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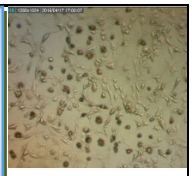

Metabolite Profiling and Antioxidant Activity of *Borassus aethiopum* Hypocotyl Fraction

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Abstract

Oxidative stress has been established among the key causes of both metabolic and cellular dysfunctions, thus developing an efficient natural antioxidant is highly required. This work profile the bioactive and examines the antioxidant capacity of the hypocotyl fractions of *Borassus aethiopum* (the palm tree) through *in vitro* and *in-vivo* assays. The phytochemical tests revealed a high content of total phenolic (875 ± 34.5 mg/g) than flavonoids (15.2 ± 0.57 mg/g) in the hypocotyl of *B. aethiopum*. A variable antioxidant activity was established by the various solvent fractions, with aqueous fraction having the highest activity *in vitro* as demonstrated its lowest IC_{50} value against DPPH radical scavenging activity (23.8 ± 0.5 µg/mL) and total antioxidant power (10.6 ± 0.2 µg/mL). Similarly, FTIR spectra revealed dominant O–H, C–H, C=O, and C–N functional groups characteristic of phenols, carboxylic acids, alkanes, and amines, confirming the presence of redox-active chemical classes. GC–MS analysis identified several antioxidant-associated metabolites, including thymol acetate, tyrosol, 3-nitrochalcone, retinal, α-ionone derivatives, 3-aminosalicylic acid, hydroquinone derivatives, diosgenin, and lupeol. The *in vivo* assessment in diabetic rat model demonstrated biochemical modulation consistent with antioxidant activity. Extract-treated groups showed reduced malondialdehyde levels (e.g., 0.54 ± 0.06 nmol/mg at 250 mg/kg) relative to diabetic controls (0.87 ± 0.16 nmol/mg), alongside increases in superoxide dismutase (0.55 ± 0.04 U/mL at 500 mg/kg) and dose-dependent alterations in catalase activity. Together, these findings demonstrate that *B. aethiopum* hypocotyl possesses a robust antioxidant signature driven by phenolic abundance, redox-active metabolites, and modulation of endogenous antioxidant enzymes, supporting its potential as a natural source of antioxidant compounds.

INTRODUCTION

Oxidative stress is one of the major causative factors in the pathogenesis of metabolic diseases, with diabetes being a significant example. This is due to the increased levels of ROS resulting from mitochondrial overloading and the activation of pro-oxidative enzymes due to sustained hyperglycemia, leading to a decline in antioxidant activity [1]. Plant-based antioxidants have long been studied for their potential in managing the problem of redox imbalance. For instance, polyphenol and flavonoid compounds have scavenged free radical substances and chelated metallic ions and have shown potential in managing diseases involving oxidative stress [2]. *Borassus aethiopum* is commonly employed in West African food and folk medicinal

practices. Recent works have reinforced the evidence that phenolics and antioxidant activity exist within extracts of different parts of the plant, as detected by DPPH, ABTS, and FRAP assays [3]. Oil and kernel extracts showed high antioxidant potential in a variety of tests [3].

Contrary to the growing body of evidence, most existing literature focuses on fruits, nuts, or other composite plant materials. The hypocotyl, for example, a part of the plant with a long history of use and phytochemical content, is poorly understood in terms of its specific antioxidant activity. Known variations within organs can significantly impact activity levels, therefore, investigating the hypocotyl extract for antioxidant activity is highly thought. This paper profile the bioactive compounds with antioxidant activity present in the hypocotyl fraction of *B. aethiopum*.

MATERIALS AND METHOD

Plant Sample Collection and Preparation

The fresh sample of *Borassus aethiopum* was collected from a botanical garden in Kano state, Nigeria. The plant sample was authenticated by a plant scientist at Bayero University, Kano, with the voucher number BUKHAN 0276. The samples were rinsed with running tap water, cut into smaller pieces, air dried at room temperature for 5 days, and milled with a blender.

Extract preparation

The powdered plant sample (780 g) was sonicated with 2.5 L of methanol in an ultrasonic bath (Model T-0205, Tulker, China) at 40 °C for 20 minutes [4]. The remaining residue was subsequently re-extracted with the same solvent three additional times to ensure optimal yield. All filtrates were pooled and concentrated using a rotary evaporator to obtain the crude methanolic extract. The resulting extract was then dried and stored in an airtight container under controlled conditions.

Experimental animals

Adult male albino rats weighing 100-110 g were procured from the Department of Human Physiology, Bayero University Kano. They were housed in polypropylene enclosures and maintained in a typical environment (a 12-h cycle of light and dark at a temperature of 25 °C ± 3 °C). The rats were fed with a rat chow and unlimited access to water.

