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Determination of Fatty Acids and Minerals from *Balanites aegyptiaca* Fruit Kernel in Heglig Forest West Sudan

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Abstract

Balanites aegyptiaca, particularly its fruit, is often used as food and in traditional medicine and herbalists throughout Africa and Asia for the treatment of many ailments. The fruit kernel has a high concentration of oil and minerals. This study aims to examine fatty acids in the oil and minerals in the fruit kernel. The Samples are collected from the Heglig forest in west Sudan for the first time, and prepared for analysis using solvent extraction and acid digestion methods. The fatty acids content is identified using a gas chromatography-mass spectrometer (GC-MS) as well and minerals are detected by adopting inductively coupled plasma optical emission spectrometry (ICP-OES). The oil contained 41.66% unsaturated fatty acids 58.33% saturated fatty acids and a variety of minerals, the highest amount belonged to Sulfur (6.9 ppm), phosphorous (2.2 ppm), and iodine (2 ppm). GC-MS analysis observed 12 different proportions of fatty acid, Linoleic acid, Stearic acid, and Oleic acid have a peak.

Keywords: *Balanites aegyptiaca*, Fatty acids, Heavy metals, Sulfur, Oleic acid.

1. INTRODUCTION

Balanites aegyptiaca (L) Del., is one of Africa's most ubiquitous yet neglected wild plants often known as "Desert date,". It can live in several habitats and can withstand a broad range of soil conditions, including clay, sand, and climate moisture levels (Chothani & Vaghasiya, 2011). There are several nomenclatures associated with *Balanites aegyptiaca*. The Heglig tree, Hingot, Lalob, Zaccone, Bedeno, Zachun, and desert date (Pushpamalar *et al.*, 2006).

The tree of *B. aegyptiaca* is thorny with 10 m in high, greenish white fragrant flowers with 5-6 mm in diameter, flowering and fruiting occur during October (Azene Tesfaye, 2015). For thousands of years, B. aegyptiaca was employed; Fruit's fleshy pulp is consumed either fresh or dry. fruit kernel is considered extremely valuable because it contains high protein content and excellent-grade oil. The kernel **is** eaten as a snack (nut), the oil that is extracted is recycled for a multitude of purposes., and the leftover cake is fed to animals. During the dry season, both fruit and kernel were commonly consumed in many nations, including Nigeria, Ethiopia, and Sudan. The stalk of the desert date is used for tool handles, utensils, food, fodder, charcoal, windbreak, gum, poles, timber, shade, and mulch (von Maydell, 1990; Lockett *et al.*, 2000, (CSL STYLE ERROR: reference with no printed form); Gullic, 2001; Kenyatta & Henderson, 2001; Fadel Elseed *et al.*, 2002; Al Ashaal *et al.*, 2010; Elfeel, 2010).

The fruit has four layers: the peel, the sugar- and saponin-rich pulp layer, and the tough woody coating that pulls away to expose the kernel. Protein, vitamins, and mineral salts are among the potent substances found in this tree The kernel contains a significant quantity of protein (up to 50%) and oil (30–60%). Additionally, it has steroids and saponins (Azene Tesfaye, 2015). The process of extracting the kernels results in the production of light yellow oil, which has been determined to contain around 42.7% linoleic acid, 31% oleic acid, and 24% saturated acid glycosides, based on compositional analysis. Fruit kernel minerals are (Nitrogen, phosphorus, potassium, Calcium, Magnesium, and iron) and protein varied from (27- 37%), The fruit also has a significant concentration of anti-nutritional components, namely tannins, and oxalate. Oxalate, an acid present in the fruit, exhibits the capacity to establish robust associations with diverse minerals, resulting in the formation of oxalate salts (von Maydell, 1990;

Chapagain & Wiesman, 2005). Crude protein, vitamin C, steroidal, saponins, Carbohydrates, and other minerals are found in fleshy pulp (64-72%). The plant consists of (38%) dry matter, the leaves, and the flowers. The fruit pulp serves as a valuable resource of essential minerals such as potassium, iron, manganese, zinc, and copper. Additionally, it contains an oil that consists of linoleic, oleic, stearic, and palmitic acids (Saetae & Suntornsuk, 2011).

