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Original Research Article

Proximate Composition, Phytochemicals Evaluation and Characterization of Aqueous Fruit Extract of *Balanites aegyptiaca* (Desert Date Palm)

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Abstract: ***Background and objectives:*** The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. *Balanites aegyptiaca* is an evergreen plant containing large varieties of chemical substances which possess important therapeutic properties and can be utilized as food supplement. The aim of the study was to carry out proximate, phytochemicals and identification of chemical compounds using aqueous fruit extract of *Balanites aegyptiaca.* ***Methods:*** The proximate and phytochemicals analysis were carried out by standard protocols of AOAC, While The phyto-constitutes of *Balanites aegyptiaca* aqueous fruit extract were determined by Gas Chromatography (Agilent 6890 series) coupled with HP-5MS column mass spectrometer. The helium was used as carrier gas at a flow of 1.0ml/min. The identification of the constituents of aqueous fruit extract was performed by matching their mass spectra and retention indices with those obtained from authentic samples and/or NSIT/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data. ***Results:*** The phytochemical analysis reveals that the aqueous fruit extract of *Balanites aegyptiaca* contained flavonoids (288.33±3.01 mg/100g), tannins (20.50±0.4mg/100g) saponin (320.90±10.28mg/100g), Glycoside (163.92±0.33), steroids (36.40±0.80mg/100g), alkaloids (78.67±1.03mg/100g) and phenols (227.43±1.01mg/100g) while Terpenoids and Anthraquinones, Anthocyanines were found to be absent. The proximate analysis of the fruits of *Balanites aegyptiaca*; revealed that the moisture content was 2.3±0.2%, crude protein 3.2±0.08%, crude fibre 16.4±0.5%, lipid content 3.1±0.7, carbohydrates 72.6±1.6% and ash content 2.4±0.2%. The GC-MS Analysis shows the presents of many important organic compounds in which three were reported to have biological activity this includes Benzene, [(methoxymethoxy)m ethyl]-, Undecanoic acid Hexadecanoic acid, ethyl ester as antifungal and antioxidant activity. ***Conclusion:*** The proximate composition indicates the nutritional potential of the *Balanites aegyptica.* The GC-MS and Phyto-chemical analysis of *Balanites aegyptiaca* shows the presence of chemical compounds for industrial, foods, additive and other pharmaceutical formulation.

Keywords: *Balanites aegyptiaca*, Fruit, GC-MS, Phytochemicals, proximate analysis.

1.0 INTRODUCTION

Balanites aegyptiaca (L.) Del. is a member of the *Balanitaceae* family. It is a multi-branched, evergreen tree found across India's arid regions. (Anon,1986) It's popular in Africa's Sudano-Sahielian area, the Middle East, and South Asia (1991, Hall and Walker). It is recognized by a variety of names, including Arabic names such as Heglig (tree), lalob (fruit); commercial names such as zaccone, zachun, desert date (dried fruit); and in India, Hindi names such as Hingot and English names such as thorn tree/desert date (Hardman and Sofowora,1991). (aduwa) in Hausa (Kirtikar and Basu,1933).

B. aegyptiaca offers a wide range of nutraceutical uses, according to Abu Al-Futuh (1993). The fruit's fleshy pulp can be consumed fresh or dried. It includes 64–72 percent carbs, as well as crude

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protein, steroidal saponins, vitamin C, ethanol, and other minerals that humans require. Mohamed*et al*., (2002) also mentioned that the seed kernel is an edible product. It has ahigh protein content and good grade oil. According to previous findings by Hall and Walker, 1991; Tesfay*et al*., 2014; Varshney and Vyas, 1982, all components of the tree, including fruits, seeds, barks, and roots, have therapeutic properties. The most important is steroidal saponins, which create diosgenin, a source of steroidal drugs such corticosteroids, contraceptives, and sex hormones, as described by Farid*et al*., 2002; Pettit*et al*., 1991. According to Tesfay*et al*., 2014, B. aegyptiaca is a multifunctional tree that supplies food, medicinal items, and fuel wood vital for subsistence living in dry and semi-arid conditions when other options are limited. Because the potential of B. aegyptiaca under management is unclear, a primary aim is to establish a picture of variety within the natural range and the capacity to produce plants with desired features, as indicated by Chothani and Vaghasiya (2006).

