



# Sorghum extract: Phytochemical, proximate, and GC-MS analyses

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## Abstract:

**Introduction.** Sorghum is available cereal seeds of African origin belonging to the *Poaceae* family. However, its metabolites and proximate composition have not studied well, which led to the under-utilization of this cereal. This research aimed to investigate the classes of phytochemical and proximate compositions of sorghum extract in order to assess its nutraceutical potential for food chemistry and dietary formulations.

**Study objects and methods.** We studied the sorghum seed oil extract obtained with the help of a Soxhlet extractor. Sorghum was purchased in Ota, Nigeria. The bioactive compounds were identified by standard methods of phytochemical screening, the nutritional content was investigated with proximate analysis, and the secondary metabolites in the sorghum extract were determined using gas chromatography – mass spectrometry (GC-MS).

**Result and discussion.** The phytochemical screening showed the presence of steroids, saponins, terpenoids, alkaloids, cardiac glycosides, and quinones in the sorghum extract. The oil yield obtained was  $11.00 \pm 0.18\%$ . The proximate analysis revealed 5.94% moisture content, 3.05% ash, 0.20% crude fiber, 11.00% fat, 5.54% protein, and 74.27% carbohydrates. The selected physicochemical parameters measured in the sorghum extract included cloud point ( $0.40^\circ\text{C}$ ), specific gravity at  $25^\circ\text{C}$  (0.81), and refractive index (1.46). The GC-MS analysis revealed the presence of 9,12-octadecadienoic acid (Z,Z)-, stigmaterol, 8-dodecen-1-ol, acetate, (Z)-, vitamin E, linoleic acid ethyl ester, and 9,12-octadecadienoic acid, methyl ester, which accounted for about 85% in the sorghum composition. Other constituents, presented at lower amounts, included 12-heptadecyn-1-ol, 1H-Imidazole-5-ethanamine, 1-methyl-, and cyclononene.

**Conclusion.** The findings of this study revealed high nutritive potential of sorghum, which make it a rich source of energy for humans and animals.

**Keywords:** Sorghum, phytochemicals, nutritional value, chromatography, proximate analysis, bioactive compounds, grain

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## INTRODUCTION

Cereals can be defined as classes of grass planted and harvested for food purposes [1]. Different types of cereals are cultivated all over the world and occupy an area of about 60%. They have notable benefits contributing to human health due to nutrients and biologically active substances in their composition [2–4].

In the present study, sorghum was of our interest. Sorghum is also called Jowar in India, Guinea corn in West Africa, and Kaoliang in China. In Nigeria, it is called Oka by the Yorubas, Dawa by the Hausas,

and Sorghum by the Igbos. There are also other nomenclatures for sorghum and its role in the food chain is well documented by Sarwar *et al.* [5].

Varoquax *et al.* reported that sorghum is highly resistant towards drought and heat, which allows it to flourish and thrive even under hot and arid environmental conditions [6]. Sorghum is known to be able to boost blood level. This is of tremendous importance to human health. For instance, women who suffer from myoma have anemia due to the excessive blood losses, especially during menstrual period.

Sorghum, with its high nutraceutical value, could help these women [7].

In Africa, where diverse species or cultivars of sorghum are cultivated, this cereal is served as an important food crop. In Nigeria, sorghum is classified into three cultivars depending on the nature and color of their seminal glands and endosperm, namely Guines, Kaura, and Farafara [8]. Nonetheless, small-scale farmers prefer Farafara to Kaura due to the fact that the former is known to have a better storage behaviour and attributes.

Sorghum is classified as a tall grass that often grows to as high as two to eight feet, occasionally being as high as fifteen feet. Generally, a whitish wax coating covers the stalks and leaves of sorghum; while specifically, stalk piths of some species are juicy and sweet [9, 10]. A well manured sorghum leaf is around 76 cm long and 5 cm wide. Panicle portion of sorghum is responsible for the production of tiny flowers which can be from loose to dense, with clusters containing 800–3000 kernels. The diversity of species is identifiable by the coloration, shape, and size of the seeds, which are smaller than wheat seeds [10].

Statistics in 2016 showed that Nigeria provided 23% of the total sorghum production in African, which made Nigeria the largest producer of sorghum in Africa [11]. Mathur *et al.* documented the emergence of *Sorghum bicolor* as a viable option for producing lignocellulosic biofuel [12]. Vanamala *et al.* reported that sorghum contains bioactive compounds that play a crucial role in its pharmacological potential and immune modulatory properties [13]. Hassan *et al.* studied the effect of ultrasonic waves and microwaves on extraction of the lipid fraction from sorghum. They revealed that these techniques increased the oil yield [14].

