

Characterization of *Senna Occidentalis* (Link.) Seed Extract

Muhammad Idris Misau^{1*}, Muhammad Zannah Lawan², Jato Isaac², Garba Kabir³,
Abdulkarim Abdulwadud Yusuf³, Ahmed Saeed Isa³, Ibrahim Maryam³

Abstract

Senna occidentalis (L.) Link, commonly known as “coffee senna” or majamfari/rai dore in Hausa, is a plant with a long history of traditional and medicinal uses against diseases such as inflammation, pains, and diabetes. This study presents the characterization of the bioactive compounds of the seed extracts. Physicochemical analysis of seed powder was carried out following standard procedures. Qualitative phytochemical contents were determined for the presence and absence of major metabolites. Quantitative phytochemical contents (total phenolic and flavonoid) were determined by Folin-Ciocalteu, aluminum chloride colorimetric assay, and total alkaloidal content by titrimetric methods, while saponins, tannins, proteins, oxalates, anthraquinones, and cardiac glycoside contents were determined by spectrophotometric procedures. The seed extract was also characterized using gas chromatography-mass spectrometry (GC-MS) and X-ray diffraction (XRD). Phytochemical content of aqueous extract revealed the presence of alkaloids, saponins, flavonoids, anthraquinones, tannins, cardiac glycosides, oxalates, and phenolics. The GC-MS analysis showed the presence of 17 bioactive components with a retention time of 5.52 to 29.43 min, mainly amide-containing compounds and fatty acids with their respective mass-to-charge ratios (m/z) in aqueous extract such as bicyclo [3.1.0] hexan-3-one (RT: 15.78 min, PA: 27.78%, m/z : 96 mol/g). Quantitative phytochemical analysis showed that the seed had a total phenolic content of 1375.5 mg GAE/100 dry weight, a total flavonoid content of 441.2 mg RE/100 g dry weight, a total alkaloids value of –25.64%, a total saponin value of 15.01%, and a total tannin value of –50.92%. The XRD showed the presence of urea, syn 77 (11%), toxic components such as graphite 23 (11%), muscovite 0.1 (9%), and cristobalite 0.4 (11%). The study showed that *S. occidentalis* seed contains bioactive compounds that are beneficial and harmful (dianthrone, an anthraquinone-derived compound that affects mitochondrial function) to the body when used for medicinal purposes. Characterization of these compounds from the seed thus provides important fingerprints for standardization, detoxification, and optimization conditions of the seeds for safe medicinal applications.

*Author for Correspondence

Muhammad Idris Misau
E-mail: immuhd@atbu.edu.ng

¹Professor, Department of Chemical Engineering, Federal Polytechnic, N’Yak, Sendam LGA, Plateau State, Nigeria

²Scholar, Department of Chemical Engineering, Faculty of Engineering & Engineering Technology, Abubakar Tafawa Balewa University, Bauchi, Nigeria

³Professor, Department of Chemical Engineering, Faculty of Engineering & Engineering Technology, Abubakar Tafawa Balewa University, Bauchi, Nigeria

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INTRODUCTION

Senna occidentalis (L.) Link, commonly known as “coffee senna”, “Rai dore or Majamfari” in Hausa, is a plant with a long history of traditional and medicinal uses against diseases such as inflammation, pains, and diabetes. The study presents characterization of the bioactive compounds of the seed extracts. Coffee *Senna* seeds contain a plethora of bioactive compounds, including flavonoids, phenolic acids, and anthraquinones, which have been shown to possess significant therapeutic potential. The extraction and

characterization of these bioactive components are crucial for developing effective medicinal applications, particularly for conditions such as arthritis, myalgia, and malaria (AMM) [1, 2].

The bioactive components extracted from *Senna occidentalis* seeds have demonstrated various pharmacological activities. Studies have highlighted their potential in treating inflammatory conditions, microbial infections, and oxidative stress-related diseases. For instance, anthraquinones have shown significant anti-inflammatory and antimicrobial properties, making them useful in treating arthritis and infections [3, 4]. Recent pharmacological studies have provided deeper insights into the anti-inflammatory, antimicrobial, and antioxidant activities of the extracts, supporting their potential use in treating various ailments [5, 6].

The extraction of bioactive compounds from *Senna occidentalis* seeds employs various methods, with Soxhlet extraction being one of the most prevalent. This technique uses both polar and non-polar solvents like aqueous solutions, ethanol, and n-hexane, which significantly affect the yield and composition of the extracted components [7, 8]. Recent advancements have also seen the development of greener extraction methods aimed at improving sustainability and efficiency [9].

