Journal of Biological Sciences and Bioconservation
Volume 7, Number 1, 2015
ISSN: 2277-0143

# ISOLATION AND CHARACTERIZATION OF STEROIDS IN THE ROOT BACK OF *Balanite egyptiaca*

**M.U. Muhammad., M. Musa., L. Abubakar. and A. Shehu**
**Department of Chemistry**
**Shehu Shagari College of Education, Sokoto State, Nigeria**
**E-mail:** mumshag2012@gmail.com

## ABSTRACT

A steroids is a types of organic compounds that contains a characteristics arrangement of four cycloalkanes rings that are joined to each other, steroids are use in the field of medicinal chemistry due to their therapeutic values. The root bark of *Balanite egyptiaca* plant was investigated phytochemically using standard procedure. The dark gummy residues were subjected to column chromatography, TLC, IR, GC-MS and NMR analysis. The results of phytochemical screening indicate the presence of Tannins, Flavonoids, Cardiac glycosides, Saponins and Steroides. The dark gummy residue were also subjected to column chromatography and gave two fractions which each on TLC shows two spots on chromatogram. The IR indicated the presence of different functional groups such as O-H, =C-H, C≡C, C=O and C-H. The GC-MS and NMR analysis indicated the presence of Esterone, Estra-1, 3, 5-(10)-trien-3-ol, Ethinyl Estradiol which are classified as contraceptics and were use in pharmaceutical companies for formulation of drugs.

**Keywords**; *Balanite egyptiaca,* Isolation, Characterization, Steroids

## INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insect, fungi and herbivorous mammals. Many of these phytochemical have beneficial effect on long term health when consumed by humans, and can be used for effective treatments of human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Tapsell and Lai, 2006). A medicinal plant is any plant which one or more of its organ contain substances that can be used for therapeutic

purposes or precursors for the synthesis of useful drugs (Amrit, 2004). The use of in plants medicines predates writing human history. Ethno-botany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicines which were derived from "Ethno- medical" plant sourced; 80% of these have had an ethno medical use identical or related to the current use of active elements of the plants (Fabricant and Farnsworth, 2001).

**Steroids**

Steroids are terpenoids lipids characterized by the steranne or steroid nucleus: a carbon skeleton with four fused rings, generally arranged in a 6-6-6-6 fashion. Steroids vary by the functional groups attached to these rings and the oxidation state of the rings. The specificity of their different biological actions is due to the various groups attached to a common nucleus. When alcohol group (OH) are attached, steroids should properly be called sterols (e.g. cortisol), whereas ketone groups (C=O) make them sterones (e.g. aldosterone). Steroids comprise of a large group of substances that mediate a very varied set of biological responses. The most widely spread steroid in the body is cholesterol, an essential component of cell membranes and starting point for the synthesis of other steroids is sex hormones, adrenal cortical hormones, and the bile salts. Steroids (E.g. glucocorticoids mineral corticoids, androgens, estrogens and progestagens) have major responsibilities as hormones, controlling metabolism, salt balance, development and function of sexual organs as well as other biological differences in sexes. Steroids in form of bile salt (e.g. Salt or cholic and deoxycholic acid and their glycine and taurine conjugates) assist in digestive processes, while another steroid is a vitamin (calcitriol) that takes partin calcium control. Steroids (naturally occurring or synthetic) such as methyl prednisolone, hydrocortisone, glucocortisteroids, corticosteroids, squalamine, oestrogens, androgens are also used for the treatment of various diseases such as allergic reactions, arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production. In addition, synthetic steroids (e.g. mifepristone) that mimic the action of progesterone are widely used as oral contraceptive agents. Other synthetic steroids (e.g. oxandrolone) are designed to mimic the stimulation of protein synthesis and muscle-building action of naturally occurring androgens. Steroids, such as nandrolone, dromostanolone, stanozolol, are often used illegally to increase the performance of competitive athletes of almost all age groups. They are banned in most sports competitions such as the Olympic game (Goldman, 1984).