Since sorghum seed oil and its defatted extracts are widely used in Africa, the aim of this study was to evaluate the nutraceutical potential, phytochemical components, and secondary metabolites of sorghum from Ota (Nigeria).

## STUDY OBJECTS AND METHODS

**Sample collection and preparation.** Sorghum seeds were purchased from a local market in Ota, Nigeria. The seeds were washed, air-dried, and finally dried in a Thermofisher vacuum oven until constant weight was achieved. The seeds then were finely powdered with a mechanical blender. Prior to extraction, the powdered seeds were protected from sunlight, dust, as well as other particulate matter to avoid oxidation and microbial contamination.

**Oil extraction.** 200 g of the powdered seeds of sorghum was weighed, carefully wrapped in Whatman filter paper, and mounted up on a Soxhlet extractor. One liter of petroleum ether was transferred into a round bottom flask connected with a thimble with the sample in. When the extraction process completed, the petroleum ether solvent was removed with an IKA® RV 10 rotary evaporator, and the sample was stored in a refrigerator.

**GC-MS analysis condition.** Agilent 7890B GC/5977 MS was utilized for the GC-MS analysis of the extract using the given conditions: column – HP 5 capillary (60 m×0.25 mm×0.25 µm); oven temperature program – the column was held initially at 50°C for 1 min after injection, then ramped to 300°C at 7°C per minute and held for 14 min; injector temperature – 250°C; detector (MS) temperature – 275°C; carrier gas – helium; inlet pressure – 40.65 psi; linear gas velocity – 39 cm/s; column flow rate – 2.7 mL/min; split ratio – 10:1; injection volume – 1 µL. The components were identified by retention time determination on the capillary column as well as by matching mass spectra with the data of the NIST mass spectral library.

**Phytochemical tests.** *Terpenoids.* 0.30 g of the seed powder was carefully transferred into a 250 mL beaker and extracted with 30 mL of chloroform for 2 h. 2 mL of trichloromethane and 3 mL of concentrated sulphuric acid were added to 5 mL of the extract, thereby forming a layer. Reddish brown color at the interface confirmed the presence of terpenoids [15].

*Cardiac glycosides* were determined by two methods. According to the Raymond method, 50% C<sub>2</sub>H<sub>5</sub>OH was gradually added to the extract in a test-tube, followed by 0.10 mL of 1% ethanolic m-dinitrobenzene. The resulting mixture then was titrated with 20% NaOH. Violet coloration confirmed the presence of active methylene group. According to the Killer Killiani method, the extract was solubilized in 1% FeSO<sub>4</sub> in 5% glacial acetic acid, followed by the addition of concentrated H<sub>2</sub>SO<sub>4</sub>. The development of blue coloration indicated the presence of deoxy sugar.

*Quinones.* Diluted NaOH was added to 1 mL of the sorghum extracted. Blue green or red coloration implied the presence of quinones [16].

*Saponins.* The extract sample was vigorously mixed with 5 mL of distilled H<sub>2</sub>O. The frothing was mixed with few drops of olive oil and shaken vigorously. The appearance of foam demonstrated the presence of saponins.

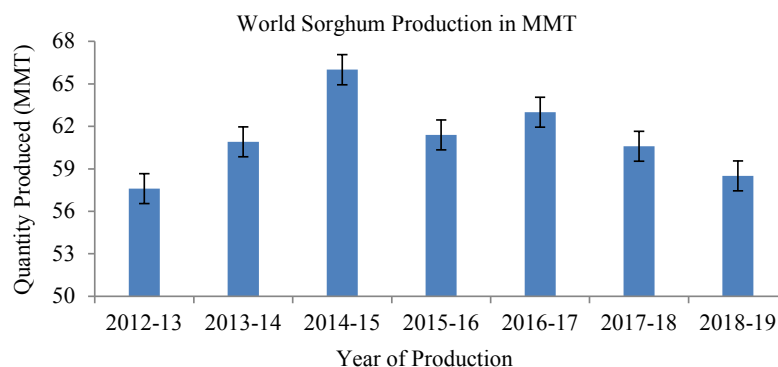
*Steroids.* 2 mL of acetic anhydride was introduced into 0.5 mL of the extract, followed by the addition of 2 mL of sulphuric acid. Change in the extract coloration from violet to blue or green indicated the presence of steroids [16].

