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Chemical Constituents of *Borassus Aethiopum* Methanol Leaf and Fruit Extract

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A B S T R A C T

This study was conducted to assess the vitamin, amino acid composition and bioactive constituents of Borassus aethiopum methanol leaf and fruit extract. Fresh leaves and fruits of B. aethiopum were collected from Karim Lamido Local Government Area, Taraba State, Nigeria. The samples were processed for various analyses, including High-Performance Liquid Chromatography (HPLC) for vitamin and amino acid content. Amino acid analysis of the fruit revealed Tyrosine as the most abundant, with a mean concentration of 61.773 ppm, accounting for 51.9% of the total amino acid content. Phenylalanine was detected at 46.706 ppm (39.3%), Threonine at 6.975 ppm (5.9%), and Asparagine at 3.491 ppm (2.9%). The vitamin analysis showed that Vitamin B1 (Thiamine) had the highest concentration at 676.201 ppm (91.1%), followed by Folate (Vitamin B9) at 62.549 ppm (8.4%). Trace amounts of Vitamin K (1.991 ppm, 0.3%) and Vitamin E (1.355 ppm, 0.2%) were also detected. For the leaf extract, phytochemical analysis revealed significant concentrations of flavonoids (14.2%), alkaloids (8.4%), oxalates (2.30 mg/100g), phenolic compounds (2.80 ppm), and saponins (1.6%). Vitamin analysis of the leaf extract using HPLC detected vitamin K (8.159 ppm, 3.2%), vitamin B1 (108.259 ppm, 42.6%), and folate (137.813 ppm, 54.2%). The antioxidant activity of the sample was evaluated using the DPPH radical scavenging assay. At a concentration of 1000 µg/mL, the sample demonstrated a significant inhibition of DPPH radical activity with a percentage scavenging of 73.46%. This activity decreased with lower concentrations, showing 67.00% at 500 µg/mL, 57.14% at 250 µg/mL, 50.17% at 125 µg/mL, and 31.02% at 62.5 µg/mL. The IC50 value, representing the concentration required for 50% inhibition of DPPH radicals, was determined to be 231.03 µg/mL, indicating a moderate antioxidant potential of the sample. These findings highlight the nutritional and bioactive potential of B. aethiopum, with significant anti-inflammatory properties, suggesting its potential use in managing inflammation-related conditions.

Keywords: Vitamins, Amino Acids, phytochemicals, *Borassus aethiopum*, methanol extract, *DPPH Radical Scavenging*

1. Introduction

Plants have long been recognized as valuable sources of bioactive compounds with potential therapeutic, nutritional, and industrial applications (Babbar *et al.,* 2015; Patra *et al.,* 2018; Banwo *et al.,* 2021; Shrinet *et al*., 2021). Among these plants, *Borassus aethiopum*, commonly known as the African fan palm, *Giginya* in Hausa and *Vheng* among the Jenjo People of Karim-Lamido L.G.A. in Taraba State, Nigeria has been traditionally utilized for its medicinal and nutritional properties in various African communities (Bolade and Bello, 2006; Gruca *et al*., 2015; MJ and Adjei, 2015; Ishwah *et al.,* (2022). Recent interest has focused on uncovering the phytochemical composition of *B. aethiopum* and its potential health benefits, particularly in relation to its anti-inflammatory, antioxidant, and nutritional properties.

Phytochemical analysis of plant species plays a critical role in identifying compounds that contribute to health benefits. *B. aethiopum* is rich in vitamins, amino acids, and secondary metabolites such as flavonoids, alkaloids, phenolic compounds, and saponins, all of which contribute to its bioactivity (Arthur, 2019; Aina and Ampitan, 2022). Amino acids, such as tyrosine and phenylalanine, are known to play significant roles in protein synthesis and metabolic processes, while vitamins like Thiamine (B1), Folate (B9), Vitamin K, and Vitamin E contribute to immune function, antioxidant activity, and cellular repair mechanisms (Boubakri *et al.,* 2016; Barrouin-Melo *et al.,* 2018; Morris and Mohiuddin, 2020; Mishra *et al.,* 2021; Suraiya *et al.,* 2022; Theodosis-Nobelos *et al.,* 2024). Additionally, the presence of flavonoids and phenolic compounds in the plant highlights its potential as an anti-inflammatory and antioxidant agent, which could be beneficial in the management of inflammation-related disorders (Leyva-López *et al.,* 2016; Spagnuolo *et al.,* 2018; Yatoo *et al.,* 2018; Al-Khayri *et al.,* 2022).

