Bioactive Landscape of Desert Date (*Balanites aegyptiaca*): An Extensive Phytochemical Characterization, Spectroscopic Analysis and its Cytotoxic Properties

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ABSTRACT
Balanites aegyptiaca is an economically and culturally important fruit-bearing tree widely distributed in Africa and the Middle East. Despite its traditional applications and potential medicinal value, it remains an underutilised plant species with limited comprehensive evaluation of its phytochemical composition, extraction efficiency and biological activities. This study aimed to evaluate the extraction efficiency, phytochemical constituents, chemical profile and acute toxicity of Balanites aegyptiaca extracts obtained using ethanol and methanol. The plant extracts were prepared using ethanol and methanol as solvents, and extraction yields were determined. Qualitative phytochemical screening was carried out to identify major secondary metabolites, while Fourier Transform Infrared (FT-IR) spectroscopy, Gas Chromatography-Mass Spectrometry (GC-MS) and UV-Visible spectroscopy were employed to characterise the chemical constituents and functional groups present in the extracts. Acute toxicity assessment was conducted using Drosophila melanogaster as a biological model to evaluate concentration-dependent effects on survival, locomotion and development. The extraction yields were 23.3% and 24.7% for ethanol and methanol extracts, respectively, indicating slightly higher extraction efficiency of methanol. Phytochemical analysis revealed the presence of saponins, tannins, alkaloids, flavonoids, phenolic compounds, steroids, terpenoids and cardiac glycosides in both extracts, with methanol showing greater affinity for terpenoids and steroids. FT-IR analysis confirmed the presence of functional groups associated with alcohols, alkanes, carbonyls and phenolic compounds, while GC-MS identified approximately 38 compounds comprising alkanes, fatty acids, phenolics and silyl derivatives. UV-Visible analysis showed a prominent absorption peak at 665 nm, suggesting the presence of chlorophyll-related compounds. Toxicity evaluation demonstrated dose-dependent cytotoxic effects, with higher concentrations (250–750 mg/10 g) causing increased mortality and developmental abnormalities, whereas lower concentrations (50–100 mg/10 g) showed no observable toxic effects. The findings indicate that Balanites aegyptiaca contains diverse bioactive compounds with potential pharmaceutical and nutraceutical applications; however, concentration-dependent toxicity should be considered in future utilisation and dosage optimisation studies.

KEYWORDS: *Balanites aegyptiaca,* Acute toxicity tests, *Drosophila melanogaster*, Cytotoxicity.

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I. INTRODUCTION

Africa has a diverse range of plant species, many of which are thought to contain considerable amounts of chemicals with potential health benefits and the ability to alleviate hunger. Despite the abundance of botanical resources, numerous plant species remain underutilized or neglected by both Western society and local inhabitants (Van Wyk, 2015). *Balanites aegyptiaca* (L.) Del is a notable example of such underutilized plants in Africa. (Zara'u et al., 2014). In Nigeria, *Balanites aegyptiaca* is most widely recognized by its Hausa name "aduwa" (or "adua"), which is also commonly used across several northern communities where the plant is abundant in the savannah and Sahel zones. In the south-western region in Nigeria, where the plant is less native and less commonly encountered, Yoruba speakers often adopt a descriptive form such as "igi aduwa" (meaning "aduwa tree"), borrowing directly from the Hausa term rather than using a distinct indigenous name.

Balanites aegyptiaca (L.) Del., commonly known as the desert date, is a widely distributed medicinal plant in arid and semi-arid regions of Africa and Asia, with extensive ethnomedicinal applications in the treatment of various ailments. Previous studies have reported the presence of diverse bioactive constituents such as alkaloids, flavonoids, saponins and phenolic compounds, alongside documented antioxidant, antimicrobial, and anti-inflammatory activities (Gargano et al., 2005; Hassan et al., 2017). Despite these findings, there remains limited information on the cytotoxic potential and detailed spectroscopic characterization of the aerial parts of the plant, particularly using integrated analytical techniques. This study, therefore, aims to investigate the phytochemical composition, cytotoxic effects, and spectroscopic profile (FT-IR, UV–Vis and GC–MS) of *Balanites aegyptiaca* aerial extract.

By providing a comprehensive evaluation of its chemical constituents and biological activity, this research seeks to contribute to the existing body of knowledge and explore the potential of the plant as a source of bioactive compounds for pharmaceutical and therapeutic applications. *Balanites aegyptiaca*, commonly known as 'Desert date', is a prickly shrub or tree that can grow to a height of 10 meters. It is widely dispersed in dry land areas of Africa and South Asia. Traditionally, it has been used in the treatment of various

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ailments such as jaundice, intestinal worm infections, wounds, malaria, syphilis, dysentery, constipation, diarrhea, hemorrhoids, stomach pain and fever. The plant is rich in protein, lipids, carbohydrates, alkaloids, saponins, flavonoids, and organic acids. *Balanites aegyptiaca,* a member of the Zygophyllaceae family, is a widespread yet often overlooked wild species in dryland regions of Africa and South Asia. (Sands, 2001). This tree is indigenous to most of Africa and parts of the Middle East. In India, it is most commonly found in regions such as Rajasthan, Gujarat, Madhya Pradesh and the Deccan (Kumar & Parveen, 2013). It grows especially well in Senegal, where it is considered one of the most prevalent tree species. *Balanites aegyptiaca* can thrive in various habitats and exhibits tolerance to a wide range of soil types, from sandy to heavy clay, as well as varying amounts of climatic moisture.

Its crown is normally spherical, either in a single compact mass or in multiple separate masses. The trunk is small and often branches out near its base. The bark varies in color from dark brown to grey and is deeply fissured. The branches are armed with stout yellow or green thorns that can grow up to 8 cm in length. The leaves consist of two separate leaflets, obovate in shape, asymmetric, and measuring 2.5 to 6 cm in length (Sands, 2001). The juvenile leaves are bright green, leathery and covered in fine hairs. The flowers grow in fascicles on the leaf axils and are fragrant with a yellowish-green color (Abdel Rahman et al*.,* 2018).

