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Determination of Nutrient and Phytochemical Profile of Basil (*Ocimum gratissimum*) Leaves and Seeds

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Abstract

The sustainable use of plant parts such as basil (*Ocimum gratissimum)* leaves and seeds is beneficial as they are believed to possess various nutritional and therapeutic values. The study investigated the nutrient and phytochemical profile of basil leaves and seeds. Specifically, the study analysed the proximate composition (moisture, crude protein, fat, ash, crude fibre and carbohydrate); vitamin content (pro-vitamin A, vitamin C and vitamin E) and phytochemical content (flavonoid and phenol) of basil leaves and seeds. Samples were processed into flour using different methods. The proximate, vitamin and phytochemical compositions of the samples were analysed in triplicates using standard methods. All data were presented as means ± standard deviations. Proximate analysis results showed that the protein content of basil seeds ranged from 14.90 - 14.93%. The Ash content of the samples varies from 4.28 - 10.45%. The concentration of crude fibre in basil leaves and seeds ranged between 14.20 - 15.77%. The vitamin analysis result showed that the pro-vitamin A content of the samples ranged from 11.28- 170.70µg/100g. Analysis of phytochemical content showed that the value of flavonoids in the samples varied from 16.67 -22.78mg/100g. The phenol content of the samples ranged between 21.71 and 23.74mg/100g. Results showed that basil leaves and seeds contain significant quantities of nutrients and phytochemicals. The findings of the study indicated that basil leaves and seeds could be used as raw materials in food and pharmaceutical industries to manufacture spices, beverages and nutraceuticals.

Keywords: Basil, Leaves, Seeds, Proximate, Vitamins, Phytochemicals

Introduction

Medicinal plants possess different parts with varying concentrations of nutrients and phytochemicals. These nutritional and medicinal properties can come from many different parts of a plant including stems, leaves, roots, seeds, fruits, and flowers (Rabizadeh et al., 2022). Nutrients are very important to life since they provide energy, function as building blocks and are necessary to regulate chemical processes. Macronutrients and micronutrients are essential dietary elements required by humans in varying quantities throughout life to perform a range of physiological functions (Gibney et al., 2009).

Phytochemicals are nonnutritive, bioactive chemical compounds found in plant foods, which are considered to be beneficial to human health. They include alkaloids, flavonoids, phenol glycosides and others (Rabizadeh et al., 2022). These bioactive compounds of plants exhibit multiple therapeutic activities including anti-microbial, anti-oxidative, anti-inflammatory, and anti-cancer activities (Ranjha et al., 2020).

Globally, the incidence of non-communicable diseases and their associated pathological complication is growing rapidly (World Health Organisation, WHO, 2022). Non-communicable diseases are a diverse group of chronic diseases that are not transmissible from one person to another but are typically caused by unhealthy behaviours (WHO, 2017). Examples of these non-communicable diseases are cardiovascular diseases, diabetes, cancers, hypertension, chronic respiratory diseases and others. WHO (2022) reported that these chronic diseases kill 41 million people each year, equivalent to 74% of all deaths globally. Non-communicable diseases have been projected to be a leading cause of morbidity and mortality in Nigeria by 2030 (WHO, 2017).

An unhealthy diet is one of the major causes of these chronic diseases which contributes to oxidative stress and excessive free radical production (Halliwell & Gutteridge, 2007; Amit & Priyadarsini, 2011). It has been identified that a lack of essential nutrients can affect proper growth and cognitive development (Allen et al., 2006). An unhealthy diet, typically high in sugar, saturated fat, and salt and low in vegetables and fruits, can contribute to the development of non-communicable diseases (NCDs) (Francesco et al., 2019). This can often lead to diet-related non-communicable diseases such as diabetes. The consequences can be devastating, these include irreversible impairment to physical and cognitive development, severe visual impairment or blindness, xeropthalmia, convulsion, neurological disorders, pellagra, beriberi, rickets, impaired cognitive function, anaemia, morbidity, mortality and substantial losses in individual and national productivity (Allen et al., 2006; WHO, 2014; Francesco et al., 2019).

Nutrition therapy is important in preventing, and managing existing diseases and slowing the rate of development of disease complications of these chronic diseases (American Diabetic Association, (ADA), 2013). A healthy diet can reduce the risk of cardiovascular diseases, cancer, diabetes and other conditions, Utilization of these medicinal plants is important to prevent the widespread and minimize the occurrence of diet-related non-communicable diseases in adults. Research has demonstrated that nutrition plays a crucial role in the prevention of chronic disease (Akinmoladun et al., 2007). Plant components such as leaves and seeds are considered to have therapeutic potential for the management of chronic diseases such as diabetes mellitus, hyperlipidemia, or cancer (Akinmoladun et al., 2007; Johnson & Melissa. 2015). Healthy foods can promote health, improve general well-being, and reduce the risk of developing certain illnesses (WHO, (2002).

