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COMPARATIVE ASSESSMENT OF PHYTOCHEMICAL AND PROXIMATE COMPOSITION OF OCIMUM BASILICUM AND OCIMUM GRATISSIMUM LEAVES EXTRACTED WITH ETHANOL AND AQUEOUS SOLVENTS

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ABSTRACT
The purpose of this study was to investigate the extraction, proximate analysis, and total phenolic and flavonoid content of the plants *Ocimum gratissimum* and *Ocimum basilicum* that are used in Kaduna State's Kaduna North Local Government region. Quercetin was employed as a reference, and the absorbance was measured at 510 nm. The Folin-Ciocalteau assay was used to assess the total phenolic content, and the Aluminum chloride colorimetric assay was used to measure the total flavonoids spectrophotometrically. Protein, carbohydrate, and fat were the subjects of the proximate analysis. The ethanolic extract of *Ocimum basilicum* had the highest TPC value (18.942), while the aqueous extract of *Ocimum gratissimum* had the lowest TPC value (2.58). Similarly, the aqueous extracts of *Ocimum gratissimum* had the highest TFC value (111.873), while the ethanolic extract had the lowest, both of which are highly significant at $p<0.05$. The high concentration of this phytochemical suggests that the leaves may contain bioactive compounds with potent biological activity that might be isolated, as well as significant compounds.

Keywords: *Ocimum basilicum, Ocimum gratissimum,* proximate and phytochemical.

INTRODUCTION

The *O. basilicum* plant is utilized as food, medicine, and spice in Nigeria. Numerous compounds found in plants used in traditional folk medicine have both nutritional and therapeutic value (Edeoga and Eriate *et al.,* 2001; Edeoga et al., 2003; Osuagwu & Nwosu, 2006; Osuagwu, 2008; Osuagwu *et al*., 2010). According to Okwo (1993) and Sofowora (1993), plants possess bioactive compounds or metabolites that exert physiological effects against bacteria and other microorganisms and are also employed as precursors for the production of effective pharmaceuticals.
With over 150 species, the genus *Ocimum* is widely distributed in temperate climates worldwide. Pandey and colleagues (2014). Antiemetic properties of *O. basilicum* and *O. gratissimum* have been documented (De Albu Guergue *et al*., 2007; Ahmed *et al*., 2014). Intensive work on the varietal diversity of o basilicum has shown that most cultivars are distinct in their characteristic aroma and coloration (Spence, 2024). Because they contain a variety of bioactive components, *O. basilicum* and *O. gratissimum* are frequently utilized as therapeutic agents in both traditional and modern medicine (Naquvi *et al*., 2012). Numerous studies have been conducted, but none have examined the plant's proximate and phytochemical characteristics in my area. This is because environmental conditions do affect plants, which is why this research was necessary.

MATERIALS AND METHODS

Collection, preparation, and authentication of plant material

The plants were collected from the Kaduna North region and brought to the botanical laboratory in the Department of Biological Sciences at Kaduna State University. There, they were recognized and verified using the voucher number 9012 for *Ocimum basilicum*. Using a mortar and pestle, plant leaf samples were collected, dried in the shade at room temperature, finely ground, sieved, and stored in a plastic container.

Sample extraction

O. gratissimum and *O. basilicum* samples weighing 50 g/200 mL and 50 g/120 mL for ethanol and 50 g/350 mL and 50 g/200 mL for aqueous (water) were stored for 48 hours before being extracted using the percolation method. What Man filter paper No. 1 was used to filter the mixture. To obtain a crude extract expressed as a percentage of the initial sample weight, the filtrate was dried in a small beaker under reduced pressure. The beaker was then covered with foil paper and kept in the dark.
Percentage % yield of extract = Weight of Extract (g)/Weight of Sample (g) x 100

Quantitative phytochemical analysis

Using established techniques, the leaves were quantitatively examined for the existence of flavonoid and phenolic content.

Determination of phenolic content

The Folín-Ciocalteu method was used to determine the total phenolic content of Ocimum leaves. After precisely weighing 20 mg of the sample, it was dissolved in 10 mL of 50% methanol. Using the same methanol, the volume was increased to 20 mL to reduce the solution's concentration to 1 mg/mL. Place 20 ul of the sample in a 96-well plate, add 150 ul of the Folin-Ciocalteu reagent, and let it stand at room temperature for three to five minutes.
An absorbance reading at 765 nm is obtained when 80 µL of a saturated sodium carbonate solution (7.5% w/v) is covered with silver foil and left at room temperature for an hour. During this time, CO_2 is released by periodic shaking. The calibration curve was made using gallic acid (5–50 ug/ml). Gallic acid was produced in methanol at varying concentrations (0.2 to 0.7 mg/ml), and the result was represented as milligrams of Gallic acid equivalent (GAE) per gram (g) of material. Every sample was examined three times. (Alhakmani and colleagues 2013).

