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Nutrient and Phytochemical Compositions of Aqueous Extracts of Black Pepper (*Piper guineense*) Seed, Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*) Rhizome

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Abstract

The study examined nutrient and phytochemical compositions of aqueous extracts of commonly consumed local spices; (*Piper guineense*) seed, turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) rhizome. Specifically, the study analysed proximate composition (moisture, protein, fat, ash, crude fibre and carbohydrate) of black pepper seed, turmeric and ginger rhizomes aqueous extracts and phytochemical content (saponin, flavonoid, alkaloid, phenol, terpenoid) of black pepper seed, turmeric and ginger rhizomes aqueous extracts. They were processed into flour using different standard methods. Four hundred grammes of each of the flour were soaked in 800ml distilled water for 24 hours and filtered. The filtrates were concentrated with oven dryer at 50°C until crude extracts were obtained. The extracts were reconstituted with distilled water using standard stock concentration (1gram of extract to 10ml of distilled water) prior to chemical analysis. Proximate and phytochemicals contents of the aqueous extracts were analysed using standard methods. Data obtained were analysed using means and standard deviations. Proximate analysis results showed that protein content of black pepper extract was highest (3.17%) while ginger extract had the lowest (1.26%) value. Ash content of turmeric extract recorded the highest (3.41%) while ginger extract had the lowest (1.40%). The concentration of crude fiber in black pepper extract was highest (2.96%) while turmeric extract had the lowest (2.56%) value. Among phytochemicals analysed, black pepper extract recorded the highest in alkaloid value (3.70mg/100g) while turmeric extract had the lowest value (2.00mg/100g). The concentration of flavonoid in turmeric extract was highest (4.53mg/100g) while black pepper had the lowest (2.80mg/100g).

Keywords: Proximate, Phytochemicals, Aqueous, Nutrient, Extracts, Spices, Analysis

Introduction

Phytochemicals are a wide variety of nonnutritive, bioactive chemical compounds found in plant foods, which are considered to be beneficial to human health. Examples of well-known phytochemicals are the flavonoids, phenolic acids, alkaloids, curcumin and carotenoids. Phytochemicals found in plant foods are powerful substances that can prevent or reduce the oxidative stress of the physiological system. Consumption of plants with nutrients and phytochemicals play some crucial roles in promoting health by improving the nutritional value of diet and preventing several chronic conditions (Duyff, 2017).

Aqueous extract of plant can be defined as extort obtained by isolation of bioactive compounds in medicinal plants using water as an extraction medium. It is prepared by evaporating a watery solution of the soluble principles of a plant matrices to a semisolid or solid consistency. Aqueous extracts of spices such as *Curcuma longa*, *Piper guineense* and *Zingiber officinale* are rich resources of nutritive and non – nutritive substances which play some crucial roles in maintaining optimal immune response that can sustain and promote health. These plant-based foods beyond micro- and macronutrients, play important roles in prevention, reduction and management of nutrition related diseases. Spices and their extracts are important ingredients in our daily diet. Though they are used in small quantities but have multitude of properties which can positively impact human health (Hamilton, 2018).

Thus, healthy dietary lifestyle patterns can have positive impact on people's health throughout their lifetime. Unhealthy diets have been shown to be associated with oxidative stress while reducing the antioxidant defense status (Tan, Norhaizan & Liew, 2018). Oxidative stress plays a crucial role in the development of numerous human diseases (Rajendran, Nandakumar & Rengarajan, 2014). Tan, Norhaizan and Liew (2018) noted that oxidative stress affects carbohydrate, lipid and protein metabolism. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced continuously in the body via oxidative metabolism, mitochondrial bioenergetics, and immune function (Roleira, Tavares-da-Silva & Varela, 2015). It is usually linked with inadequate intake of nutritive and non nutritive bioactive substances relative to nutritional requirements. The body system requires nutrients and phytochemicals in order to function optimally. Nutritional problems are associated with many chronic diseases such as diabetes mellitus, cancer, cardiovascular diseases and other nutrition related diseases (Rajendran, Nandakumar & Rengarajan, 2014). Good nutrition practice can reduce, prevent, control and eliminate these diseases.