Therapeutic activity of the plant has been reported in studies ranging from the stem bark and leaves (Chothani and Vaghasiya, 2011; Abdel Rahman et al*.,* 2018; Tsafe et al*.*, 2019). Various parts of the plant, particularly the fruits, exhibit multiple biological and pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial and cytotoxic activities (Al-Malki et al*.,* 2015). This research specifically focuses on the aerial parts of the plant to evaluate their cytotoxic activity. The significance of this study lies in the limited existing research on the phytochemical composition, cytotoxic potential, and characterization of *Balanites aegyptiaca* aerial extract. Research on *Balanites aegyptiaca* is important because natural products are increasingly being investigated as potential sources of new medications and treatments. Investigating the phytochemical components of *Balanites aegyptiaca* aerial extracts may reveal new bioactive compounds with therapeutic potential, possibly leading to the development of new drugs or treatments (Atanasov et al*.,* 2021).

Furthermore, examining the cytotoxic effect of the extract is essential. The limited number of extensive studies on the characterization of *Balanites aegyptiaca* aerial extracts highlights the need for this research to bridge the existing knowledge gap. A comprehensive understanding of its chemical characteristics is crucial for maximizing the therapeutic potential of *Balanites aegyptiaca* in modern medicine. (Abdel Rahman et al*.,* 2018).

II. METHODOLOGY

A. Materials, Apparatus and Equipment

The materials, apparatus and equipment used in this research comprised a range of reagents, laboratory tools and analytical *instruments* necessary for extraction, phytochemical screening, and cytotoxicity studies. Chemical reagents included ethanol, methanol, distilled water, sodium hydroxide solution (10%), hydrochloric acid (dilute and 1% aqueous), ferric chloride solutions (2% and 10%), Dragendorff's, Mayer's and Wagner's reagents, chloroform, acetic anhydride, concentrated sulfuric acid, ammonia solution (10%), agar-agar, baker's yeast, yellow corn flour, and methyl paraben (nipagin). Apparatus and basic laboratory equipmentemployed were porcelain mortar and pestle, scissors, spatula, muslin cloth, Whatman No. 1 filter paper, test tubes, conical flasks, measuring cylinders, water bath, cotton wool, transparent ruler, glass and plastic vials and polyethylene bags. Specialized equipment included an aquarium for culturing *Drosophila melanogaster*, a rotary evaporator (Heidolph G3), FT-IR spectrometer (Agilent Cary 630), UV-Visible spectrometer (Agilent Cary 60) and GC-MS spectrometer (Agilent GC 7890B coupled with MSD 5977A), all of which were utilized for extract concentration and detailed spectroscopic characterization. Software used for data analysis in cytotoxicity was the GraphPad software (version 2023, Prism 10). Extraction was performed using a solvent-to-sample ratio of 1:5 (w/v) for nine days under cold maceration conditions with intermittent agitation. Filtrates were concentrated using a rotary evaporator and water bath maintained at 45°C. Statistical analysis of extraction yields obtained from triplicate experiments indicated that the difference between ethanol (23.3%) and methanol (24.7%) extracts was not statistically significant ($p > 0.05$), suggesting comparable extraction efficiencies under the experimental conditions employed.

B. Study design and Analytical approach

In order to investigate the cytotoxicity of *Balanites aegyptiaca* aerial extract, a crude extract of the sample was obtained through cold extraction, the crude extract was then subjected to preliminary phytochemical screening using standard procedures. Spectroscopic characterization was conducted to investigate the functional groups present, the bioactive constituents, and as well the to confirm the preliminary phytochemical screening conducted, using FT-IR, UV-Vis and GC-MS spectroscopic analysis respectively. The cytotoxicity of the crude extract was then evaluated using Acute Toxicity Study: *Drosophila melanogaster* (Harwich strain) was utilized for this study, of which the mortality rate, negative geotaxis and eclosion assay were carried out respectively. The FT-IR analysis was conducted using an Agilent Cary 630 FT-IR spectrometer over a spectral range of 4000–650 cm^{-1}, at a resolution of 4 cm^{-1} with 32 scans per sample. UV-Vis analysis was carried out using an Agilent Cary 60 UV-Visible spectrophotometer within the wavelength range of 200–800 nm using methanol as blank. GC-MS characterization was performed using an Agilent GC 7890B coupled with MSD 5977A equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), helium carrier gas at 1.0 mL min^{-1}, injection volume of 1 µL, oven temperature programmed from 50°C to 300°C at 10°C min^{-1} and mass scan range of m/z50–550. Compounds were identified using the NIST spectral library with a similarity index of ≥80%. The cytotoxicity of the crude extract was evaluated using Drosophila melanogaster (Harwich strain). Six treatment groups were established: control, 50, 100, 250, 500 and 750 mg extract per 10 g diet. Each treatment was

conducted in triplicate, with 20 flies per replicate (n = 60 flies per treatment group).

C. Sample Collection

The aerial part of *Balanites aegyptiaca* was obtained from its natural habitat in a Fulani settlement located at the outskirts of Sabon Yalwa along Kaduna/Zaria Road, Kaduna state. The plant samples were authenticated and given a voucher number from the Herbarium Section, Department of Biological Sciences, Kaduna State University (KASU), Kaduna, Nigeria.

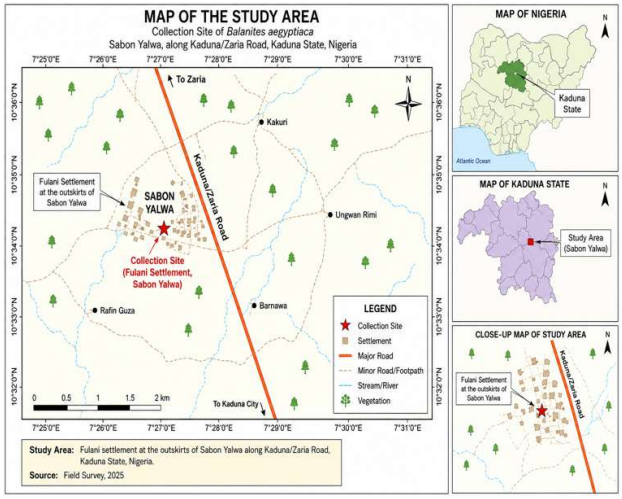

Figure 1. Location of the study area showing the collection site of ***Balanites aegyptiaca*** in Sabon Yalwa along Kaduna–Zaria Road, Kaduna State, Nigeria. Source: Authors' field survey and map design (2025).