Balanites leaves, flowers, and fruit pulp are high in protein, K, Fe, Mn, Zn, and Cu. According to (Hall and Walker, 1991), (Chothani and Vaghasiya 2011), the fleshy pulp of both unripe and ripe fruit is edible and eaten dry or fresh, the fruit is used as sweetmeats in Ghana, alcoholic liquor in Nigeria, and a soup component in Sudan. Young leaves and fragile shoots are prepared as a vegetable by boiling, pounding, then frying or adding fat. Flowers are used as a supplement to meals in the western region of Africa. According to Hall and Walker (1991). According to (Chothani and Vaghasiya, 2011), the kernels yield edible oil that is utilized in cooking. Because the oil remains stable when heated and has a high smoking point, its free fatty acid concentration is minimal. It has a pleasant aroma and flavor. The leaves are eaten raw or cooked, the oily seed is boiled to make it less bitter and eaten combined with sorghum, and the blooms can be eaten. The fruit may be fermented to make alcoholic drinks. The seed contains seed oil, which is used as a cooking oil. The seed cake that remains after the oil is extracted is typically utilized as animal feed. *Balanites aegyptiaca* is utilized in a variety of folk remedies all around the world. This plant has grown in popularity and is now used to treat a variety of ailments and problems. According to Wilson*et al*., 2009, the fruit is used as an oral hypoglycemic medication in Africa's Sahara area. Furthermore, according to Hall and Walker (1991), the fruits are often employed as a purgative, antiparasitic, and schistosomicide. According to Chapagain and Wiesman (2006), the stem, root, and leaf extracts of B. aegyptiaca have often been employed as numerous traditional folk remedies, particularly in the treatment of parasites, sore throat, constipation, and eye discomfort. Previous research on the therapeutic properties of *B.aegyptiaca* has revealed anthelminthic, antivenin, anticancer, antioxidant, mosquito larvicidal anti-inflammatory, antidiabetic (Tesfaye, 2015), wound healing, hepatoprotective, hypocholesterolemic, diureticcontraceptive, and antiviral activities in various parts of *Balanites* extracts Gaur*et al*., 2008.Aqueous extract of fruits showed spermicidal activity as reported by Speroni*et al*., 1998 without local vaginal irritation in human being antidiabetic, treatment of jaundice. The aim of this study is to carry out proximate, phytochemicals and characterisation of chemical compounds of aqueous fruit extract of *Balanites aegyptiaca.*

2 METHODS

2.1 Sample collection and preparation

Fresh *Balanites aegyptiaca* fruits were harvested concurrently in Tudun wada local government, Kano state, Nigeria. Prior to examination, the materials were identified and authenticated in the herbarium division of Kano University of Science and Technology Wudil's biological science Department, and then shade dried for around two weeks. The dried berries were steeped in distilled water for 48 hours, filtered, and the extract evaporated to dryness before being extracted with distilled water.

2.2 Phytochemical Screening

2.21Qualitative Analysis on Phytochemical Constituent of *Balanites aegyptiaca* fruits

Balanites aegyptiaca aqeous extract were weighed and dissolved in 50ml of distilled water. The mixture was shaken gently and allowed to dissolve for about five (5) minutes the solution was then subjected to the following qualitative tests.

Qualitative phytochemical analysis the extracts was tested for the presence of bioactive compounds by using following standard methods (Sofowora, 1993, Trease and Evans 1989, Harborne 1973).

2.2.2Test for Alkaloids

Few drops of 1% HCl were added to the filtrate to which 5 drops of freshly prepared Dragendorff‟s reagent was added. Formation of a precipitate indicated the presence of alkaloids.

2.2.3Test for Anthraquinones

The filtrate (5ml) was hydrolysed with diluted Conc. H_2SO_4. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

2.2.4 Test for polyphenols

Yellow precipitates obtained by the addition of 3 drops of lead acetate solution (5%) to the filtrate indicated the presence of phenolic compounds (Velevan, 2015).

2.2.5 Test for Tannins

The dried powdered sample (0.5g) was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. Formation of

brownish green or a blue-black colouration indicated the presence of tannins

2.2.6 Test for Saponins

The powdered sample (2g) was boiled in 20 ml of distilled water in a water bath and thenfiltered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drop s of olive oil and shaken vigorously; formation of emulsion indicates the presence of saponnins.