Additionally, the polarity of a solvent affects the extraction efficiency and the type of bioactive compounds that can be isolated. Polar solvents are typically more effective at extracting polar compounds, including flavonoids, glycosides, and phenolic acids, which are known for their medicinal properties [10]. Non-polar solvents, on the other hand, are more suited for the extraction of non-polar compounds, such as essential oils and fatty acids, which are integral in various therapeutic and aromatic applications [11].

However, water is non-toxic, non-flammable, and environmentally benign, making it a safer alternative to organic solvents for both the operator and the environment [12]. Water is inexpensive and readily available, reducing the overall cost of the extraction process [13]. Water's polarity allows it to selectively extract polar bioactive compounds, which are often responsible for the medicinal properties of plant materials [14]. Aqueous extracts often exhibit enhanced bioactivity due to the presence of water-soluble phytochemicals, which can improve the efficacy of medicinal formulations [15]. Water is compatible with green extraction technologies, such as ultrasound-assisted extraction and microwave-assisted extraction, which can further enhance extraction efficiency and sustainability [16].

Finally, characterization of the extracted bioactive compounds is essential for identifying and quantifying the constituents within *Senna* coffee (*Senna occidentalis*) seeds using advanced analytical techniques; thermogravimetric analysis (TGA) assesses the thermal stability and composition of extracts by measuring weight loss upon heating [17]. Gas chromatography-mass spectrometry (GC-MS) separates and identifies volatile and semi-volatile organic compounds [18].

This paper tends to report the findings from the characterization of *Senna Occidentalis* seed that result in identifying the bioactive components present, aiming at identifying the toxic components to enhance their medicinal applications.

MATERIALS AND METHODS

Collection and Identification Plant Materials

Senna occidentalis dried pods were collected from a forest in Maiduguri, Nigeria, and the seeds were collected in a clean container. They were taken to the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Borno State, for taxonomic identification and authentication. A voucher specimen number of UMM/FPH/FAA/009 was deposited for the coffee *Senna* (*Senna occidentalis*) seeds in the herbarium of the Department of Pharmacognosy.

Preparation and Characterization of *Senna Occidentalis* Seeds

The seeds were cleaned thoroughly to remove dirt, debris, and other impurities. This is typically done by washing the seeds with distilled water and allowing them to air dry for 72 hours. The dried seeds

weighing 3000 g were grind into fine powder using a mechanical grinding machine (HM95, Japan), weighed, and extracted using distilled water (aqueous), ethanol, and n-hexane. The seed extracts and powder were then concentrated in vacuum and characterized using various techniques to provide valuable information about their thermal behavior, crystalline structure, chemical compositions, and phyto-chemical constituents [19].

Phytochemical Screening of *Senna Occidentalis* Seeds (SOS)

The phytochemical screening of *S. occidentalis* aqueous, ethanol, and n-hexane seed extract was carried out following the procedures described by [20–24], Phytochemical constituents of some Nigerian medicinal plants, 2021 [25] to determine the presence and absence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, anthraquinones, cardiac glycosides, phenolics, and proteins.

Proximate and Ultimate Analysis of SOS Powder

The method for proximate analysis was carried out to determine the nutritional components of moisture content, ash content, crude protein, crude fat, crude fiber, and nitrogen-free extract (NFE) of the SOS [26, 27].

The moisture content was determined by weighing 5 grams of the powdered seed and placing it in a pre-weighed moisture dish. Then, dry the sample in an oven at 105°C for 6 hours. Finally, the percentage of moisture content was determined using Equation (1).

$$\text{Moisture Content (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 10 \quad (1)$$

The ash content was obtained by weighing 2 grams of dried seed powder and placing it in a crucible. Thereafter, incinerate the sample in a muffle furnace at 550°C for 6 hours until a constant weight was achieved. The ash content was calculated using Equation (2).

$$\text{Ash content (\%)} = \frac{\text{Weight of Ash}}{\text{Initial Weight}} \times 100 \quad (2)$$

The crude protein percentage was evaluated by digesting 1 gram of seed powder with concentrated sulfuric acid using a Kjeldahl digestion apparatus. After which, distilled the digested sample and titrated the distillate with 0.1 N hydrochloric acid. Equation (3) was used to determine the nitrogen content by the Kjeldahl method and multiplying by a factor of 6.25.