D. Sample Preparation

The aerial parts of the plant (*Balanites aegyptiaca*) were air-dried for six weeks, then transported to the National Steel Raw Materials Exploration Agency, Kaduna, where it was pulverized and stored in an airtight container.

E. Extraction

The extraction method described by Tsafe et al., (2019) was followed with minor modifications. The pulverized aerial parts of *Balanites* aegyptiaca were soaked in ethanol (1:5 w/v) in a sealed container for nine days of cold extraction with occasional stirring. The extract was filtered through a muslin cloth and Whatman no. 1 filter paper. It was then concentrated using a water bath at 45°C and a rotatory evaporator, followed by shade air-drying. The leftover residue was discarded. The extraction was performed using two different polar solvents, ethanol and methanol. The resulting extracts were stored in labeled containers for future analysis. The percentage yield was calculated using Equation (1):

$$\%\, Yield = w_1/w_2 \times 100 \quad (1)$$

where w_1 = weight of the extract and w_2 = initial weight of the sample

F. Preliminary Phytochemical Screening

The phytochemical screening was conducted using both qualitative and quantitative approaches. Data collected included qualitative responses such as colour changes, precipitate formation, and frothing characteristics associated with standard phytochemical tests. The presence and relative intensity of phytochemical constituents were recorded using a semi-quantitative scoring system (−, +, ++, +++).

G. Qualitative screening

The test sample (crude aerial extract) was subjected to preliminary phytochemical screening using standard protocols, according to Evans, 2009. The extract was tested for the presence of steroids, alkaloids, tannins, flavonoids, saponins, phenolics, glycosides, and terpenoids, following the standard methods described by Evans (2009).

H. Spectroscopic Characterization

Fourier Transform-Infrared Spectroscopy (FT-IR) and UV-Visible Spectroscopy (UV-Vis) analyses were conducted at the Multipurpose Laboratory, Ahmadu Bello University, Zaria, Kaduna Nigeria, following the spectral analysis method described by Ogbiko et al., (2018). The phytocompounds were characterized using Gas Chromatography Mass Spectrometry (GC-MS) at the Analytical Laboratory, Department of Chemistry, Faculty of Science, Yobe State University.

1) FT-IR Spectroscopy

The functional groups present in the extract were identified using an FT-IR (Fourier Transform Infrared) Spectrometer. This was done by measuring the amount of infrared radiation absorbed by each type of bond in the extracts' molecule, using an FT-IR spectrophotometer. The FT-IR analysis was performed using an Agilent Technologies Cary 630 FT-IR spectrometer at the Multipurpose Laboratory, Ahmadu Bello University, Kaduna, Nigeria. The dried crude extract was prepared using the direct sample (ATR) method without further modification. Spectra were recorded over a scanning range of 4000–650 cm^{-1} at a resolution of 4 cm^{-1}, with 32 scans per sample to improve signal-to-noise ratio. Background correction was carried out prior to each measurement. The obtained spectra were analyzed by identifying characteristic absorption bands corresponding to specific functional groups.

2) UV-Vis Spectroscopy

The Ultraviolet-Visible spectroscopy was used to measure the absorbance of light by the extract's molecules in the UV and visible region of the electromagnetic spectrum. This was achieved using a UV-Vis spectrometer. The UV–Visible spectroscopy was conducted for the crude extract using an Agilent Technologies Cary 60 UV–Vis spectrophotometer at the same facility. The crude extract was diluted in methanol to obtain an appropriate concentration and the solution was placed in a 1 cm path length quartz cuvette. Absorbance measurements were recorded over a wavelength range of 200 - 800 nm with methanol used as the blank. The spectral peaks obtained were analyzed to determine the presence of chromophoric compounds and conjugated systems within the extract.

3) GC-MS Spectroscopy

The GC-MS works by producing a beam of gas ions from the extract molecule (analyte), the resulting mixture of ions were sorted out according to their mass to charge (m/z) ratios using magnetic field. A digital output was then produced in the form of peaks, from which the charge to mass ratio and the intensity (abundance) of the ions detected for each compound were determined. The GC–MS analysis of the crude sample was carried out using an Agilent Technologies GC 7890B coupled with MSD 5977A mass selective detector at the Analytical Laboratory, Department of Chemistry, Yobe State University.

The extract was filtered and diluted in analytical-grade solvent prior to injection. Separation was achieved using a capillary column (HP-5MS, 30 m × 0.25 mm internal diameter × 0.25 µm film thickness)**.** Helium was employed as the carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 1 µL in split mode. The oven temperature was programmed from 50°C (held for 2 min) to 300°C at 10°C/min**,** with a final hold time of 10 min. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, with a scan range of m/z 50–550. Identification of compounds was performed by comparing the obtained mass spectra with those in the NIST mass spectral library, and only compounds with a similarity index of ≥80% were reported.

4) Determination of Cytotoxicity Activity (Acute Toxicity Study)

i. *Drosophila melanogaster* stock and culture

Wild type *Drosophila melanogaster* (Harwich strain) fruit flies were obtained from the National Species Stock Centre (Bowling Green, OH, USA). The flies were bred and maintained on cornmeal medium containing brewer's yeast (1% w/v), agar-agar (1% w/v), and nipagin (0.08% v/w) as a preservative, at constant temperature of (24 ± 2 °C) and 70% humidity under a 12-hour dark/light cycle.

ii. Toxicity studies (LC_{50}) and incapacitation action test of *Balanites aegyptiaca* aerial part crude extract on *Drosophila melanogaster*

Mortality study (*LC_{50}*)

The toxicity study was evaluated with slight modifications to the method described by Iorjiim et al., (2020). The fruit flies were divided into six groups, with each group receiving a different concentration of the *Balanites aegyptiaca* extract. The concentrations (50 mg, 100 mg, 250 mg, 500 mg, and 750 mg) were incorporated into 10 g of the flies' diet. These diets were then transferred into empty, sterilized treatment vials. The first group served as a normal control (NC), containing only 10 g of the flies' diet with no extract added. The experiment was prepared in three replicate vials per group, with each vial containing twenty fruit flies of both sexes. The *Drosophila melanogaster* flies were introduced into the vials, and mortality was recorded every 24 hours for a total of seven days.

iii. Determination of negative geotaxis

A negative geotaxis experiment, as previously reported by Gargano et al., (2005), was performed to assess the flies' locomotor performance. Flies from normal control (NC) and treatment groups were transferred into separate vials. Each group of flies were tapped to the bottom of the vial, and the number that rose beyond 6 cm in 6 seconds was recorded. This count was then calculated as a percentage of the total number of flies in the vial.

iv. Reproductive Ability (Eclosion assay)

Twenty adult fruit flies of both sexes (males and females), aged 1 to 4 days, were transferred into treatment vials. Each vial contained 10 g of the flies' diet supplemented with different concentrations of the crude extract. The extract concentrations used were 50 mg, 100 mg, 250 mg, 500 mg, and 750 mg. A normal control (NC) group received only 10 g of the standard diet without extract. The flies were allowed to feed for three days before being removed from the vials. The vials were incubated until day 10, when the eclosion assay was conducted. The assay assessed reproductive success by counting the number of pupal cases in each vial, including the normal control, as described by Mark et al., (2021).