*Tannins.* 10 mL of bromine water was introduced into 0.5 g of the extract sample. Decolorization of Br<sub>2</sub>/H<sub>2</sub>O showed the presence of tannins.

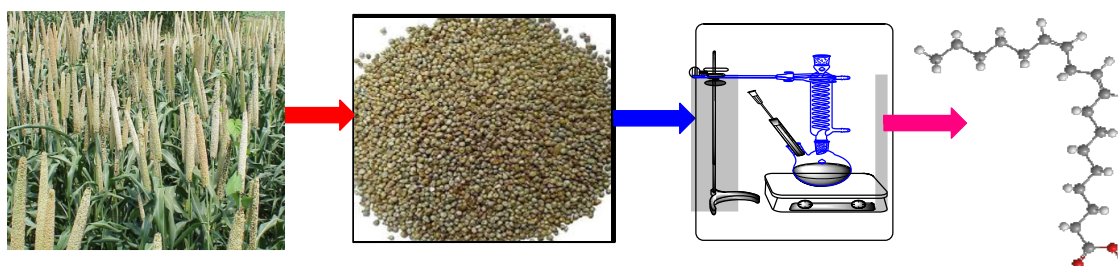
*Proximate determination.* Proximate analysis was carried out using combination of techniques and methodologies earlier reported. For instance, crude protein and moisture contents were determined using the method by Ajani *et al.*, carbohydrate by Owoye *et al.*, Molisch's test by Gangwal *et al.*, Biuret test by Suneetha *et al.*, and total ash by Abdulkadir *et al.* [2, 3, 17–19].

## RESULTS AND DISCUSSION

Sorghum has been identified and rated as the fifth cereal crop of greatest significance globally [20, 21].



**Figure 1** Statistics of world sorghum production from 2012 to 2019 [24]



**Figure 2** Stages of sorghum processing to identify secondary metabolites

Sorghum is used in food and feed production, in wallboards, fences, biodegradable packing material, as well as for ethanol production [22, 23]. According to the data in [24], before the outbreak of COVID-19, the trend in the world sorghum production from 2012 to 2019 fluctuated between 57 to 59 million tons (Fig. 1). Based on the world sorghum production data, there was a drastic increase in 2015 which was as a result of the increase in sorghum usage by the Chinese in livestock feed meal. This made them to purchase large volumes of sorghum from the USA.

In our previous works we studied seed oils and their extracts, namely from *Caryota mitis* L., *Adenanthera pavoninalinn* L., and sandbox tree (*Hura crepitans* L.) [2, 3, 25]. Continuing our research, this work featured the phytochemical screening, proximate determination, and GC-MS analysis of extract from sorghum from Ota (Nigeria) in order to investigate its nutraceutical potential and add more secondary metabolites to the organic structure database.

Seeds of sorghum were harvested from the plant. It was crushed and mounted on Soxhlet extractor to obtain the oil while the remaining defatted component was herein referred to as the crude extract. The processing stages of the sorghum to identify the secondary metabolites is demonstrated in Fig. 2.

**Phytochemical screening.** The phytochemical screening of the sorghum extract under study was performed by using standard methods as reported in our previous work [25]. The qualitative phytochemical

constituent composition was determined in the sorghum seed oil obtained with the help of two different solvents: petroleum ether and ethanol (Table 1). Saponin availability in the sorghum extracts was established with foam test and Froth test. In the extract obtained with petroleum ether saponins were not detected, while the extract obtained with ethanol contained saponins.

Saponins contain an agent with surface activity due to the sugar units which are very soluble in water. Although, the saponin units in saponins have high

**Table 1** Phytochemical screening of sorghum extract

Phytochemicals	Type of test	Petroleum ether extract	Ethanol extract
Steroids	Salkowski	–	+
Saponins	Foam test/Froth	–	+
Tannins	Ferric chloride	–	–
Terpenoids	Acidified chloroform	+	+
Alkaloids	Dragendroff	+	–
Cardiac glycosides	Killer Killiani	+	+
Phenol	Ferric chloride	–	–
Oxalates	Acid digestion	–	–
Quinone	Dilute NaOH	+	+

The symbol (–) represents absence, while symbol (+) represents presence

lipophilicity, which make them soluble in fat [26]. DE Bruijn found that a wide variety of leguminous plants contains diverse saponins; for instance, five classes of saponins were reported in soya beans [27]. This explained why the extracts obtained with ethanol had positive saponin test. Saponins play a significant role in the reduction of plasma cholesterol as a result of the effective inhibition of cholesterol absorbing capacity in the intestinal tract of experimentally investigated animals [28].