Total flavonoid

The total flavonoid content was determined using the method described by [5]. A volume of 0.5 mL of a 2% AlCl solution in ethanol was added to 0.5 mL of the crude extract. After one hour at room temperature, the absorbance was measured at 420 nm using the spectrophotometer. The formation of yellow coloration showed the presence of flavonoids. The samples of the extract were estimated at a final concentration of 1 mg/mL. using the given equation based on the calibration curve y =0.025x, + C R^2 = 0.9812, Where x was the absorbance and was the quercetin equivalent,

Total phenolics

The total phenolic content in the extract was determined by the modified Folin-Ciocalteu method [6]. The crude extract (1 mg/mL) was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of sodium carbonate. The mixtures were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development, and absorbance was then measured at 765 nm using a spectrophotometer. Total phenolic content was expressed as mg/mL tannic acid equivalent using the following equation based on the calibration curve: y = 0.1216x, where x was the absorbance and y was the tannic acid equivalent (mg/mL).

DPPH Assay

The DPPH radical scavenging assay was performed with slight modifications literature [7]. Accurately, 2.0 mL of 4 mg/mL sample extract (crude and the fraction) solution was mixed with 2.0 mL of 0.1 mM freshly prepared DPPH solution (in methanol). After incubation in the dark for 30 min at room temperature, the absorbance of the different sample extract solutions was measured as follows at 517 nm using ascorbic acid as a positive control.

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where
Ac- Control reaction absorbance
As-Test specimen absorbance.

Solvent-Based Fractionation Using Column Chromatography

Fractionation of the crude extract was carried out using column chromatography. A glass chromatography column was packed with silica gel, which served as the stationary phase. The column was carefully packed to ensure uniform bed height and avoid air pockets, and pre-conditioned with a non-polar solvent before sample loading. Approximately 5-10 g of crude plant extract was adsorbed onto a small quantity of silica gel and allowed to dry to form a free-flowing powder. This mixture was gently loaded onto the top of the packed column. The column was then eluted sequentially with solvents of increasing polarity in the following order: n-hexane, chloroform, ethyl acetate, ethanol, and distilled water. Each solvent was applied gradually and in sufficient volume (typically 50–150 mL per stage, depending on column size) to allow thorough elution. Lastly, the eluents were collected in separate fractions, which allows the solvents to evaporate, and were further used in the determination of antioxidants, and characterization, which include FTIR and GC-MS.

In Vivo Antioxidants Analysis

Serum Catalase Activity

Catalase activity in serum samples was assessed spectrophotometrically by measuring the decomposition of hydrogen peroxide [8]. Briefly, 100 µl serum was incubated with 1.0 mL of 65 mM hydrogen peroxide substrate in sodium-potassium phosphate buffer (pH 7.4) at 37 °C for 3 minutes. The reaction was halted by addition of a titanium tetrachloride reagent, which binds residual hydrogen peroxide to form a yellow complex. Absorbance of the complex was measured at 405 nm using a spectrophotometer. Catalase activity was then calculated based on a standard curve prepared using known concentrations of hydrogen peroxide, with values expressed in kilounits (kU).

Superoxide Dismutase (SOD) Activity

SOD activity was quantified using the WST-1 method, which involves inhibition of WST-1 (water-soluble tetrazolium salt-1) formazan dye formation by superoxide radicals generated from xanthine and xanthine oxidase [9]. The assay mixture contained WST-1 reagent, xanthine substrate, xanthine oxidase enzyme, and assay buffer. Serum samples were introduced into the reaction mixture and incubated at 37 °C for 20 minutes. Absorbance was measured at 440 nm to determine the degree of inhibition by SOD. The enzyme activity was expressed in units per milliliter (U/mL) and calculated using the formula provided in the manufacturer's protocol based on percentage inhibition.

Determination of Malondialdehyde

Serum malondialdehyde levels were quantified using the thiobarbituric acid reactive substances (TBARS) assay, a well-established spectrophotometric method for evaluating lipid peroxidation [10]. Serum sample (0.2 mL) was mixed with 3 mL of 1% phosphoric acid and 1 mL of 0.6% TBA solution. The mixture was heated in a boiling water bath at 95 °C for 45 minutes to facilitate the reaction. After cooling on ice for 10 minutes, the samples were centrifuged at 10,000 rpm for 5 minutes to remove precipitated proteins and debris. The absorbance of the supernatant was measured at 532 nm using a UV-visible spectrophotometer. A standard curve was generated using serial dilutions of 1,1,3,3-tetramethoxypropane (TMP), which hydrolyzes to MDA under assay conditions. MDA concentrations were expressed as nanomoles per milligram of protein (nmol/mg protein), with protein content determined using the Biuret method.

RESULTS

Total phenolic and total flavonoid contents of *B. aethiopum*

In **Fig. 1**, the total phenolic and flavonoid contents expressed as mg/g of *B. aethiopum* hypocotyl extract was presented. The result shows that the hypocotyl contain significantly higher total phenolic content of 875 ± 34.5 mg/g than flavonoids (15.2 ± 0.57 mg/g).