III RESULTS AND DISCUSSION

A. Percentage Yield of *Balanites aegyptiaca* (L.) Del. Ethanol and Methanol Aerial Part Extract

The extraction efficiency of *Balanites aegyptiaca* aerial parts was evaluated using two different organic solvents: ethanol and methanol. The choice of solvent plays a critical role in the quantitative recovery of plant metabolites, as it determines the range of compounds solubilized based on polarity. The results of the extraction process are summarized in Table 1 below.

Table 1 Percentage yield of Balanites aegyptiaca aerial crude extract with different solvents

***Balanites aegyptiaca* aerial extract**	**Percentage yield (%)**
Ethanol extract	23.3%
Methanol extract	24.7%

As shown in Table 1, the methanol extract produced a slightly higher yield (24.7%) compared to the ethanol extract (23.3%). This finding suggest that methanol has a slight advantage over ethanol in terms of extraction efficiency. The slightly higher yield obtained with methanol indicates that it is more effective at extracting the desired compounds from the plant material. Although the difference is small, it suggests that methanol may be better suited for solubilizing and extracting the bioactive components due to its distinct chemical properties compared to ethanol.

Methanol's effectiveness in extracting these compounds may be attributed to its high polarity. This polarity difference influences how well each solvent can dissolve various phytochemicals present in *Balanites aegyptiaca*. Methanol, due to its unique solubility profile, may be able to extract or dissolve a greater range of chemicals than ethanol. This is in agreement with the findings of Azwanida et al., (2015).

However, the modest difference in yield suggests that both solvents are relatively efficient in the extraction process.

B. Phytochemical Screening Results for *Balanites aegyptiaca* (l.) Del. Ethanol and Methanol Aerial Part Extract

Qualitative phytochemical screening was performed to identify the primary classes of secondary metabolites present in the aerial parts of *Balanites* aegyptiaca. This analysis is fundamental to understanding the therapeutic potential of the plant, as these bioactive compounds are responsible for its medicinal properties. The comparative results for both ethanol and methanol extracts are presented in Table 2.

Table 2 Phytochemical screening results of the crude extract

Phytochemicals	Ethanolic extraction	Methanolic extraction
Saponins	++	++
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Terpenoids	+	++
Phenolic	++	++
Glycosides	+	+
Steroids	+	++

++ Highly present + Moderately present

The table provides valuable insights into the plant's medicinal properties and how different extraction methods affect the yield of bioactive compounds. Both extracts revealed a rich array of phytochemicals essential for recognizing the plant's therapeutic benefits. Specifically, this study identifies saponins, tannins, alkaloids, flavonoids, phenolic compounds, steroids, terpenoids and cardiac glycosides in both extracts, showcasing a strong base of therapeutic properties. The methanol extract stands out with significantly higher levels of terpenoids and steroids compared to the ethanol extract, indicating that methanol is more effective in extracting these specific compounds. In contrast, the ethanol extract contains moderate levels of terpenoids and steroids, showing that while it still has these beneficial compounds, their concentrations are lower than those in the methanol extract. This difference highlights the importance of choosing the right solvent for extraction, as it significantly affects the concentration of specific phytochemicals. These findings align with previous research conducted by Wakawa et al., (2018a, 2018b), which also identified alkaloids, flavonoids, tannins, saponins, terpenes, steroids, cardiac glycosides, phenols, balsam and renins in various parts of *Balanites aegyptiaca* using different solvents.

This phytochemical screening reveals the extensive medicinal potential of Balanites aegyptiaca. The presence of numerous bioactive compounds in both extracts suggests that the plant could be a valuable source of natural remedies for a range of health conditions. The variations in phytochemical profiles between the methanol and ethanol extracts highlight the importance of extraction methods in maximizing the plant's therapeutic efficacy, paving the way for further research into its health benefits and applications in both traditional and modern medicine.

C. FT-IR Spectra of *Balanites aegyptiaca* Aerial Part Extract

Fourier Transform Infrared (FT-IR) spectroscopy was utilized to identify the functional groups present within the crude extract of *Balanites aegyptiaca*. By measuring the absorption of infrared light at specific frequencies, the chemical bonds and molecular structures of the extract was characterized as shown in figure 1 below.

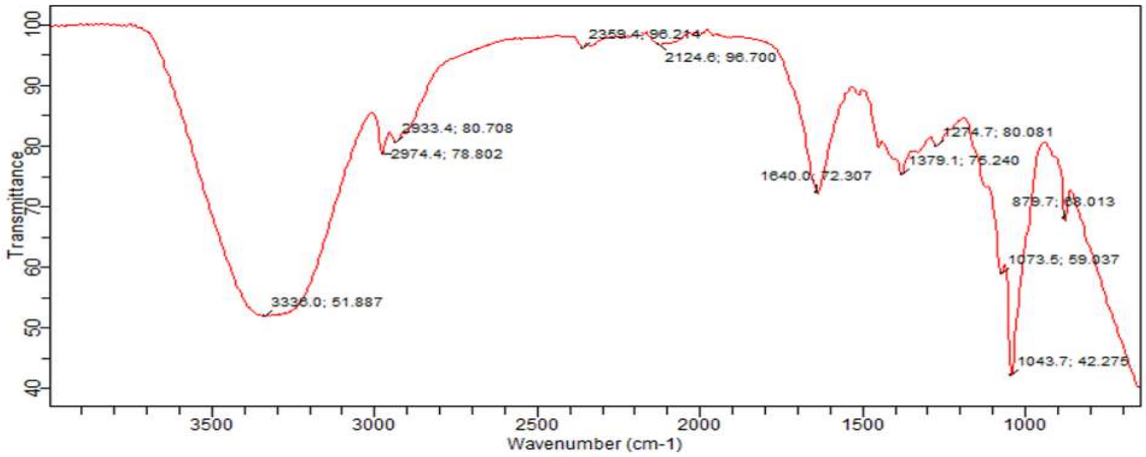

Figure 1 FT-IR spectra of *Balanites aegyptiaca* (L.) Del. aerial part crude extract