Balanites aegyptiaca kernel is utilized as an orally administered antidiabetic medication in traditional Egyptian medicine and also has been used for several ailments throughout history (Kamel *et al.*, 1991; Gnoula *et al.*, 2008; Abou Khalil *et al.*, 2016). The use of B. aegyptiaca in medical practice is widespread, such as intestinal worm infection, constipation, syphilis, malaria, and hemorrhoids (Chothani & Vaghasiya, 2011). Also, the essential oil of (Lalob) has been extensively used in cosmetics, perfumes, and as a food flavor; Furthermore, it is worth noting that this substance has medical qualities that make it suitable for therapeutic purposes. Additionally, it exhibits antibacterial and antioxidant activities, rendering it applicable in the field of agro-alimentary practices (Burt, 2004; Rasheed *et al.*, 2021).

Balanite kernel oil inhibited *Candida albicans* and *Staphylococcus aureus* due to its high concentrations of β-sitosterol, linoleic, stearic, and oleic acids. Further research suggests that the kernel oil of *Balanites aegyptiaca* has a veridical course of action against the Herpes simplex virus type1. In addition to its effectiveness as an antihelminthic agent against Schistosoma mansoni and Fasciola gigantica (Al Ashaal *et al.*, 2010).

Many scientific studies have found that Balanite extracts from desert dates have antibacterial, anti-inflammatory, antidiabetic, antioxidant, anticancer, hepatoprotective, and molluscicidal properties (Mariod & Ahmed, 2022). According to reported research (Mokhtar *et al.*, 2021) desert date oils are evaluated for their effectiveness versus *Tribolium castaneum Herbst.* Before being tested on lesions using the film residue technique at various doses and periods, chloroform, hexane, and ethanol were used to extract the oil. Chloroform and hexane had a 100% death rate at the same doses (Mokhtar *et al.*, 2021).

Plants require an intricate equilibrium of mineral nutrients for their growth and effective reproduction, necessitating the mobilization of these elements from the soil matrix and their subsequent absorption by the roots in the form of metal ions (Mehra & Farago, 2008), metal ions are transported through the plant's vascular system from its roots. Metals are naturally prevalent in soil, but mining, agriculture, sewage treatment, and significant industrial operations have increased metal pollution in the environment, for example As, Al, B, Cd, Cr, Ag, and Pb which may potentially be harmful to humans and plants (Ozyigit *et al.*, 2021).

Certain metals, including Zn, Mn, Ni, Cu, and Co, are classified as micronutrients that play a critical function in plant growth. Additionally, certain metals, such as Fe, K, Cu, Ca, P, N, and Mn, are essential for human nutrition and are important for maintaining optimal bodily functions (Burt, 2004; Rasheed *et al.*, 2021) *B. aegyptiaca* fruit kernel and fruit pulp It consists of different proportions from potassium, phosphorous, manganese, magnesium, iron, copper, and other elements (Chapagain & Wiesman, 2005).

The goal of this research is to investigate the fatty acid profile of the oil and minerals content of the seed kernel originating from B. aegyptiaca species indigenous to the Heglig forestry in Sudan for the first time. To do this, a combination of GC-MS and ICP-OES techniques are used, marking the first examination of these components in this particular species and location.

2. MATERIALS AND METHOD

2.1. Chemicals

The *B. aegyptiaca* fruit is collected from (Heglig area, South Kordofan State, Sudan, State district 9°59'55.3"N29°19'41.5"E at an elevation of 20 m a.s.l) during growing seasons March 2022. Khartoum's National Centre for Research's Medicinal and Aromatic Plants Research Institute verified the sample. The fruit kernel oil is extracted after crushed and ground to powder after dried.