Ocimum gratissimum is a widely known and commonly consumed medicinal plant. The plant belongs to the Genus of *Ocimum Lamiaceae* (basil) and is a species of *Ocimum gratissimum*. is a scented shrub with lime-green leaves (United States Department of Agriculture, 2008). It is the most abundant of the genus *Ocimum* and its common names are clove Basil, Scent leaf, Fever plant or Tea bush. *Ocimum gratissimum* plant is a perennial plant that is widely distributed in the tropics of Africa and Asia (Cohen, 2014). It is woody at the base and has an average height of 1-3 meters. *Ocimum basilicum* is an annual plant found widely in the tropical, subtropical and temperate regions of the world (Cohen, 2014).

The leaves are edible portions of plants that are consumed as food by humans. It is popularly known as a scent leaf. The leaves are broad and narrowly ovate, usually 5-13cm long and 3-9cm wide. Basil seeds are dark brown in colour and with round shapes. The leaves can be used fresh or dried to add a distinctive flavour and aroma to foods. They are highly appreciated for their pleasant aroma and are used as seasoning, and medicine and are eaten as a vegetable (Osuagwu et al., 2010). Basil leaves and seeds are good sources of essential nutrients such as carbohydrates, protein, fat, vitamins and minerals, and excellent sources of phytochemical and dietary fibre (Jimoh & Oladiji, 2005; Zahran et al., 2020). They possess nutritional and therapeutic potentials that can enhance immunity. They have been reported to have anti-carcinogenic effects, anti-inflammatory, hypoglycemic and antioxidant potential (Hamid et al., 2010; Egata, 2021).

Studies have shown that basil seeds can be used in the manufacture of beverages, liqueurs, vinegar, drinks, and teas, and are used in the food, pharmaceutical, and cosmetic industries (Hosseini-Parvar et al., 2010; Hajmohammadi et al.,2016; Naji-Tabasi & Razavi, 2017). The seeds are commonly added to bakery products as a source of dietary fibre for technological purposes (Hajmohammadi et al., 2016; Rezapour et al ., 2016). The mucilage extracted from basil seeds has been widely studied and has good functional properties such as emulsifying, foaming, thickening, stabilizing, viscosity, and gelling properties (Nazir et al., 2017; Bravo et al., 2021). Seeds of basil have been used as traditional medicine for the treatment of dyspepsia, ulcers, diarrhoea and other illnesses. Studies have shown that leaves and seed extracts of *ocimum gratissimum* exhibit many nutritional and medicinal values (Shuaib et al., 2015; Keith, 2018; Bravo et al.,2021). Basil seeds contain some powerful antioxidant vitamins and minerals which have diuretic, antipyretic, antispasmodic, analgesic hepatoprotective and anti-inflammatory effects (Uhegbu, et al., 2012; Zahran et al., 2020).

Basil leaves and seeds are considered to be underutilized since they can offer alternative and cheaper sources of macro and micronutrients as well as antioxidant potential. They are easily cultivated and grown in Nigeria. Good management of these natural resources is very crucial in the family to achieve a healthier lifestyle since synthetic drugs have several side effects besides many benefits they offer. Analysis of these edible medicinal plant parts can play a crucial role in assessing their nutritional significance (Pandey et al., 2006). This study therefore aimed to provide information on the nutrient and phytochemical compositions of the leaves and seeds of basil. This will help to justify and validate its usage and suitability as a medicinal plant with various antioxidant properties.

Objectives of the Study

The general objective of the study was to investigate the nutrient and phytochemical profile of Basil (*Ocimum gratissimum)* leaves and seeds. Specifically, the study determined:

1. the proximate composition (moisture, protein, fat, ash, crude fibre and carbohydrate) of *Ocimum gratissimum* leaves and seeds;
2. the vitamin composition (pro-vitamins A, vitamin C and vitamin E) of *Ocimum gratissimum* leaves and seeds
3. the phytochemical content (flavonoid and phenol) of O*cimum gratissimum* leaves and seeds.

Materials and Methods

***Design of the Study*:** The study adopted an experimental research design. The proximate, vitamins and phytochemical content analysis were conducted in the food and analytical laboratory of the Nutrition and Dietetics/Home Science and Management Department, University of Nigeria, Nsukka.