The resulting spectra are illustrated in Figure 1, with corresponding functional group assignments detailed in subsequent analysis. The FT-IR Spectra of the crude extract of the plant in Figure 1 shows several significant peaks of the aerial plant extract from *Balanites aegyptiaca* (desert date). This shows the presence of various functional groups such as a large peak at 3336 cm^{-1}, which corresponds to O-H stretching vibrations found in alcohols and phenolic compounds. This demonstrates that hydroxyl groups are involved in hydrogen bonding. A sharp peak at 2974 cm^{-1} indicates C-H stretching vibrations associated with alkanes, as well as the existence of aliphatic hydrocarbons. Carbonyl groups, such as ketones, aldehydes, or carboxylic acids, show a prominent peak at 1640 cm^{-1}. A peak at 1043 cm^{-1} indicates the presence of phenolic chemicals in the extract, which are associated with C-O stretching. These spectral characteristics provide detailed information about the functional groups in the extract, indicating a complex mixture of alcohols, alkanes, carbonyl compounds, and phenols. This information is critical for understanding the extract's chemical composition and potential bioactive characteristics.

D. UV-Vis Spectroscopy of *Balanites aegyptiaca* (L.) Del. Aerial Part Extract

The chemical composition in *Balanites aegyptiaca* through several notable absorption peaks is as shown in Table 3. *Ultraviolet*-Visible (UV-Vis) spectroscopy was employed to further characterize the extract by identifying pigments and compounds with conjugated electronic structures. This technique provides a "fingerprint" of the extract's absorption characteristics across the visible and UV spectrum. The specific absorption values recorded during the analysis are provided below.

Table 3 UV-VIS peak values of *Balanites aegyptiaca* (L.) Del. aerial part crude extract

Peaks	Wavelength (nm) range	Wavelength (nm)	Absorbance
First peak	650-700	665.00	0.220
Second peak	600-650	607.00	0.054
Third peak	500-550	536.00	0.071
Fourth peak	500-550	505.00	0.080

From Table 3, The wavelengths and absorbance of the first, second, third and fourth UV-VIS peaks analysis of the *Balanites aegyptiaca* aerial part extract is as shown. The table provides insights into its chemical composition through several absorption peaks. The most prominent peak is shown at 665.00 nm, with an absorbance of 0.220, indicating the presence of chlorophyll-related compounds (Rachma & Veinardi, 2010), which is required for photosynthesis and is responsible for green coloration in plants. This implies that the extract may contain a high quantity of potential flavonoids and polyphenolic chemicals, which can absorb in this wavelength range due to their extended conjugated structures. At 607.00 nm, the lower absorption value of 0.054 indicates the existence of additional chemicals in modest levels. Additional peaks at 536.00 nm and 505.00 nm, with absorbance values of 0.071 and 0.080, respectively, suggest the presence of other pigments or chemical components. The lower absorption values at these wavelengths, compared to the peak at 665.00 nm indicate either a lower concentration or different types of absorbing compounds. Overall, the spectrum shows a rich variety of pigments and chemicals, with chlorophyll-related compounds being the most prominent component, indicating the extract's extensive chemical composition. Further studies are needed to accurately identify and quantify these molecules; however, these findings indicate the existence of important photosynthetic pigments and other minor ingredients (Strack et al*.,* 2003).

E. GC-MS Spectroscopy of *Balanites aegyptiaca* (L.) Del. Aerial Part Extract

Gas Chromatography-Mass Spectrometry (GC-MS) was performed to provide a detailed identification of the specific volatile and semi-volatile compounds within the extract. This technique allows for the separation of complex mixtures and the identification of individual molecules based on their mass-to-charge ratios. The compounds identified through this analysis are listed in Table 4.

Table 4 Compounds identified in the GC-MS analysis of the crude extract

Peak	R.T	Formula	Name
1	4.3	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	4.5	$C_{12}H_{26}$	Dodecane
3	5.8	$C_5H_6N_2O$	4(1H)-Pyrimidinone, 6-methyl
4	6.3	$C_{14}H_{30}$	Tetradecane
5	6.8	$C_9H_{13}NO$	p-Hydroxyamphetamine
6	7.0	$C_{12}H_{23}NO_2$	1-Hexyl-1-nitrocyclohexane
7	7.3	$C_{11}H_{24}O$	C11H24O 2-Undecanol
8	7.9	$C_{13}H_{18}O_2$	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ether
9	8.8	$C_9H_{13}NO_3$	Epinephrine
10	9.6	$C_{11}H_{22}O_2$	Undecanoic acid
11	9.8	$C_{10}H_{16}N_2O$	1-Methyl-3,6-diazahomoadamantan-9-one
12	10.0	$C_{20}H_{32}O_3$	15(S)-Hydroxy-(5Z,8Z,11Z,13E)-eicosatetraenoic acid
13	11.1	$C_{11}H_{22}O_2$	Undecanoic acid

