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ARTICLE

Antioxidant Potential and GC-MS Characterization Of the Seed Oil Extract of African Yam Bean *(Sphenostylis stenocarpa)*

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Abstract

Sphenostylis stenocarpa (African yam bean), a tropical underutilised legume, has gained research interest due to its nutritional and potential health benefits. Six different accessions of *S. stenocarpa* were examined in this study using aqueous and 70% acetone extraction methods to assess their performance as natural antioxidants. Seeds were sourced from IITA's Genetic Resources Center and cultivated at the Ahmadu Bello University Institute for Agricultural Research farm facility in Zaria. They were air-dried, ground into fine powder and subjected to Soxhlet solvent extraction. Antioxidant assays, including hydroxyl radical scavenging activity (HRSA), were performed according to standard protocols. Results showed that at a concentration of 500 µg, the *S. stenocarpa* extract exhibited antioxidant activity of 33.3%, at 250 µg and 500 µg at 41.6%. Bioactive compounds were identified in the samples through Fourier Transform Infrared Spectroscopy (FTIR). The presence of aldehydes, carboxylic acids, esters and aromatic rings were confirmed in the functional group analysis. GC-MS analysis revealed multiple chemical compounds possessing antioxidant activity. The analysis identified 9,12-octadecadienoic acid (Z, Z) as the most abundant, at 42.12%, followed by n-hexadecanoic acid at 30.56% and octadecanoic acid at 4.34%. These results suggested that *S. stenocarpa* possesses significant antioxidant activity and could serve as a natural protective agent. Additional investigation of its bioactive components and their potential applications within food production and drug discovery is needed to maximize its potential benefits, support food security and health initiatives.

Keywords: antioxidant activity, phytochemical screening, hydroxyl, DPPH assay, Total Phenolic Content (TPC)

1.0 Introduction

Cell protection occurs when reactive nitrogen and oxygen species are neutralized by antioxidant compounds [1]. These reactive species cause oxidative damage in the body, leading to the onset of persistent and progressive diseases. Antioxidants act as key agents in preventing disease by suppressing oxidative processes [2]. These are widely used as reactive species responsible for several diseases. Foods are rich in phenols and flavonoids, which are major sources of natural antioxidant protection. Free radicals and reactive oxygen species can be eliminated by natural substances from food with antioxidant properties, preventing oxidative reactions that lead to cellular damage [3]. The tropical West African plant, *Sphenostylis stenocarpa*, commonly known as African yam bean (AYB), is an underutilized legume. Its edible seeds and tubers serve as food ingredients typically made into porridge, though prolonged cooking time poses a practical challenge. The dry seeds contain protein, fat, ash and carbohydrates in about 26%, 10%, 4% and 60%, respectively [4].

Oxidative stress, arising from the excessive generation of reactive oxygen and nitrogen species (ROS/RNS), plays a critical role in the development of numerous chronic diseases, including cancer, cardiovascular disorders and neurodegenerative conditions. Although synthetic antioxidants are commonly employed to mitigate oxidative damage, increasing concerns regarding their toxicity and long-term safety have necessitated the search for safer, naturally derived alternatives. Consequently, attention has shifted toward plant-based sources of antioxidants, particularly those that are underutilized and may possess untapped bioactive potential [5]. *Sphenostylis stenocarpa* is a tropical legume indigenous to West Africa, valued for its high nutritional composition, including significant levels of protein, carbohydrates and essential minerals. Despite its importance in local diets and its adaptability to diverse environmental conditions, AYB remains largely underexploited in scientific research, particularly with respect to its phytochemical composition and functional bioactivity [6]. Existing studies have primarily focused on its nutritional attributes and have reported the presence of phenolic compounds with moderate antioxidant activity [6, 7]. However, these studies are limited in scope, as they do not provide detailed chemical characterization of the seed oil extract or establish clear relationships between specific bioactive compounds and observed antioxidant effects [6, 7].

The major problem addressed in this study is the lack of comprehensive information on the chemical constituents of AYB seed oil and their contribution to its antioxidant activity. Previous investigations have not sufficiently integrated quantitative antioxidant evaluation with advanced analytical techniques capable of identifying and profiling individual compounds. This gap limits the full understanding and potential utilization of AYB in food, nutraceutical and pharmaceutical applications [8]. Therefore, there is need for a more robust analytical approach that combines antioxidant assays with detailed molecular characterization. In response to this need, the present study employs an integrated methodology involving hydroxyl radical scavenging activity assays alongside Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC-MS) analysis to evaluate and characterize the seed oil extract of AYB. This combined approach enables the identification of functional groups and specific bioactive compounds, thereby providing a clearer understanding of the relationship between chemical composition and antioxidant activity.

