). They were labeled, packaged and stored in a plastic air tight container under refrigeration for analysis.

Chemical Analysis: **S**ome vitamins, minerals and antinutrient compositions of these samples were determined. All the analyses of the sample were done in triplicates using standard methods.

Antioxidant vitamin content determination of the samples

Pro vitamin A (Beta-carotene): Pro-vitamin A content was determined using the spectrophotometric method as described by Onwuka (2005). 0ne gram of the sample was weighed out followed by addition of 3ml of absolute ethanol. A precipitate of protein was formed, and the extract of vitamin with 5mL of heptanes layer taken to corvette and read at 450nm against a blank of heptanes in a spectrophotometer.

Vitamin C (Ascorbic acid): Vitamin C was determined by the method described by Association of Official Analytical Chemist, AOAC (2010). Two grammes of each sample was weighed into 250ml flat bottom flask and dissolved with 2 ml of distilled water. Trichloroacetic acid (TCA) was added and colour will be developed with 2, 6—dichloroindophenol. The colour (pink) developed was read.

Vitamin of E (tocopherol): Vitamin E was determined by the method of AOAC (2010). Two grams to the sample was weighed into a flat bottom flask. Ten milliliter of absolute alcohol and 20ml of tetraoxosulphate IV acid (H2SO4) was added. A clear solution was formed .Ten milliliter of the solution was pipetted into a test tube and heated in a water bath at 90 degree centrigrade for 30minutes. This was allowed to cool and absorbance was read in a spectrophotometer at 470nm wavelength.

Mineral Content Determination

Iron (Fe), Magnesium (Mg), Potassium (k) Copper (Cu) and Zinc content : The content of the samples were determined using the using the atomic absorption spectrophotometer method as described by AOAC (2010). Two grammes of the samples were ignited in muffle furnace at 55°C for 6 to 8 hours. The ash of the samples were dissolved with hydrochloric acid. The solution was shaked vigorously for 40 minutes. The different wavelengths at absorbance was measured. The absorbance reading for iron was read at 510nm wavelength when the colour of the solution turned pink. The absorbance reading for magnesium and potassium were taken at 560nm wavelength. The absorbance for copper was read at 520nm wavelength while that of zinc was taken at 530nm.

Quantitative Determination of phytochemicals

Total steroid content: This was determined by spectrophotometer method as described by Mahdu et al. (2016). Five grammes of the sample was transferred into 10 ml volumetric flasks. In addition, H2SO4 acid (2ml) and iron (III) chloride (0.5% w/v, 2 ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated over water-bath at 70 ± 2o°C for 30 minutes with occasional shaking. The volume was made up to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Total tannin content: The Folin- Denis method as described by AOAC (2010) was used for the determination tannin content. Five grammes of the sample was extracted with 300ml diethyl ether for 20 hours at room temperature. The residue was boiled for 2 hours with 100ml distilled water, cooled and filtered and the extract was adjusted to a volume of 100ml in a volumetric flask. Then, calorimetrically using Folin-Denis reagent, the tannins content was determined by measuring the absorbance of the solution at wavelength of 760nm.

Total glycoside content: This was determined by spectrophotometric methods as described by Solich et al. (1992). Ten percent (10%) ethanol extract was mixed with 10 mL newly prepared Baljet's solution (95 mL of 1% picric acid + 5 mL of 10% NaOH). After an hour, the liquid was diluted with 20 millliter distilled water, and the absorbance was measured with a spectrophotometer at 495nm.

Data analysis Techniques**:** The data was analyzed using mean and standard deviation. Results of three replicates were used. Analysis of variance (ANOVA) was used to determine statistically significant differences between the means. A probability value of < 0.05 was considered statistically significant.

RESULTS

Table 1: Antioxidant Vitamin composition of the samples (mg/100g)

Sample Pro	Vit A (µg/100 g)	Vit. C	Vit. E
Black Pepper	75.43^{a}±0.39	20.69^{a}±0.10	7.08^{a}±0.05
Turmeric	322.24^{c}±2.77	20.62^{a}±0.03	7.93^{b}±0.05
Ginger	177.16^{b}±0.44	27.73^{b}±0.44	7.49^{a}±0.41

n=3, values are represented as mean ± standard deviation means with the different superscript are statistically different (p< 0.05)

Table 1 shows the antioxidant vitamin composition of the samples. pro vitamin A content of turmeric was significantly higher (322.24 µg /100g) compared to ginger (177.16µg/100g) and black pepper (75.43µg /100g) Vitamin C content of ginger was significantly higher (27.73mg/100g) compared to black pepper(20.69mg/100g) and turmeric (20.62mg/100g. Vitamin E content of turmeric was significantly higher (7.93 mg/100g) compared to black pepper (7.08mg/100g) and ginger (7.49 mg/100g).