***Procurement of Materials*:** Two hundred (400g) of Basil (*Ocimum gratissimum*) leaves and seeds were procured from Home Garden, Edem Ani Nsukka Enugu State, Nigeria. O*cimum gratissimum* leaves and seeds were identified at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

***Preparation of samples for chemical analysis*:** The leaves (400g) were separated from the stems, sorted to remove defects, washed twice with distilled water to remove dirt and sand, drained in a plastic sieve, cut into smaller sizes, dried in an oven at a temperature of 40 °C for 4 hours and ground into flour using a blender (Soyona Japan).

The seeds (400g) were sorted by separating them from the stalk, stems and leaves, washed, drained and were oven- dried at 40°C for 4 hours, ground into flour using a blender (Soyona Japan). The ground samples (both the leaves and the seeds) obtained were packaged in a plastic container air-tight container, labelled and stored at room temperature for laboratory analysis.

***Chemical Analysis*:** This involved proximate, vitamin and phytochemical analysis of the two samples.

***Proximate analysis*:** The proximate analysis of the Ocimum *gratissimum* leaves and seeds for moisture, ash, fat, crude protein and carbohydrate were determined as described by the Association of Official Analytical Chemists, AOAC (2010).

Moisture content: Two grams of the samples were placed in each of the aluminum dishes. These in turn were transferred to a thermostatically controlled oven at a temperature of 105^{O}C and normal atmospheric pressure. Drying was continued until a constant mass was obtained.

Amount of moisture= initial mass (mass of empty container + wet sample) -- final mass (mass of empty cup + dry sample)

Fat content: The fat content of the samples was determined using the Soxhlet extraction method. The defatted samples were removed and the solvent recovered. The flask and the content were reweighed. The flask was cooled in a desiccator and weighed.

***Ash content*:** Two grams of the samples were weighed into a crucible which was previously ignited and cooled before weighing. The crucible and its contents were heated on a muffle furnace at a temperature of 55^{O}C then transferred into desiccators and cooled for 15-30 minutes. It was then weighed and the percentage of ash was calculated.

***Crude fibre content*:** Two grams of the sample were weighed into a crucible and placed in a hot 200ml of 1.25% tethraoxosulphate (H_2SO_4) and boiled for 30 minutes.

***Crude protein content*:** Twenty grams (20g) of the samples were weighed and carefully transferred to a Kjeldahl flask. Titration was against standard 0.1N(normal solution) of HCl. The difference in weight between oven-dry weight and weight after incineration was taken as the fibre content of the sample.

***Carbohydrate content*:** This was determined by the difference method; 100 – Total % content of other components. CHO=100- (%crude protein + %Ash + %crude fibre + %moisture +% fat)

Vitamin Determination

***Pro-vitamin A (β-Carotene)*:** The procedure of Jakutowicz et al. (1997) was used. One gram of the sample was weighed. Then the proteins were first precipitated with 3ml of absolute ethanol b/4 the extraction of vita with 5ml of heptane. The test tube containing this was shaken vigorously for 5 minutes. On standing, 3ml from the heptane layer was taken up in a cuvette and read at 450nm against a blank of heptane. The standard was prepared, read at 450nm and pro-vit. A was calculated.

Determination of Vitamin C & Vitamin E: The AOAC (2010) method was used. Five grammes (5g)of the sample were weighed into a flat-bottom flask and 60 ml TCA/Acetic acid solution was added. The mixture was left for about an hour before it was filtered. The filtrate was made up to 200ml. Ten millilitres 10ml was taken for titration with 0.05% 2, 6-dichlorophenol indophenols. The vitamin C content was calculated.

Vitamin E absorbance of the solution was then taken at 520nm against a blank

Phytochemical determination

Total flavonoid content: This was determined according to the method of Harborne (I973). Five grams of each of the samples was boiled in 50ml of 2M HCl solution for 30 minutes under reflux. The dried crucible was reweighed and the difference in the weight was then the quantity of flavonoid content in the sample.

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

Statistical Analysis

Data obtained in this study were analyzed using IBM SPSS Statistics version 22. Descriptive statistics such as means and standard deviation were used to present results.

Results

Proximate composition of leaves and seeds of *Ocimum gratissimum*

Table 1 presents the proximate composition of the leaves and seeds of *Ocimum gratissimum*. The protein content of the samples ranged between 14.90% to 14.93% where the fresh seeds were higher (14.93%) and the leaves (14.90%). The moisture content of the leaves was higher (10.66%) than the seeds (4.86%). The fat content of the seeds was higher (28.49%) than the leaves (5.63%). The Ash content of the samples ranged between 4.28 - 10.45% where the leaves recorded a higher value (10.45%) than the seeds (4.28%). The crude fibre content of the samples ranged between 14. 20 – 15.77%. The concentration of the leaves was higher (15.77%) than the seeds (14.20%). The carbohydrate value of the leaves was higher (42.57%) than the seeds (33.35%).