**Botany of Plant**

*Balanite egyptiaca* a member of *Zygophyllaceae* or *Balanitaceae* family (Ndoye, *et al;2004*).It's common name is Desert date, belongs to the kingdom *Plantae,* division *Magnoliophyta*, class *Magnoliosida*, order *Sapindales,* family *Zygophyllaceae,* genus *banites de*, synonyms *ximenia aegyptiaca, agialida senegalensis van tiegh* etc. It has many common names. In Arabic it is known as *lalob*, *hidjihi*, *inteishit*, and *heglig* (*hijlij*). In Hausa it is called *aduwa,* in Swahili *mchunju* and in Amharic *bedena* (Yves Guinand and Dechassa Lemessa,2009), Hindi name is Hingot (JP Yadav, Manju Panghal (2010). It is found in the driest part of tropical Africa and grow to an altitude of 100m high that is the area with mean annual rain fall of about 250-400mm and temperature of 20-30$^0$c. It is multi-branched Spinyissured. Branches shrub or tree up to about 10m tall, Crow spherical in one or several masses. Trunk short and often branching from near the base. Bark dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8cm long. Leaves with two separate leaflet; leaflet avovate, asymmetric, 2.5 to 6cm long, bright green, leathery, with fine hairs when young, flowers in fascicles in the leaf axils, and are fragrant, yellowish-green (pack,2010).

**Traditional Uses**

Traditionally *balanite* powder with the addition of piper betle leaf, the juice is taken once with water for 9 day, soon after the menstruation to avoid unwanted pregnancy (vijigiri and Sharma 2010). In egyptian folk medicine, the fruit are used as an oral hypoglycemic. (kamel, 1998) and antidiabetic; an aqueous extract of the fruit is used in Sudanese folk medicine in the treatment of jaundice. It is also used in food preparation and herbal medicine, especially in Africa and some developing countries (obidah *et al*, 2009). The fresh leaf of the plant *Acalypha* is pounded with small amount of root of *Balinite aegyptiaca* and *Cissus guadrangularis*, and then soaked in water for an hour or two, decanted and administered intranasal and orally. Latex of the plant is used in epilepsy, administered through intranasal route. Sticks are used as tooth brush. Fruit are used to treat dysentery and constipation. The seed oil is used to treat tumors and wounds, as laxative; also it is used in treatment of hemorrhoids, stomach aches, jaundice, yellow fever, syphililis and epilepsy. (Ojo *et al* 2006). The bark is used in the treatment of round worm infections and as a fish poison. The aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity. (Zarroug and Bashir 2001)

## MATERIALS AND METHODS

### Collection and Treatment of Sample

The plant materials (root bark) were collected between $5^{th}$-$6^{th}$ of January, 2013 at Badon Hanya village of Wamakko Local Government area of Sokoto state, Nigeria. The plant was identified at Botany Unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant part were separated manually and then washed with distilled water to remove sand and other impurities. The samples were air dried under shed, and then grounded into fine powder with the aid of pestle and mortar. The powder obtained from plant part were then sieved and stored in polythene bag until required for use.

### Extraction of Plant Materials

The powdered plant sample (100g) was packed in the thimble made from thick filter paper, which is loaded into the main chamber of the sohxlet extractor. The soxhlet was then equipped with condenser. The ethanol was heated to reflux. The ethanol vapours travels up a distillation arm, and then flood into the chamber housing the thimbles of solid. The condenser ensures that the ethanol vapour cools, and drips back into the chamber housing the solid material. The chamber containing the solid materials slowly fills with warm ethanol. Some of the desired compound then resolves in the warm ethanol. When the soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running down to the distillation flask. The cycles were allowed to repeat many times, for six hours. The extracts were left on the laboratory for two days to obtain a dark gummy residue (20.50g).

### Phytochemical Screening of the Active Fraction

Each of the active fractions was subjected to phytochemical analysis as described by Elolemy *et al.,* (1994); Sofowara A. (1993); Harbone J.B (1998).