Table 2: Mineral composition of the Sample (mg/100g)

Minerals	Black Pepper	Turmeric	Ginger
Zinc	0.97^{b}±0.02	0.92^{a}±0.01	1.02^{c}±0.04
Magnesium	303.61^{c}±1.44	241.00^{b}±1.00	194.73^{a}±3.90
Calcium	357.67^{b}±3.21	167.67^{a}±1.53	162.00^{a}±3.46
Iron	0.34^{a}±0.00	0.29^{a}±0.04	0.51^{b}±0.04
Copper	46.47^{c}±1.74	12.20^{b}±0.13	3.43^{a}±0.09
Potassium	747.33^{c}±2.52	676.67^{a}±3.06	714.67^{b}±4.73
Manganese	25.84^{b}±0.29	23.03^{a}±0.05	23.71^{a}±0.54
Sodium	15.97^{b}±0.48	16.87^{c}±0.38	13.54^{a}±0.57
Phosphorus	125.34^{a}±1.16	214.00^{c}±4.00	198.33^{c}±1.53

n=3, values are represented as mean ± standard deviation means with the different superscript are statistically different (p< 0.05)

Table 2 shows the mineral composition of the samples. Magnesium content of black pepper (303.61 mg/100g) was significantly higher than that of turmeric (241.0 mg/100g) and ginger (194.73 mg/100g). Calcium content of black pepper was significantly higher (357.67 mg/100g) compared to turmeric (167.67 mg/100g) and ginger (162.0 mg/100g). The value of zinc in ginger was significantly higher (1.02 mg/100g) compared to black pepper (0.97mg/100g) and turmeric (0.92 mg/100g). The concentration of iron in black pepper was significantly higher (0.34mg/100g) compared to turmeric (0.29mg/100g) and ginger (0.51 mg/100g). Copper content of black pepper was significantly higher (46.47 mg/100g) than that of turmeric (12.20mg/100g) and ginger (3.43 mg/100g). Potassium content of the samples ranged from 676.67 to 747.33 mg/100g. The value of potassium in black pepper was significantly higher (747.33mg/100g) compared to ginger (714.67mg/100g) and turmeric (676.67mg/100g). The concentration of manganese in black pepper was significantly higher (25.84mg/100g) compared to ginger (23.71mg/100g) and turmeric (23.03mg/100g). Sodium was highest in turmeric (16.87 mg/100g) while black pepper and ginger contained 15.69mg/100g and 13.54mg/100g respectively. Phosphorus content of turmeric was significantly higher (214 mg/100g) than that of black pepper (125.34mg/100g) and ginger (198.33mg/100g).

Table 3: Phytochemical composition of the samples (mg/100g)

Sample	Steroid	Tannin	Glycoside
Black Pepper	1.80^{b} ±0.15	6.27^{b}±2.19	1.28^{a}±0.35
Turmeric	0.99^{ab} ±0.61	4.67^{ab}±0.27	6.93^{b}±0.05
Ginger	0.35^{a} ±0.25	3.50^{a}±0.30	1.39^{a}±0.41

n=3, values are represented as mean ± standard deviation means with the different superscript are statistically different (p< 0.05)

Table 3 shows the phytochemical composition of the samples. The steroid contents of the samples ranged between 0.35 to 1.80mg/100g. The concentration of steroid in black pepper was significantly higher (1.80mg/100g) compared to turmeric (0.99mg/100g) and ginger (0.35mg/100g). Tannin content of black pepper was significantly higher (6.27mg/100g) compared to turmeric (4.67mg/100g) and ginger (3.50mg.100g). Glycoside content of the samples ranged between 1.28 to 6.93mg/100g. Glycoside content of turmeric was significantly higher (6.93mg/100g) than that found in ginger (1.39mg/100g) and black pepper (1.28 mg/100g).

Discussion of findings

The findings of study on Table 1 showed the antioxidant vitamin composition of the spices. The pro vitamin A content of the spices were higher (75.43µg/100g to 322.24 µg/100 g) when compared with works by Okonkwo and Ogu (2014) with value range (7.08 to 14.83µg/100 g) where Pro vitamin A in black pepper was 7.08 µg/100g and ginger (14.83 µg/100g). The variation could be attributed to differences in climatic conditions. Studies have shown that agro- climatic locations along with temperature and rainfall have significant effects on the plant constituents (Nsuala, Kamatou, Sandasi, Enslin & Viljoen, 2017). Pro vitamin A in the diet are effective antioxidants for promoting immune system health, good vision and epithelial cell differentiation and can inhibit the development of certain diseases (Jomova & Valko, 2013; Roleira et al., 2015).

Antioxidant Vitamin C in the spices ranged from 20.62mg/100g to 27.73mg/100g. Vitamin C contents of these spices were lower in concentration when compared with work by Okonkwo and Ogu (2014) which reported higher vitamin C content value (292.62 to378.62mg/100g) where black pepper had 292.62mg/100g and ginger (378.62mg/100g). The discrepancy could be attributed to genetic variation, agronomic practices which may influence the environmental conditions and nutrients in the soil (Yesenia et al., 2021). Numerous studies have shown that spices are rich sources of antioxidants vitamin C which can scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation, promotes growth and repair of tissues, essential for the synthesis of collagen and carnitine (De La Torre et al., 2015; Olusoji, Adelowo & Taiwo, 2023; Ines et al., 2023).

