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Comparative Evaluation of the Proximate, Phytochemical and Antioxidant Properties of Selected Leafy Vegetables (Bitter, Pumpkin and Spinach Leaves)

Mohammed Jibrin[1]*, Ruth Musa Micah[1] & Folashade Habibat Omotehinwa[2]

[1]Department of Chemistry, Nasarawa State University, Keffi, Nasarawa state, Nigeria
[2]Department of Chemistry, Federal University of Health Sciences, Otukpo, Benue state, Nigeria

Abstract

The study is aimed at comparative evaluation of the proximate, phytochemicals and antioxidant properties ofthree leafy vegetables (*V. amygdalina, Spinaciaoleracea* and *Telfairiaoccidentalis*). The study was carried out using standard analytical procedures. The proximate analysis revealed highest percent moisture (5.08±0.13%), crude protein (19.69±0.87%), carbohydrate (38.31± 1.42%) and metabolizable energy (244.24±3.82 KJ/100g) in pumpkin, highest percent ash (15.20 ± 0.86 %) and crude fat contents (8.30±0.18%) in spinach, while bitter leaf recorded the highest percent crude fibre (20.01 ± 1.20%). The phytochemical analysis revealed the presence of flavonoid, saponin, alkaloid, tannin and phenol which ranged from 8.67±0.11 mg/100 g to 18.98± 0.15 mg/100g, 0.9±0.00 mg/100g to 1.21± 0.10 mg/100g, 8.87±0.21 mg/100 g to 11.65±0.68 mg/100g, 0.81 ± 0.01 to 10.32± 1.2 mg/100g and 1.36 ±0.01 mg to 4.24±0.01 mg/100g, respectively. The antioxidant activities revealed highest percent inhibition (71.20%) in bitter leaf. However, based on the analysis of variance on the results obtained, the study revealed that there were significant differences in most of the analyzed parameters at 95% confidence level. Therefore, this study inferred that the nutritional and phytochemical contents of the leafy vegetables samples varied significantly and their consumption in a diet will be responsible for maintaining good health and proper functioning of the body system.

Keywords: Proximate, phytochemicals, antioxidant, leafy vegetables

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Corresponding author
M. Jibrin ✉
jibrilmohammed50@gmail.com

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Introduction

The nutritional and health benefits of eating green leafy vegetables have been demonstrated by several studies [1–3]. Thus, the nutrients (protein, minerals, fibers, etc) included in leafy vegetables can be absorbed by the body and employed as protective and regulating substances [2]. Additionally, they hold a significant position among other food crops since they give the body plenty of several different vitamins and minerals [3]. However, for the underprivileged portion of the population, green leafy vegetables are an affordable yet high-quality source of nourishment, particularly in areas where malnutrition is common [1, 4]. According to literature, leafy vegetables are great for calorie-conscious individuals who can satiate their hunger without consuming a lot of carbohydrates because they are low in calories and provide very little utility energy [5–6]. Furthermore, according to Udochukwu *et al.*, they contain phytochemicals which several of these phytochemicals are anti-nutrients that lower the bioavailability of vitamins and minerals. While certain types of these phytochemicals have been utilized in traditional medicine to treat a variety of ailments in people [7].

Moreover, in Nigeria, starchy foods like rice, yam, and cassava flour are often consumed with cooked green vegetables as a soup or seasoning [2]. Additionally, in order to add flavor, taste, color, and aesthetic appeal to diet, these dishes can be made with a combination of several vegetables [8]. However, information on the statistical comparative evaluation of the nutritive, phytochemicals and antioxidant properties of these leafy vegetables (bitter leaf, pumpkin and spinach) is still scanty. Therefore, this study is aimed at statisticalevaluation of the proximate, phytochemical and antioxidant properties of selected leafy vegetables (Bitter Leaf, Spinach and fluted pumpkin) commonly consumed in Nigeria.

Materials and Methods

Sample collection and preparation

Fresh samples of three different vegetable leaves namely; spinach (*Spinaciaoleracea*), and pumpkin (*Telfairiaoccidentalis*) and bitter (*V. amygdalina*) leaves were collected directly from different farms in Keffi LGA of Nasarawa State. They were thoroughly washed to remove soil particles and other impurities. After washing, the samples were air dried by spreading them on polythene bags for two weeks. Finally, the dried samples were ground into smaller sizes or powder using a mortar and pestle and stored in polythene bags for further analyses.

Determination of proximate compositions

Moisture, ash, crude fiber, crude protein (% N x 6.25), and ether extract (EE) were determined for proximate compositions using the standard procedures outlined by the Association of Official Analytical Chemists [9].

