# ANTIOXIDANT POTENTIAL AND GC-MS CHARACTERIZATION OF THE SEED OIL EXTRACT OF AFRICAN YAM BEAN (*Sphenostylis stenocarpa*)

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**ABSTRACT**

The African yam bean (*Sphenostylis stenocarpa*), a tropical underutilized legume, has gained research interest due to its nutritional and potential health benefits. This study investigated the antioxidant activity of aqueous and 70% acetone extracts of six accessions of *Sphenostylis stenocarpa* to evaluate their potential as natural antioxidants. The seeds were obtained from the Genetic Resources Center, International Institute of Tropical Agriculture (IITA) and cultivated at the Research Farm of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. They were air-dried, ground into fine powder and subjected to solvent extraction using the Soxhlet apparatus. Antioxidant assays, including hydroxyl radical scavenging activity (HRSA), were conducted following standard protocols. Results showed that at a concentration of 500 µg, the *S. stenocarpa* extract exhibited an antioxidant activity of 41.6%, while at 250 µg, the activity was 33.3%. Fourier Transform Infrared (FTIR) analysis identified functional groups corresponding to carboxylic acids, aldehydes, esters and aromatic rings; indicating the presence of bioactive compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of various bioactive compounds, including *n*-hexadecanoic acid (30.56%), 9,12-octadecadienoic acid (Z,Z) (42.12%), and octadecanoic acid (4.34%), known for their antioxidant properties. The findings suggested that *S stenocarpa* possesses significant antioxidant activity, making it a potential source of natural antioxidants. Further research into its bioactive components and their applications in food and pharmaceutical industries is recommended to enhance its utilization and promote food security.

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

## INTRODUCTION

Antioxidants are substances that scavenge or slow down the activities of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which inhibit oxidative mechanisms that lead to chronic and degenerative diseases [1,2]. Recently, the use of antioxidants has been encouraged due to the implication of ROS and RNS in virtually all diseases. Phytochemicals such as phenol and flavonoids, which are widely present in foods have been shown to possess anti-oxidative power, thereby making food the natural source of these antioxidants. Evaluation of the antioxidant activities of natural substances has been of interest in recent years. Antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases. [Adewale *et. al.,* 2012]

African yam bean (*Sphenostylis stenocarpa*)

The African yam bean (AYB) (*Sphenostylis stenocarpa*) as seen in Figure 1, is a tropical underexploited grain legume, is grown in West Africa; as a security crop by peasant farmers, for both its edible seeds and tubers. The seed is usually cooked as a local porridge mixed with other ingredients. The mature seed contains on dry weight basis, 25.67% protein, 9.81% fat, 3.87% ash and

60.65% carbohydrates. It has a characteristic problem of being hard-to-cook and is largely underutilized as a minor grain legume, even when it is an important food crop in tropical Africa [Olaiya *et. al.,* 2016].


Figure 1. Image of AYB plant and seed [Olaiya *et. al.,* 2016].

AYB will continue to be a valuable constituent of African peasant agriculture. Rural producers of bean pudding (moi-moi) and bean cake (akara) sometimes replace cowpea with African yam bean [3]. Its inherent ability to adapt to diverse environments might have contributed largely to its survival and widespread use. Underutilized legumes increase the demand for plant protein in lieu of expensive animal protein [4]. The different accessions of AYB (Figure 2.) ranks well among neglected crops and can contribute to food security if its genetic resources are saved for utilization in breeding and improvement [3].

The seed is a highly priced food legume in Nigeria owing to high crude protein content, with an enduring reputation in the food and culture of the Igbos and the Yorubas [5]. There is no known work on its innate antioxidative properties. This research examines the effect of antioxidant capacity of the yam bean phenolics as well as their characterisation using GC-MS.

Figure 2 Different species of AYB [Adebowale *et. al.,* 2009].

## MATERIALS AND METHODS

### Collection of the Plant Material

AYB seeds were obtained from the Genetic Resources Center, International Institute of Tropical Agriculture (IITA) and planted at the Research Farm of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria during the 2022/2023 planting season. The plant was authenticated at the herbarium section of the Department of Biological Sciences, Nigerian Defence Academy, Kaduna. The seeds were air dried under shade, then ground to a fine powder by using vibrating cup mill machine. The ground sample was stored in an airtight glass jar until use [6].