2.2.7 Test for Flavonoids

Dilute ammonia solution (5 ml) were added to a portion of the aqueous filtrate of thepowdered sample followed by addition of concentrated H_2SO_4. A yellow colouration observed in each filtrate indicated the presence of flavonoids. The yellow colouration disappeared on standing

2.2.8 Test for Terpenoids (Salkowski Test)

The filtrate (5 ml) was mixed in 2 ml of chloroform; 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.2.9 Test for Cardiac Glycosides (Keller-Killani test)

The filtrate (5 ml) was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then treated with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides

2.2.10 Test for Anthocyanins

The aqueous filtrate (2 ml) was added to 2 ml of 2N HCl and ammonia. The appearance of reddish-pink turned blue-violet indicated the presence of anthocyanins

2.2.11 Test for phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.3 Quantitative Analysis of Phytochemical Constituents of *Balanites aegyptiaca*fruits

2.3.1. Determination of total Flavonoids

Total flavonoids content was determined by aluminium chloride method described by (Kumar *et al*., 2008) with minor modification. 0.5 ml of the sample was mixed with 0.3 ml of 5% sodium nitrite. After 5 min 0.3 ml of 10% aluminium chloride was added. After 6 min, 2.0 ml of 1 M sodium hydroxide was added and the total volume was made up to 5.0 ml with distilled water. The absorbance of the mixture was measured at 510 nm against a reagent blank. Catechol was used as standard. The flavonoid content was expressed as milligram of catechol equivalence (CAE) per gram of extract.

2.3.2 Determination of Alkaloids

The sample (5 g) was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hr. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed (Velevan, 2015).

2.3.3 Determination of Tannins

The sample (500mg) was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10min (Velevan, 2015).

2.3.4 Determination of Saponin

The samples were ground and 20 g of each were placed into a conical flask and 100cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hr with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight (Velevan, 2015).

2.3.5 Determination of Cardiac Glycosides

A tincture of the sample was prepared by preparing 10% extract in 70% alcohol by shaking 1g of pulverized mint with 10ml 70% alcohol. The mixture was left overnight with occasional shaking for 2hr and then filtered. 10ml of the purified filtrate transferred in to a dry stopped Erlynmeyer flask was added to 10ml of baltet's reagent. The blank was prepared at the same time using 10ml of distilled water instead of the purified filtrate and 10ml of baljet's reagent. They were made to stand for 1hr, for maximum colour development. The solutions were diluted with 20ml of distilled water and mixed. The intensity of the colour obtained was measured at 495nm using a suitable

spectrophotometer. The colour was stable for several hours. The difference between experiment and blank (E-B) is equal to the original reading. The percentage total glycoside was calculated using the absorptivity of digitoxin = 170, similarly treated at 495 nm as follows:
% Total cardiac glycoside = (A x 100 / 17) g% Calculated as digitoxin.

Where A = absorbance of the colour at 495nm.

2.3.6 Determination of Total Phenols by spectrophotometric method

FolinCiocalteau's technique was used to determine the total phenolic content of different leaf extracts of *Balanites aegyptiaca* (Singleton and Rossi, 1965). Folin-reagent Ciocalteau's was applied to 1.0 ml of the sample. After 3 minutes, add 1.0 ml of saturated Na_2CO_3 (35%) to the aforementioned mixture and dilute to 10 ml with purified water. After 90 minutes in the dark, the tubes' absorbance was measured at 725 nm against a reagent blank. Gallic acid was used as standard. Results were expressed as milligrams of Gallic Acid Equivalence (GAE) per gram of extract.