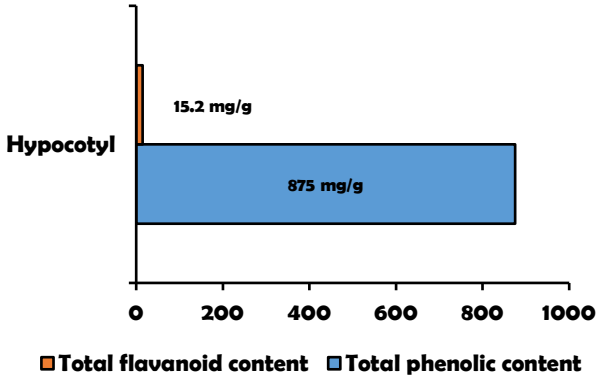

Fig. 1. Total phenolic and flavonoid contents of hypocotyl of *B. aethiopum*. Data are presented as mean ± SD (n=3).

DPPH radical scavenging activity of hypocotyl fractions of *B. aethiopum*

The DPPH radical scavenging activity of *B. aethiopum* was presented in **Table 1**. The solvent fraction of the hypocotyl showed a variable inhibition of the DPPH radical, as indicated by their IC_{50} value. The various solvent fractions of the hypocotyl showed great inhibitory activity against the DPPH radical. Aqueous fraction of the hypocotyl revealed the highest DPPH radical scavenging activity with lowest IC_{50} (10.58 ± 0.22 µg/mL). This was significantly lower than all other solvents fractions and crude extract but significantly higher than the gallic acid standard (0.0001 ± 0.00 µg/mL).

Total antioxidant capacity (TAC) of *B. aethiopum*

The total antioxidant capacity of *B. aethiopum* varied significantly among the various solvent fractions (**Table 2**). The TAC of the hypocotyl fractions was significantly higher than the crude extract except for aqueous fraction, which was found to have lower IC_{50} values.

Table 1. DPPH radical scavenging activity of hypocotyl of *B. aethiopum.*

Sample	Hypocotyl IC_{50} (µg/mL)
N-hexene	618.05 ± 5.99*
Chloroform	22223.8 ± 14.85**
Ethyl-acetate	105.19 ± 0.16***
Ethanol	20141.4 ± 14.03****
Aqueous	10.58 ± 0.22*****
Crude extract	646.5 ± 156.9******
Standard (gallic acid)	0.0001 ± 0.00

Values are presented as mean ± standard deviation (n = 6). Within each column, means with different numbers of asterisks are significantly different ($p < 0.05$).

Table 2. Total antioxidant capacity of hypocotyl of *Borassus aethiopum.*

Fraction	Hypocotyl IC_{50} (µg/mL)
N-hexene	121.6 ± 0.57*
Chloroform	380.6 ± 1.40**
Ethyl-acetate	291.3 ± 0.76***
Ethanol	75.16 ± 1.40****
Aqueous	23.8 ± 0.50*****
Crude extract	47.6 ± 0.70******
Standard (gallic acid)	0.0001 ± 0.00

Values are presented as mean ± standard deviation (n = 6). Within each column, means with different numbers of asterisks are significantly different ($p < 0.05$).

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectrum analysis of the aqueous fraction of *Borassus aethiopum* hypocotyl identified key absorption peaks at 3238, 2925, 1596, 1454, 1045, 937, and 899 cm^{-1} (**Table 3**). These peaks collectively indicate the presence of carboxylic acids, alkanes, primary amines, and aromatic compounds. Specifically, characteristic vibrational modes such as O–H stretching and bending (for carboxylic acids), C–H stretching and twisting (for alkanes), N–H bending and C–N stretching (for primary and aliphatic amines), and C–H vibrations (for aromatic compounds) were observed (**Fig. 2**).

Table 3. Functional groups identified in aqueous fraction of *B. aethiopum* hypocotyl by FTIR.

Absorbance	Class of compound	Functional group	Intensity
3238	O–H stretch	carboxylic acids	Medium
2925	C–H stretch	alkanes	Medium
1596	N–H bend	primary amines	Medium
1454	C–H bend	alkanes	Medium
1045	C–N stretch	aliphatic amines	Medium
937	O–H bend	carboxylic acids	Medium
899	C–H	aromatics	Strong

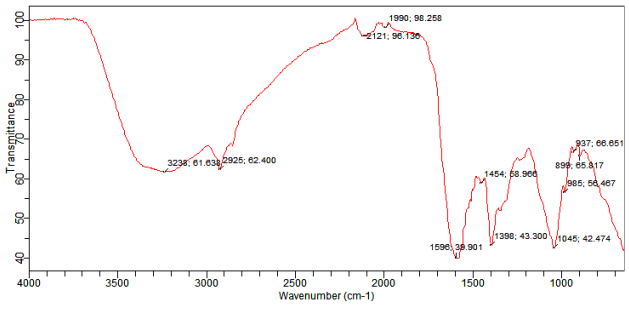

Fig. 2. FTIR spectrum of aqueous fraction of *B. aethiopum* hypocotyl.