Chemicals of analytical grade are obtained from ISOLAB, Germany except Sodium Hydroxide pellets purchased from ALPHA CHEMIKA, India, SRM 3280-multi vitamin tablets from Sigma Aldrich, USA.

2.2 Oil Extraction

The powdered fruit kernel is subjected to extraction by immersing it in an enclosed glass container for 48 hours. The solvent employed is n-hexane. The oil is extracted by evaporating a mixture of the filtrates at a lower temperature and pressure.

2.3. Sample preparation for elemental analysis

The examination of mineral content in the powdered dry fruit kernel is conducted using the acid digestion technique, using concentrated nitric acid at a temperature of 300°C. The concentration of major minerals and trace minerals was ascertained via ICP-OES.

2.4. GC-MS Analysis Condition

The essential oil is subjected to analysis using GC-MS "Simadzu Company, QP2010-Ultra" instrument equipped with quipped with a split/split less injector, a capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). Operating conditions were as follows: 1.61 ml\min flow rate of helium gas; 10°C/min heating rate from 60°C to

300°C in the column, with interface temperature 250 °C and 300°C injection port

Split mode is used to introduce the sample into the system and afterward subjected to analysis using scan mode within the m/z range of 40-500. The charges to ratio are determined, and the whole process took a total of 29 minutes. The fatty acids are specified by matching their mass fragmentation (m/z) and retention time to a library of known compounds. The findings are duly documented.

2.5. ICP-OES Analysis Condition

The ICO-OES analyzer 7000 Shimadzu is used for elemental analysis. The instrument parameters are adjusted as follows: In this experimental setup, nitrogen is used as a collision gas 0.6 L/min flow rate, while argon is utilized as a plasma gas and auxiliary gas with a flow rate of 9 L/min and 0.6 L/min, respectively. And 1.1 kW radio frequency built with ISDS software. For element detection, SRM 3280-multi vitamin tablets are used as a standard reference material.

3. RESULTS AND DISCUSSION

3.1. GC-MS analysis

GC-MS analysis of fatty acids from the *Balanites aegyptiaca* oil is performed, and the amount of oil extracted is 53.7% (wt/wt), This agrees with prior research showing a significant concentration of nutritious oil in the plant (54.5-57%wt/wt) (Mohamed *et al.*, 2002; Al Ashaal *et al.*, 2010). The components were originally identified by comparing retention time with the NIST MS library. The identification of twelve distinct fatty acids is made and reported in Table 1. And Figure 1 displays the total ion chromatograms (TIC).