2.4 PROXIMATE ANALYSIS

Nutritional Analysis: The Association of Official Analytical Chemists (AOAC) standard techniques (AOAC, 1984) were used to assess the moisture, crude protein, crude fat, total ash, and crude fibre contents of each sample. Moisture content was assessed by heating 2.0g of each fresh sample to a constant weight in a crucible in an oven set to 105 degrees Celsius. The dry matter was utilized to calculate the other values. The Kjeldahl technique was used to calculate crude protein (percent total nitrogen x 6.25) using 2.0g samples; crude fat was produced by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60°C) as the extractant. The ash content of 10.0g samples was evaluated after they were incinerated for 5 hours at 550°C in a muffle furnace. Crude fibre was prepared by incinerating the residue in a muffle furnace at 550°C for 5 hours after digesting 2 42.0g of sample with H SO and NaOH. The moisture content of each sample was measured by heating 2.0g of each sample to a constant weight in a crucible in a 105°C oven. Each analysis was performed three times. By subtracting the sum of the percentages of the other constituents from 100%, the total quantity of carbs in the sample was calculated. The total carbs content of the sample is determined by this number.

2.5 Gas Chromatography – Mass Spectroscopy (GCMS) Analysis of *Balanites aegyptiaca* Aqueous Fruit Extract.

The phyto-constitutes of *Balanites aegyptiaca* aqueous fruit extract were determined using Gas Chromatography (Agilent 6890 series) coupled with an HP-5MS column mass spectrometer at 30°C column temperature and was heated to 300°C at 10°C for 5 minutes. Helium was employed as the carrier gas at a flow rate of 1.0ml/min. The elements of water melon rind aqueous extract were identified by comparing their mass spectra and retention indices to those obtained from actual samples and/or NSIT/Wiley spectra libraries, utilizing various search methods (PBM/NIST/AMDIS) and accessible literature data (Kulkarni *et al.,* 2015).

3.0 RESULTS AND DICUSSON

3.1 Quantitative Phytochemical Analysis of Aqueous Fruit Extract of *Balanites aegyptiaca*

The result of the Quantitative phytochemicals analysis revealed the concentrations of the secondary metabolites analyzed to be as follows: flavonoids 288.33+3.01 mg/100g), tannins (20.50+0.45 mg/100g), saponins (320.90+10.28 mg/100g), alkaloids (78.67+1.03mg/100g), steroid (36.40+0.80), glycoside (163.92+0.33mg/100g), phenols (227.43+1.01mg/100g)were as terpenoids, reducing sugars, anthraquinones, Anthocyanines were absent(Figure1). The presence of these secondary metabolites adds to the fruits of *Balanites aegyptiaca's* therapeutic properties. Saponins have anti-inflammatory and anti-microbial properties. While flavonoids have been shown to have a variety of beneficial effects, including anti-inflammatory, estrogenic, and antibacterial action. In modern medicine, alkaloids offer great pharmacological potential, including analgesic (e.g., morphine), antihyperglycemic and antibacterial properties . Secondary metabolites from plants acted as radical scavengers, with beneficial biological effects in the treatment of cardiovascular disorders. The fruits of *Balanites aegyptiaca* contain phenols (Gamo *et al.,* 2020).

While the proximate analysis of the fruits of *Balanites aegyptiaca;* revealed that, the moisture content was found to be 2.3±0.2%, This value is very low compared to that of Umooh *et al* (1998) and Ashaye *et al.,* (2005), whose reported higher moisture content of matured fruits. Crude protein was found to be 3.2±0.08%, Proteins are essential component of diet needed for survival of animals and humans, their basic function in nutrition is to supply adequate amounts of required amino acids in nutrition (Pugalenthal *et al.,* 2004). Protein deficiency causes growth retardation, muscle wasting, edema, abnormal swelling of the belly and collection of fluids in the body (Perkins – Veazie *et al.,* 2005). Furthermore, crude fibre is the organic residue remaining after the inorganic components of the foodstuff sample have been broken down by acid and alkali treatment. *Balanites aegyptiaca* fruit has 16.40.5% crude fiber. This finding differs from that of Faznira and Seri (2014), who found 0.00 percent crude fiber in apple, cider vinegar. The lipid content of *Balanites aegyptiaca* fruit was 3.10.7%, which was very low; hence, the fruit may not be a good source of oil-soluble vitamins. The results were higher than those for fluted pumpkin pod and pulp, which were 0.50 and 0.30

g/100 g, respectively (Essien *et al.*, 1992). Carbohydrates, is among the three main nutrients in foods, which provide energy sources for human body and constitute the structure and content of many cells (Falcone *et al.*, 2007; Chiu *et al.*, 2018). *Balanite aegyptiaca* fruit has a carbohydrate content of 72.61.6%, which is quite high when compared to other fruits. Because of its high carbohydrate content, it has a sweet flavor and can be a useful source of energy (Adepoju *et al.*, 2006). The amount of ash discovered was 2.40.2%. The ash content value was comparable to that of most fruits (Brain and Alan, 1992), however it was lower than that reported by (Amoo and Lajide, 1999 and Bello *et al.*, 2008). Theash contents of *B.aegyptiaca* fruits obtained gives an idea about the inorganic content of the samples from where the mineral content could be obtained (Figure 2).