$$\text{Crude Protein (\%)} = \text{Nitrogen Content} \times 6.25 \quad (3)$$

The crude fat content was determined by weighing 2 grams of seed powder and placing it in a Soxhlet extractor. Extraction was made using petroleum ether for 6 hours. The crude fat content was obtained using Equation (4).

$$\text{Crude Fat (\%)} = \frac{\text{Weight of Extracted Fat}}{\text{Initial Weight}} \times 100 \quad (4)$$

The crude fiber was evaluated by digesting 2 grams of defatted seed powder with 1.25% sulfuric acid and 1.25% sodium hydroxide and then filtered, dried, and weighed the residue. Also, incinerated the residue in a muffle furnace at 600°C for 2 hours. The crude fiber content was calculated using Equation (5).

$$\text{Crude Fiber (\%)} = \frac{\text{Weight of Residue} - \text{Weight of Ash}}{\text{Initial Weight}} \times 100 \quad (5)$$

Nitrogen-free extract (NFE) was the portion of the SOS grinded powder that remained after subtracting the nitrogenous compounds (primarily proteins) from the total dry matter. It represents the carbohydrate fraction, including sugars, starches, and other non-nitrogenous compounds. NFE is an important parameter in nutritional analysis as it provided an estimate of the energy-yielding carbohydrates in the SOS grinded powder. Therefore, the NFE was performed using Equation (6).

$$NFE (\%) = (Moisture + Ash + Crude Protein + Crude Fat + Crude Fiber) \quad (6)$$

Ultimate analysis was conducted to determine the elemental composition of SOS, powder such as carbon, hydrogen, nitrogen, and sulfur. The oxygen content was obtained by difference. 2 mg of SOS powder was loaded into the combustion chamber of the CHNS elemental analyzer, and the furnace is heated to 850°C under a constant flow of helium and oxygen. Upon combustion, the sample generates uniform compound gases of the elements C, H, N, and S.

These combustion products, such as CO₂, H₂O, and NO₂, were measured using gas chromatography, and thus the ratio of the elements in the original sample is determined. C, H, and N and S can all be determined simultaneously; the data is usually collected by TrueSpec LECO software. The oxygen content is thereafter calculated using Equation (7).

$$O\% = 100\% - (Ash - C - H - N - S) \quad (7)$$

Gas Chromatography-Mass Spectrometry (GC-MS) of SOS Extracts

The GC-MS analysis of *S. occidentalis* seeds was carried out in an Agilent 7890A (Agilent Technologies, UK) GC coupled to an MS detector. The operational temperature was 50°C which was later optimized to 200°C.

The flow rate was 2 µL/min, while the total runtime was 25 min. Sample injection was carried out by auto-injection; the carrier gas was helium [22, 5].

Thermogravimetric Analysis (TGA)

The TGA was carried out to determine the effects of heat on the seed powder. Various chemical compositions of the seed, such as organic constituents, were also determined using the TGA [27–29]. The TGA was performed to describe the characteristics of the inorganic constituents in the seed powder in relation to temperature. A small amount of the powder weighing 10 g was used for the analysis under standard atmospheric nitrogen and a 20 mL/min flow rate using the Shimadzu TGA apparatus with a temperature range of 100 to 1000°C and a 10°C/min heating rate.

X-ray diffraction (XRD)

The crystal structure of the seed powder was determined using the XRD following previously described methods [30–34]. The X-ray diffraction (XRD) patterns of the powdered seeds were recorded on an Empyrean range multi-purpose diffractometer (Malvern Panalytical Ltd., UK) at a wavelength of 0.15406 Å.

RESULTS AND DISCUSSION

Results

Proximate and Ultimate Composition of Seed Powder

The results from the proximate analysis of seeds showed that nitrogen-free extract was the highest with a value of 46.96%, followed by crude protein content of 18.59% w/w. The ultimate analysis further showed that carbon and oxygen compositions were 45.93% w/w and 39.19% w/w, respectively (Table 1).

Table 1. Proximate and ultimate compositions of SOS.