Aqueous extracts of spices can perform many functions in the body. They function as nutrients which provide energy, build and repairing body tissues, regulate body processes as well protecting the body against the effects of ageing. Phytochemicals in aqueous extracts are bioactive and non nutritive substances in plant which have therapeutic potentials and disease protective properties (Bi, Lim & Henry, 2017). Plants are endowed with various phytochemical molecules such as terpenoids, phenolic, tannins, flavonoids, alkaloids, and other metabolites which are rich source of free radical scavengers (Campos-Vega, & Oomah, 2013). They possess numerous antioxidant benefits such as anti – inflammatory, anti- cancerous, anti-diabetes, anti-lipidemic, antimicrobial, anti-viral and other health benefits (Rajendran, Nandakumar & Rengarajan, 2014).

Determination of the major nutrients and phytochemicals in foods are of great importance since they can help in the identification of nutritional content, chemical composition and qualities in food. Turmeric is a golden yellow spice that is used as a colouring agent with medicinal properties. The plant belongs to the *Zingiberaceae* family. Black pepper is a spice plant from the family *Piperaceae* with medicinal potentials. It acts as a bioenhancer since it has unique ability to increase bioavailability or effectiveness of other bioactive compounds (Delecroix, Abaïdia, Leduc, Dawson & Dupon, 2017). Ginger is a spice plant from the family *Zingiberaceae* and is also noted for its wide range of therapeutic and medicinal potentials. These functional foods are used as both flavoring and seasoning ingredients in food preparation.

Studies have reported that aqueous extracts of turmeric, black pepper and ginger have the ability to enhance the synthesis of intracellular antioxidants which can inhibit cytokinase, chemo kinase, and cyclooxygenase (COX) and lipoxygenase (Nimse & Pal, 2015; Imani et al., 2015; Chopra, Dhingra, Kapoor & Prasad, 2017). Epidemiological studies stated that the regular consumption of plant and their extracts can reduce the risk of chronic diseases associated with oxidative damage (Zhang et al., 2015; Evuen, Okolie & Apiamu, 2022). Phytochemicals isolated from plant sources have been used for the prevention and treatment of disease. Aqueous extraction or extraction of source material with water is safer, less toxic, economical and more bioavailable because of the various processing method involved (Silva- Correa, 2021).

Malnutrition as a result of food insecurity is prevalent in developing country such as Nigeria (World Health Organisation, WHO, 2021). Utilization of aqueous extracts of available indigenous spices such as turmeric, black pepper and ginger would help to solve nutritional problems. However, food analytical studies on the chemical composition and quality of these aqueous extracts are scarce. Therefore, there is great need for comprehensive documentation of the proximate and phytochemical composition of these aqueous extracts in order to scientifically validate their usage in treatment and management of diseases.

Objectives of the Study

The general objective of the study was to examine the nutrient and phytochemical compositions of aqueous extracts of black pepper *(Piper guineense)* seed, turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) rhizome.

Specifically, the study determined:

1. proximate composition (moisture, protein, fat, ash, crude fibre and carbohydrate) of black pepper seed, turmeric and ginger rhizomes aqueous extracts
2 phytochemical constituents (saponin, flavonoid, alkaloid, phenol, terpenoid) of black pepper seed, turmeric and ginger rhizomes aqueous extracts .

Materials and Methods

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

Procurement: Three kilogrammes of black pepper seed (*Piper guineense*) turmeric (*Curcuma longa*), and ginger (*Zingiber officinale*) rhizome were procured from Ogige local market Nsukka, Enugu State, Nigeria.

Identification of samples: The samples were identified in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Preparation of samples: Turmeric and ginger rhizome were sorted to remove debris and defected rhizomes. The rhizomes were carefully washed with distilled water to remove dirt and sand. They were allowed to drain in a plastic sieve. The rhizomes were peeled, cut into small sizes, oven dried at a temperature of 40 °C for 24 hours, milled into flour using a high speed electric blender (Soyona Japan), packaged in a plastic air tight container, labeled and stored at room temperature for analysis.