### Chromatographic Separation of Crude Extract

A piece of glass wool was inserted at the bottom of glass column, and then 40g silica gel powder (80-120$\mu$m) was packed into the column and glass wool inserted just above the silica gel surface. The column was washed with 50:30:20 ratios of chloroform, ethanol, petroleum ether, and 10g of sample was transferred onto the column and eluted with the same ratio. Fractions were collected at 10$cm^3$ per fractions which were used to test for steroids, UV, IR and TLC.

### Thin Layer Chromatography (TLC)

Commercially prepared TLC glass plate (8-20cm) was used. A sharp line was drawn

with a pencil 2cm from one end of the plate. The sample (s) to be analyzed was spotted on the line drawn on the plates using capillary tube and then allowed to dry. The dry plates were placed into the TLC tank containing chloroform, methanol (8:4.5) solvent mixture and the tank was covered. The solvent rose up the plate by the capillary action. When the solvent front was just about 1cm to the upper end of the plate, the plate was removed and a tick line was drawn to mark the position of the solvent front. The plate were allowed to dry and the spots were developed with sulphuric acid and the Rf values of the spots were measured.

**IR**

Fourier transform infrared spectroscopy (FTIR) experiments were performed using shimadzu (model) instrument control by IR solution software set at spectra re-solution of $4cm^{-1}$ .The sample was rob on disc. The prepared sample window was scanned between 400-$400cm^{-1}$ 25times and the mean printed.

**UV- Visible Spectrometry**

Each of the fractions was subjected to UV-visible analysis and their wavelength of absorption taken on Shimadzu UV-Visible analysis and their spectrometer 1650PC series.

***GC-MS (Gas Chromatography- Mass Spectroscopy)***

Gas chromatography mass spectroscopy (GC-MS) experiments were performed using Shimadzu (QP2010 Model). The instrument settings were column oven and injection temperature at $60.0^0C$ and $25.00^0C$ respectively, injection mode was split, the total flow and column flow were set at 6.2ml/min and 1.67ml/min respectively, the flow control mode set at linear velocity. The ion source and interference temperature were set at $200.00^0C$ and $250.00^0C$ and the solvent cut time was set at 2.5minutes, and total run time of 35minutes

**NMR**

The nuclear magnetic resonance spectroscopy experiments were performed using variance $NMR^1$ (mercury-200BB). The sample were dissolve in $CdCl_3$ and run at ambient temperature with relax delay time of 1.00sec, pulse 45.0 degree and the acquisition time was 1.300secs. The total time was 10 minute 5 seconds and powered by 39dB.

## RESULT AND DISCUSSION

## RESULTS

**Table 1: Results of Phytochemical Screening of Dark Brown Gummy Residue from Root Bark**

| Chemical composition | Dark brown gummy residue |
|---|---|
| Tannins | ++ |
| Saponins | +++ |
| Flavonoids | + |
| Cardiac glycosides | + |
| Steroids | +++ |
| Anthraquinones | - |
| Alkaloids | - |

**Key: + = Just present ++ = Appreciable present +++ = Much more present**
**- = Absent**

**Table 2: Test for steroids on fraction**

| Chemical composition | Fraction 1 | Fraction 2 |
|---|---|---|
| Saponins | ++ | ++ |
| Steroids | ++ | ++ |

**Key: ++= Appreciable Present**

**Table 3: TLC results of Residue, Fraction 1 and Fraction 2**

| Fraction | Solvent system | No of component(s) | $R_f$ value |
|---|---|---|---|
| Residue | Chloroform: ethanol: petroleum ether (3:2:1) | 2 | 0.52 |
| Fraction 1 | Chloroform: ethanol: petroleum ether (3:2:1) | 1 | 0.16 |
| Fraction 2 | Chloroform: ethanol: petroleum ether (3:2:1) | 1 | 0.18 |