GC-MS Profile of Aqueous Hypocotyl Fractions of *B. aethiopum*

The result of GC–MS analysis of the aqueous fraction *B. aethiopum* hypocotyl presented in **Table 5** shows a complex combination of phenol, terpenoids, aldehydes, chalcones, and steroidal compounds**,** each listed with their respective peak number, retention time (RT), percentage area, compound names, and their molecular weights, with varied retention times. The

GC-MS chromatograms (**Fig. 4a-4j)** identified leading compounds that gave some of the highest peak areas as lupeol, (431,802.64) with a retention time of 15.8 min, as well as multiple peaks of diosgenin with retention times of 11.8, 15.1, and 16.6 min, with relatively high areas (298,195.31). Also relatively high was the area given by aldehyde-retinal (388,474.24) with a retention time of 8.7 min. Secondary peaks with moderate intensities were found for 2-methyl hydroquinone (256,582.13) with a retention time of 17.5 min, α-ionone aldehydes (176,148.09) with a retention time of 13.8 min, 3-nitrochalcone (176,317.84) with a retention time of 8.0 min, as well as tyrosol (106,860.59) with a retention time of 16.8 min.

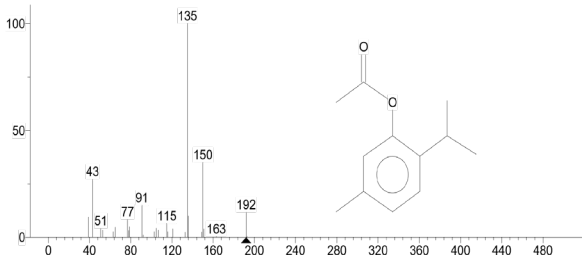

Fig. 4a. Thymol Acetate

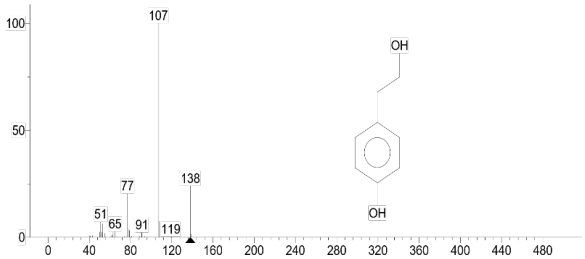

Fig. 4b. 4-hydroxybenzeneethanol.

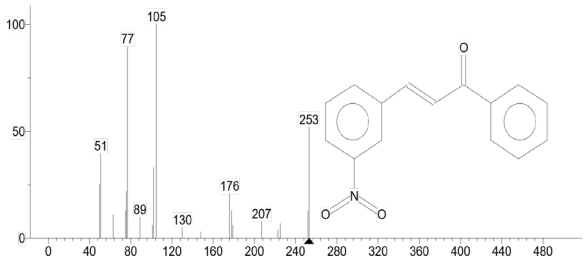

Fig. 4c. 3-Nitrochalcone.

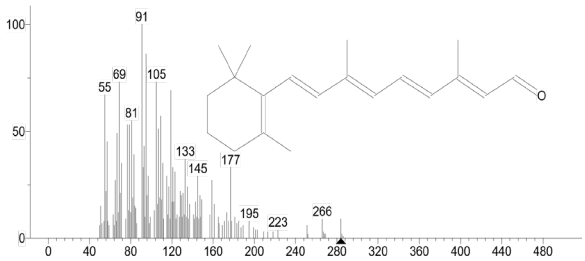

Fig. 4d. Retinal (Vitamin A aldehyde).

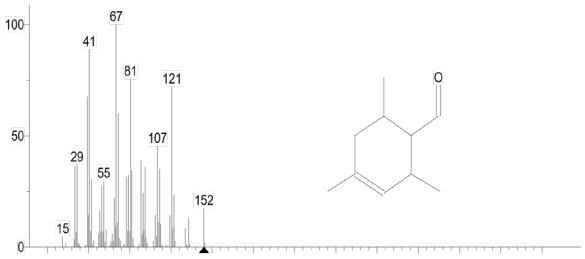

Fig. 4e. 3-Cyclohexene-1-carboxaldehyde, 2,4,6-trimethyl- (α-Ionone aldehyde derivative).

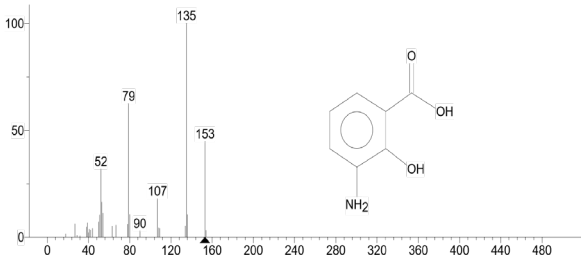

Fig 4f. 3-Aminosalicylic acid.