Cardiac glycosides are present in the both petroleum ether and ethanol extracts. These valuable secondary metabolites are able to enhance myocardial contraction, treating thereby congestive heart failure [29]. Cardiac glycosides also indirectly effect on vascular resistance [30]. Thus, the presence of cardiac glycosides in sorghum could be exploited for their medicinal potential. The presence of cardiac glycosides in the sorghum extracts was detected by Raymond and Killer Killiani tests; the latter effectively transformed 2-deoxy-sugars of cardiac glycosides by distinctive coloration, which made the qualitative and quantitative monitoring easier [29]. Molecules of cardiac glycosides are capable of inhibiting  $\text{Na}^+/\text{K}^+$ -ATPase [31].

Both petroleum ether and ethanol extracts of sorghum contained terpenoids. Terpenoids form a group of compounds, the majority of which occur in the plant kingdom. Simpler mono- and sesquiterpenes are the main constituents of essential oils [32]. Because of their sweet smell, these essential oils are used in perfumery in cosmetic chemistry [33]. Quinone was also present in the tested sorghum extracts. Naturally, quinone plays an important role in transduction and accumulation of energy, which is necessary in such processes as respiration and photosynthesis [34]. Alkaloids were found in the sorghum extracts, while tannins, phenol, and oxalates were not detected in the both ethanol and petroleum ether samples.

**Proximate and physico-chemical analyses.** The proximate analysis results are presented in Table 2. The importance of oil in human dietary intake cannot be overestimated. Its biological availability and fatty acid profile depends in most cases on environmental, crop, and genetic conditions [35]. The Soxhlet extraction with n-hexane as a solvent revealed the crude fat of the extract of  $11.00 \pm 0.34\%$ . This was a higher yield than the fat content ( $9.32\%$ ) reported from the distiller dried grain (DDG) sorghum [36].

The ash content in the sorghum extracts under study was  $3.05 \pm 0.11\%$ , which is in accordance with the results obtained by Mohammed *et al.* who studied nutritional composition of three commonly consumed varieties of sorghum [8]. The ash content may be affected by the nature and amount of ions in the soil from which plants draw nutrients. In our work, the ash content is within acceptable limit ( $< 5\%$ ) [37]. Soil composition has a partial but direct effect on ash

**Table 2** Physicochemical and proximate determination of sorghum extract

Parameters	Obtained values
Proximate determination parameters	
Moisture content, %	$5.94 \pm 0.18$
Ash content, %	$3.05 \pm 0.11$
Crude fiber, %	$0.20 \pm 0.07$
Protein, %	$5.54 \pm 0.15$
Crude fat, %	$11.00 \pm 0.34$
Carbohydrates, %	$74.27 \pm 0.85$
Organic matter, %	$91.01 \pm 0.93$
Selected physicochemical parameters	
Refractive index	1.46
Density, g/mL	0.81
Cloudy, °C	0.40

Values are mean  $\pm$  SD for triplicate measurement

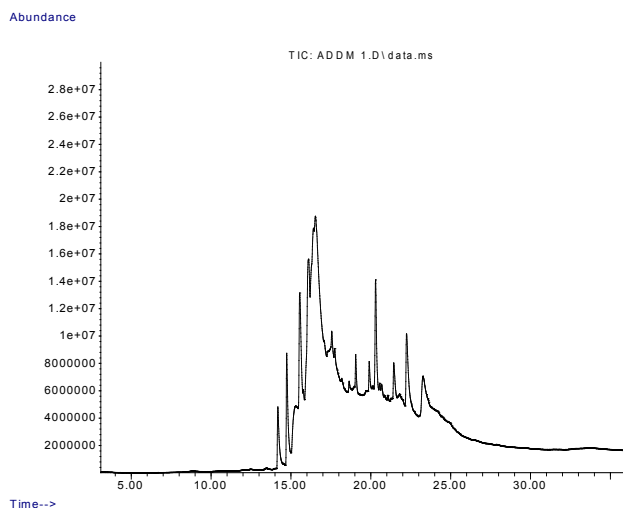
content, as the ash content of seeds may partially be a function of the soil composition on which the plants grow [38, 39].