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14	11.4	$C_7H_8O_3$	3,5-Dihydroxybenzyl alcohol
15	12.5	$C_7H_{10}O_2$	2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one
16	12.8	$C_7H_{10}O_2$	2-Cyclopenten-1-one, 2-hydroxy-3,4-dimethyl
17	13.1	$C_{12}H_{22}O_2$	Isobutyraldehyde, bis(2-methylallyl)acetal
18	13.6	$C_9H_8O_2$	trans-Cinnamic acid
19	14.5	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
20	14.9	$C_{18}H_{28}O_2$	2-Cyclopropen-1-ol, 1,2-dicyclopentyl-3-(1-methylethyl)-, acetate
21	15.6	$C_{19}H_{25}NO_5$	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxol yl)ethyl]pentyl ester
22	16.1	$C_{12}H_{22}O_4$	Dodecanedioic acid
23	16.6	$C_{20}H_{25}N_3O$	Lysergic acid diethylamide
24	17.2	$C_{21}H_{27}NO_2$	Methadone N-oxide
25	17.8	$C_{15}H_{24}O_2Si$	Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane
26	18.0	$C_{12}H_{18}N_2O_2$	8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione
27	18.4	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
28	19.7	$C_{19}H_{38}O_2$	Methyl stearate
29	20.2	$C_{18}H_{34}O_2$	Oleic Acid
30	20.8	$C_{19}H_{34}O_2$	Methyl 2-octylcyclopropene-1-heptanoate
31	22.2	$C_{20}H_{38}O_2$	Ethyl Oleate
32	26.7	$C_{10}H_6N_2OS_2$	Quinomethionate
33	27.9	$C_{17}H_{30}OSi$	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane
34	28.2	$C_{10}H_{28}O_4Si_3$	Silicic acid, diethyl bis(trimethylsilyl) este
35	29.6	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate
36	30.1	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate
37	31.6	$C_{10}H_{28}O_4Si_3$	Silicic acid, diethyl bis(trimethylsilyl) ester
38	31.8	$C_{12}H_{34}O_4Si_4$	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane

As shown Table 4 above, the GC-MS analysis identified approximately 38 distinct compounds, indicating a diverse chemical profile. These chemicals were detected during a retention time range of 4 to 32 minutes. The analysis identified several alkanes, including dodecane and tetradecane, which are simple hydrocarbons found in both natural and synthetic materials. Undecanoic acid and oleic acid, two well-known lipid and oil components, were also discovered. The study also discovered complex organic compounds, such as 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethenone and 1-Methyl-3,6-diazahomoadamantan-9-one, which have intricate structures and unique functional groups. Phenolic substances like p-Hydroxyamphetamine and 3,5-Dihydroxybenzyl alcohol were found, indicating phenolic hydroxyl groups. In addition, the analysis revealed many silyl and siloxane derivatives, including Trimethyl[4-(2-methyl-4-oxo-2-pentyl) phenoxy]silane, indicating the method's potential to detect organosilicon compounds. Alcohols, including 2-Undecanol, and carbonyl-containing compounds, such as epinephrine 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one, were also identified. These findings are consistent with the FT-IR interpretation, which also found alcohols, phenols, alkanes, and carbonyl groups in the extract. The consistency of the GC-MS and FT-IR results ensures a full and accurate analysis of the extract's chemical composition. These findings align with those of Hassan et al., (2017), who identified fatty acids such as oleic acid and hexadecanoic acid from the GC-MS analysis of the leaves and fruit mesocarp of *Balanites aegyptiaca* (L.) Del.

F. Cytotoxicity analysis (Acute toxicity study) of *Balanites* aegyptiaca (L.) Del. aerial part extract on *Drosophila melanogaster*

In order to evaluate the safety and biological activity of the extract, acute toxicity studies were conducted using *Drosophila* melanogaster as a model organism. This study employed mortality, negative geotaxis (locomotion) and eclosion (developmental) assays across a range of concentrations (50 mg/10 g to 750 mg/10 g of diet). The observed biological responses are visualized in Figures 2, 3, and 4.

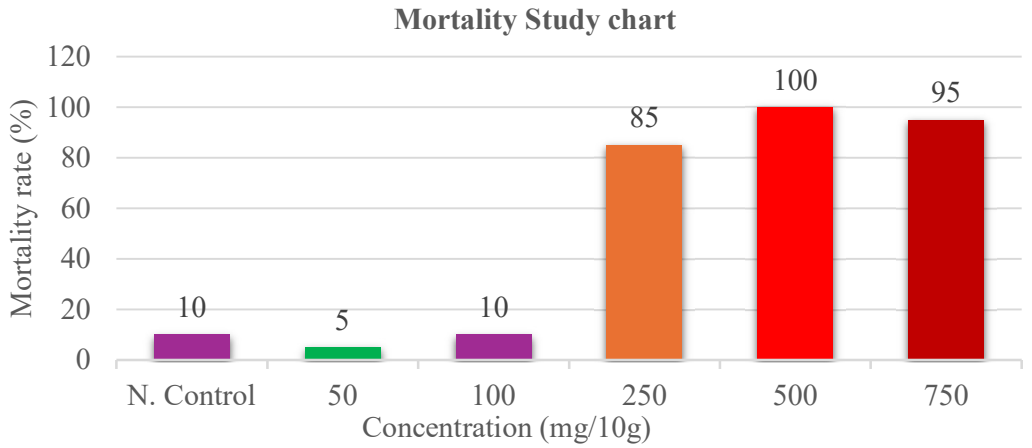

Figure 2 Mortality study chart of *Balanites aegyptiaca* (L.) Del. extract on *Drosophila melanogaster* (Azwanida, 2015)

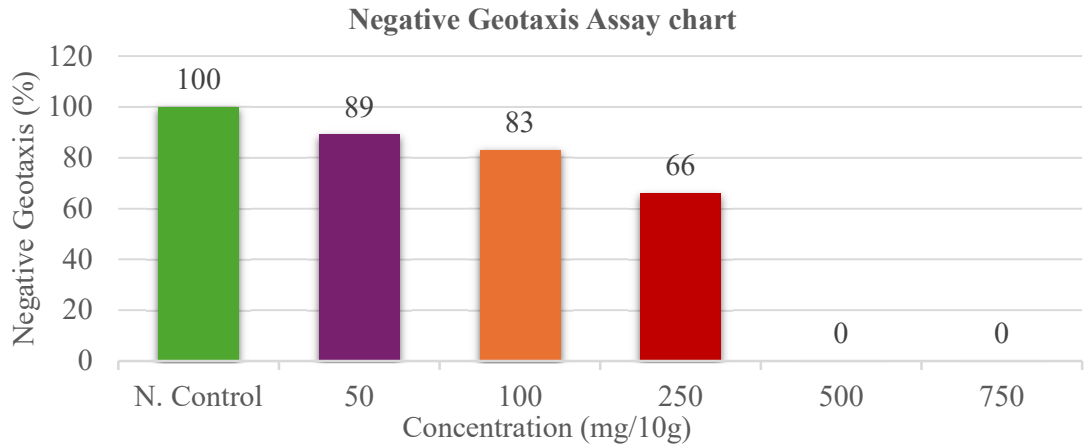

Figure 3 Negative Geotaxis chart of *Balanites aegyptiaca* (L.) Del. extract on *Drosophila melanogaster* (Al-Malki, 2015)