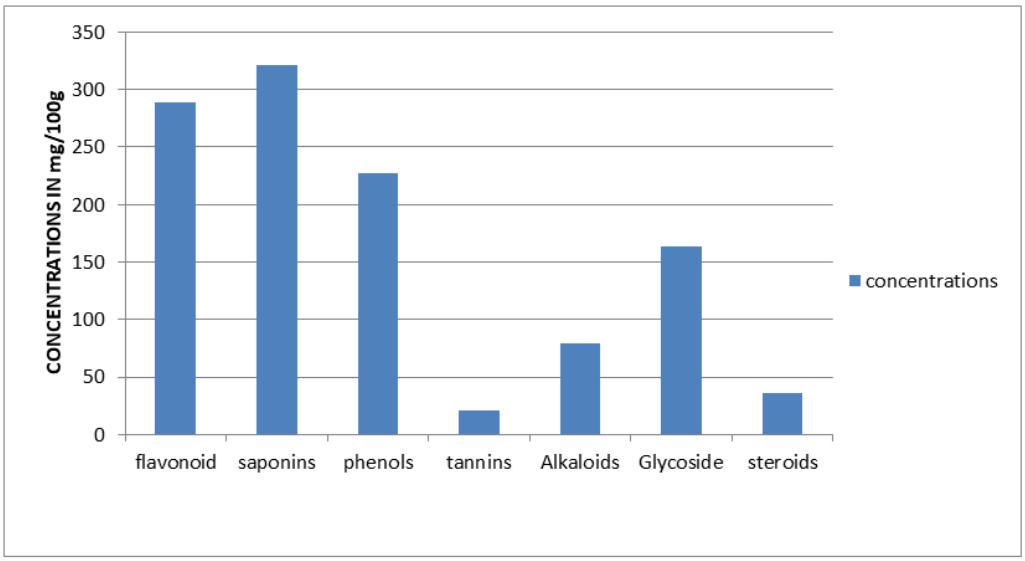


Figure 1: Quantitative phytochemicals analysis of aqueous fruit extract of *Balanites aegyptiaca*