By using the difference, the total carbohydrate content was calculated. The calorific values (KJ/100g) were calculated by summing up (% proteins x 2.44) + (% carbohydrates x 3.57) + (% lipids x 8.37) for each sample.

Each parameter in the samples was calculated in triplicate, reported as in percentage, and its standard deviation was computed and displayed as indicated in the Tables. All the reagents used were of analytical grade.

Phytochemical screening

The phytochemical compositions of the leafy vegetable extracts were screened to identify and quantify for the presence of tannins, saponins, alkaloids, flavonoids and phenol, using standard analytical procedures reported by Chukwuma and Ejikeme [10] with slight modifications.

Antioxidant activity

The free-radical antioxidant activities of the samples were investigated using 2,2-diphenyl-1-picrylhydrozyl radical (DPPH) in methanol as adopted by Jibrin *et al.* [11] with slight modifications. Thus, 1 mL of 1 mM of DPPH in methanol was prepared under darkness condition and 2 mL of this solution was added to 1 mL of various concentrations (0.2 - 1.0 mg/ml) of the extracts. Vitamin C was used as a standard. The absorbance was taken after 30 minutes at 517 nm and Percentage DPPH radical scavenging activity was calculated by the following equation,

% inhibition = $[A_{control} - A_{extract}) / (A_{control})] \times 100$

Statistical analysis

Data were presented in mean and standard deviation and were also subjected to analysis of variance (ANOVA) for comparative analysis using R-statistical software (R-4.2.3).

Results and Discussion

Proximate composition

The result of the proximate compositions of the selected vegetables (bitter leaf, pumpkin and spinach) is presented in Table 1.

From the Table (1), the moisture contents of the samples fell within the range of 3.38 to 5.08%. Statistically, the values indicated that there was no significant difference between bitter leaf and spinach samples but there was with the pumpkin sample at 95% confidence level. According to literature, moisture content determines the shelf-life of any food substance, the higher the moisture content the shorter the shelf-life while the lower the moisture content the higher the shelf-life [12-13]. However, the values obtained for the moisture contents are low when compared with 6.97% reported for spinach [14], 43.18±0.59 reported for fluted pumpkin [6] and 18.47% reported for bitter leaf [5]. This implies that the studied samples would have longer shelf life than those reported in the literature. Also, the difference between the values of the reported samples and studied samples could be attributed to different factors such as operating conditions, samples treatment and possibly environmental factors.

From the study, the values of ash contents fell within the range of 11.10 to 15.20% which also indicated no significant difference between bitter leaf and spinach but showed significant difference with the fluted pumpkin at95%confidence level. However, with the exception of fluted pumpkin, the values of the ash contents were found to be in agreement with 16.65% reported for bitter leaf [1], 15.86% for *Vernoniacolorate* and 15.29% for *Moringaoleifera* [15]. Ash content is a reflection of the mineral contents preserved in the leafy vegetables [16]. This implies that more minerals are expected in the spinach and bitter leaf samples than the fluted pumpkin. According to literature, proteins are building block units and are needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA [3, 5, 7]. This study revealed that the protein contents of the leafy vegetables fell within the range of 13.13 to 19.69%. Based on the analysis of variance, the values were found to be significantly different from each other at95%confidence level. The values were also found to be higher than the protein contents reported for *Momordicafoecide* (4.6%) leaves and *Lesiantheraafricanas* (13.1%) [16]. This suggests that the studied samples are better sources of protein than those reported in the literature. This study also revealed 28.13% crude fiber in bitter leaf, 20.01% in spinach and 18.17% in fluted pumpkin. However, statistical analysis indicated that there was a significant difference among the samples at 95% confidence level. The values were also found to be slightly lower than 12.11 - 33.00% for the leafy vegetables (*Amaranthushybridus, Andasoniadigitata, Ceibapatendra, Hibiscus sabdariffa* and *Vignaunguiculata*) except for *Ceibapatendra* which is the least value (12.11%) reported by Asaolu *et al.* [14]. Investigations revealed that fiber cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Another findingalso revealed that a high dietary fiber consumption is linked to improved insulin sensitivity, which suggests it may help prevent and manage Type 2 diabetes [3, 8]. This suggests that the substantial amount of fiber obtained from the leafy vegetable samples could help in keeping the digestive system healthy and functioning properly when consumed in a diet. The crude fat values were found to be 5.70% in bitter leaf, 8.30% in spinach and 7.10 in pumpkin respectively. The result showed a significant different among the samples at 95% confidence level. The values were also found to be in agreement with the crude fat values obtained from *Talinumtriangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthushybridus* (4.80%), *Calchorusafricanum* (4.20%) [17]. Jibrin *et al.* [18] reported that a diet that provides 1–2% of a person's energy as fat is adequate for them because consuming too much fat has been linked to various cardiovascular disorders.