Table 1: Proximate composition of leaves and seeds of *Ocimum gratissimum*

Sample	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Crude fibre (%)	Carbohydrate (%)
Leaves	14.90±0.78	10.66.78±0.38	5.63±0.27	10.45±0.03	15.77±0.04	42.57±0.79
Seeds	14.93±0.70	4.86±1.21	28.49±0.79	4.28±0.76	14.20±0.28	33.35±0.52

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

Vitamin composition of the leaves and seeds of *Ocimum gratissimum*

Table 2 presents the vitamin composition of the leaves and seeds of *Ocimum gratissimum.* The pro-vitamin A content of the samples ranged between 11.28 to170.7 µg/100g. The leaves contained a higher concentration of pro-vitamin with a value of 170.7 µg/100g than the seeds (11.28mg/100g). Vitamin C content of the samples ranged between 11.15 to 12.01mg/100g where the seeds recorded 11.15mg/100g and the leaves (12.01mg/100g). Vitamin E content of the samples varied from 0.17 to 0.31g/100g where the seeds had a higher concentration (0.17mg/100g) than the leaves (0.31g/100g).

Table 2: **Vitamin composition of the leaves and seeds of *Ocimum gratissimum***

Samples	Pro-vitamin A (µg/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)
Leaves	170.7±2.67	12.01±0.04	0.17±0.05
Seeds	11.28±0.09	11.15±1.17	0.31±0.00

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

Mineral composition of leaves and seeds of *Ocimum gratissimum* (mg/100g)

Table 3 presents the phytochemical composition of the leaves and seeds of *Ocimum gratissimum*. It shows that the flavonoid content of the samples ranged between 16.67 to 22.79mg/100g where the value of the seeds was higher (22.79mg/100g) than the leaves (16.67 mg/100g). The phenol concentration of the seeds was higher (23. 74mg/100g) than the leaves (21. 7mg/100g).

Table 3: Mineral composition of leaves and seeds of *Ocimum gratissimum*(mg/100g)

Samples	Flavonoid	Phenol
leaves	16.67±0.23	21.71±0.01
Seed	22.79±0.13	23.74±0.01

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

Discussion

The crude protein content of the leaves and seeds of the basil plant in the study ranged between 14.90 to 14.93%. This is not in line with works by Bravo et al. (2021) where the crude protein of basil leaves and seeds ranged between 11.4–22.5%. The differences in crude protein content could be attributed to genetic variation and climatic or regional soil differences. Proteins in these samples could act as enzymes, hormones and antibodies that are involved in numerous metabolic processes in the body. Dietary proteins are responsible for bodybuilding, formation of bones, teeth, hair and the outer layer of skin, maintaining the structure of blood vessels and other tissue, and can help in cell division as well as growth (Mgbeje et al., 2019).

The moisture content of the samples was within the range (4.86 –10.66%). The findings are not in agreement with the moisture content reported by Olumide et al. (2019) with a value range of 13.50 -14.50%. The moisture content of food is an index of shelf life stability and quality (Shahzadi et al., 2005). Moisture or water is a universal solvent which dissolves other substances and carries nutrients and other materials throughout the body, thus, making it possible for every organ to perform its function effectively (Reichardt & Timm, 2020).

Ash content of the samples varied between 4.28% and 10.45%. This is higher when compared with the findings by Ishola et al. (2017) who reported a lower value range (4.54 - 4.38%). Ash content in food samples is 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 (Chinedu et al ., 2018).

The crude fibre contents of the leaf and seed of *Ocimum gratissimum* ranged from 14.20 – 15.77%. The findings do not agree with the findings by Bravo et al. (2021) who reported crude fibre content with a value range of 7.11 to 26.2 %. Crude fibre could serve as a good dietary fibre source. Several studies have reported that fibre facilitates the absorption of trace elements in the gut, and can lower the risk of coronary disease, hypertension, constipation, diabetes, and colon and breast cancer (Adepoju et al., 2012; Graf et al., 2015). Fibre diets are an important component of diabetes management, resulting in improvements in measures of glycaemia control, blood lipids,

The fat content of the samples ranged between 5.63% - 28.49%. The result varies with the findings by Ishola et al. (2017) who reported lower fat content with value range (6.34 - 6.5%). This difference in composition could have been a result of differences in the nutrients of the soil, the age of the plant at harvest, environmental factors, method of cultivation, seasonal variation and time of harvesting. 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 can help in cognitive development, and overall cell function and protect the body's organs. blood pressure and play a useful role in the synthesis and repair of vital cell parts (Duyff, 2017).