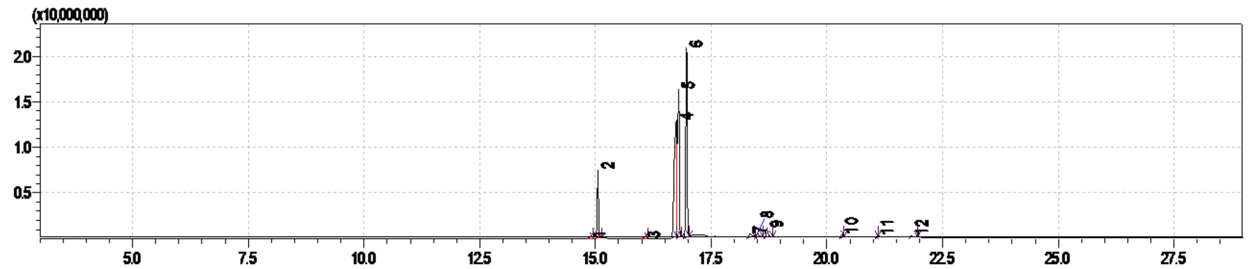

Figure 1: Total GC chromatograms of *Balanites aegyptiaca* oil

Table 1: The names of fatty acid and GC details of *Balanites aegyptiaca* oil

Peak no.	Name	Molecular formula	Ret Time	Area%
1.	9-Hexadecenoic acid (Palmitoleic acid)	$C_{16}H_{30}O_2$ C16:1	**14.857**	0.05
2.	Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$ C16:0	**15.054**	8.24
3.	Heptadecanoic acid (Margaric acid)	$C_{17}H_{34}O_2$ C17:0	**16.040**	0.03
4.	9,12-Octadecadienoic acid(Z-Z) (Linoleic acid)	$C_{18}H_{32}O_2$ C18:2	**16.745**	33.86
5.	9-Octadecenoic acid (Z) (Oleic acid)	$C_{18}H_{34}O_2$ C18:1	**16.800**	24.20
6.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$ C18:0	**16.967**	29.58
7.	11,14-Eicosadienoic acid	$C_{20}H_{36}O_2$ C20:2	**18.335**	1.11
8.	cis-11-Eicosenoic acid (gondoic acid)	$C_{20}H_{38}O_2$ C20:1	**18.500**	0.77
9.	Eicosanoic acid (Arachidic acid)	$C_{20}H_{40}O_2$ C20:0	**18.700**	1.53
10.	Docosanoic acid (Behenic acid)	$C_{22}H_{44}O_2$ C22:0	**20.322**	0.30
11.	Tricosanoic acid (Tricosylic)	$C_{23}H_{46}O_2$ C23:0	**21.090**	0.02
12.	Tetracosanoic acid (Lignoceric acid)	$C_{24}H_{48}O_2$ C24:0	**21.825**	0.31

Table 1 shows the total fatty acids discovered from B. aegyptiaca seed oils hexane extracts, the oil has 41.66% unsaturated fatty acids and 58.33% saturated fatty acids.

Were linoleic acid being a major unsaturated one at 33.86% and oleic acid at 24.2%, but palmitoleic acid, gondoic acid, and 11,14-Eicosadienoic acid are the lowest ratio 0.05, 0.77, 1.11% respectively. The other seven fatty acids are saturated and no branched-chain fatty acids are observed. By comparing these results with (Mohamed *et al.*, 2002) they characterize 75% of unsaturated fatty acids from the kernel, while in an alternative article (ABDEL-RAHIM *et al.*, 1986), extract 63.2% from pulp and fruit kernel.

The results of the main four fatty acids are briefly discussed below:

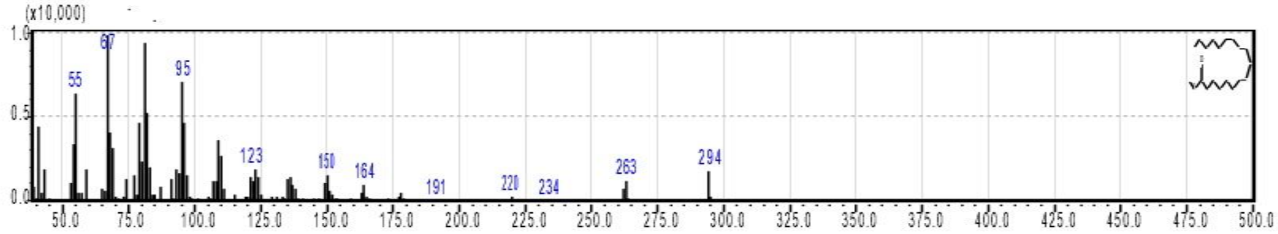

Figure 2: 9, 12-Octadecadienoic acid (Linoleic acid) mass spectrum

The compound 9,12-Octadecadienoic acid has the greatest area percentage of 33.86% at a retention time of 16.745 s, as seen in Table 1 of the GC chromatogram. This finding suggests that *Balanites aegyptiaca* oil contains the highest quantity of this particular fatty acid. Figure 2 displays the mass spectrum of Linoleic acid, whereby the signal seen at m/z 294 corresponds to the molecular ion M^+ $[C_{19}H_{34}O_2]^+$ as methyl ester. The observed signal of linoleic acid at m/z 263 is traced back to methoxyl group losses.