Parameters	Value
Proximate Contents (% w/w)	
Moisture content	7.96
Ash content	4.42
Crude protein	18.59
Crude fat (lipid)	8.43
Crude fiber	13.63
Nitrogen-free extract (NFE)	46.96

Ultimate Contents	
Carbon	45.93
Hydrogen	6.43
Nitrogen	3.21
Sulphur	0.50
Oxygen	39.19

Phytochemical Contents of SOS

The qualitative phytochemical screening of SOS extracts revealed the presence of alkaloids, flavonoids, anthraquinones, tannins, saponins, and proteins (Table 2).

$$Y = 0.002x + 0.08998 \quad (8)$$

$$R^2 = 0.592 \quad (9)$$

$$Y = 0.001x + 0 \quad (10)$$

$$R^2 = 0. \quad (11)$$

Table 2. Qualitative phytochemical profiles of SOS extracts.

S.N.	Constituent	Test	Observation	n-hexane	Ethanol	Aqueous
1	Phenols	Ferric chloride	Green color	–	+	+
2	Saponins	Frothing	Froth formation	–	+	+
3	Proteins	Ninhydrin	Purple color	+	+	+
4	Alkaloids	Dragendorff	Orange or red precipitate	–	+	+
5	Flavonoids	Shinoda	Pink color	+	+	+
6	Tannins	Gelatin	White precipitate	–	+	+
7	Oxalate	Ammonium hydroxide	White precipitate	–	+	+
8	Anthraquinones	Ammonium hydroxide	Formation of red color	–	+	+
9	Cardiac glycosides	Bromine water	Yellow precipitate	–	+	+

Note: + means present and – means absent.

Table 3. Quantitative phytochemical contents of *S. occidentalis* seed extracts.

Phytochemical	Value/Unit
Total phenolics	1375.5 mg GAE/100 g dry weight
Total flavonoids	441.2 mg RE/100 g dry weight
Total alkaloids	–25.64%
Total saponins	15.01%
Total tannins	–50.92%
Total proteins	18.59 %
Total oxalates	13.26 %
Total cardiac glycosides	269.43 %
Total anthraquinones	0.34 %

On the other hand, the quantitative phytochemical contents (Table 3) revealed the total phenolic content (1375.5 mg GAE/100 g dry weight, total flavonoid content (441.2 mg RE/100 g dry weight), total alkaloid (–25.64%), saponins (15.01%), tannins (–50.92%), proteins (18.59%), oxalates (13.26 %), cardiac glycosides (269.43 %), and anthraquinones (0.34%). Total phenolic content (TPC) and total flavonoid content (TFC) were obtained from the gallic acid and rutin calibration curves, respectively, from, for example [2, 20–24, 35, 36]. In Figure 1, the graph depicts the GC-MS profiles of *S. occidentalis* extracts.

XRD Patterns of *S. occidentalis* Seed Powder

The seed powder X-ray diffraction showed that expansion lattice was easily noticed when the seed powdered samples were heated to 600°C. Moreover, with an increase in temperature, the peaks became narrower and sharper (Figure 2), which agrees with [30, 31, 34].