The protein content was determined to be  $5.54 \pm 0.15\%$ , which was within the range of earlier reported values, namely  $4.82 \pm 2.39\%$  for white sorghum and  $6.06 \pm 0.40\%$  for red sorghum. The moisture content of the sorghum tested was  $5.94 \pm 0.18\%$ , which indicated moderate shelf-life of sorghum [19].

In addition, the investigated sorghum herein had the fiber content of  $0.20 \pm 0.07\%$ . Dietary fiber is valuable in digestion, hormone production, and cardiovascular health. This also assists in the reduction of low-density lipoprotein cholesterol due to its bile reabsorbed reduction capability in the intestinal tract. Fibers in food prevent excess starch in the body and regulate metabolic conditions such as diabetes and hypercholesterolemia [40].

The carbohydrate content, which generally referred to the readily digested carbohydrates like sugar, starch, as well as organic acids, amounted for  $74.27 \pm 0.85\%$ . High carbohydrate content supplies energy for the metabolic process, thus stabilizing health status of the consumers [41]. The proximate analysis showed that the studied sorghum extract contained  $91.01 \pm 0.93\%$  of total organic matter.

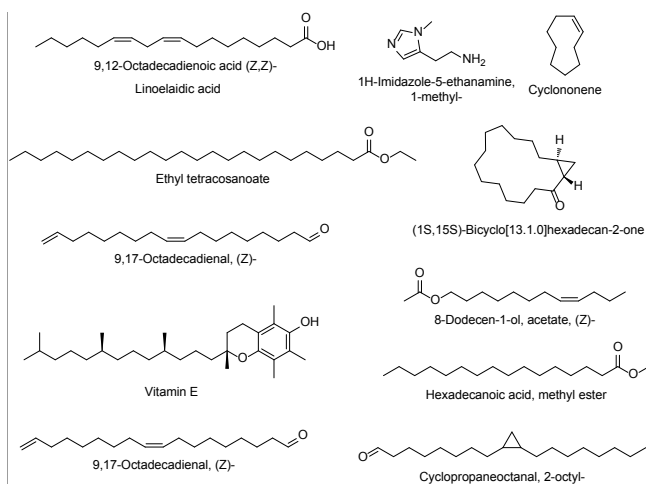
The physicochemical parameters of the sorghum extract included its refractive index, density, and cloud point (Table 2). According to the refractive index value, the oil was of a good quality, so it can be used for homogeneous binary mixture formation. In their research, Ospina *et al.* reported that the above mentioned parameters are characteristics for fast and cheap testing of the purity of essential oils [42]. The density of the seed oil of sorghum was  $0.81 \text{ g/mL}$ , which was lower than that of *C. mitis* ( $0.93 \text{ g/mL}$ ) and *A. pavonina* ( $0.85 \text{ g/mL}$ ) in our previous works [2, 3]. This also implies that the oil of sorghum was less viscous than that of *A. pavonina* and *C. mitis*.



**Figure 3** GC-MS chromatogram of sorghum extract

**GC-MS analysis.** Based on the GC-MS analysis, Fig. 3 demonstrates the chromatogram of the sorghum extracts under study. The chromatogram allows us to compare spectra of each composition and the NIST library data. The molecular structures of the identified constituents are shown in Fig. 4, with 9,12-Octadecadienoic acid, methyl ester being the predominant fatty acid.

The mass spectrum chromatography assay showed that the major constituents of the sorghum included organic acids, esters, sterols, tocopherols, and fatty aldehyde. Organic acids alone accounted for about 72% of the sorghum composition. Overall, the most abundant compounds are 9,12-Octadecadienoic acid (Z,Z)-, Stigmasterol, 8-Dodecen-1-ol, acetate, (Z)-, vitamin E, Linoleic acid ethyl ester and 9,12-Octadecadienoic acid,



**Figure 4** Structural identification of sorghum extract components analyzed chromatographically

and methyl ester, which accounted for about 85% of the sorghum composition.

Among the organic acids, 9,12-Octadecadienoic acid (Z,Z)-, Linoleic acid ethyl ester and hexadecenoic acids were contained in high concentrations. Hexadecanoic acids had three isomers, namely hexadecanoic acid, methyl ester; hexadecanoic acid, ethyl ester; and n-Hexadecanoic acid, making up about 5% of the composition.