This suggests that the vegetables samples are good for consumption since they had low fat contents. The result showed that the carbohydrate values ranged from 29.16 to 37.80% which showed no significant difference between spinach and pumpkin leaves but showed a statistical difference with bitter leaf at 95% confidence level. These values were found to be very low when compared with 82.8% reported by Okon *et al.* [19] for sweet potatoes leaves but higher than 20% reported for *Sennaobtusfolia* [20]. Thus, high carbohydrate content contributes to the energy value of any given food substance. This suggests that the studied samples will contribute higher energy values than *Sennaobtusfolia* but lower than sweet potatoes leaves. The calorific values obtained from this study fell within the range of 196.05± 1.61 to 244.24±3.82 which showed no significant difference between spinach and pumpkin but showed a statistical difference withthe bitter leaf sample. However, the calorific values were found to very low when compared with 1337.7-1536.43 KJ/100g for velvet tamarind (*Dialiumguineense*) (seed, pulp and shell) reported by Jibrin *et al.* [18], 1352.70 ± 2.10 to 1594.80 ± 3.50 KJ/100 g for Baobab (pulp and seeds) reported by Hashim *et al.* [21] and 1801.05 ± 2.15 to 2139.58 ± 4.55 KJ/100 g for blood plum (pulp and seeds) reported by Aremu *et al.* [22]. This confirms the reported literature which describes leafy vegetables as low calorific values containing foods [5, 23]. This also suggests that comparatively, the studied samples cannot be considered as good sources of energy.

Table 1: Proximate compositions of bitter, pumpkin and spinach leaves samples

Parameters (%)	Bitter leaf	Spinach	Pumpkin
Moisture content	3.78± 0.10^{a}	3.38 ± 0.01^{a}	5.08± 0.13^{c}
Ash content	15.10± 1.30^{a}	15.20± 0.86^{a}	11.65± 1.41^{c}
Crude fiber	28.13 ± 1.67^{a}	20.01 ± 1.20^{b}	18.17± 1.10^{a}
Crude fat	5.70 ± 1.10^{a}	8.30± 018^{b}	7.10± 1.13^{c}
Crude protein	18.13 ± 1.01^{a}	15.31 ± 1.27^{b}	19.69± 0.87^{c}
Carbohydrate	29.16± 1.05^{a}	37.80± 0.56^{b}	38.31± 1.42^{b}
Energy	196.05± 1.61^{a}	241.80 ±0.86^{b}	244.24±3.82^{b}

Each value represents the mean ± standard deviation of triplicate determinations values within the same row with different superscripts are significantly different at 95% confidence level.

Table 2: Phytochemical analysis of bitter leaf, pumpkin and spinach

Phytochemicals (mg/100g)	Better leaf	Spinach	Pumpkin
Flavanoids	18.98 ± 0.15^{a}	8 .67 ± 0.11^{b}	11.84 ± 0.23^{c}
Phenols	4.24 ± 0.01^{a}	2.16 ± 0.02^{b}	1.36± 0.01^{c}
Alkaloids	11.32± 0.16^{a}	6.65± 0.68^{b}	9.87 ± 0.21^{c}
Saponnins	1.03 ±0.01^{a}	0.97 ± 0.00^{a}	1.21 ± 0.10^{a}
Tannins	10.32± 1.2^{a}	1.02 ± 0.12^{b}	0.81 ± 0.01^{b}

Each value represents the mean ± standard deviation of triplicate determinations; value within a row with different superscripts are significantly different at 95% confidence level.

Phytochemical screening

The phytochemical compositions of the selected leafy vegetables (bitter leaf, spinach and pumpkin) are shown in Table 2.