The carbohydrate content of the leaves and seeds of *Ocimum gratissimum* ranged between 33.35 - 42.57%. Carbohydrate in human diets is a key source of energy (Keim et al., 2014). 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 of the study in Table 2 showed the antioxidant vitamin composition of the samples. The pro-vitamin A content of the samples ranged between 11. 28μg/100g to 170. 28 μg/100g. Pro vitamin A in the diet can act as antioxidants which are potential scavengers of free radicals in the body and can protect the body against ill health and ravages of aging (Granato et al., 2018). They are essential for gene expression growth, cell differentiation and integrity of epithelial tissues, reproduction immune system function, contribute to healthy skin colour, support normal vision, protect human lymphocytes from damage by singlet oxygen and lower the risk for several degenerative disorders, including various types of cancer, cardiovascular or ophthalmological diseases (Gibney et al., 2009).

Antioxidant Vitamin C in the samples ranged from 20.62mg/100g to 27.73mg/100g. Vitamin C are a powerful antioxidant which has positive effects in the treatment of numerous chronic diseases such as cancer, diabetes, and cardiovascular diseases (Mgbeje et al., 2019). Studies have shown that vitamin C in the basil plant can scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation, promoting the growth and repair of tissues, and essential for the synthesis of collagen and carnitine (Keith, 2018; Egata, 2021).

Vitamin E in the diet can act as an antioxidant which can inhibit, and prevent oxidation in fats or lipids and can help protect body tissue from damage caused by free radicals which can harm cells, tissues, and organs (Traber & Atkinson, 2007). Studies have reported that this fat-soluble vitamin in the diet can break the free radical chain reaction, help prevent cellular damage in the liver, protect enzymes and hormones, as well as the polyunsaturated fatty acids of biological membranes and low-density lipoprotein against oxidation by oxygen radicals (Awah & Verla, 2010; Traber & Atkinson, 2007; 2016; Zahran et al., 2020).

The findings of the study in table 3 showed that the flavonoid content of the samples ranged between 16.67 to 22.79mg/100g. This is not in line with works by Tarayrah et al. (2022) with a value range (of 9.0 to 34.2mg/100g). The presence of flavonoids in the samples supports their use in the treatment and management of diabetes since flavonoids act as antioxidants in biological systems (Nimse & Pal, 2015). Flavonoids are an important part of the human diet that can influence the quality and stability of foods by acting as flavourings, and colourants with antioxidants, anti- inflammatory and anti-diuretic effects (Takemoto & Takemoto, 2018). Many studies have suggested that flavonoids exhibit biological activities including antiallergenic, antiviral, anti-inflammatory and vasodilating actions((Takemoto & Takemoto, 2018; Tarayrah et al., 2022).

Phenol content of the samples was lower (21.71 to 23.74mg/100g) when compared with works byTarayrah et al. (2022) with value range (51.0 to 58.2mg/100g) where the leaves recorded 51.0mg/100mg and seeds (58.20 mg/100g). Numerous studies have shown that basil seeds and leaves are excellent sources of antioxidants with their high content of phenolic compounds which are therapeutically useful in the management of diseases(Uhegbu et al., 2012; Rajendran et al., 2014; Omodamiro & Jimoh, 2015; Njoku et al., 2011). They are associated with skin protection, brain function, blood sugar and blood pressure regulation, in addition to antioxidant and anti-inflammatory activity (Hamid et al., 2010; Zahran et al., 2020; Bravo et al., 2021).

Conclusion

Nutrient and phytochemical analysis of plants is very important in assessing their nutritional significance. It shows that the leaves and seeds of *Ocimum gratissimum* could be good sources of nutrients, antioxidants vitamins and phytochemicals that are very important in the prevention and maintenance of nutrition-related diseases. In conclusion, it indicates that basil seeds and leaves are natural sources of nutrients, antioxidants and phytochemicals that can prevent and manage many nutrition-related diseases.

Recommendations

The following recommendations were made based on the findings of this study:

1. further studies should be carried out on the anti-nutritional composition of the seeds and leaves of *Ocimum gratissimum* to help the general public know their safe limits or levels.
2. home makers should be encouraged to incorporate *Ocimum gratissimum* seeds and leave flour into recipes such as cakes, drinks, biscuits and food preparations to prevent nutrient deficiencies.
3. nutrition education programmes should be mounted in communities on the utilization of basil leaves and seeds in the prevention and management of nutrition-related diseases.

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