Furthermore, while earlier research has established the presence of antioxidant activity in AYB [8, 9], there remains a need to identify the dominant compounds responsible for this activity and to assess their potential relevance in industrial applications. This study addresses these gaps by providing detailed chemical profiling and linking the findings to possible applications in food preservation and drug development. Therefore, the aim of this study is to evaluate the antioxidant potential and chemically characterize the seed oil extract of *Sphenostylis stenocarpa*. Specifically, the study seeks to determine the antioxidant activity of the extract using hydroxyl radical scavenging assays, identify the functional groups present through FTIR spectroscopy, characterize the bioactive compounds using GC-MS analysis and relate these findings to the observed antioxidant properties and their potential applications.

Figure 1. Image of AYB plant and seed [13].

AYB plays an important role in subsistence farming systems. It is prepared in various traditional dishes, including moi-moi (bean pudding) and akara (bean cake). It isoften used as a substitute for cowpea [10]. Its adaptability to diverse conditions has been instrumental for its survival, cultivation, and popularity. AYB is an affordable alternative to plant-based protein compared to animal-based proteins [4, 11]. Various AYB accessions (Figure 2) are considered important yet neglected crops that could enhance food security through conservation of genetic material for crop improvement [3].

Figure 2; Different species of AYB [4].

2.0 Materials and Methods

2.1 Collection of the Plant Material

AYB seeds sourced from IITA's Genetic Resources Centre in Ibadan were grown at Ahmadu Bello University Agricultural Research facility in Zaria during the 2022/2023 rainy season. The Nigerian Defence Academy, Department of Biological Sciences, Kaduna confirmed the botanical identity of the plant through herbarium examination. Harvested seeds were air dried to constant mass and mechanically pulverised to obtain a homogeneous powder, which was preserved in airtight glass containers pending analysis.

2.2 Sample Extraction

The extraction of *S. stenocarpa* seed was done after the method discussed in section 2.1 [12]. 250 ml of *n*-hexane was employed per extraction cycle and the system was operated under reflux for 16 hours. Upon the completion of extraction, the solvent phase was concentrated by thermal evaporation, yielding the crude seed oil extract. Oven evaporation of n-hexane resulted in a 2-3 ml of AYB seed extract remaining in the flask. The yield was calculated using equation 1.

$$\% \, Yield \;=\; \frac{weight\ of\ extract}{weight\ of\ sample} \quad 100\% \qquad \text{........ (1)}$$

2.3 Measurement of Antioxidant Activity

Assessment of hydroxyl radical scavenging activity (HRSA) for the AYB was conducted using the laboratory protocols discussed in [12]. 250 ml of the oil extract was added to 1 ml iron ethylenediaminetetraacetic acid (EDTA), 0.5 ml dimethyl Sulfoxide (DMSO) and 0.5 ml ascorbic acid respectively, in a vial. The mixture was incubated for 15 minutes at 80-90°C, cooled and treated using 1 ml of ice-cold trichloroacetic acid and 3 ml of Nash reagent. The process was completed after the exposure of the mixture for 15 minutes. The same procedure was repeated for 500 µg of the AYB seed extract. The absorbance from both compositions was at 412 nm. The % HRSA was calculated using equation 2 [10].

$$\% HRSA \;=\; 1 - \;\frac{Absorbance\ of\ extract}{Absorbance\ of\ control\ sample} \; 100\% \qquad \text{...... (2)}$$

2.4 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

A detector composed of deuterated triglycerine sulphate, possessing a detectivity value of 10^9 cmHz^$^{1/2}$ W^{-1} was used for the spectrum analysis [13]. The samples were prepared by placing specimens between KBr plates. The spectra were obtained in the 4000-400 cm^{-1} middle region at 4 cm^{-1} resolution using 8-128 scans in absorbance mode at room temperature.

2.5 Gas Chromatography Mass Spectrometry (GC-MS) Analysis of *S. stenocarpa* Seed Extract

Chemical profiling of the extract was performed using the gas chromatography-mass spectrometry. Compound separation occurred on a capillary column measuring 30 m in length and 0.25 mm in diameter, with a bonded stationary phase eliminating the need for solid support. The separated compounds were pyrolyzed at 450-615°C, then analysed using full scan mode (m/z 15 – 650) with 70 eV electron impact ionization, causing fragmentation into molecular and fragment ions. The resulting mass spectra were highly reproducible, allowing for compound identification through spectral library matching.