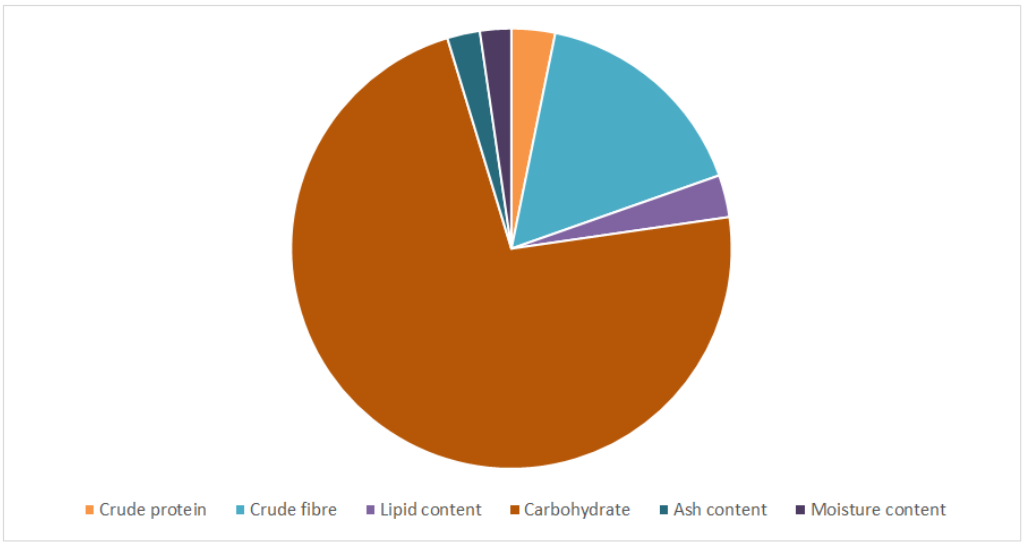


Figure 2: Proximate composition of *Balanites aegyptiaca* aqueous fruit extract

3.2 Gas Chromatography-Mass Spectrophotometric of Aqueous fruit extract of *B. Aegyptiaca*

The GCMS analysis of aqueous fruit extract of *B. Aegyptiaca* shows the presence of various organic compounds (i.e aliphatic hydrocarbons, fatty acids, aromatic componds e.t.c) at different peaks having many importants medicinal and industrial uses, this compounds Such as [(methoxymethoxy)m ethyl]-, Undecanoic acid Hexadecanoic acid, ethyl ester. e.t.c and they found to be important source of industrial raw materials (Figure 3, Table 1).

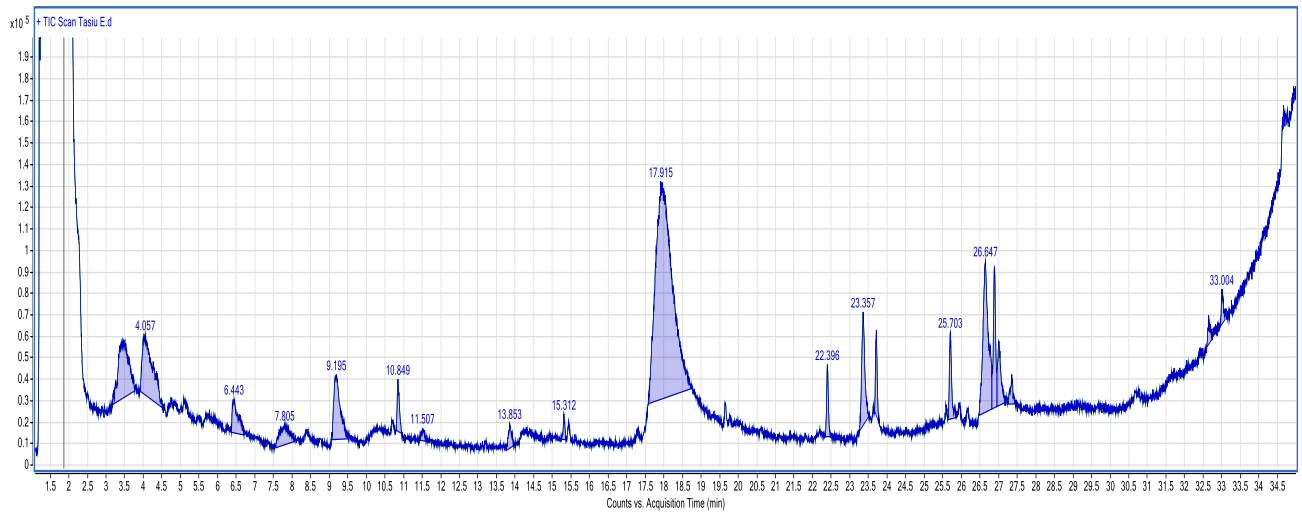
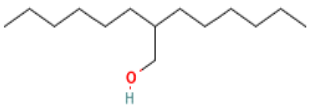
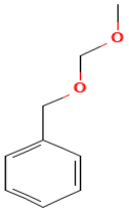
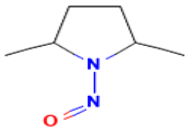
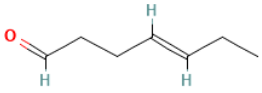
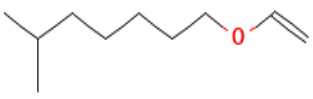
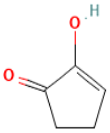
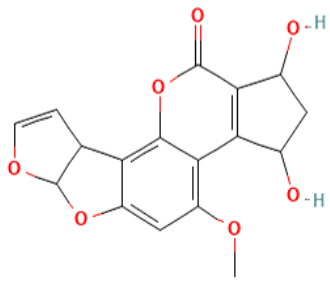
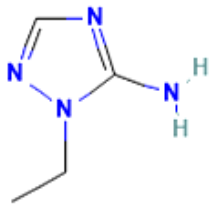
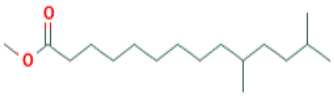
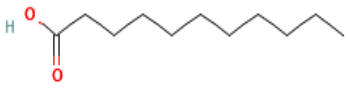
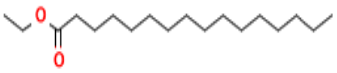
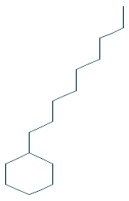
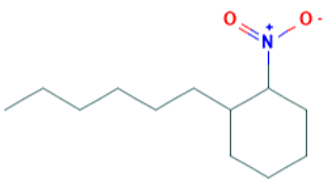
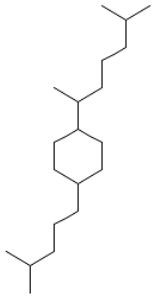
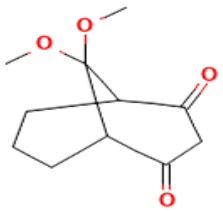
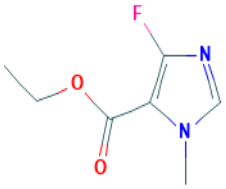