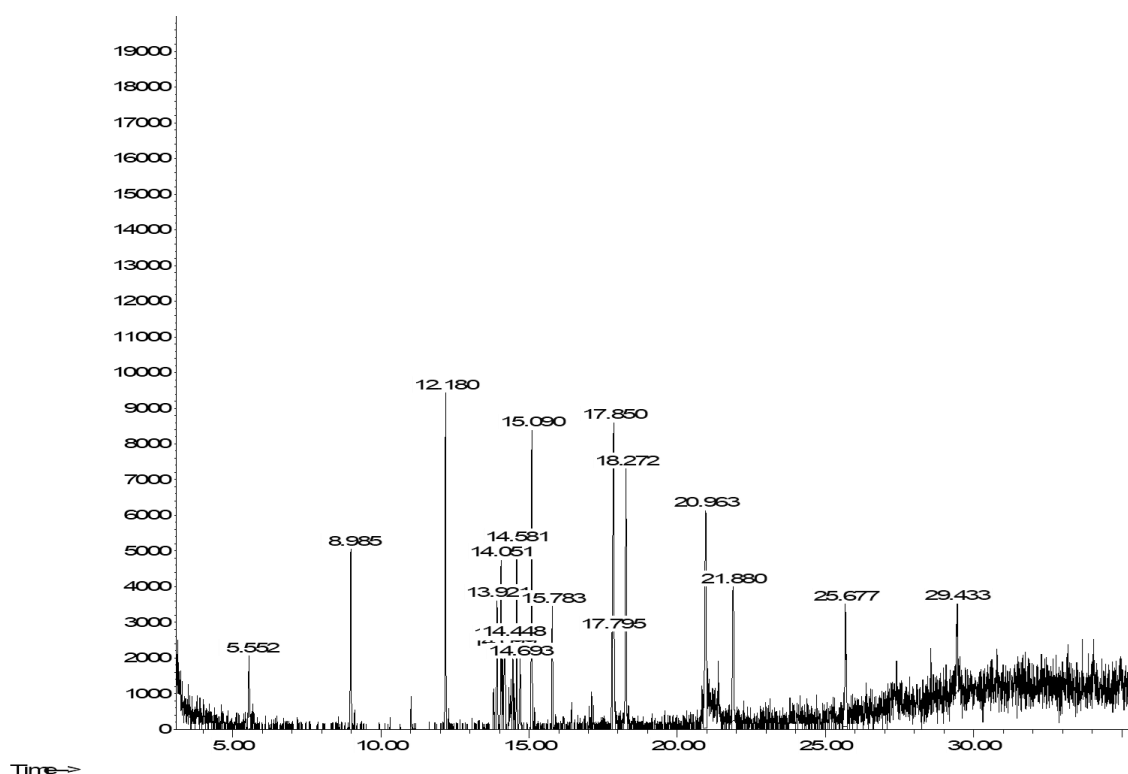


Figure 1. GC-MS profiles of *S. occidentalis* extracts.

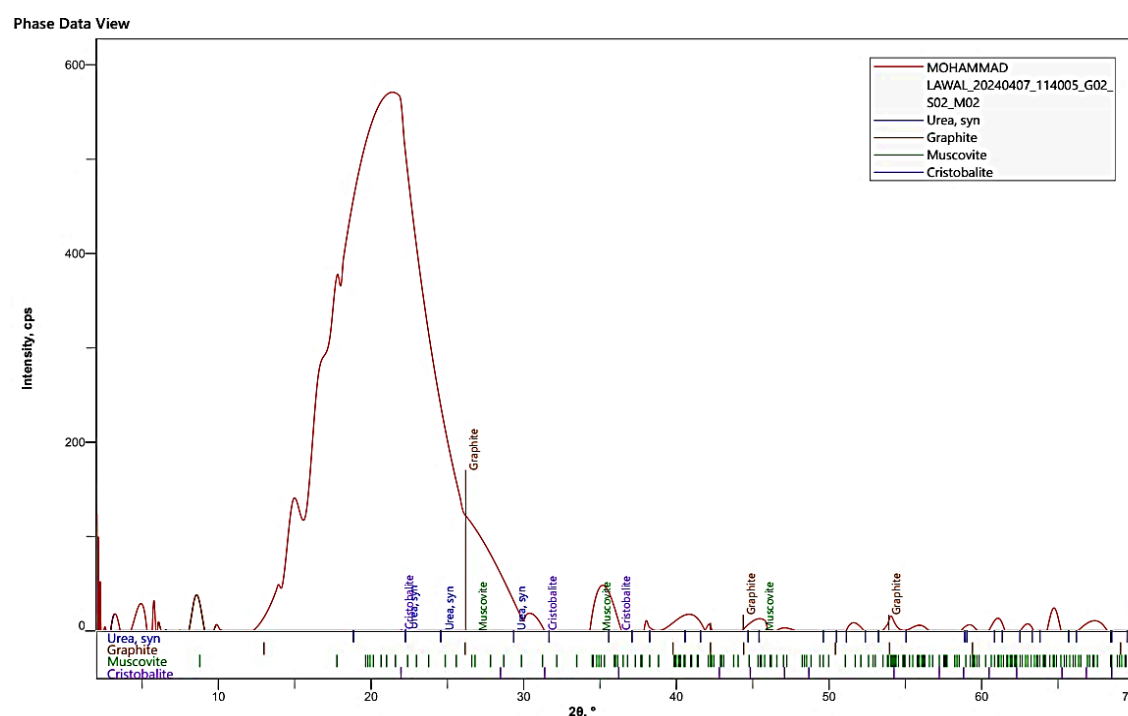


Figure 2. XRD pattern of *S. occidentalis* seed powder.

DISCUSSION

The seeds of coffee senna are sometimes used as a coffee substitute. Its seeds are also used to solve many health issues, such as antibacterial, antifungal, anti-inflammatory, boost immunity, and many more. This is due to the presence of vital components such as proteins, fatty acids, and alpha-tocopherols. Medicinal plants are the primary source of drugs in herbal medicine (HM), which have

been incorporated in the processes leading to the production of orthodox medicines because of the raw materials derived from plants morphological parts (Sofowora, 2019). Many phytochemicals have been isolated from plants for use by humans in healthcare systems; however, some of these drugs from plants have been withdrawn because of their acute or chronic toxicity as well as certain side effects [37].