3.0 RESULTS AND DISCUSSION

3.1 Percentage Yield of Extract

Table 1. Percentage yield of extract from 330 g of the sample

Initial weight (g)	Final weight (g)	% Yield
330	15.2	4.60

Table 1 shows the percentage yield of *S. stenocarpa* seed oil extract. A % yield of 4.60% was obtained for *S. stenocarpa*.
The moderate yield obtained in this study suggests that while the oil content is limited, the extract may be concentrated in bioactive compounds, which is supported by subsequent GC-MS results showing high proportions of fatty acids such as linoleic and palmitic acids. Furthermore, variations in yield compared to other studies may arise due to differences in geographical origin, seed variety and extraction conditions, which are known to affect lipid recovery in plant materials [13]. Therefore, the yield reported here provides a useful baseline for evaluating extraction efficiency and optimizing future extraction protocols.

3.2 Measurement of Antioxidant Activity

Table 2. % Hydroxyl Radical Scavenging Activity (HRSA) of *S. stenocarpa*

Sample	Absorbance at 412 nm	% Antioxidant	% HRSA
Control	0.60	-	-
Sample (250 ug)	0.40	33.3	66.6
Sample (500 ug)	0.35	41.6	58.3

Table 2 shows the percentage level of hydroxyl radical scavenging activity at different concentrations for *S. stenocarpa* seed oil extract. The control (no treatment) column indicated the hydroxyl radical level in the absence of the oil extract treatment. The antioxidant activity at 500 µg has an absorbance of 0.35 at 412 nm, and the control has an absorbance of 0.60 at the same wavelength (412 nm). The oil extract was characterised by 41.6% of antioxidant activity and 58.3% HRSA. Similarly, the antioxidant activity of the oil extract of *S. stenocarpa* at 250 µg concentration has an absorbance of 0.40 at 412 nm. The 'control' has an absorbance of 0.60 at 412. However, the antioxidant activity of 33.3% and 66.6% was observed for the sample at 250 µg.

3.3 Fourier Transform Infra-red (FTIR) Spectroscopic Analysis

Figure 3 shows the analysis of the AYB seed oil extract. The Fourier Transform Infra-red (FTIR) infrared spectrum displayed absorption peaks corresponding to various functional groups. Carboxylic acid COOH groups showed strong absorption at 2915.5 and 2843.7 cm^{-1}. Alkyne C≡C stretching appears at 2357.6 and 2330.0 cm^{-1}. The carbonyl C=O stretching of aldehydes, esters, and ketones are detected at 1752.7 cm^{-1}. Additional spectra include the aromatic C=C bonds at 1529.0 cm^{-1}, amine C-N stretches at 1280.4 cm^{-1}, alcohol, carboxylic acid and ether C-O stretches at 1064.8 cm^{-1}, and alkene C=H at 880.0 cm^{-1}.

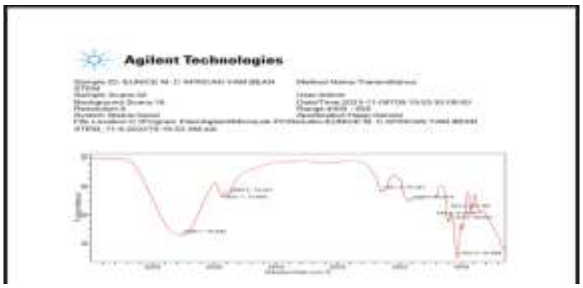

Figure 3; Fourier Transform Infra-red (FTIR) Spectroscopic Analysis

3.4 Gas Chromatogram Mass Spectrometer (GC-MS) Analysis of *S. stenocarpa* Seed Extract

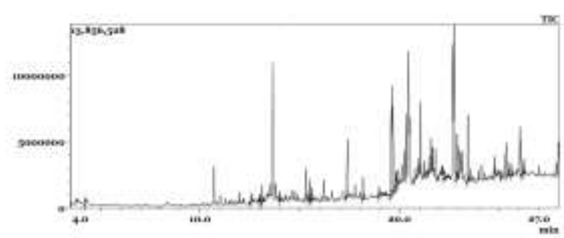

Figure 4: A figure showing the chromatogram of *S. stenocarpa* seed extract

Figure 4 shows the chromatogram of *S. stenocarpa* seed extract. The profile in Table 3 confirmed the presence of 15 major components. The identified compounds include 2,4-Decadienal (E,E) at 0.39%, Dodecanoic acid 2-phenylethyl at 0.16%, 1,3-Benzenedicarboxylic acid at 0.45%, Oxirane 2,2-dimethyl-3-(3,7,12) at 0.11%, 7-Hexadecenal (Z) at 0.62%, Docosanoic acid at 0.09%, 9-Octadecenoic acid (E) at 0.04%, Bis(2-ethylhexyl) phthalate at 0.25%, Pentatriacontane at 0.12%, 9,12-Octadecadienoic acid (Z,Z) at 42.12%, Palmitoleic acid at 0.66%, 4,8,12,16-Tetramethylheptadecane at 0.13%, and Hexanedioic acid bis(2-ethylhexyl) at 0.06%.