This study aims to analyse the vitamin, amino acid and bioactive constituents of *B. aethiopum* fruit and leaf methanolic extracts, providing insights into its chemical composition and potential medicinal uses. By employing High-Performance Liquid Chromatography (HPLC) to quantify its essential compounds.

2. Materials and Methods

2.1 Plant Collection and Processing

Fresh *Borassus aethiopum's* fruits and leaves were harvested from its natural habitat in the Karim Lamido Local Government Area of Taraba State. After collection, the fruit and leaf samples were sorted, cleaned, and transported in sacks to the Biological Science laboratory at Taraba State University, Jalingo.

The collected fruits and leaves were chopped separately into small pieces and air-dried in the shade for two weeks until fully dehydrated. The dried samples were then ground into a fine powder. About The 100 g of the powdered materials were soaked in 800 mL of methanol for 72 hours, with occasional stirring to facilitate extraction. The mixture was filtered using Whatman's No.1 filter paper, and the resulting filtrate was concentrated using a rotary evaporator.

2.2 Vitamin Analysis and Amino Acid Identification and Quantification

Vitamins and amino acids were identified by comparing their retention times to those of known standards. The quantitative content of each vitamin and amino acid were determined by measuring the peak areas in the HPLC chromatogram. The analysis was carried out using reverse-phase High-Performance Liquid Chromatography (HPLC) (Agilent 1100 HPLC System, USA)-The HPLC system employed a Reprosil 100 C8 mn 5um 4.6 x 150mm column, UV/VIS detection, and a mobile phase of 100% Methanol at a flow rate of 1 ml/min. The content of bound amino acids was calculated by subtracting the free amino acid content from the total amino acid content (Chen *et al*., 2010; Feshchenko *et al*., 2021).

2.3 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was performed using the method described by Dzoyem and Eloff (2015). Briefly, 900 µL of DPPH solution (0.2 mM) prepared in methanol and mixed with 100 µL of plant extract sample at various concentrations (12.5 to 1200 µg/mL). After incubation at room temperature for 30 min, the absorbance of the mixture was measured at 517 nm using Varian Cary 400 Scan UV/Visible Spectrophotometer. Ascorbic acid was used as a positive control, methanol as a negative control, and extract without DPPH as a blank. The percent of inhibition of DPPH radical scavenging (%I) was calculated using the formula: $\%I = (\text{Absorbance Control} - \text{Absorbance Sample})/\text{Absorbance Control}) \times 100$. The concentration of plant extracts necessary to scavenge 50% of radicals (IC_{50}) was calculated by plotting inhibition percentages against concentrations of sample.

2.4 Statistical Analysis

Statistical analysis was performed using SPSS version 27.0. Descriptive statistics, such as means and standard deviations, were calculated for compound concentrations. Related-Samples Wilcoxon Signed Rank Test test statistic and Bayesian Correlation between vitamin contents of the leaf and fruit methanol extract was employed to determine significant differences ($p < .05$) was considered significant.

3. Results

Table 1 present the phytochemical composition *B. aethiopum* methanol leaf extract. Among the phytochemicals analyzed, flavonoid has the highest mean concentration at 14.2%, indicating its substantial presence in the sample. Additionally, alkaloids showed notable concentrations of 8.4%, Oxalate was 2.30mg/100g, Phenolic concentration was 2.80ppm and Saponins showed a relatively low concentration at 1.6%.

Table 2 presents the vitamin contents of *B. aethiopum* methanol leaf extract analyzed using High-Performance Liquid Chromatography (HPLC). Three vitamin compounds were detected at various retention time and concentration. Vitamin K, was detected at a retention time of 1.628 min with a concentration of 8.159ppm and constitutes 3.2% of total sample. Vitamin B1 (Thiamine), Folate (Vitamin B9), and Vitamin K. Thiamine was detected at a retention time of 2.505 minutes with a concentration of 108.259 ppm, contributing to approximately 42.6% of the total vitamin content. Folate, was identified at a retention time of 2.897 minutes, showing a significantly higher concentration of 137.813ppm and constituting 54.2% of the total vitamin content. The analysis indicates a higher concentration of Folate(B9), followed by Thiamine and Vitamin K.

Table 3 and figure 1 are the result of amino acid content in *Borassus aethiopum* methanol fruit extract. Among the amino acid analyzed, Tyrosine has the highest mean concentration at 61.773 µg and representing 51.9% indicating its substantial presence in the fruit sample. Phenyl Alanine was detected concentrations of 46.706 µg, representing of 39.3% of total amino acid content. Threonine in the sample was found to be 6.975 µg and 5.9%, and Asparagine showed a relatively low concentration at 3.491 µg and 2.9% respectively.