The result of the phytochemical analysis revealed the presence of flavonoids, tannins, phenols, saponins and alkaloids at different concentrations as shown in Table 2. However, this study agrees with most literatures on the presence of these phytochemicals at different concentrations in the studied samples [24-26]. The result also revealed that there was a significant difference in most of the phytochemicals presence among the samples. Thus, the concentrations of flavonoids ranged from 18.98 ± 0.15to 8 .67 ± 0.11 mg/100g which shows a significant difference among the samples at 95% confidence level with bitter leaf having the highest concentration. Research has demonstrated that flavonoids may enhance the body's production of detoxifying enzymes, decrease inflammation, and inhibit the growth of malignancies [26-27]. This suggests that consumption of bitter leaf among the samples will more advantageous due its high flavonoids content and its roles in the body. According to research, tannins have been linked to a variety of physiological and anti-microbial activities, including the stimulation of phagocytic cells, host-mediated tumor activity, and a broad spectrum of anti-infective effects [27-28]. However, this research revealed that the concentrations of tannin fell within the range of 0.71 ± 0.01 to 10.32± 1.2 mg/100g which showed high significant difference between bitter leaf and the other samples. The high concentration of tannin found in bitter leaf sample is in consistent with the value (9.62 mg/100g) obtained by Udochukwu *et al.* [24] in his study on the phytochemical analysis of *Vernoniaamygdalina* and *Ocimumgratissimum* extracts and their antibacterial activity on some drug resistant bacteria. This suggests that the bitter leaf sample is a better source of tannin compare to the other samples. As a result, consumption of bitter leaf may be a source of bioactive compounds useful in the treatment and prevention of cancer in the body [29]. According to Abdullahi and Santhose [30], the antioxidant properties of phenols are far stronger than those of vitamins C and E. This studyrevealed that the concentrations of phenols fell within the range of 4.24 ± 0.01to 1.36 ± 0.01 mg/100g. However, the result showed that there was a significant difference among the samples at 95% confidence level. Highest concentration of phenol was observed in bitter leaf which also suggests that it's a good source of phenols. The result also revealed a significant difference in the values of alkaloids among the samples at 95% confidence level. According to Olasupo *et al.* [26], alkaloids constitute a significant class of naturally occurring medications that are used to treat congestive heart failure. Because of their extreme toxicity and pronounced bitter taste, alkaloids are employed by plants as a defense mechanism against invertebrate pests, microbial diseases, and herbivores [24, 27]. Furthermore, according to Adetunji *et al.* [31], alkaloids possess antispasmodic, antibacterial, therapeutic, and antimalarial characteristics, making them the most effective phytochemical. Highest concentration of alkaloid was found in bitter leaf.

This implies that bitter leaf is good source of alkaloids and its consumption will help in the treatment of some ailments as mentioned above. According to literature, bioactive chemicals known as saponins have the ability to reduce the stomach's absorption of glucose and cholesterol [24, 26, 29]. In addition, a family of naturally occurring substances called saponins complexes with cholesterol to create holes in bilayers of cell membranes. Consequently, these substances may be employed as agents that lower or inhibit cholesterol [31]. However, this study revealed that the saponins levels in the samples fell in the order; bitter leaf > pumpkin > spinach which showed no significant difference among the samples.

Antioxidant analysis

Figure 1 shows the comparative plot ofantioxidant capacities of the selected leaf vegetable samples using DPPH as free radical scavenger. The antioxidant capacities of the leafy vegetable samples showed a significant difference at 95% confidence level.

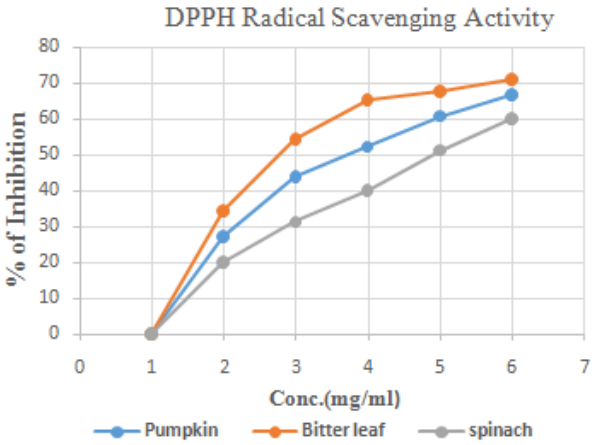

Figure 1: Comparative plot of antioxidant capacities of the selected leaf vegetable samples

From the figure, it could be observed that as the concentration of the extracts increases, the inhibition efficiencyincreases for all the samples. However, these trends agree with most literatures [30, 32, 33]. Also from the figure, highest percentage inhibition was observed in bitter leaf. This could be attributed to the high percentage compositions of phytochemicals presence in the bitter leaf compared to the other samples as shown in Table 2.

Conclusion

This studyevaluated the proximate, phytochemicals and antioxidant properties of three selected leafy vegetables (bitter leaf, pumpkin and spinach) on comparative basis. However, the values obtained from the study were subjected to analysis of variance (ANOVA). Based on the statistical analysis, the study revealed that there were significant differences among the samples at 5% confidence level. Therefore, this study inferred that the nutritional and phytochemical contents of the leafy vegetables samples varied significantly and their consumption in a diet will be responsible for maintaining good health and proper functioning of the body system.

Conflict of interest: The authors hereby declared no conflict of interest in this research work

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