Black pepper seed were sorted to remove debris. The seeds were washed, drained, oven dried at 40°C for 4 hours, milled into flour form using high speed blender (Soyona Japan), packaged in a plastic air tight container, labeled and stored at room temperature for analysis.

Preparation of aqueous extracts: Four hundred grammes (400g) of each of the flour were soaked in 800ml distilled water for 24 hours and filtered using Whatman no. 42 filter paper. The filtrate were concentrated to dryness with oven dryer set at 50 °C until crude extracts were obtained. The extracts were properly stored in an air-tight container for further studies. The extracts were reconstituted with distilled water using standard stock concentration (1gram of extract to 10ml of distilled water) prior to chemical analysis.

***Chemical Analysis*: A**nalysis of samples was carried in triplicates using standard methods. The proximate and phytochemical analysis were conducted in the Food and Analytical Laboratory of Nutrition and Dietetics/Home Science and Management Department, University of Nigeria, Nsukka.

Determination of Proximate Composition: The proximate composition (moisture, protein, fat, ash, crude fibre and total carbohydrate) of the aqueous extracts of black pepper seed, turmeric and ginger rhizomes were determined using the method of Association of Official Analytical Chemist (AOAC, 2010).

Moisture content: Approximately 5g of each aqueous extract was introduced into the weighed crucible and the weight determined using a weighing balance. The crucible containing the extract was placed in a hot air oven at a temperature of 95 -100^{o}C under pressure less than or equal to 100mmHg. The crucible and its content were cooled in a desiccator and weighed**.**

Crude protein: The micro Kjedahl method was used for crude protein determination. Twenty grams (20g) of each of the aqueous extract was weighed and carefully transferred to a kjeldahl flask. Titration was against standard 0.1N (concentration in terms of normality of the acid) HCl. The same procedure also was carried out for the blank experiment. Titration was against standard 0.1N HCl. Crude protein content of the aqueous extract was calculated.

Fat content: Fat content of the aqueous extracts were determined by using soxhlet method as described by AOAC (2010). The defatted extract were removed and the solvent recovered. The flask and the content were reweighed. The flask was cooled in a desiccator and weighed.

Crude fiber content: Ten grammes of each of the aqueous extract were weighed into a crucible and placed in a hot 200ml of 1.25% tethraoxosulphate (H_2SO_4) and boiled for 30 minutes were weighed into a crucible and placed in a hot 200ml of 1.25% tethraoxosulphate (H_2SO_4) and boiled for 30 minutes The residue was transferred into a muffle furnace for two hours and fiber content calculated.

Ash content: Aqueous extract of 2g was weighed into a crucible. Each of the extract was transferred into a pre-heated Muffle furnace at 50°C. At this temperature light grey ash was obtained. The ashy extract was cooled in a desiccator, weighed and recorded.

Carbohydrate content: The total carbohydrate content was determined by difference 100 – Total % content of other components. Carbohydrate = 100 – (%protein + %fat + %ash + crude fibre + %moisture).

Quantitative Determination of Phytochemical Constituents of the aqueous extracts

Total Alkaloid content: The alkaloids content of the sample was determined using the method of Harborne (1973). Five grammes of the extract was weighed into a 250ml beaker and 200ml of l0% acetic acid in ethanol was added to it, covered and allowed to stand tor 2 hours. The residue was dried in an oven which is the alkaloid. It is then reweighed and the alkaloid content was calculated.

Total flavonoid content: Flavonoid content was determined according to the method of Harborne (I973). Five grammes of each of the aqueous extract was boiled in 50ml of 2M HCI solution for 30min under reflux. The dried crucible was reweighed and the difference in the weight was then the quantity of flavonoid content in the extract.