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Determination of Total flavonoid content

The aluminum chloride colorimeter method was used to determine the total flavonoid content of Ocimum leaf extracts. Before the experiment started, a stock solution containing 1 mg/mL quercetin was prepared. (combined with 0.83–0.33 mg/ml quercetin standard solution). Twenty milligrams of the sample were precisely weighed and dissolved. 20 ul of extracts were combined. Each 96-well plate received 15 µL of sodium nitrite (5% $NaNO_2$) (w/v) and was allowed to sit at room temperature for 6 minutes. After adding 15 ul of 10%w/v $AlCl_3$ to the well and waiting another 6 minutes, each sample received 80 ul of sodium hydroxide (4% NaOH) and was incubated for another 15 minutes. The absorbance at 510 nm was measured following incubation.

In milligrams of quercetin equivalent per gram of material, TFC was calculated. Using a standard curve created with a standard quercetin solution, the result was represented as milligrams of quercetin equivalent per gram of material. Three separate analyses were performed on each sample (Zhichen and colleagues, 1999).

Proximate Analysis

Protein, carbohydrate, and fat levels were determined by proximate analysis using the technique described by the Association of Official Analytical Chemists (AOAC) in 1990 (Ceirwyn, 1995).

Carbohydrate analysis

The Anthrone test is used to estimate the total amount of carbohydrates in the sample quantitatively. After 65 mg of the material was deproteinized using cold acetone, 10 volumes of 0.75% Anthrone in 84% w/w sulfuric acid were added, and the mixture was heated for 10 minutes at 100°C. For the evaluation, absorbance was measured at 540 nm. Three duplicates of each value were recorded.

Determination of fat

A boiling tube was filled with 0.1g of the sample. After adding 10 milliliters of concentrated HCl, the liquid was placed in a boiling water bath until the solids dissolved and the solution turned brown. After cooling, it was removed and put into a separating funnel. 5 mL of pet ether and 10 mL of ethanol were added, and the mixture was agitated to dissolve. After that, it was left to stand for a few minutes in order to separate. The ether layer was transferred into a clean, dry conical flask (w1) after being weighed. A water bath was used to evaporate the extracts. (W2) - (W1) / 0.1 x 100 was used to calculate the fat weight after it had been dried at 105°C in an oven, chilled, and weighed. Values were noted in triplicate.

Determination of protein

The Biuret reagent test, briefly explained, was used to determine protein content. 500mL of 0.2 Mol/liter sodium hydroxide is used to dissolve 3g of copper sulphate ($CuSO_4.5H_2O$) and 9g of sodium potassium tartarate. 5g of potassium iodide is then added, and the volume is increased to 1liter using 0.2 Mol/liter sodium hydroxide. Pipette 1 mL of the provided sample into a different test tube after pipetting 0.0, 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard into the series of labelled test tubes. Fill each test tube with 1 milliliter of liquid. The blank is a tube containing one milliliter of distilled water. Next, 3 mL of the biuret reagent was added to each test tube, including the blank and unknown. The tubes were shaken or vortexed to mix the contents, then heated to 37°C for 10 minutes. After cooling the material to room temperature, the absorbance at 540 nm was measured relative to a blank. Three copies of each value were recorded. The protein concentration (mg/mL) was calculated using the formula Y = 0.0425X – 0.002.

Statistical Analysis of Data

The mean and standard deviation of the collected data were used for statistical analysis in a Microsoft spreadsheet, including the Student's t-test.

RESULTS AND DISCUSSION

Phytochemical composition of crude extract of ethanol and aqueous extract of *Ocimum basilicum and Ocimum gratissimum* leaves.

By quantitatively screening the extracts of the two leaves, the phytochemical composition of the ethanolic and aqueous extracts of *O. basilicum* and *O. gratissimum* leaves was determined. The ethanolic extract had the highest value for the phenolic content, while the aqueous extract revealed the highest amount of flavonoid content in the two leaves, as shown.

Table 1: Displays the Phytochemical Analysis of the Extracts of *O. gratissimum* and *O. basilicum.*

The phytochemical components of the plants show total phenolic content values of 17.357% and 18.942%. *O. gratissimum* and *O. basilicum* aqueous extracts had respective values of 2.58% and 2.87%, with *O. basilicum* exhibiting the greatest value. These results are significant at $p<0.05$. The ethanolic extract of *O. gratissimum* had the lowest TFC, at 96.935%, which was highly significant at $p < 0.05$. In comparison, the aqueous extracts had the highest TFC, at 111.893%, which was significant at $p < 0.05$. *O.grarissimum* Ethanolic Extracts had the highest value of 43.33% for tannin, while the lowest was recorded in O.basilicum Ethanolic extracts with 9.73%. O. basilicum aqueous extract had the highest value for alkaloid, 13.14%. In comparison, *O. gratissimum* had the lowest value of 0.11% for saponins, O. gratissimum had the highest value of 0.27%, and the lowest was recorded in the aqueous extract, which had a negative value, indicating a minute presence in the leaf extracts. The present results are in contrast to those of Vlase *et al (2014), who discovered* that the ethanolic extract of *O. basilicum* contained 175.5 mg GAE/g of total phenolic content. However, another found that 80% aqueous methanol had a total phenolic content of 45.4 mg GAE/g.