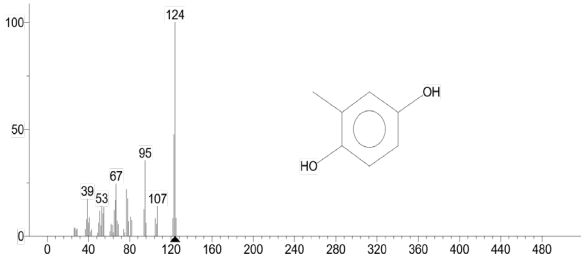

Fig. 4g. 1,4-Benzenediol, 2-methyl- (2-Methylhydroquinone).

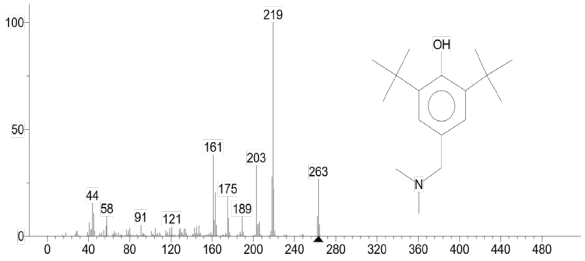

Fig. 4h. 2,6-di-tert-Butyl-4-(dimethylaminomethyl)phenol.

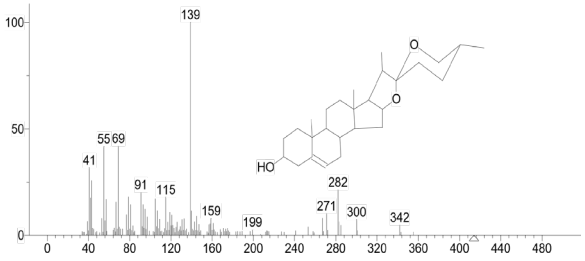

Fig. 4i. Diosgenin.

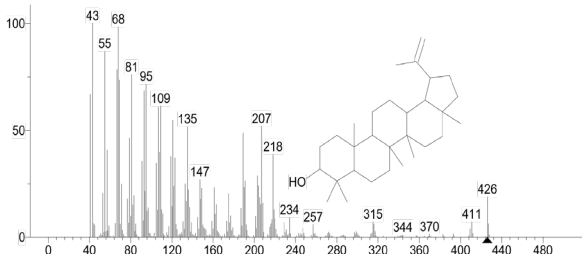

Fig. 4j. Lupeol.

Table 5. Metabolites from aqueous fraction *B. aethiopum* hypocotyl profile using GC-MS.

Peak No.	Retention Time (RT)	Area (%)	Compound Name	MW g/mol	Notes on Antioxidant Evidence
1	5.7	97,024.61	Thymol acetate	192.26	Thymol derivatives are known antioxidants (phenolic structure).
2	7.0	106,860.59	Tyrosol (4-hydroxybenzeneethanol)	138.17	Strong antioxidative phenolic; common in olive extracts.
3	7.9	176,317.84	3-Nitrochalcone	253.26	Chalcones have reported radical-scavenging activity.
4	8.7	388,474.24	Retinal (Vitamin A aldehyde)	284.44	Vitamin A family compounds are well-documented antioxidants.
5	9.0	176,148.09	α-Ionone aldehyde derivative	150.22	Ionones exhibit antioxidant and anti-radical properties.
6	9.3	104,825.83	3-Aminosalicylic acid	153.14	Salicylic acid derivatives have strong antioxidant properties.
7	9.7	256,582.13	2-Methylhydroquinone	124.14	Hydroquinones are classical antioxidants via redox activity.
8	11.4	87,983.64	2,6-Di-tert-butyl-1,4-benzenediol	222.33	A sterically protected hydroquinone, widely used as an antioxidant stabilizer.
9	11.8	139,036.63	Diosgenin	414.63	Diosgenin has reported antioxidant and anti-inflammatory activity.
10	15.1	187,448.93	Diosgenin	414.63	Same compound; repeated peak.
11	16.6	298,195.31	Diosgenin	414.63	Same compound; repeated peak.
12	15.8	431,802.64	Lupeol	426.73	Triterpenoid with strong antioxidant activity.

Serum antioxidants activity

The activities of superoxide dismutase (SOD) and catalase (CAT) in serum in **Table 6** reveal a complex, dose-dependent antioxidant response to *Borassus aethiopum* treatment in type 2 diabetic rats. Diabetic control rats showed significantly reduced SOD (0.32 ± 0.03 U/mL) and CAT (20.91 ± 0.06 kU) activities compared to normal controls (0.44 ± 0.03 U/mL and 28.81 ± 0.82 kU, respectively), indicating oxidative stress due to hyperglycemia. Treatment with *Borassus aethiopum* at 250 mg/kg improved both SOD (0.42 ± 0.04 U/mL) and CAT (23.82 ± 2.09 kU) activities, suggesting antioxidant support. Interestingly, the 500 mg/kg dose yielded the highest SOD activity (0.55 ± 0.04 U/mL), but catalase dropped sharply (11.1 ± 1.89 kU), and at 1000 mg/kg, both enzymes were suppressed (SOD: 0.32 ± 0 U/mL; CAT: 9.88 ± 1.80 kU), indicating possible pro-oxidant or toxic effects at high doses.