Total phenol and terpenoid content: Total phenol and terpenoid were determined according to the method of Edeoga, Okwu and Mbabie (2005). Five grammes of the extract was pipette into a 50ml flask, and then 10 ml of distilled water was added. 3 ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were added. The extracts were made up to mark and left to react for 30minutes for colour development. Total phenol content was measured at 505nm. Total terpenoid absorbances and prepared extract was determined in a spectrophotometer at 420nm.

Total saponin content: This was estimated using the method of Obadoni and Ochuko (2001). Two grammes of the aqueous extract was placed in 200ml of 20% ethanol. The mixture was filtered and the residue re-extracted with 200ml of 20% ethanol. The combined butanol extract was washed using 5% aqueous NaCL and evaporated to dryness to precipitate crude saponins which was weighed (Saponin content = weight of sample before extraction — loss in weight after extraction).

Data Analysis Techniques: This was carried out using statistical product for service solution (SPSS) version 23. The data obtained was presented as mean ± standard deviation. Results of three replicates were used.

Findings of the study

Table 1: Proximate composition of *Piper guineense, Curcuma longa and Zingiber officinale* aqueous extracts (%)

Sample	Protein	Ash	Crude fibre	Fat	Moisture	Carbohydrate
Black pepper Extract	3.17 ±0.12	2.76±0.06	2.96±0.05	2.93±0.06	72.92±0.08	15.26±0.01
Turmeric Extract	2.87±0.75	3.41±0.29	2.56±0.24	2.00±0.17	72.95±0.77	16.21±0.59
Ginger Extract	1.26±0.21	1.40±0.04	2.80±0.35	1.66±0.04	74.47±0.19	18.34±0.72

n=3, values are represented as mean ± standard deviation

Table 1 shows the proximate composition of black pepper *(Piper guineense),* turmeric *(Curcuma longa) and* ginger *(Zingiber officinale)* aqueous extracts. The protein content of the extracts ranged between 1.26 to 3.17%. Black pepper extract had the highest (3.17%) while had ginger extract had the lowest (1.26%) protein value. Ash content of the extracts varied between 1.40 - 3.41%. Turmeric extract recorded the highest (3.41%) while ginger extract had the lowest value (1.40%). The crude fiber content of the extracts ranged between 2.56 - 2.96%. Black pepper extract recorded the highest (2.96%) in concentration while turmeric extract had the lowest value (2.56%). Fat content of the extracts varied from 1.66 to 2.93%. Black pepper extract had the highest (2.93%) while ginger extract had the lowest (1.66%) fat value. The moisture content of the extracts ranged between 72.92 - 74.47%. Ginger extract had the highest (74.47%) value while black pepper extract had the least value (72.92%). Carbohydrate content of the extracts ranged between 15.26-18.34%. Ginger extract had the highest score (18.34%) while black pepper extracts had the lowest (15.26%) value.

Table 2: Phytochemical composition of *Piper guineense, Curcuma longa and Zingiber officinale* aqueous extracts (mg/100g)

Samples	Alkaloids	Flavonoids	Phenols	Saponins	Trepenoid
Black pepper Extract	3.70±0.54	2.80±0.55	3.27±2.19	1.11±0.31	3.43±0.07
Turmeric Extract	2.00±0.40	4.63±0.61	6.67±0.67	1.73±0.59	4.04±0.07
Ginger Extract	2.32±0.53	3.33±0.25	6.10±0.30	2.01±0.40	3.64±0.59

n=3, values are represented as mean ± standard deviation

Table 2 presents the phytochemical composition of the aqueous extracts. Alkaloids content of the aqueous extract ranged between 2.00mg/100g and 3.70mg/100g. Black pepper extract had the highest (3.70mg/100g) followed by ginger extract (2.32mg/100g) and turmeric extract which had the lowest value (2.00mg/100g). Flavonoid contents of black pepper, turmeric and ginger extracts were 2.80mg/100g, 4.63mg/100g, 3.33mg/100g, respectively. Phenols content of the aqueous extract ranged between 3.27mg/100g and 6.67mg/100g and turmeric extract recorded the highest (6.67mg/100g), followed by ginger extract (6.10mg/100g) and black pepper extract which had the lowest (3.27mg/100g) value. Saponin content of the extracts ranged between 1.11mg/100g to 2.01mg/100g. Ginger extract had the highest (2.01mg/100g) in saponins content while black pepper extract had the lowest value (1.11mg/100g). The terpenoid content of the aqueous extracts ranged between 4.04mg/100g and 3.43mg/100g. Turmeric extract had the highest value (4.04mg/100g) while black pepper extract recorded the lowest value (3.43mg/100g).