The results presented in Figure 3 and Table 2 reveal that the seed oil extract of *Sphenostylis stenocarpa* contains a diverse array of bioactive compounds, with major constituents such as 9,12-octadecadienoic acid (linoleic acid), n-hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid) occurring in significant proportions. The predominance of these fatty acids, particularly linoleic acid, which is widely reported to possess antioxidant, anti-inflammatory and cardioprotective properties, suggests that the observed antioxidant activity of the extract is strongly linked to its chemical composition [13]. The presence of additional minor compounds further indicates a possible synergistic effect contributing to the overall bioactivity of the extract.

Table 3. Profile of compounds from GC-MS Analysis of *S. stenocarpa seed extract*

S/N	RT	Names of Compounds	Molecular Formula	Molecular weight	Area%
1.	8.982	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152	0.39
2.	9.601	2-Dodecenal, (E)	$C_{12}H_{22}O$	182	0.06
3.	11.676	2,6,10-Dodecatrien-1-ol, 3,7,11	$C_{15}H_{26}O$	222	0.05
4.	12.114	Dodecanoic acid	$C_{12}H_{42}O_2$	200	0.15
5.	12.455	2,2,4-Trimethyl-1,3-pentanediol	$C_{16}H_{30}O_4$	286	0.32
6.	14.447	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.98
7.	15.760	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.22
8.	16.731	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.17
9.	17.189	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.05
10.	17.598	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	30.56
11.	18.365	Oleic Acid	$C_{18}H_{34}O_2$	282	0.14
12.	18.973	9,12-Octadecadienoic acid (Z,Z)	$C_{19}H_{34}O_2$	294	0.18
13.	19.053	9-Octadecenoic acid, methyl est	$C_{19}H_{36}O_2$	296	0.30
14.	19.846	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	42.12
15.	19.919	9,12-Octadecadienoic acid (Z,Z)	$C_{22}H_{42}O_2$	338	13.11

The implications of these findings are substantial for both food and pharmaceutical applications. In the food industry, the identified compounds, especially unsaturated fatty acids, could serve as natural antioxidants for food preservation, helping to delay lipid oxidation and extend shelf life [14]. In pharmaceutical and nutraceutical contexts, these bioactive constituents may be explored for the development of plant-based therapeutic agents targeting oxidative stress-related diseases [14]. Furthermore, the identification of industrially relevant fatty acids highlights the potential of *S. stenocarpa* seed oil as a raw material in cosmetics, functional foods and bioactive formulations.

The relevance of these results to the present study lies in their direct support of the study's aim, which is to evaluate the antioxidant potential and chemically characterize the seed oil extract. The GC-MS analysis provides molecular-level evidence of compounds known for antioxidant activity, thereby validating the results obtained from the hydroxyl radical scavenging assay. This establishes a clear relationship between the chemical profile of the extract and its functional antioxidant properties, strengthening the scientific basis of the study.

To a considerable extent, these findings are consistent with previous studies on *S. stenocarpa* and other leguminous seeds, which have reported the presence of fatty acids such as linoleic and palmitic acids as dominant constituents with notable antioxidant and biological activities [15]. Similar GC-MS-based studies on plant seed oils have also identified these compounds as key contributors to antioxidant capacity, thereby supporting the reliability and reproducibility of the present results [13, 14, 15]. However, variations in the relative abundance of compounds may occur due to differences in geographical origin, extraction methods and environmental conditions, which could explain minor discrepancies with earlier reports.

CONCLUSION

The results of this study demonstrate that *Sphenostylis stenocarpa* seed oil contains significant bioactive compounds, particularly linoleic acid, palmitic acid, and stearic acid, which are known to contribute to antioxidant activity. This confirms its potential as a natural source of antioxidants with applications in food preservation, nutraceutical development and pharmaceutical formulations targeting oxidative stress–related diseases. The findings are directly relevant to the study as they establish a clear link between the chemical composition of the extract and its observed antioxidant properties.

This study contributes new knowledge by providing detailed GC-MS characterization of AYB seed oil and integrating it with antioxidant activity data, thereby strengthening the understanding of its structure–activity relationship. It also refines existing knowledge by highlighting the seed oil fraction - an area previously underexplored - as a valuable source of bioactive compounds. Overall, this study reinforces the potential of this underutilized legume as a functional resource for health and industrial applications while opening avenues for further targeted research.

Nevertheless, there are still areas that require further investigation. Future studies should focus on isolating and purifying the major bioactive compounds to evaluate their individual antioxidant capacities and mechanisms of action. In addition, in vivo studies and toxicity assessments are necessary to validate their safety and efficacy for therapeutic applications. There is also a need to explore other extraction techniques that may improve yield and preserve sensitive bioactive compounds.

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