Table 4 and figure 2 presents the vitamin contents of *Borassus aethiopum* methanol fruit extract analyzed using High-Performance Liquid Chromatography (HPLC). Four vitamin compounds were detected at various retention time and concentration. Vitamin B1, was detected at a retention time of 2.363 min and has the highest concentration of 676.201ppm and constitutes 91.1% of the total sample. Folate (Vitamin B9), was detected at a retention time of 2.897 min with a concentration of 62.549ppm, contributing to approximately 8.4% of the total vitamin content. Vitamin K content was

detected at 1.827min representing 1.991ppm representing 0.3% of the total vitamin content. Vitamin E was detected at a retention time of 5.540min with a concentration of 1.355ppm and percentage composition of 0.2%.

Fig. 3 shows the antioxidant capacity of extracts from *B. aethiopum* methanol leaf extract. The best antioxidant capacity is at a concentration of 1000ug/mL (73.46%) and the lower antioxidant activity was at a concentration of 62.5ug/mL (31.02%). The IC_{50} was estimated to be equal to 231.03 µg/mL.

Table 1: Phytochemical Composition of *Borassus aethiopum* of methanol Leaf extract.

Flavonoids (%)	**Alkaloids** (%)	**Saponins** (%)	**Oxalate** (mg/100g)	**Phenolic** (ppm)
14.2	8.4	1.6	2.30	2.80

Table 2: Vitamin Contents of *Borassus aethiopum* methanol leaf extract using HPLC

S/N	**Vitamin**	**Retention Time (min)**	**Response**	**Amount (ppm)**	**Amount (%)**
1	Vitamin K	1.628	326.395	8.159	3.2
2	Vitamin B1	2.505	160.003	108.259	42.6
3	Folate(B9)	2.897	558.188	137.813	54.2
		Total		254.231	100.0

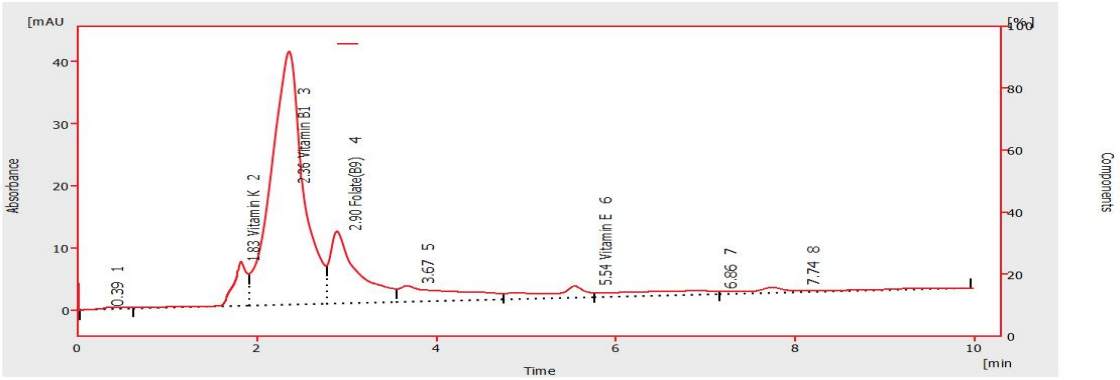

Figure 1: Chromatogram of HPLC Analysis of Vitamin Content of *Borassus aethiopum* methanol fruit extract.

Table 3: Vitamin Contents of *Borassus aethiopum* methanol fruit extract using HPLC.

S/N	Vitamin	Retention Time (min)	Response (Peak Area)	Amount(ppm)	Amount (%)
1	Vitamin K	1.827	79.649	1.991	0.3
2	Vitamin B1	2.363	999.409	676.201	91.1
3	Folate(B9)	2.897	253.343	62.549	8.4
4	Vitamin E	5.540	65.233	1.355	0.2
		Total		742.096	100.0