The serum malondialdehyde (MDA) concentrations across experimental groups, however, reflect the oxidative stress status in type 2 diabetic rats treated with *Borassus aethiopum*. Diabetic control rats exhibited elevated MDA levels (0.87 ± 0.16 nmol/mg protein) compared to normal controls (0.75 ± 0.12), indicating increased lipid peroxidation due to hyperglycemia-induced oxidative stress. Treatment with the standard drug reduced MDA significantly (0.52 ± 0.13), suggesting effective antioxidant protection. Similarly, *Borassus aethiopum* at 250 mg/kg yielded comparable MDA reduction (0.54 ± 0.06), while the 500 mg/kg and 1000 mg/kg doses showed slightly higher MDA levels (0.67 ± 0.35 and 0.65 ± 0.12, respectively), though still lower than the diabetic control. These results suggest that *Borassus aethiopum* exerts a protective effect against lipid peroxidation, with optimal antioxidant activity observed at lower doses.

Table 6. Serum antioxidant enzymes and malondialdehyde levels in type 2 diabetic rats treated with aqueous fraction *B. aethiopum* hypocotyl.

Groups	Malondialdehyde (nmol/mg protein)	Superoxide Dismutase Activity (U/mL)	Catalase Activity (kU)
Normal Control	$0.75 \pm 0.12^{*}$	0.44 ± 0.03	28.81 ± 0.82
Diabetic Control	0.87 ± 0.16	$0.32 \pm 0.03^{*}$	$20.91 \pm 0.06^{*}$
Drug Control	$0.52 \pm 0.13^{**}$	$0.34 \pm 0.04^{*}$	$25.75 \pm 2.50^{*}$
250 mg/kg Extract	$0.54 \pm 0.06^{**}$	$0.42 \pm 0.04^{*}$	$23.82 \pm 2.09^{*}$
500 mg/kg Extract	$0.67 \pm 0.35^{*}$	$0.55 \pm 0.04^{**}$	$11.10 \pm 1.89^{**}$
1000 mg/kg Extract	$0.65 \pm 0.12^{*}$	$0.32 \pm 0.00^{*}$	$9.88 \pm 1.80^{**}$

Values are presented as mean ± standard deviation (n = 6). Within each column, means with different numbers of asterisks are significantly different ($p < 0.05$).

DISCUSSION

Phenolics and flavonoid

The findings in this study revealed that the total phenolic content, a measure of compounds renowned for their free radical scavenging properties, was substantially high in the hypocotyl. The high concentrations of phenolic content in the *B. aethiopum* hypocotyl fraction highlight the substantial presence of key bioactive compounds. These analytes are paramount for their potent antioxidant capabilities, primarily through their ability to scavenge free radicals and mitigate oxidative stress [11]. Given that oxidative stress is a fundamental pathological mechanism underlying the development and progression of various chronic diseases, including diabetes and its associated complications, the richness of these compounds in the hypocotyl underscores its significant potential as a therapeutic or functional food. This indicates that the hypocotyl could play a valuable role in combating oxidative damage, thereby offering protective effects against metabolic disorders and supporting overall cellular health. Similarly, [12] reported that the extract exhibited a concentration-dependent increase in total phenolic content, with a measured value of 73.65 mg tannic acid equivalent (TAE) per gram of extract. Additionally, [13] reported that the total phenolic content (TPC) of *B. aethiopum* was moderately present, which directly associates its compounds with antioxidant activity. Inconsistently, [14] reported that flavonoids represented the predominant class of phytochemicals in the extract, while phenolic compounds were present in much lower amounts. This distribution suggests a higher abundance of flavonoid-based constituents compared to other phenolic derivatives in the analyzed sample.

DPPH radical scavenging activity

Amongst the *in vitro* assays for the determination of antioxidant properties is the DPPH antiradical assay, which was employed to evaluate the antioxidant activity of hypocotyl fraction of *B. aethiopum*. As indicated by IC_{50} values, lower values correspond to stronger antioxidant activity. The aqueous fraction of the hypocotyl showed significantly lower IC_{50} value than others solvent fractions. These results suggest that the hypocotyl contains potent antioxidant compounds that are extractable by some polar solvents, aligning with earlier findings on its higher total phenolic and flavonoid content. As expected, the standard antioxidant compound (Gallic acid) demonstrated exceptional potency across all comparisons, serving as a benchmark for