Discussion of Findings

The crude protein content of the aqueous extracts in the study were higher (1.26 to 3.17%) than the crude protein reported by Okonkwo and Ogu (2014) with value range 5.86 -11.50% where black pepper extract recorded 5.86%. Wang et al. (2020) stated that the discrepancy in protein content could be attributed to genetic variation, climatic or regional soil differences. Protein in these aqueous extracts could be good sources of nutrition for body building and immune boosters and can help in cell division as well as growth (Okeke & Elekwa, 2006). Protein is vital for various body functions such as body development, maintenance of fluid balance, formation of hormones and enzymes (Ahaotu & Lawal, 2019).

The moisture content of the aqueous extracts of black pepper, turmeric and ginger were within the range (72.9% – 74.47%). This is in line with moisture content reported by Ahaotu and Lawal (2019) with value range (72.00 -76.02%) where turmeric aqueous extracts recorded 76.02%. Moisture or water is a universal solvent which dissolves other substances, carries nutrients and other materials throughout the body, thus, making it possible for every organ to perform its function effectively (Wang et al., 2020). However, in terms of natural product stability and shelve life, high moisture content tends to promote the growth of microorganism, contamination and chemical degradation (Wang et al., 2020).

Ash content of the aqueous extracts ranged from 1.40-3.41%. This is lower when compared with the findings by Imo et al.(2018) which reported higher values (6.51 -7.03%) on ash content where black pepper extracts recorded 6.51%. Ash content in food samples are a reflection of the mineral contents. Minerals are inorganic- substances which are essential in human nutrition for metabolism and overall chemical processes in the body. Studies have shown that dietary ash plays an important role in regulating blood sugar as well as maintaining acid-alkaline balance (Da Silveira Dos Santos et al., 2014; De La Torre, Gassara, Kouassi, Kaur & Belkacemi, 2015).

The crude fiber contents of the extracts ranged from 2.56 - 2.96%. The findings does not agree with the findings by Uzomba, Amaralam and Obinna (2019) that reported higher crude fiber content with value range (15.23 -.15.50%) where turmeric aqueous extract had 15.23%. Crude fiber could serve as good dietary fiber sources. Several studies been reported that fiber facilitates absorption of trace elements in the gut, can lower risk of coronary disease, hypertension, constipation, diabetes, colon and breast cancer (Alissa & Ferns, 2012; Gropper, Smith & Carr, 2018). Moreso, soluble fiber has beneficial effects on glycemic and lipid metabolism, prevents constipation, appetite satisfaction and the movement of food through the digestive system.

Fat content of the extracts ranged between 3.78% - 5.80%. The result varies with the findings by Uzomba, Amaralam and Obinna (2019) who reported higher fat content with value range (16.00 - 16.89%) where black pepper extracts recorded 16.00%. The variation might be as a result of specie differences, climatic condition and processing methods used (Yesenia et al., 2021). However, dietary fats are important because of their high energy value and can help in absorption of the fat-soluble vitamins (Vitamin A, E, D and K). Essential fatty acids contained in the fat of natural plant foods can help to improve heart rhythm, brain and cognitive development, overall cell function and protect the body’s organs. blood pressure and play useful role in the synthesis and repair of vital cell parts (Duyff, 2017).

The carbohydrate content of the aqueous extracts ranged between 15.26 - 18.34%. Carbohydrate values of the aqueous extracts were slightly similar with works by Abdulsalam et al. (2017)

with carbohydrate value range (16.23 to 16.37%) where ginger extract recorded 16.23.00% and turmeric extract, 16.37%. Carbohydrate in the diet can provide the body with energy for daily activities and is vital for regulation of nerve tissues. Ahaotu and Lawal (2019) reported that a low carbohydrate diet could reduce the risk of cardiovascular diseases and control diabetes among obese patients.