Vitamin E in these spices showed that they are powerful antioxidant vitamins that can help the body to fight toxins, germs and boost the immune system as well as fulfill several roles in maintaining cellular homeostasis, scavenge free radicals, chelate metal ions and function as chain breakers in the lipid peroxidation cycle (Lobo et al., 2010; Ines et al., 2023).

The result of study on table 2 revealed the mineral composition of the spices. Potassium, calcium, magnesium and phosphorus concentration were found in appreciable quantities in all the samples. Potassium is an essential nutrient and has an important role in the synthesis of amino acids and can help in the secretion of insulin in the pancreas (Gropper et al., 2018).

Calcium is reported to be essential for blood clotting, strong bones, teeth formation and as a co-factor in some enzyme catalysis and healthy communication between the brain and other parts of the body (Gropper et al., 2018). Phosphorus content of the extract samples ranged from 29.40mg/100g to 18.52mg/100g. Phosphorus has been reported to be good for bones and teeth formation. It contributes to energy production by participating in the breakdown of carbohydrates, protein and fats. It is needed for growth, maintenance, repair of tissues and cells, production of genetic materials and maintenance of acid-base balance (Miller & Welch, 2013). In humans, magnesium is involved in the formation of bones and teeth, serves as catalyst in energy producing reactions within the cells (Gropper et al., 2018). Magnesium plays crucial role in lipid membrane stabilization, replication and metabolic processes (Gropper et al., 2018).

The samples contain considerable amount of zinc, iron, copper, sodium and manganese. Iron is an essential trace element that is required in small amounts by the body. It helps the red blood cells transport oxygen to all parts of the body and is used by cells and tissues throughout the body for essential metabolic activities (Miller & Welch, 2013). Zinc plays a role in cell division, in numerous chemical reactions within the body, cell growth, vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions, wound healing, and the breakdown of carbohydrates (Miller & Welch, 2013).

Manganese in these spices are essential for the metabolization of cholesterol, carbohydrates, and protein. Manganese is also necessary for normal brain and nerve function (Gropper et al., 2018). Copper plays a role in production of red blood cells, maintaining nerve cells and the immune system. It plays an important role in oxidation-reduction reactions and in scavenging free radicals. Sodium regulates the acid/alkali balance along with other minerals such as potassium, calcium and magnesium. Deficiency of sodium in the diet could lead to improper fluid balance throughout the abody (Miller & Welch, 2013).

The findings on phytochemical composition of the spices showed that steroid ranged between 0.35 to 1.80mg/100g. The steroid content of the spices were slightly similar when compared with works by Akem et al. (2016) with steroid value range (0.26 to 1.63mg/100g) were ginger recorded 0.60mg/100g and pepper (1.63mg/100g). Steroid in the diet is necessary for many physiological functions including growth, development, brain function, energy metabolism, homeostasis, reproduction, lowering cholesterol levels (Rudolph, Cornil & Mittelman-Smith, 2016; Morand & Tomás Barberán, 2019).

The tannin content of the spices ranged between 3.50 to 6.27mg/100g. Tannin content of the spices were higher in concentration when compared with works by Nwinuka, Ibeh and Ekeke (2005) which reported lower and same tannin values in all the samples (0.01mg/100g) where black pepper had 0.01mg/100g and ginger (0.01mg/100g). The variation could be attributed to differences in climatic conditions (Nsuala, Kamatou, Sandasi, Enslin & Viljoen, 2017). Studies have reported numerous biological activities of tannin which include anti-cancerous, anti-allergic, anti-inflammatory, anti-helminthic and anti-microbial, cardioprotective, antitumor, antibacterial, antiviral and immune-modulatory effects (Kartik et al., 2019; Rashid et al., 2019).

The spices contained glycosides which can play some essential roles in the diet such as analgesic, anti-arrhythmic, regulation of glucose metabolism, cardioprotective, purgative effect (De La Torre et al., 2015; Williams, 2021).

Conclusion

The study provided some information on the antioxidant vitamin, mineral and phytochemical composition of the *Curcuma longa, Zingiber officinale* and *Piper guineense* spices. These spices contain appreciable quantities of micronutrients and bioactive compounds which are needed by human in fighting nutrition related diseases. The findings indicated that these local spices are promising sources of natural antioxidants and micronutrients. It strongly suggest that these spices should be used in preventing and managing nutrition related diseases.

Recommendations

Based on the findings of the study, the following recommendations were made:

1. further research on the safe levels of the phytochemicals in the spices should be carried out.
2. further human studies should be carried out on the spices in order to explore therapeutic benefits of these phytochemicals in treatment and prevention of diseases.
3. patients suffering from degenerative diseases should be encouraged to use these local medicinal spices to prevent and manage their condition since they posses antioxidant nutrients and phytochemicals with therapeutic potentials.
4. incorporation of these local spices into recipes such as cake, drinks, biscuits and in food preparations will help to enrich these products and help prevent nutrient deficiencies.

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