**Table 4: IR Results of Residue**

| Frequency ($cm^{-1}$) Literature range | Frequency ($cm^{-1}$) Sample | Assignment | Functional group |
|---|---|---|---|
| 3640-3610 | 3639.80 | O-H | Alcohols |
| 3100-300 | 3093.92 | =C-H | Aromatics |
| 3000-2850 | 2897.18 | C-H | Alkane |
| 3300-2500 | 2634.85 | O-H | Carboxylic acid |
| 1720-1705 | 1708.99 | C=O | Ketones |
| 1750-1730 | 1743.52 | C=O | Ester |
| 2260-2100 | 2253.22 | C≡C | Alkynes |

**Table 5: IR Results of Fraction 1**

| Frequency ($cm^{-1}$) Literature range | Frequency ($cm^{-1}$) Sample | Assignment | Functional group |
|---|---|---|---|
| 3640-3610 | 3610.86 | O-H | Alcohols |
| 3100-3000 | 3099.71 | =C-H | Alkane |
| 3300-2500 | 2644.49 | O-H | Carboxylic acid |
| 3500-3200 | 3441.12 | O-H | Alcohol, phenols |
| 1720-1705 | 1708.99 | C=O | Ketones |
| 1750-1730 | 1743.52 | C=O | Ester |
| 2260-2100 | 2253.22 | C≡C | Alkynes |

**Table 6: IR Result of Fraction II**

| Frequency ($cm^{-1}$) Literature range | Frequency ($cm^{-1}$) Sample | Assignment | Functional group |
|---|---|---|---|
| 3640-3610 | 3624.37 | O-H | Free hydroxyl |
| 3100-3000 | 3074.63 | =C-H | Alkene |
| 3000-2850 | 2889.46 | C-H | Alkane |
| 3300-2500 | 2654.14 | O-H | Carboxylic acid |
| 3500-3200 | 3433.40 | O-H H-bend | Alcohol & phenol |
| 1720-1705 | 1708.99 | C=O | Ketones |
| 1750-1730 | 1743.52 | C=O | Ester |
| 2260-2100 | 2253.22 | C≡C | Alkynes |

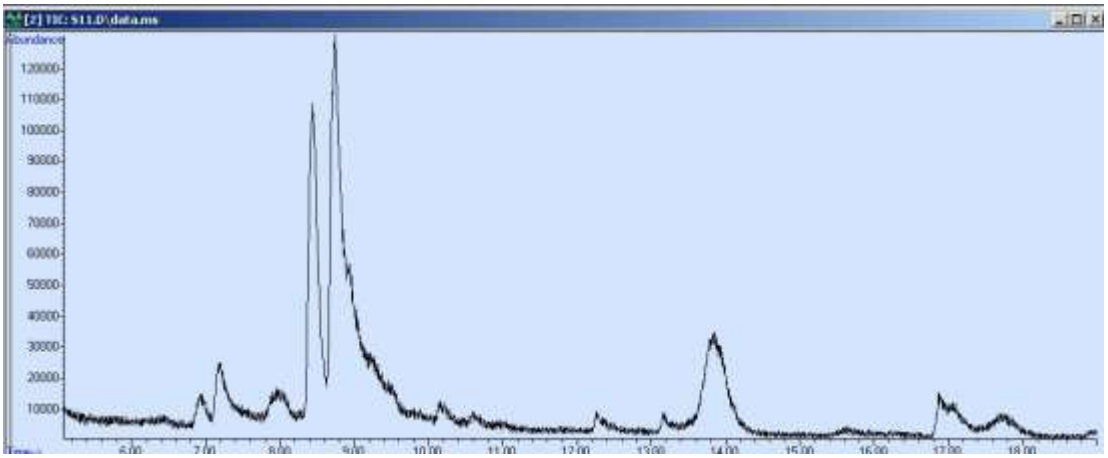
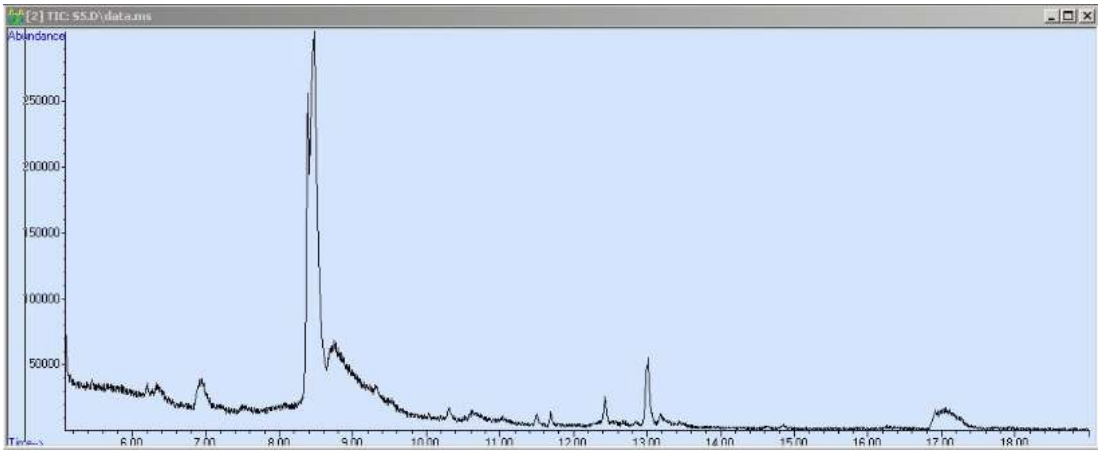