### Sample Extraction

The extraction of *S. stenocarpa* seed was done through the method [6] with a little modification [6]. About 250 ml of n-hexane was added to a round bottom flask, which was attached to a Soxhlet extractor connected to a heating mantle. The ground AYB powder was loaded into the thimble, which was placed inside the Soxhlet extractor. The side arm was lagged with glass wool. The solvent was heated using the heating mantle, for evaporation and condensation to occur. Once the level of solvent reached the siphon it poured back into the flask and the cycle continued for a total of 16 hours. The n-hexane was evaporated using an oven, leaving a small yield of extracted AYB seed material (about 2 to 3 ml) in the glass bottom flask. The yield was calculated using the formula below:

$$\%\ Yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times 100\%$$

### Antioxidant Assay

Hydroxyl radical scavenging (HRSA) of AYB was estimated [7]. The oil extract (250 ml) was taken and 1ml of iron EDTA solution, 0.5ml of EDTA solution, 1ml of DMSO and 0.5ml of ascorbic acid were added to a glass vial. The mixture was incubated in a water bath at 80 to 90°C for 15 min. After incubation, 1ml of ice-cold trichloroacetic acid and 3ml of Nash reagent were added, then the reaction mixture was incubated at room temperature for 15 min. The procedure was repeated with a volume of 500 µg of the AYB seed extract. The absorbance from both compositions was read at 412 nm. The % HRSA was calculated by the following formula:

$$\%\ HRSA = 1 - \frac{Absorbance\ of\ extract}{Absorbance\ of\ control\ sample} \times 100\%$$

### Fourier Transform Infra-red (FTIR) Spectroscopy

The FTIR spectra was recorded in the middle infrared (4000 $cm^1$ to 400 $cm^1$) with a resolution of 4 cm-1 in the absorbance mode for 8 to 128 scans at room temperature. 25ml of the samples for FTIR analysis was

placed between the KBr plate. The spectra were measured using a deuterated triglycerinesulphate detector (DTGS) with a specific detectivity of 109 cmhz1/2 w-1 .

**Gas Chromatography Mass Spectrometry (GC-MS) of *S. stenocarpa* seed extract**

A 30 cm ´ 0.25 mm capillary column was used, 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.

**RESULTS AND DISCUSSION**

Percentage Yield of Extract The percentage yield of *S. stenocarpa* seed oil extract of was 4.60%, deduced from an initial weight of 330 g and final weight of 15.2 g.

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

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

Antioxidant Assay

Table 1 shows %HRSA

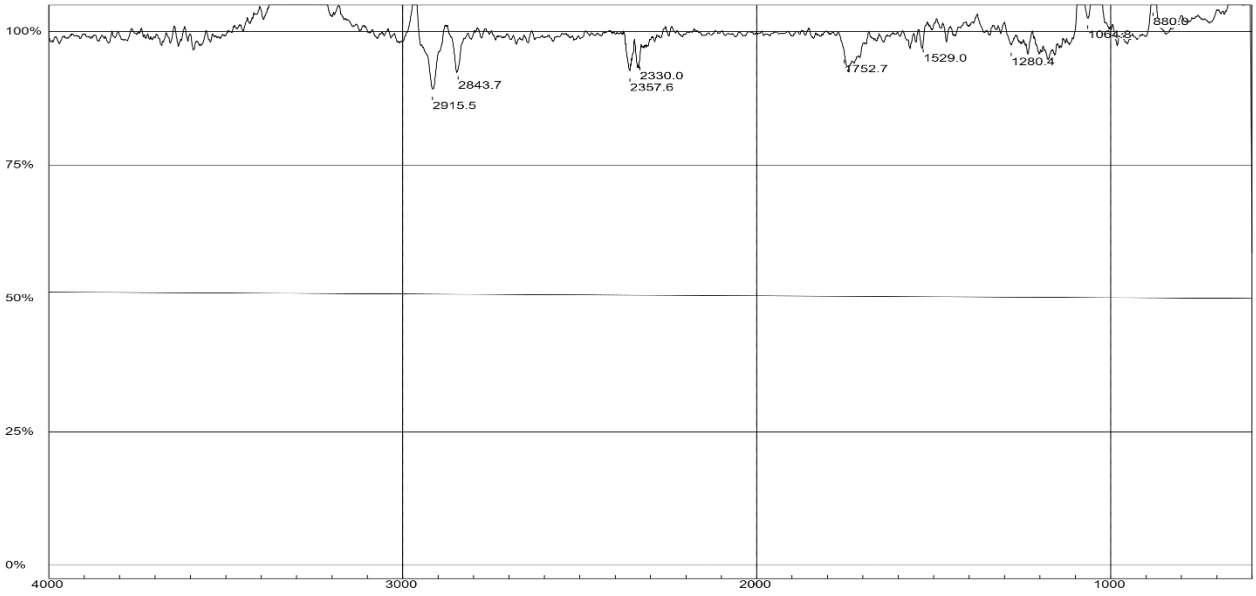
| | Absorbance at 412nm | %Anti-oxidant | %HRSA |
|---|---|---|---|
| Control | 0.60 | - | - |
| Sample (250ug) | 0.40 | 33.3 | 66.6 |
| Sample (500ug) | 0.35 | 41.5 | 58.3 |