evaluating plant extract efficacy. According to [15], the IC_{50} value for DPPH radical scavenging activity was reported to be 231.03 µg/mL, suggesting that the sample possesses moderate antioxidant potential based on its capacity to inhibit 50% of the free radicals at this concentration. Similarly, [13] reported that *B. aethiopum* exhibited a significant DPPH radical scavenging activity, indicating a moderate level of antioxidant efficacy. [12] demonstrated that the DPPH radical scavenging activity of the extract was concentration-dependent, with an effective concentration (EC_{50}), indicating strong antioxidant potential. [16] found that the DPPH radical scavenging assay and reducing power assay showed promising antioxidant potential of the extract at 0.5 mg/mL. Subsequently, *B. aethiopum*, particularly the hypocotyl with its higher phenolic and flavonoid content shows a strong DPPH radical scavenging activity, which reinforce its potential as a natural source of antioxidants to combat oxidative damage and support overall health, including in diabetes management.

Total antioxidant capacity

The total antioxidant capacity (TAC) assays suggested that the hypocotyl fraction of *B. aethiopum* contain bioactive constituents with radical-scavenging potential, but their effectiveness is influenced by the polarity of the solvent used. The aqueous fraction demonstrated the highest antioxidant activity among all fractions tested, suggesting the presence of water-soluble antioxidants such as polyphenols or amino acid derivatives in the hypocotyl. According to [12], the extract exhibited a total antioxidant activity of 329.4 mg of vitamin C equivalent per gram of extract, demonstrating a concentration-dependent antioxidant response. [17] reported that the total antioxidant capacity of *B. aethiopum* tissue was significantly lower than that of the standard ascorbic acid, indicating comparatively weaker antioxidant potency. The plant possesses a strong ability to scavenge harmful free radicals, thereby protecting cells and tissues from oxidative damage.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectral analysis of aqueous fraction of *B. aethiopum* hypocotyl provides invaluable insights into their diverse chemical constituents and functional groups, offering a foundational understanding of their potential biological activities. Across the examined fraction presented in **Table 3**, several common functional groups indicative of bioactive compounds were identified. These include prominent and broad absorption bands consistent with O–H stretching vibrations, suggesting the widespread presence of alcohols and/or phenols, which are well-known for their antioxidant and anti-inflammatory properties [18]. The hypocotyl aqueous fractions particularly emphasized the presence of carboxylic acids, further supported by O-H bending bands, often linked to phenolic acids and organic acids with radical-scavenging capabilities. Nitrogen-containing functional groups were also consistently observed across all fractions. Medium-intensity N-H bends and C-N stretches (characteristic of primary and aliphatic amines) were prominent. These indicate the presence of diverse nitrogenous phytochemicals such as amino acids, peptides, or alkaloid derivatives, which are frequently associated with enzyme inhibition, anti-inflammatory, antimicrobial, and even neuroactive properties [18].

Furthermore, aliphatic hydrocarbon chains (alkanes), indicated by C-H stretching and bending vibrations, were found in the aqueous fractions of the hypocotyl, likely originating from fatty acid residues or lipid-soluble constituents. The hypocotyl aqueous fraction further showed aromatic structures (C-H out-of-plane bending), implying the existence of phenolic compounds like flavonoids, tannins, or lignans, reinforcing its antioxidant and pharmacological potential. [19]. [20] revealed a significant absorption band for intermolecularly linked hydroxyl (O-H) groups, corresponding to cellulose. This observation provided evidence that the hydroxyl groups were engaged in hydrogen bonding. Similarly, [21] results showed the presence of N-H, O-H, and C-O stretching, indicating the presence of various functional groups.

This comprehensive FTIR analysis highlights the remarkable phytochemical complexity and diversity within different fractions of *B. aethiopum*. The rich profile of functional groups, including various forms of hydroxyls, carbonyls, amines, and aromatic structures, underscores the plant's significant therapeutic potential. These findings are crucial for guiding further targeted isolation, characterization, and pharmacological investigations of specific bioactive compounds responsible for the observed biological activities, particularly their anti-inflammatory and antidiabetic properties [19].

GC-MS Profile of hypocotyl aqueous fraction of *B. aethiopum*

The GC-MS profile of the aqueous fraction of *B. aethiopum* hypocotyl shows a chemically heterogeneous mixture dominated by triterpenoids, steroidal sapogenins, phenolic/hydroquinone-type compounds, chalcone-related species and small oxygenated terpenoid/aldehyde components, a distribution that suggests multiple biosynthetic origins and potential for multifaceted bioactivity [22]. Lupeol was found to be one of the most dominant peaks in the chromatogram (RT: 15.8, Area: 431,802.64) that represents a large triterpenoid component. This finding is in agreement with the fact that lupeol was found abundantly in many medicinal herbs, often being reported as a dominant pentacyclic triterpenoid component in phytochemical analyses [22].