**Table 7: GC-MS results for Dark gummy residue**

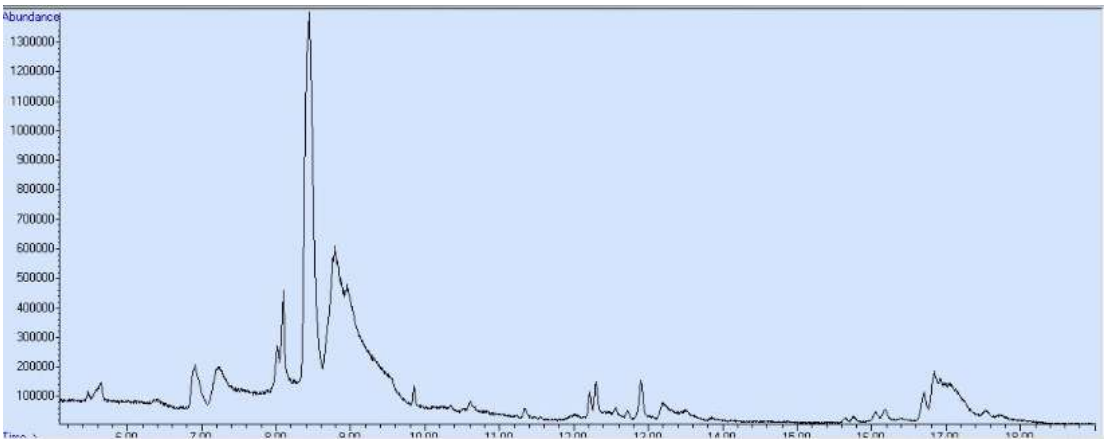
| Peak Number | Retention Time (Min.) | Base Peak | Molecular ion peak |
|---|---|---|---|
| 1. | 4.18 | 95 | 108 |
| 2. | 5.80 | 84 | 127 |
| 3. | 8.14 | 101 | 144 |
| 4. | 9.77 | 97 | 125/126 |
| 5. | 13.36 | 111 | 181/182 |
| 6. | 13.63 | 112 | 155 |
| 7. | 18.34 | 87 | 158 |
| 8. | 19.09 | 76 | 270 |
| 9. | 20.51 | 73 | 256 |
| 10. | 22.31 | 55 | 296 |
| 11. | 23.36 | 55 | 284 |
| 12. | 27.48 | 149 | 430 |