Linoleic acid is an omega-6 fatty acid one of the most crucial necessary fatty acids for the human body and plays a crucial part in promoting heart health and reducing cholesterol (Lown, 2014).

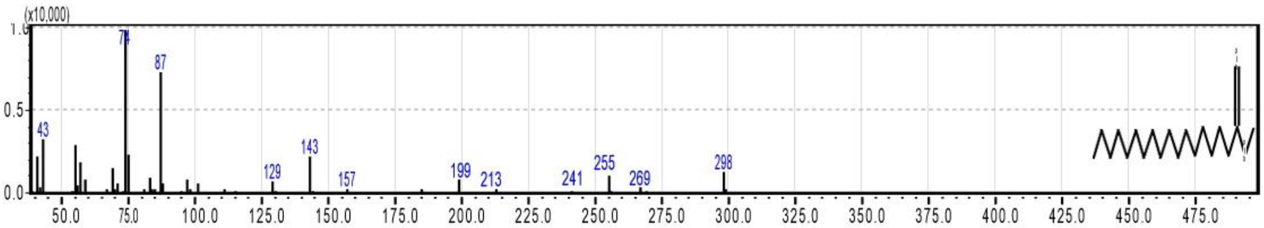

Figure 3: Octadecanoic acid (Stearic acid) mass spectrum

In the GC chromatogram, it is revealed that stearic acid constituted the second most prominent component in B. aegyptiaca oil. This is shown by a peak with a retention time of 16.967 s, which accounted for 29.58% of the total area under the curve. As illustrated in Figure 3 the mass spectrum of stearic acid, the molecular ion of the methyl ester form M+ $[C_{19}H_{38}O_2]^+$ appears at m/z 298, and the signal seen at m/z 269 is indicative of the compensatory effect resulting from the elimination of an ethyl group. The primary fragment seen at m/z 74 may be attributed to the presence of a methyl ester group.

Stearic acid as ester, being a prevalent saturated fatty acid, is often generated by the hydrolysis of animal fat or the hydrogenation of cottonseed or vegetable oil. Stearic acid finds use in several industries such as soap manufacturing, cosmetics production, and lubricant formulation, as well as serving as softening and releasing agents (Hiremath *et al.*, 2019).

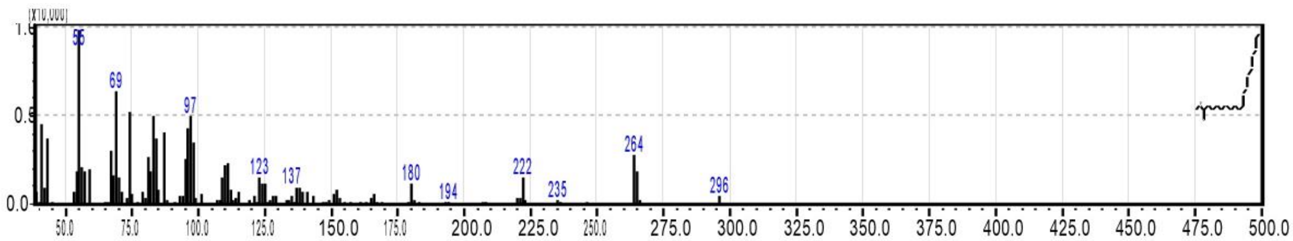

Figure 4: 9-Octadecenoic acid (Oleic acid) mass spectrum

The third primary constituent in B. aegyptiaca oil is 9-octadecenoic acid, as shown by its presence in the GC chromatogram. This is indicated in Table 1, where it is noted to have a retention time of 16.8s and an area under the peak of 20.2%. The mass spectrum of oleic acid is illustrated in Figure 4; The m/z 296 signal is identified as methyl ester of oleic acid M^+ $[C_{19}H_{36}O_2]^+$. The m/z of 264 is associated with the elimination of a methoxy group.