Chemically, plants contain different classes of phytochemicals that are either safe to use or toxic for human's health [38]. To encourage the use of these toxic medicinal plants for the treatment of diseases in traditional medicine (TM), there is the need for detoxification and purification. The processes of detoxification and purification of toxic materials from plants for medicinal applications have been well practiced in natural products and chemical science research in recent times.

Evaluation of physicochemical parameters such as proximate analysis helps to assess the nutritional composition and overall quality of the seeds [39], while ultimate analysis reveals the elemental compositions of seeds. This analysis provides information on the chemical compositions and energy contents of seeds. In the present study, the moisture content with a value of 7.96% w/w falls within the acceptable limit of not greater than 10%. Because the moisture content greater than 10% will make drug materials susceptible to microbial contamination as well as excess values for additional unwanted products for the buyers.

Similarly, crude fiber stimulates digestion and encourages the production of important intestinal bacteria in animals. The amount of crude fiber in the animal feed thus basically depends on the purpose of the feed. In human healthcare systems, not a high amount of crude fiber is needed to avoid persistent diarrhea. In this study, the high amount of crude fiber (13.63%) and crude proteins (18.59%) are signs that *S. occidentalis* seeds might possess certain health hazards when the levels of these parameters are high. It has been reported that crude fiber content describes the plant cell wall components such as cellulose, hemicellulose, and lignin, which are usually not or barely digestible, and thus the portion of the feed that is not energetically usable by the animals. For instance, a high number of crude proteins have been reported to have accelerated liver cirrhosis in experimented animals. Also, high levels of crude proteins may mean that the liver or bile duct is damaged or diseased [35]. Furthermore, the high amount of nitrogen-free extract (46.96%) means that the seeds have a high amount of amide-containing compounds, which could be toxic for use in disease treatment. The toxic nature of *S. occidentalis* seeds was further confirmed by the presence of a high number of certain phytochemicals, such as alkaloids, anthraquinones, and saponins. The seeds are the most toxic part of the plant. The main toxic component of the *S. occidentalis* seed is dianthrone, an anthraquinone-derived compound that affects mitochondrial function.

Despite the high amount of certain toxic phytoconstituents contained in the seeds of coffee *Senna* in this study, high levels of phenolic contents (1375.5 mg GAE/100 g dry weight) and total flavonoid contents (441.2 mg RE/100 g dry weight) in the seeds show the medical relevance of *S. occidentalis* seed. For instance, phenolics and flavonoids have been used as potential antioxidants, anti-inflammatory agents, and anticancer agents [40]. The roles of these phenolics and flavonoids in this study were not different from the already reported roles. For better detoxification of plant materials for medicinal uses, it is also very crucial to characterize such plant materials, which is the first step to achieving safe use of such plant materials [41]. In this, characterization of *S. occidentalis* seeds ensures the production of monographic fingerprints for quality assurance of the seeds and its authentic chemical profiling. Many techniques used in this study for the characterization of the seeds, such as GC-MS, HPLC, XRF, and XRD, are powerful techniques with their own advantages. For instance, the XRD technique provides insight into the crystal structure and polymorphism of the seeds, aiding in the understanding of their physical properties. The study shows that *S. occidentalis* seeds contain 17 bioactive compounds, which are mainly fatty acids, as revealed by the GC-MS. The TGA of the seeds has minimum decomposition at 800°C and total weight loss of 18%, as well as maximum decomposition at 1000°C and weight loss of 82.1%. Furthermore, the high concentration of lead (Pb) in the seeds, as

shown by XRF analysis, further confirmed the toxicity of the coffee *Senna* seeds to humans. This is because lead has been reported to cause major organ damage in the body, such as liver, kidney, and heart, whether ingested in a trace amount or a large amount [30].

CONCLUSION

This study showed that *Senna occidentalis* seeds contain vital bioactive compounds that are very useful in human healthcare. However, some of these phytoconstituents have been reported to be toxic to the body when used. This was seen from their higher concentration as obtained. It is therefore essential to detoxify the seeds before use as therapeutic aid. The study further showed that characterization of the seeds provides a potential guide towards detoxification and subsequent use as therapeutic agents in disease conditions to prevent any toxic effects resulting from usage.

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