**Table 8: GC-MS results for fraction I**



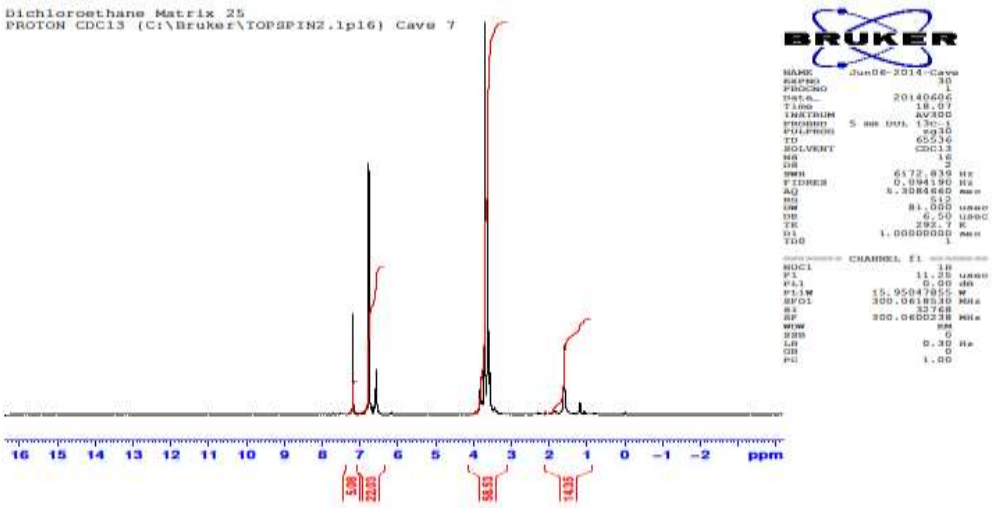

| Peak Number | Retention Time (Min.) | Base Peak | Molecular ion peak |
|---|---|---|---|
| 1. | 4.61 | 84 | 113 |
| 2. | 5.77 | 84 | 127 |
| 3. | 8.15 | 101 | 144 |
| 4. | 9.76 | 97 | 125/126 |
| 5. | 13.63 | 112 | 155 |
| 6. | 14.02 | 57 | 142 |
| 7. | 17.95 | 87 | 144 |
| 8. | 19.10 | 74 | 270 |
| 9. | 20.53 | 73 | 256 |
| 10. | 22.32 | 55 | 296 |
| 11. | 23.38 | 55 | 282 |
| 12. | 27.31 | 98 | 314 |
| 13. | 27.48 | 149 | 391 |