Oleic acid is an omega-9 unsaturated fatty acid, Oleic acid is the predominant fatty acid found in human adipose tissue and ranks second in terms of quantity among all human tissues, with palmitic acid being the most prevalent. One of the notable impacts shown by the substance is its inherent antioxidant qualities since it can directly modulate the production and functionality of antioxidants (Zeece, 2020).

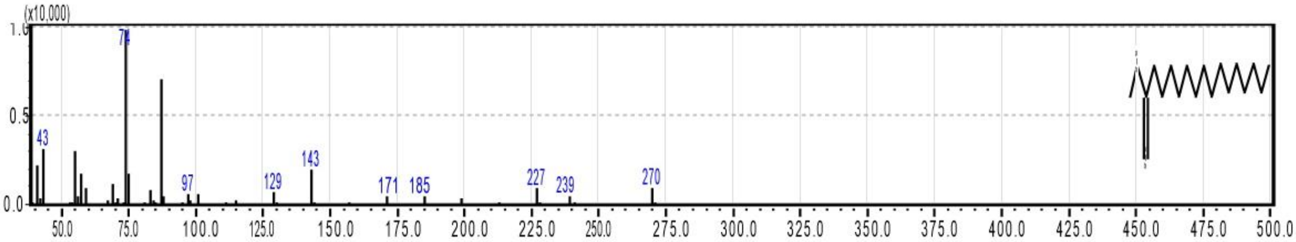

Figure 5: Hexadecanoic acid (Palmitic acid) mass spectrum

The fourth major constituent present in B. aegyptiaca oil is hexadecanoic acid or palmitic acid, as shown by its appearance in the GC chromatogram at a retention time of 15.054 and an area under the peak of 8.24%. As shown in Figure 5 the mass spectrum of palmitic acid is ester form, M+ is detected at m/z 270, while the loss of a methoxy group is evident at m/z 239. Additionally, the major fragment at m/z 74 may be caused by the presence of the methyl ester group.

Palmitic acid, being the predominant saturated fatty acid present in the human body, serves as a precursor to longer fatty acids. Palmitic acid is often used as an acid and as the sodium palmitate salt in many applications, including but not limited to the fields of food, soaps, cosmetics, and industrial mold-releasing agents. Several studies have shown that palmitic acid is associated with elevated levels of LDL and total cholesterol (Carta *et al.*, 2017). Notably, the World Health Organization (WHO) has recently declared that there exists compelling evidence linking palmitic acid to an increased risk of cardiovascular disease (WHO, 2003).

When comparing the findings presented above with the study done by (Mokhtar *et al.*, 2021) on B. aegyptiaca in the Alsondodab zone of the Khartoum state, it is observed that the ratio of Linoleic acid is found to be higher in both studies. However, there is a difference in the percentage, with the plant in the Heglig area exhibiting a percentage of 63.66% compared to 33.86% in the investigation conducted by Mokhtar *et al.*, Furthermore, the concentration of Hexadecanoic acid is reported to be 15.86% higher in the B. aegyptiaca plant in the Heglig region compared to the 8.24% reported by Mokhtar *et al.*, in their study. In the current research, it is reported that the amounts of Octadecanoic acid and 9-octadecenoic acid in the Alsondodab region were 0.83% and 3.95% respectively, which are lower than those found in B. aegyptiaca in the Heglig zone. The observed discrepancy might perhaps be ascribed to the diverse plant habitats present in these regions, alongside the experimental parameters.

3.2. ICP-OES analysis

The presence of different minerals and heavy metals is determined by ICP-OES analysis of the fruit kernel of *Balanites aegyptiaca.* The obtained results are provided in the tables below.

Table 2: The main elements in B. aegyptiaca fruit kernel

Elements	Concentration mg/L
S	6.9
P	2.2
I	2.0

Table 3: The trace elements in B. aegyptiaca fruit kernel

Elements	Conc µg/L	Elements	Conc µg/L
Al	170	Ni	6.6
Fe	78	Ca	440
K	980	Mg	93
Mn	4.7	Na	73
Tl	17	Zn	10
B	2.9	Cu	28
Ag	0.79	Se	13

B. aegyptiaca fruit kernel elemental composition is determined and is presented in Table 3. It is observed that sulfur exhibited the highest concentration (6.9 mg/l). The significant presence of sulfur in the B. aegyptiaca fruit kernel contributes to its antifungal properties, which have been used in Sudanese traditional medicine for the treatment of Dematophytosis, a fungal disease.