From **table 1**, the result of the antioxidant activity of the oil extract of (*sphenostylis stenocarpa*) at 500µg concentration showed 0.35 absorbance at 412 nm and the control showed 0.60 absorbance at 412 nm. The oil extract was also recorded to have41.6% of antioxidant activity while the control was recorded to have 58.3 % of antioxidant activity. The antioxidant activity of the oil extract of *S. stenocarpa* at 250µg concentration showed 0.40 absorbance at 412 nm and the control showed 0.60 absorbance at 412. The oil extract was also recorded to have 33.3% of antioxidant activity while the control was recorded to have 66.6% of antioxidant activity. Table 4 showed 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.

**Fourier Transform Infra-red Spectroscopy (FTIR)**

Figure 3 shows the FTIR spectrum of *S. stenocarpa* seed extract. There is strong absorption at 2915.5cm-1 and 2843.7cm-1 are due to COOH of carboxylic acids; bands at 2357.6cm-1 and 2330.0cm-1 indicate the present of CºC stretching of alkynes, the peaks at 1752.7$^{-1}$ is due to C=O of aldehydes, ester and ketones. Other peaks at 1529.0cm-1 indicates the presence of C=C of aromatic rings, 1280.4cm-1 indicates the present of C-N of amines, 1064.8cm-1 is due to C-O of alcohols, ether and carboxylic acid and the peak at 880.0cm-1 show the present of alkenes group of C=C

Figure 3. FTIR spectrum of *S. stenocarpa* seed extract




4.4 Fourier Transform Infrared Spectroscopy (FTIR) The IR spectrum of sample E at fig 3. show strongly absorption at 2915.5cm$^{-1}$ and 2843.7$^{-1}$ which is due to the COOH of carboxylic acids, 2357.6$^{-1}$ and 2330.0

indicate the present of C≡C stretching of alkynes 1752.7$^{-1}$ is due to C=O of aldehydes, ester and ketones, 1529.0$^{-1}$ due to the present of c=c of aromatic rings, 1280.4$^{-1}$ indicate the present of C-N of amines,1064.8$^{-1}$ which is due to C-O of alcohols, ether and carboxylic acid, 880.0$^{-1}$ show the present of alkenes group of C–H

**Gas Chromatogram Mass Spectrometer Analysis**

The GC-MS analysis of Figure 4 shows the chromatogram of *S. stenocarpa* seed extract. The profile in Table 2. confirmed the presences of 32 major components. Some of these compounds include 2,4-Decadienal, (E,E)- (0.39%) Dodecanoic acid, 2-phenylethyl (0.16%) 1,3-Benzenedicarboxylic acid, b (0.45%) Oxirane, 2,2-dimethyl-3-(3,7,12) (0.11%) 7-Hexadecenal, (Z) (0.62%) Docosanoic acid (0.09%) 9-Octadecenoic acid, (E) (0.04%) Bis(2-ethylhexyl) phthalate (0.25%) Pentatriacontanen (0.12%), 9,12-Octadecadienoic acid (Z,Z) (42.12), Palmitoleic acid (0.66), 4,8,12,16-Tetramethylheptadeca (0.13), Hexanedioic acid, bis(2-ethylhe) (0.06).