9,12-Octadecadienoic acid (Z,Z)-, was the predominant fatty acid. It had two double bonds (C=C), which qualified it as an unsaturated fatty acid. 9,12-Octadecadienoic acid (Z,Z)-, occurs as glycosides in plants, which corroborates the presence of cardiac glycosides in their phyto-constituents. This essential fatty acid is a functional component of human food

**Table 3** Identification of sorghum constituents (GC-MS)

Sample №	Retention time	Area Pct	Library (ID)	Molecular formula (molecular weight)
1	12.4708	0.0443	12-Heptadecyn-1-ol	C <sub>17</sub> H <sub>32</sub> O (252.44)
2	13.0716	0.0063	1H-Imidazole-5-ethanamine, 1-methyl-	C <sub>6</sub> H <sub>11</sub> N <sub>3</sub> (125.17)
3	13.4607	0.0376	Cyclononene	C <sub>9</sub> H <sub>16</sub> (124.22)
4	14.0329	0.018	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one	C <sub>16</sub> H <sub>28</sub> O (236.39)
5	14.1874	0.9545	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> (270.45)
6	14.7424	1.5527	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> (284.48)
7	15.3032	2.3082	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> (256.42)
8	15.5549	4.1409	9,12-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub> (294.47)
9	16.1042	5.17	Linoleic acid ethyl ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub> (308.50)
10	16.5391–31.8454	55.7397	9,12-Octadecadienoic acid (Z,Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> (280.45)
11	19.9093	2.4792	Tetracosanoic acid, methyl ester	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub> (382.66)
12	20.3042	3.3967	Ethyl tetracosanoate	C <sub>26</sub> H <sub>52</sub> O <sub>2</sub> (396.69)
13	20.8649 26.4667	3.3824	9,17-Octadecadienal, (Z)-	C <sub>20</sub> H <sub>38</sub> O (294.52)
14	22.2496	5.6524	Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub> (430.71)
15	23.2738	8.2181	Stigmasterol	C <sub>17</sub> H <sub>32</sub> O (252.44)
16	24.4526	5.866	8-Dodecen-1-ol, acetate, (Z)-	C <sub>14</sub> H <sub>26</sub> O <sub>2</sub> (226.36)
17	33.5963	1.0329	Cyclopropaneoctanal, 2-octyl-	C <sub>19</sub> H <sub>36</sub> O (280.49)

which takes a part in biosynthesis of prostaglandins and cell membranes. Other polyunsaturated fatty acids have recently been reported to have implication on inflammatory thrombotic condition like COVID-19 [43].

Prominent esters included ethyl tetracosanoate and 8-Dodecen-1-ol, acetate, (Z)-, which accounted for about 9% of the sorghum composition. Sterols (stigmasterol), tocopherols (vitamin E) and fatty aldehyde (9,17-Octadecadienal, (Z)-, linoleate group & Cyclopropaneoctanal, 2-octyl-) were contained in less quantities, accounting for about 8, 6, and 4%, respectively.

### CONCLUSION

The sorghum oil extract was analyzed for identification of phytoconstituents, proximate compositions, and physicochemical parameters. It was also characterized spectroscopically for the nature and structures of its secondary metabolites using GC-MS. Proximate determination showed that the sorghum sample contained beneficial amounts of nutrients, while phytochemical screening revealed the presence of bioactive essential phytochemicals.

Thus, sorghum and sorghum-based food could be of high benefit to the population with nutritional deficiencies, for example, to developing countries. This work provided a base for a comparison of nutritional

value and therapeutic potential of sorghum extract with other natural food cereal sources. Sorghum requires further research on fortification and functionalization of food with sorghum extract to decrease nutraceutical shortage in the population.

### CONTRIBUTION

Olayinka O. Ajani designed the work and wrote the original draft. Taiwo F. Owoeye collected and pretreated the sample, as well as carried out phytochemical screening. Kehinde D. Akinlabu carried out phytochemical screening. Oladotun P. Bolade ran and discussed the GC-MS analysis. Oluwatimilehin E. Aribisala carried out sample pretreatment, Soxhlet extraction, and formal laboratory analysis. Bamidele M. Durodola carried out laboratory testing and editing of the manuscript. All authors read and approved the final manuscript before submission and they are equally responsible for plagiarism.

### CONFLICT OF INTEREST

The authors state that there is no conflict of interests related to the publication of this article.

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