The data reveals that phosphorus is the second prominent mineral, with a concentration of 2.2 mg/l. This value exceeds the findings reported by (Elfeel, 2010) and indicates variations across various geographical regions. The concentration of Um Abdalla is measured to be 1.75 mg/l, Rashad is found to have a concentration of 1.63 mg/l, and Ed Alfrissan had a concentration of 1.72 mg/l in Sudan. Additionally, the plant bloom in Sokoto state, Nigeria had a concentration of 0.6 mg/l (Umar *et al.*, 2014) However, a much lower concentration of 63 mg/l was recorded in Borno State, Nigeria (Eromosele *et al.*, 1994).

The findings indicate that the fruit kernel of B. aegyptiaca has a noteworthy concentration of iodine, measuring 2.0 mg/l. Furthermore, according to the data shown in Table 4, sodium exhibits moderate amounts of 0.073 mg/l, whilst boron and nickel demonstrate trace values of 2.9 and 6.6 µg/l, respectively. Thallium is detected at a significantly elevated concentration of 17 µg/l, beyond the permissible threshold as stipulated by

China National Standards (Han *et al.*, 2023), which sets the safe limit at 0.1 µg/l.

The concentrations of calcium (Ca), magnesium (Mg), and potassium (K) are measured to be 0.44, 0.093, and 0.98 mg/l, respectively. The aforementioned results exhibited a smaller magnitude when compared to the published values documented in different geographical locations within Sudan and Nigeria (Eromosele *et al.*, 1994; Elfeel, 2010). In comparison, the concentration of Mn in the fruit kernel of B. aegyptiaca in Heglig, Sudan is found to be much lower at 0.047 mg/l, as opposed to the concentration observed in Borno, Nigeria, which is 9 mg/l (Eromosele *et al.*, 1994). In contrast, the recorded iron concentration of 0.078 mg/l in Borno, Nigeria above the published value of 0.03 mg/l (Eromosele *et al.*, 1994). However, it is found to be lower than the reported values in three distinct places in Sudan (Elfeel, 2010). These areas include the Balanites - Acacia zone with a reported iron concentration of 1.07 mg/l, the Nuba Mountains have a documented iron concentration of 0.79 mg/l, and Western Sudan with a recorded iron content of 0.97 mg/l.

Low quantities of silver, selenium, and copper are detected at 0.79, 13, and 28 µg/l, respectively. However, aluminum was identified in a more substantial quantity of 170 µg/l. This finding might perhaps be ascribed to the elevated concentrations of silver, selenium, copper, and aluminum in the Heglig area, as described in a previous study (Osman Zooalnoon & Musa, 2019). These elevated concentrations are likely a consequence of the presence of produced water in oil fields.

4. CONCLUSION

This study provides an examination of the analysis conducted on various minerals and fatty acids contained in the fruit kernel of *Balanites aegyptiaca* in the Heglig area of Sudan, marking the first instance of such inquiry. The samples are subjected to methylation to identify fatty acids using gas GC-MS, and an acid digestion approach is used to assess minerals using ICP-OES.

Study shows that the fruit kernel of B. aegyptiaca has a high concentration of sulfur. Therefore, this might be the underlying cause for the development of therapeutic properties. Along with significant amounts of other minerals including phosphorus and iodine. In addition to a substantial amount of potassium and calcium. The elemental results analysis surprisingly indicated the presence of thallium at a dangerous concentration.

The seed oil of Balanites aegyptiaca has a diverse array of fatty acids, notably including a substantial proportion of oleic acid and linoleic acid. These two fatty acids are considered necessary for human physiological functions.

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