Figure 3: Chromatogram of GC-MS of *Balanites aegyptiaca* Aqueous Fruit Extract

Table 1: Gas Chromatography-Mass Spectrophotometric of Aqueous Fruit Extract of *B. Aegyptiaca*

Peak No.	Retention Time	IUPAC Name	Molecular Formula	Structural Formula	Nature and Medical Important
1.	3.479	2-hexyl-octan-1-ol	$C_{14}H_{30}O$		Is an aliphatic alcohol, Not available
2.	4.057	Benzene, [(methoxymethoxy)methyl]-	C9H12O2		Is an aliphatic alcohol and it has antifungal activity (Chebi *et al*., 2005)
3.	6.443	Pyrrolidine, 2,5-dimethyl-1-nitroso-$_{12}$	C6H12N2O		Not available
4.	9.195	4-heptenal	C_7H0		Is a medium chain aldehyde
5.	10.849	Ether 6-methylheptyl vinyl	$C_{10}H_{20}O$		Not available
6.	11.507	2-Cyclopenten-1-one, 2-hydroxy-	C5H6O2		Not available

7.	15.312	Cyclopenta[c]furo[3', 2':4,5]furo[2,3-h][1]benzopyran-11(1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4-methoxy-	C17H14O7		Not available
8.	17.915	1H-1,2,4-Triazol-5-amine, 1-ethyl-	C4H8N4		Not available`
9.	22.396	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	C17H34O2		Not available
10.	23.357	Undecanoic acid	C11H22O2		Is a medium chain fatty acid , has antifungal activity(Rossi *et al.*, 2021)
11.	23.712	Hexadecanoic acid, ethyl ester	C18H36O2		Is a long chain fatty acid ethyl este, has antioxidant activity (Ponnamma and Manjunath, 2012).
12.	25.703	1-cyclohexylnonnene	$C_{15}H_{28}$		Not available
13.	26.647	1-hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$		Not available
14.	26.887	Cyclohexane 1-(1,5 dimethylhexyl)-4-(-4-methylpentyl	$C_{20}H_{40}$		Not available

15.	27.002	9-9-dimethylbicyclo(3,3,3 1)nona-2-4-dione	$C_{11}H_{16}O_4$		Not available
16.	33.004	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	C7H9FN2O 2		Not available

3.3 CONCLUSION

The proximate composition indicated the nutritional potential of the *Balanites aegyptica.* The GC-MS and Phyto-chemical analysis of *Balanites aegyptiaca* shows the presence of flavonoids tannins, saponnins, alkaloids, steroid, glycoside, and phenols, these phytochemicals were confirm by GC-MS analysis such as includes 2-hexyl-octan-1-ol, 4,5,9trihydroxy-dodeca-1,11-diene, 5-ethyl-3-methyl-3,4-nonadien-6-yne, 2-heptadecenal, Decanoic acid ethyl ester hence can be recommended for industrial, foods, additive and other pharmaceutical formulation.

Ethical Approval: No ethical approval required.

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