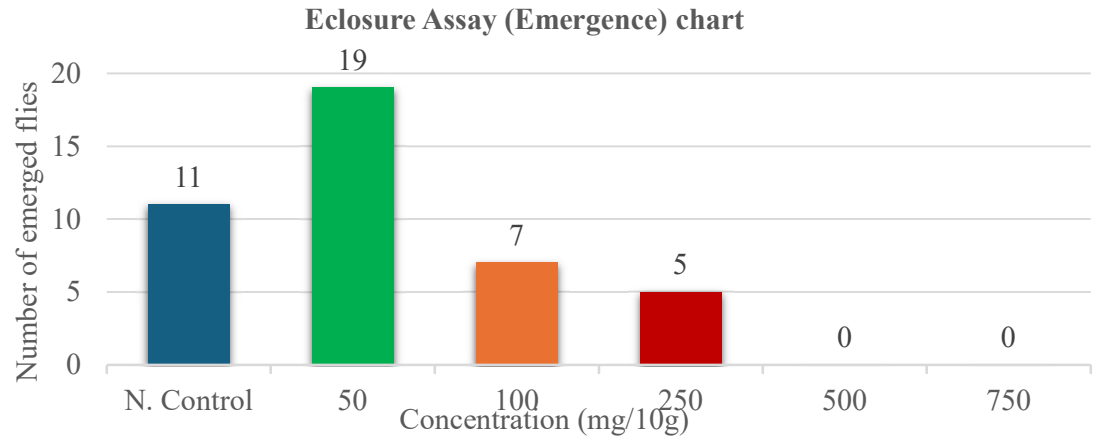

Figure 4 Chart for the Number of emerged flies in different concentrations (Al-Malki, 2015)

Figure 2 shows the mortality study chart of *Balanites aegyptiaca* (L.) Del. extract on *Drosophila melanogaster*, Figure 3 shows the Negative Geotaxis chart of *Balanites aegyptiaca* (L.) Del. extract on *Drosophila melanogaster* while figure 4 shows the Chart for the Number of emerged flies in different concentrations. Detailed acute toxicity study was conducted on *Balanites aegyptiaca* aerial part extract using *Drosophila melanogaster*. Three different assays were

employed to evaluate the effects of various extract concentrations on the flies. The assays included a mortality study, a negative geotaxis assay, and an eclosion assay. The fruit flies were divided into six groups, with each getting a different concentration of the extract in their diet: Normal Control (NC), 50 mg, 100 mg, 250 mg, 500 mg, and 750 mg per 10 grams of food. The results of these assays provide a comprehensive assessment of how different quantities of the extract affect the flies' health and behavior.

1) Mortality Study: The mortality results demonstrated an overall dose-dependent increase in toxicity of *Balanites aegyptiaca* aerial extract on *Drosophila melanogaster*. In the normal control (NC) group, a mortality rate of 10% was recorded, reflecting baseline or natural death in the absence of treatment. At 50 mg/10 g diet, mortality decreased slightly to 5%, indicating no observable toxic effect at this concentration. At 100 mg/10 g, mortality increased to 10%, suggesting the onset of mild toxicity. A pronounced increase was observed at 250 mg/10 g, where mortality rose sharply to 85%, indicating substantial toxic activity of the extract. At higher concentrations, mortality reached 100% at 500 mg/10 g and 95% at 750 mg/10 g (Figure 1). Although a slight reduction in mortality was observed at 750 mg/10 g compared to 500 mg/10 g, statistical analysis revealed that this difference was not significant ($p > 0.05$). Therefore, both concentrations can be interpreted as producing comparably high mortality effects, consistent with a plateau phase in a typical dose–response relationship rather than a true decline in toxicity at higher dose. These findings confirm the strong cytotoxic potential of the extract at elevated concentration.s.

2) Statistical Analysis: All experimental data were expressed as mean ± standard deviation (SD) of three replicates. Statistical analysis was performed using GraphPad Prism (version 8.0)**.** Differences among treatment groups were evaluated using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc multiple comparison test to determine pairwise differences between means. Statistical significance was set at $p < 0.05$. Specifically, comparisons between high-dose groups (500 mg/10 g and 750 mg/10 g) were conducted to determine whether observed differences in mortality were statistically meaningful. Where $p > 0.05$, differences were considered not statistically significant and interpreted as comparable biological effects.

3) Negative Geotaxis Assay: The negative geotaxis assay, which evaluates the flies' locomotor activity, demonstrated a gradual decline in normal behaviour as the concentration of the extract increased. In the NC group, all flies (100%) exhibited normal locomotion, suggesting no negative effects. At 50 mg, 89% of the flies exhibited normal movement, which declined to 83% at 100 mg. The trend continued with only 66% of flies maintaining normal locomotion at 250 mg. At the higher concentrations of 500 mg and 750 mg, the locomotor activity is 0% which is attributed to the mortality of these concentrations (100% and 95%). This indicates that the extract significantly disrupts the flies' movement and coordination at higher doses/concentrations.

4) Eclosion Assay: The eclosion assay evaluated the emergence of adult flies from pupae to gauge the developmental impact of the extract. In the NC group, 11 flies emerged, indicating normal development. The 50 mg group experienced an increase in the number of emerging flies, with 19 successfully hatching, suggesting that this lower concentration does not negatively affect development. In contrast, at 100 mg, only 7 flies emerged, and this number decreased further to 5 at 250 mg. In the groups exposed to 500 mg and 750 mg, no flies emerged, due to 100% and 95% mortality rates, respectively. The absence of emerging flies at the higher concentrations reveals the extract's severe impact on development and implies that high dosages are not only lethal to adult flies but also damaging to the development of pupae. Further studies employing intermediate concentrations and expanded sample sizes are recommended to validate this observation and establish a more detailed dose-response relationship.

In summary, the *Balanites aegyptiaca* aerial part extract exhibited severe cytotoxic effect on *Drosophila melanogaster*, with toxicity increasing as the concentration of the extract increases. Lower concentrations (50 mg and 100 mg) had relatively minor effects on mortality, locomotion, and eclosion. However, higher concentrations (250 mg, 500 mg, and 750 mg) led to significant increases in mortality, severe locomotion impairment, and complete inhibition of eclosion. These findings highlight the importance of carefully managing dosage when using this extract, as high concentrations can have severe toxic effects, affecting both the survival and development of the flies or in the case of a human trial. Further research is needed to identify the specific bioactive compounds responsible and to understand their mechanisms, which will be crucial in assessing the extract's safety and therapeutic potential.