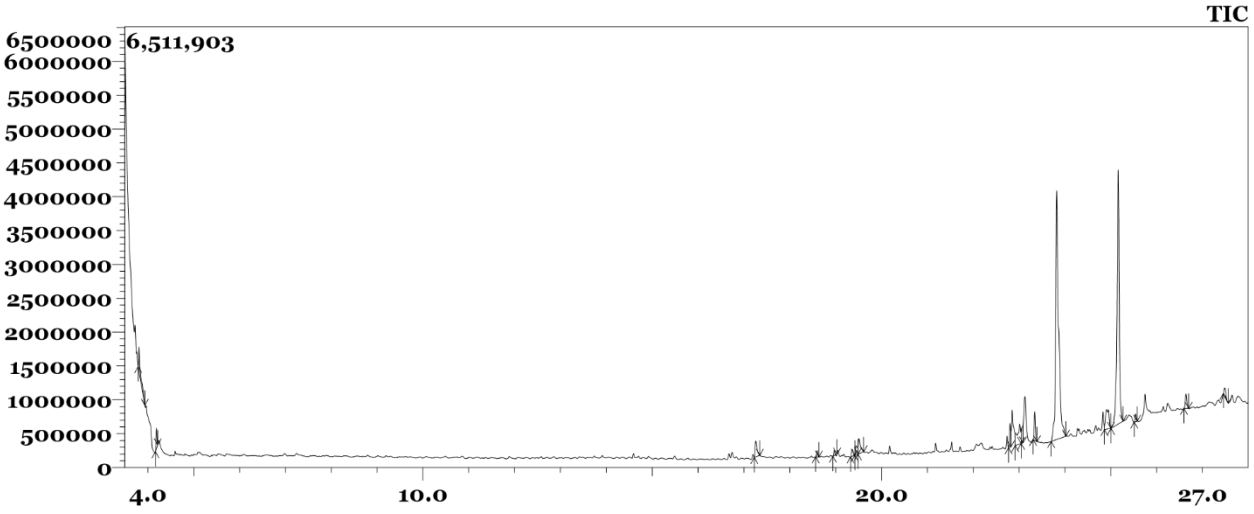



Figure 4. Chromatogram of *S. stenocarpa* seed extract

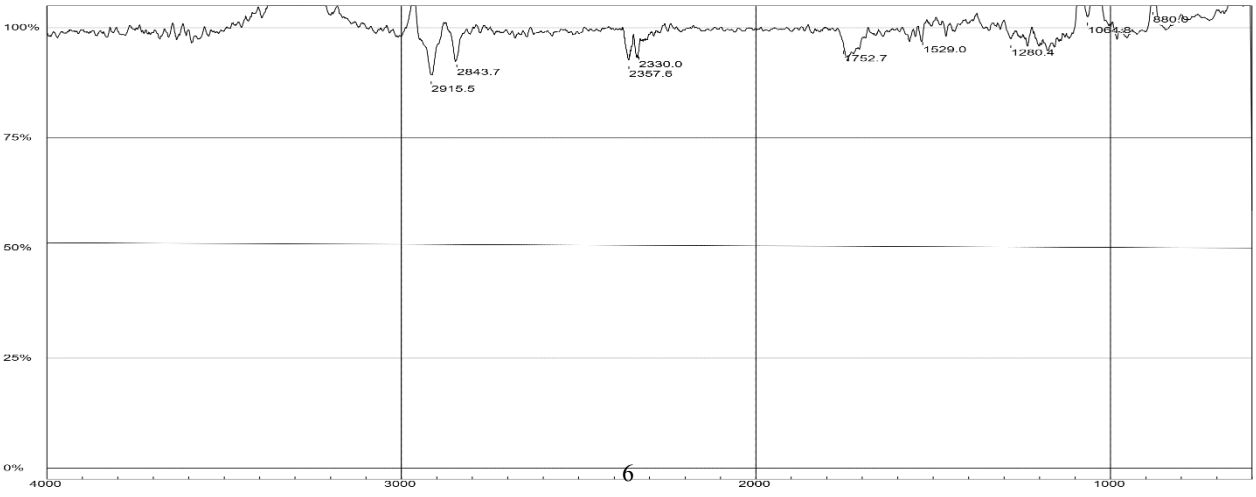


**Table 2. Profile of compounds from GC-MS Analysis of *S. stenocarpa* seed extract**

| S/N | RT | Compound Name | 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-pentanedio | $C_{16}H_{30}O_4$ | 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 |

## CONCLUSION

The investigation into the antioxidant activity of aqueous and 70% acetone extracts from six accessions of *Sphenostylis stenocarpa* has provided valuable insights into the potential of this underutilized tropical legume as a source of natural antioxidants. The results demonstrated that the seed extracts exhibited notable antioxidant activity, with 41.6% hydroxyl radical scavenging activity at a concentration of 500 µg and 33.3% at 250 µg. These findings highlight the presence of bioactive compounds within *S. stenocarpa* that contribute to its antioxidant properties, affirming its nutritional and potential health benefits. The study underscores the importance of exploring underutilized crops like the African yam bean to address nutritional deficiencies and promote sustainable agricultural practices.