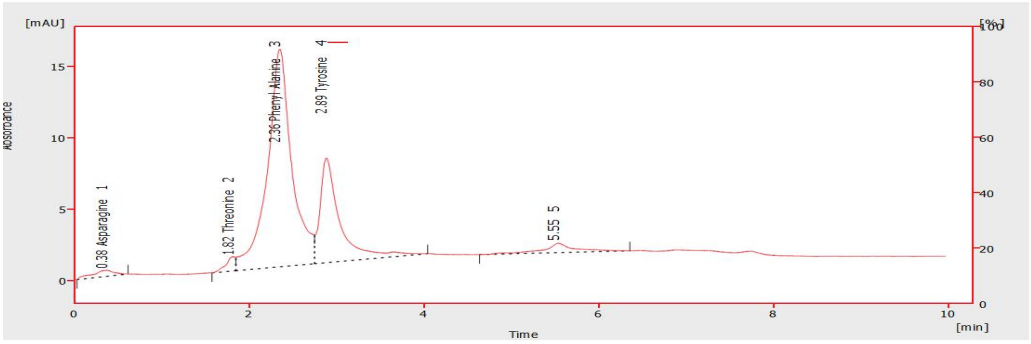

Figure 2: Chromatogram of HPLC Analysis of Amino acid Content of *Borassus aethiopum* methanol fruit extract.

Table 4: Amino acid Contents of *Borassus aethiopum* methanol fruit extract by HPLC.

S/N	Amino Acid	Retention Time (min)	Response (Peak Area)	Amount (ppm)	Amount% (%)
1	Asparagine	0.382	7.880	3.491	2.9
2	Threonine	1.820	7.548	6.975	5.9
3	Phenyl Alanine	2.358	297.783	46.706	39.3
4	Tyrosine	2.892	116.374	61.773	51.9
		Total		118.946	100.0

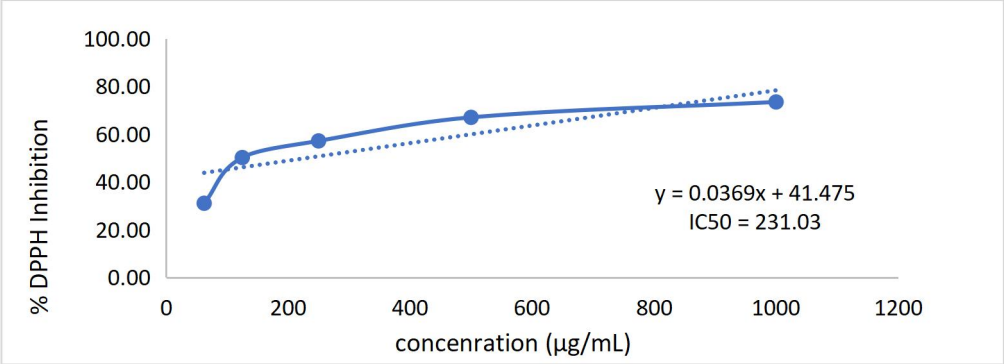

Figure 3: DPPH radical scavenging activity of *Borassus aethiopum* methanol leaf extract.

4. Discussion

The phytochemical analysis of *B. aethiopum* leaves revealed a rich composition of flavonoids (14.2%), alkaloids (8.4%), oxalates (2.30 mg/100g), phenolic compounds (2.80 ppm), and saponins (1.6%). These bioactive compounds are known for their therapeutic potential. Flavonoids and phenolic compounds possess anti-inflammatory, antispasmodic, antimicrobial, and antioxidant properties (Jakimiuk *et al*., 2022), while saponins have been used to treat cardiovascular diseases, gastric ulcers, and inflammation (Roopashree and Naik, 2019). Saponins also enhance the absorption of bioactive molecules and drugs (Liao *et al*., 2021). The oxalate content, though lower than toxic levels, suggests that high-oxalate foods should be consumed alongside calcium-rich foods to prevent mineral deficiencies (Morrison and Savage, 1999).

The vitamin composition of *B. aethiopum* leaves, determined by HPLC, showed high levels of Folate (137.813 ppm, 54.2%), followed by Thiamine (108.259 ppm, 42.6%) and Vitamin K (8.159 ppm, 3.2%). Folate plays a critical role in DNA methylation and cell division, while Vitamin K supports immune function and cellular communication (Ferland, 2012). The antioxidant activity of *B. aethiopum* leaf extract was highest at 1000 µg/mL (73.46%) and lowest at 62.5 µg/mL (31.02%), highlighting the plant's potential for mitigating oxidative stress.

The HPLC analysis of *Borassus aethiopum* fruit identified four key amino acids, with Tyrosine being the most abundant, comprising 51.9% of the total amino acid content at a concentration of 61.773 ppm. Phenylalanine was the second most prevalent at 46.706 ppm (39.3%), followed by Threonine (6.975 ppm, 5.9%) and Asparagine (3.491 ppm, 2.9%). These amino acids are crucial for various physiological functions. L-Threonine, for instance, is widely used in the food and pharmaceutical industries for its role in liver protection and detoxification (Dong *et al*., 2012; Liu *et al*., 2015; Wang *et al*.,

2019). Asparagine supports both nervous system function and liver health by binding ammonia in tissues and participating in metabolic regulation (Sirovaya *et al*., 2014).