IV CONCLUSION

The study has shown that the aerial part extract of *Balanites aegyptiaca* possesses significant medicinal potential, as demonstrated by its diverse phytochemical profile and notable cytotoxic effects. Methanol was found to be a more effective solvent than ethanol, particularly for extracting terpenoids and steroids. Spectroscopic analyses revealed the presence of a complex array of functional groups and compounds within the extract. Acute toxicity tests on *Drosophila melanogaster* (Fruit flies) showed a dose-dependent increase in toxicity, with lower concentrations having minimal impact and higher concentrations causing significant mortality and developmental issues. These findings emphasize the need for precise dosage control and support the need for further research to identify the specific compounds responsible for these effects, which is necessary for assessing the extract's therapeutic potential and safety.

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APPENDIX

Appendix A
FT-IR Spectrum of *Balanites aegyptiaca* (L.) Del. aerial part crude extract

Table A.1: FT-IR result interpretation of *Balanites aegyptiaca* (L.) Del. aerial part crude extract

Wavelength range (cm^{-1})	Wavelength of Peak (cm^{-1})	Bond	Functional group
3500-3000	3336	O-H	Alcohols
3000-2500	2974	C-H	Alkane
2000-1500	1640	C=O	Carbonyl
1500-1000	1043	C-O	Phenols

Appendix B

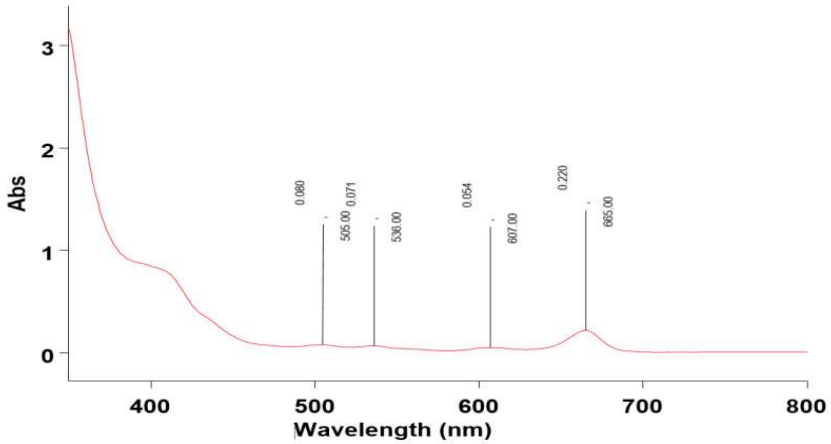

Figure A.1: UV-Vis Spectrum of *Balanites aegyptiaca* (L.) Del. aerial part crude

Appendix C

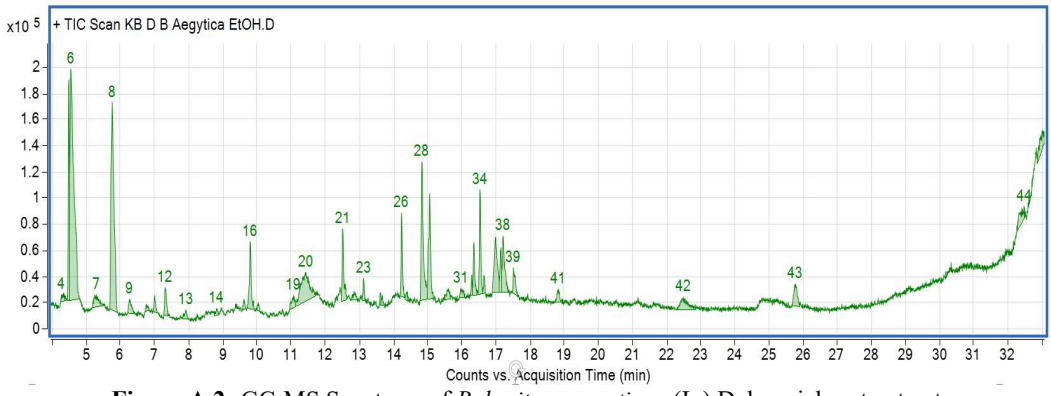

Figure A.2: GC-MS Spectrum of *Balanites aegyptiaca* (L.) Del. aerial part extract

Appendix D

Figure A.3: Three-day-old virgin *Drosophila melanogaster* (Fruit flies) in D. melanogaster Laboratory, Department of Biochemistry, Kaduna State University (KASU)

Appendix E

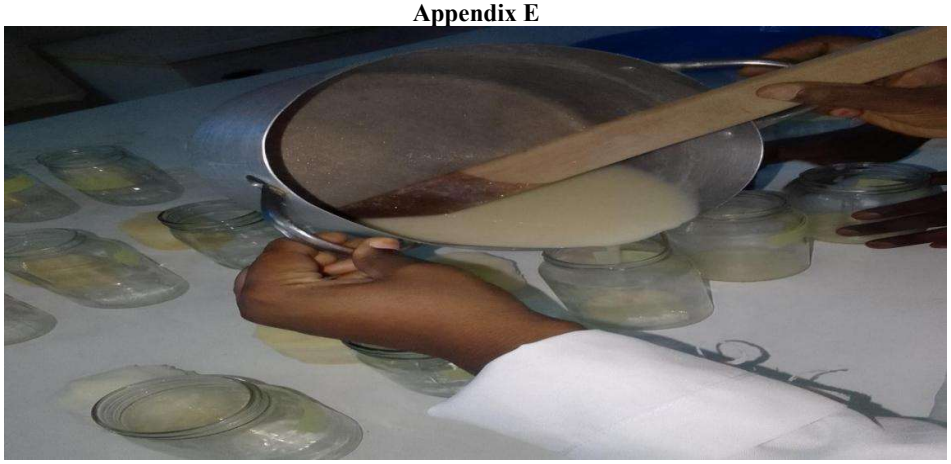

Figure A.4: *Drosophila melanogaster* (Fruit flies) diet has been prepared in D. melanogaster Laboratory, Department of Biochemistry Kaduna State University (KASU)