The findings on phytochemical composition of extracts showed that alkaloids content of extracts are moderate and ranged from 1.32mg/100g to 2.70mg/100g. Moderate levels of alkaloids are powerful pain relievers and are known as stimulants of the central nervous system. (Okoye & Ebeledike, 2013). Alkaloids are powerful group of chemicals that exhibit chemoprevention and chemotherapeutic effects. Zhu et al. (2020) stated that high level of alkaloids exerts toxicity and adverse effects to humans especially in physiological and neurological activities.

Alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents (Heinrich, Mah & Amirkia, 2021). Studies have shown that alkaloids may help to increase the antioxidant capacity of serum or plasma (Zang et al., 2015; Heinrich, Mah & Amirkia, 2021). Numerous studies have reported the analgesic, antispasmodic and antibacterial properties of alkaloids (Okoye & Ebeledike, 2013; Rajendran, Nandakumar & Rengarajan, 2014; De La Torre, Gassara, Kouassi, Kaur & Belkacemi, 2015).

The presence of flavonoid in the aqueous extracts supports its use in the treatment and management of diabetes since flavonoids act as antioxidants in biological systems (Nimse & Pal, 2015). Flavonoids are natural coloring in plant based foods with antioxidant, anti-inflammatory and diuretic effects. Studies have shown that these flavonoids in plant based foods facilitates the action of Vitamin C. The presence of these flavonoids in these extracts can serve as powerful antioxidants which prevent premature aging and can boost immune health (Nimse & Pal, 2015).

Numerous epidemiological, preclinical, and clinical studies have shown that spices and their extracts are excellent sources of antioxidants with their high content of phenolic compounds which are therapeutically useful in the management of diseases (Rajendran, Nandakumar & Rengarajan, 2014); De La Torre, Gassara, Kouassi, Kaur & Belkacemi, 2015). It slows oxidative degradation of fats and oils thereby improve the healthy nutritional value and the quality of food (Duyff, 2017). Spices are associated with skin protection, brain function, blood sugar and blood pressure regulation, in addition to antioxidant and anti-inflammatory activities (Greger, 2015).

The aqueous extracts of these plants contained saponin. The concentration of saponin in these extracts varied from 1.11 to 2.01 mg/100g. Saponins serve as natural antibiotics, which help body to fight infections and microbial invasions. They also enhance the effectiveness of certain vaccines, lower cholesterol and knock out some tumor cells, particularly lung and blood cancers (Sparg, Light & Staden, 2004). Saponin are toxic in high concentrations and may also affect nutrient absorption by inhibition of metabolic and digestive enzyme (Sparg, Light & Staden, 2004).

Conclusion

The present study has provided some information on the proximate and phytochemical composition of the aqueous extracts of *Curcuma longa, Zingiber officinale* and *Piper guineense*. Analysis of these medicinal plant extracts play a role in assessing their nutritional and bioactive significance. These aqueous extracts contain nutrients such as protein, fat, crude fiber, ash, carbohydrate and water. Moreso, they contain some phytochemicals such as alkaloids, flavonoids, saponin, terpenoid which have medicinal and therapeutic properties. It indicated these aqueous extracts may be used as sources of nutrients and bioactive molecules by humans in prevention and treatment of diseases. Moreso, in food industries for the production of health promoting foods.

Recommendations

Based on the findings of the study, it is recommendated that further studies be carried out on:

1. anti- nutritional factors of the aqueous extracts to determine their bioavailability potentials.
2. dose standardization of these aqueous extracts for ease utilization in prevention and treatment of disease.
3. free radical scavenging potentials of these aqueous extracts in order to exploit its antioxidant activity in the treatment of nutrition related diseases.
4. anti-microbial assay of these aqueous extracts in order to evaluate their protective potentials.

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