The multiple large peaks marked as diosgenin (RT: 11.8, 15.1, as well as other peaks such as RT: 16.6) with a large peak (298,195.31) that indicate a large steroidal sapogenin component in the extract. This may be ascribed to the glycosidal derivatives of diosgenin as mentioned in a previous report that identified multiple peaks of the same aglycone [23]. A high peak corresponding to retinal (RT 8.7, area 388,474.24) as well as the appearance of aldehydes associated with ionone derivatives suggest a strong carotene/apocarotenoid component. This is also consistent with the partial solubilization or degradation products of carotenoids that might be expected in a GC–MS system [24]. Several peaks associated with phenol/hydroquinone derivatives such as 2-methylhydroquinone and 2,6-di-tert-butyl-1,4-benzenediol appear in this chromatogram, associated with the strong electrophile nature associated with the phenol component, a feature that is always associated with such studies, along with natural extracts [25].

Detection of chalcone and other α, β-unsaturated carbonyl compounds, like 3-nitrochalcone, together with smaller phenolics, such as tyrosol, along with thymol acetate, shows that this fraction is composed of both simple phenolics as well as more complex polyconjugates. Such diversity in composition is often responsible for a wide range of chemical reactivity, which might correspond to multiple peaks of moderate intensity in the mid retention time regions [26].

Serum malondialdehyde, superoxide dismutase and catalase activity

Oxidative stress plays a central role in the pathogenesis of type 2 diabetes mellitus (T2DM), primarily through the overproduction

of reactive oxygen species (ROS) and impaired antioxidant defenses. The observed reduction in SOD and CAT activities in diabetic control rats aligns with findings from [27] and [28], who reported diminished antioxidant enzyme activity in diabetic patients due to chronic hyperglycemia and ROS accumulation.

The improvement in antioxidant enzyme activity at 250 mg/kg and 500 mg/kg doses of *Borassus aethiopum* suggests the presence of bioactive phytochemicals with ROS-scavenging properties. Phytochemicals such as flavonoids, tannins, and polyphenols are known to enhance antioxidant defenses by upregulating endogenous enzymes like SOD and CAT [29,30]. These compounds may act via modulation of redox-sensitive transcription factors such as Nrf2, which regulates antioxidant gene expression. However, the paradoxical drop-in catalase activity at 500 mg/kg and the suppression of both enzymes at 1000 mg/kg raise concerns about dose-dependent toxicity.

High concentrations of polyphenols can exhibit pro-oxidant behavior, especially in the presence of transition metals, leading to oxidative damage rather than protection [31]. This phenomenon underscores the importance of identifying therapeutic windows for phytomedicines, as excessive dosing may overwhelm cellular antioxidant systems and impair enzyme function [32]. Moreover, the inverse correlation between SOD and CAT activities at higher doses may reflect compensatory mechanisms or enzyme-specific regulation under oxidative stress. Studies such as [33] have shown that SOD and CAT activities are tightly regulated and may respond differently to phytochemical interventions depending on tissue type, metabolic state, and ROS burden.

The results from malondialdehyde activity assessment in this study provided added insight into the antioxidants. Malondialdehyde is a well-established biomarker of lipid peroxidation and oxidative stress, particularly in diabetes mellitus where chronic hyperglycemia accelerates reactive oxygen species (ROS) generation. The elevated MDA levels in diabetic control rats corroborate findings from [34], who reported significantly higher MDA concentrations in type 2 diabetic patients, linking oxidative stress to cardiovascular risk. The reduction in MDA following treatment with *Borassus aethiopum* aligns with the antioxidant potential of its phytochemicals particularly flavonoids, tannins, and polyphenols which are known to scavenge free radicals and inhibit lipid peroxidation [29,32]. The comparable efficacy of the 250 mg/kg dose to the drug control suggests that *Borassus aethiopum* may offer a natural alternative for mitigating oxidative damage in diabetic conditions.

However, the slightly elevated MDA levels at 500 mg/kg and 1000 mg/kg raise questions about dose-dependent responses. While still lower than the diabetic control (group II), these values may reflect a threshold beyond which the extract's antioxidant capacity plateaus or reverses. High doses of polyphenols have been shown to exhibit pro-oxidant behavior under certain conditions, potentially exacerbating oxidative stress [35]. Further, [36] emphasized that lipid peroxidation products like MDA contribute to poor glycemic control and metabolic complications in type 2 diabetes. The observed reduction in MDA with *Borassus aethiopum* treatment may therefore reflect not only antioxidant activity but also improved metabolic regulation.

CONCLUSION

The hypocotyl of *Borassus aethiopum* exhibited very good antioxidant activities evidenced by its high phenolic and flavonoid content, robust radical-scavenging activity among fractions, and confirmation of metabolites associated with antioxidant properties. The evidenced *in vivo* antioxidant activity indicates that it enhances endogenous antioxidant defenses as well as diminishes oxidative damage. This suggest that the hypocotyl is a promising natural source of antioxidant compounds with potential applications in functional foods and phytotherapeutic formulations.

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