Analytical techniques, including Fourier Transform Infrared (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS), further elucidated the chemical composition of the extracts. FTIR analysis confirmed the presence of functional groups such as carboxylic acids, aldehydes, esters, and aromatic rings, which are indicative of bioactive compounds with antioxidant capabilities. The GC-MS analysis of African Yam Bean *S. stenocarpa* seed oil successfully identified its chemical constituents. The IR spectrum show the present of functional groups such as the COOH of carboxylic acids, CºC stretching of alkynes, C=O of aldehydes, ester and ketones, C=C of aromatic rings, C-N of amines, C-O of alcohols, ether and carboxylic acid, alkenes group of C-H. GC-MS analysis identified key compounds, including n-hexadecanoic acid (30.56%), 9,12-octadecadienoic acid (Z,Z) (42.12%), and octadecanoic acid (4.34%), known for their antioxidant and health-promoting properties. These findings provide a robust foundation for understanding the chemical basis of *S. stenocarpa*'s antioxidant activity and its potential applications.

The significant antioxidant activity of *S. stenocarpa* suggests its potential as a valuable resource in the food and pharmaceutical industries, where natural antioxidants are increasingly sought after for their safety and efficacy. Incorporating *S. stenocarpa* into functional foods, dietary supplements, or pharmaceutical formulations could enhance its utilization, contributing to food security and public health, particularly in tropical regions where the crop is cultivated. Moreover, the study highlights the need to promote underutilized legumes to diversify agricultural systems and reduce reliance on conventional crops, thereby supporting sustainable development goals.

This study demonstrated the efficacy of GC-MS in characterizing bioactive compounds present in *S. stenocarpa* seed oil, paving the way for further research into its potential applications in food, pharmaceutical and industrial sectors

## REFERENCES


[1] Cardador-Martínez, A., Loarca-Piña, G., & Oomah, B. D. (2002). Antioxidant activity in common beans (*Phaseolus vulgaris L.*). *Journal of Agricultural and Food Chemistry*, 50(24), 6975-6980. https://doi.org/10.1021/jf020296n

[2] Olaiya, C. O., Soetan, K. O., & Akinmusire, O. O. (2016). Evaluation of the antioxidant activity of flavonoids in medicinal plants. *Journal of Medicinal Plants Research*, 10(30), 451458. https://doi.org/10.5897/JMPR2016.6252

[3] Adewale, B. D., Daniel, I. O., Aremu, C. O., Adebisi, M. A., & Oyelade, O. J. (2012). Genotypic variability and stability of some nutritional properties of African yam bean (Sphenostylis stenocarpa Hochst ex A. Rich. Harms). *International Journal of Agriculture and Food Science Technology*, 3(4), 317-326.

[4] Adebowale, Y. A., Afolabi, T. J., & Lawal, O. T. (2009). Comparative study of the functional properties of mucuna bean (*Mucuna pruriens*) and soybean (*Glycine max*) flour. *Food Hydrocolloids*, 23(4), 1209-1214. https://doi.org/10.1016/j.foodhyd.2008.08.014

[5] Asoiro, F. U., & Ani, A. O. (2011). Determination of some physical properties of African yam bean (Sphenostylis stenocarpa) seeds. *Pertanika Journal of Science & Technology*, 19(2), 389-396.

[6] García-Ayuso, L. E., Monente, C., Torija, M. E., Garrido, I., & Lebrón-Aguilar, R. (2017). Extraction of bioactive compounds from coffee pulp using microwave-assisted extraction. *Food Chemistry*, 221, 198-207. https://doi.org/10.1016/j.foodchem.2016.10.081

[7] Halliwell, B., & Gutteridge, J. M. C. (1981). Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Letters*, 128(2), 347-352. https://doi.org/10.1016/0014-5793(81)80114-7