The vitamin analysis of *B. aethiopum* fruit revealed that Vitamin B1 (Thiamine) had the highest concentration at 676.201 ppm, representing 91.1% of the total vitamin content. Folate (Vitamin B9) was detected at 62.549 ppm (8.4%), while Vitamins K and E were present in smaller quantities at 1.991 ppm (0.3%) and 1.355 ppm (0.2%), respectively. Vitamin K plays a vital role in immune regulation and has been linked to the management of inflammatory diseases and cancer (Namazi *et al*., 2019). In the nervous system, Vitamin K supports cellular communication and sphingolipid metabolism (Ferland, 2012).

Folate is essential for DNA synthesis and repair, but excessive folic acid intake can lead to unmetabolized folate, potentially contributing to cancer development by promoting cancer cell survival and altering nutrient metabolism (Gruber, 2016). On the other hand, folate deficiency may result in DNA hypomethylation, increasing the risk of disease. Vitamin E, an essential antioxidant, is crucial for maintaining cellular function and regulating enzymatic activity. Although deficiencies are rare, inadequate Vitamin E can cause neurological and immune dysfunction. Vitamin E supplementation during cancer treatment may also help alleviate the side effects of chemotherapy, particularly those involving oxidative stress (Zhou, 2005).

Comparing the vitamin contents of the leaf and fruit revealed that Related-Samples Wilcoxon Signed Rank Test test statistic (7.000) is not significant at the 0.05 level (Asymptotic Sig. = 0.465). This indicates that there is no significant difference in the medians of the leaf and fruit data. The Bayesian Correlation between the leaf and fruit on the other hand showed a positive correlation for both posterior mode and mean of the correlation coefficient (.617 and .326, respectively), suggesting a positive correlation between leaf and fruit data.

The phytochemical composition of *B. aethiopum* aligns with this global trend, showcasing its potential as a source of bioactive compounds. Phenolic and flavonoid compounds, which are abundant in *B. aethiopum*, are among the most important phytochemicals, offering a range of benefits, including anti-inflammatory, antioxidant, and antimicrobial activities (Ndam *et al*., 2016; Rasool *et al*., 2020; Jakimiuk *et al*., 2022). The presence of saponins, though in relatively low concentrations, underscores the traditional use of *B. aethiopum* in treating various health conditions. Saponins are known to reduce inflammation and enhance drug absorption, making them valuable in medicinal applications (Roopashree and Naik, 2019; Liao *et al*., 2021). Oxalate, while considered an anti-nutrient, was present at safe levels, though caution is advised in diets rich in oxalates to prevent mineral deficiencies. Consuming oxalate-rich foods with calcium sources such as dairy can help mitigate the risk of oxalate-induced deficiencies (Morrison and Savage, 1999).

The rich composition of vitamins, amino acids, and phytochemicals in *B. aethiopum* supports its potential use in nutraceutical, pharmaceutical, and medicinal applications. The high levels of antioxidants and anti-inflammatory compounds make it a promising candidate for further study in the prevention and management of diseases related to oxidative stress and chronic inflammation.

5. Conclusion

The present study highlights the rich nutritional and bioactive potential of *B. aethiopum* fruit and leaf methanolic extracts. The HPLC analysis identified key amino acids such as Tyrosine and Phenylalanine in the fruit, alongside vitamins like Thiamine (Vitamin B1) and Folate (Vitamin B9), which were present in significant amounts. These compounds are essential for various metabolic functions, including protein synthesis, liver protection, and immune regulation. The leaf extract was found to contain high levels of flavonoids, alkaloids, and phenolic compounds, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. The antioxidant activity of *B. aethiopum* leaf extract, coupled with the presence of vitamins and phytochemicals, underscores the plant's potential role in combating oxidative stress and inflammation. Furthermore, the safe levels of oxalates and the therapeutic potential of saponins suggest that *B. aethiopum* can be safely incorporated into traditional and modern medicinal applications. The findings indicate that *B. aethiopum* possesses significant nutritional and therapeutic value, making it a promising candidate for further research and development in the fields of nutraceuticals, pharmaceuticals, and functional foods. Future studies should explore the mechanisms of action of its bioactive compounds and investigate its efficacy in clinical applications.

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