Additionally, it was discovered that *O. basilicum* had a comparable total phenolic content of 96.4 mg GAE/g. The results of this investigation are supported by the observation made by Hamed et al. (2017) that the aqueous extract of *O. basilicum* contained 32.7 mg of catechol per g of total flavonoid content.

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Table 1: Quantitative phytochemical components of the extract from different solvents

Components	Phenolics (%)	Flavonoids (%)	Alkaloids (%)	Tannins (%)
Ethanol				
O. gratissimum	17.357 ± 0.107	96.9353 ± 0.095	0.38 ± 0.02	43.33 ± 0.26
O. basilicum	18.942 ± 0.184	109.5397 ± 0.130	0.20 ± 0.09	9.73 ± 4.23
Aqueous (Water)				
O. gratissimum	2.58 ± 0.027	111.873 ± 0.308	0.11 ± 0.05	12.83 ± 1.34
O. basilicum	2.867 ± 0.045	107.9147 ± 0.095	13.46 ± 0.08	21.50 ± 5.95

Proximate composition of crude extract of ethanol and aqueous extract of *Ocimum basilicum and Ocimum gratissimum* leaves.

The aqueous extract of *O. gratissimum* had a mean and standard deviation of 4.92% (4.92 ± 0.06), which was very significant at p<0.05, whereas the ethanolic extract had a higher protein value of 34% (34 ± 0.09). In contrast, the ethanolic extract of *O. basilicum* has a higher protein content (8.68%; 8.68 ± 0.03) than the aqueous extract. The ethanolic extract of the two leaves contains a significant amount of protein. The carbohydrate content of the ethanolic extract of *O. gratissimum* and *O. basilicum* is 7.23% (7.23 ± 0.02) and 5.76% (5.76 ± 0.01), respectively, higher than the aqueous extracts of *O. gratissimum* and *O. basilicum* (4.48% (4.48 ± 0.05) and 2.44% (2.44 ± 0.01), which are highly significant at p<0.05. This is comparable to the value of *O. gratissimum* 6.63% (Oluwole *et al*., 2019). The carbohydrate content was higher than the value of some leafy vegetables consumed in Nigeria, such as *Vernonia amagdalina* (8.65%), *O. gratissimum* (1.22%), and *Hibiscus sabdarifa* (15.79%), according to reports. The fat percentage shows that the ethanolic extract has higher fat values than the aqueous extracts of *O. gratissimum* and *O. basilicum*, which had fat percentages of 30% and 27.3%, respectively, compared to 8.33% and 10% for the aqueous extracts. These values are higher than the fat content of other Nigerian vegetables, which are 0.45% in *A. cruentus*, 0.32% in *C. olitorius*, and 0.21% in *A. argenta*, according to Onwordi *et al .,* (2019), while the seeds of basil contain 10% protein, 5.6% mineral, 33.0% fat, and 43% carbohydrates (Nazir *et al*., 2017). This shows that both the ethanolic and aqueous extractions are statistically significant, and that the ethanolic extract has more carbohydrates than the aqueous extract.

Table 2: Proximate composition of the leaves of *O. basilicum* and *O. gratissimum*

Sample	Protein (%)	Carbohydrate (%)	Fat (%)
Ethanol			
O. gratissimum	34.0 ± 0.09	7.23 ± 0.02	30.0
O. basilicum	79.3 ± 0.50	5.76 ± 0.01	27.3
Aqueous (Water)			
O. gratissimum	4.92 ± 0.06	4.84 ± 0.05	8.33
O. basilicum	8.68 ± 0.03	2.44 ± 0.01	10.0

Values are represented as mean and standard deviation, and fat is represented as a percentage.

Conclusion

Phenol and flavonoids, which are active components in the usage of the plants as medicines and spices, were detected in the phytochemical composition. The plant was a good source of nutrients due to its protein and carbohydrate content, and its fat content indicated the presence of vital oils. Statistically, there was no difference between the two leaves in any of the extracts examined from the phytochemical and proximate composition.

Recommendation

When it comes to phytochemical and proximate composition, O. basilicum and O. gratissimum have significant medicinal benefits; consequently, using these leaves in our meals will help shield the body from nutrient deficiencies.

Conflict of interest

There was no conflict of interest among the authors regarding the publication of this article.

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