**Table 9: GC-MS results for fraction II**

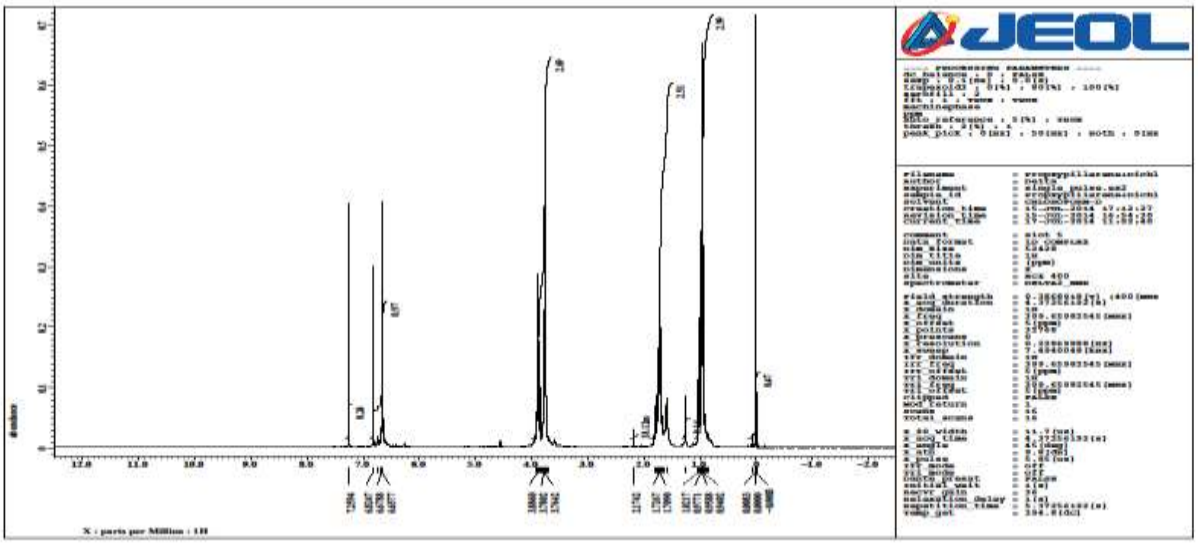



| Peak Number | Retention Time (Min.) | Base Peak | Molecular ion peak |
|---|---|---|---|
| 1. | 5.79 | 84 | 127 |
| 2. | 8.15 | 101 | 144 |
| 3. | 8.78 | 84 | 126 |
| 4. | 9.78 | 97 | 126 |
| 5. | 13.38 | 111 | 181/182 |
| 6. | 13.63 | 112 | 155 |
| 7. | 14.21 | 57 | 143 |
| 8. | 20.51 | 60 | 256 |
| 9. | 22.33 | 55 | 296 |
| 10. | 23.38 | 55 | 282 |
| 11. | 27.48 | 149 | 391 |
| 12. | 29.08 | 98 | 411 |

## Table 10: $^{1}H$ NMR Results




## Table 11: $H^{1}$ NMR Results



## DISCUSSION

A soxhlet extraction was carried out in order to extract steroids from the root bark of *Balanite egyptiaca* in which a dark brown gummy residue was obtained that averagely weight 20.50g after allowing it to concentrate for two days as. The residue was tested for phytochemical composition and results revealed the presences of Tannins, saponins, flavonoids, steroids, cardiac glycoside. The result of the analysis is in line with the findings of Daya *et al.,*2011 who reported the presence of steroidal saponing, about 1% glycosides and major sapogenin and yamogenin in their research

### Column chromatography

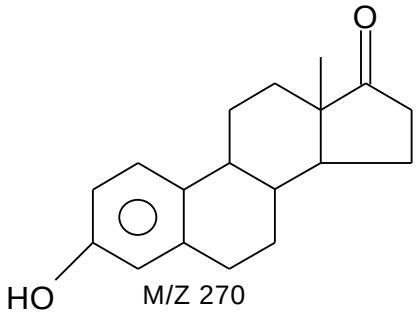
The residue obtained after extraction was subjected to column chromatography and resulted in two fractions which were allowed to concentrate for 3 days and yield light gummy yellow and Dark gummy yellow which were classified in to fraction 1&2 respectively. The residue and the fractions are also subjected to TLC, FTIR, GC-MS and NMR in an attempt to identify the structure (s) of the compound (s) isolated.

### TLC

Both residue and fractions were also subjected to TLC in order to identify the number of component(s). So the TLC results of the residue gave two spots, appearance of different spots indicate that the residue contains a mixture of steroids which are different from one another by the side chain or functional group attached to the chain. But fraction 1 shows only one spot while fraction 2 shows one spot with tailing. Therefore, the TLC result obtained from both residue and fractions were inline since each fraction resulted in one spot and two spots from residue.

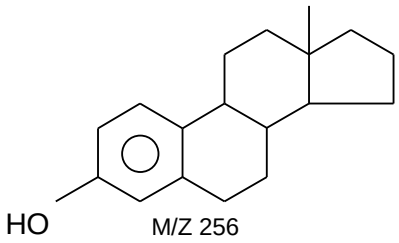
### FTIR

The IR analysis for the residue, fractions 1 &2 was carried out. However, the result indicate the absorption frequency at 3639.89, 3610.86 and 3624.37$cm^{-1}$ for O-H (alcohol), as well another absorption at 2634.85, 2644.49 and 2654.40$cm^{-1}$ for COO-H (carboxylic acid) while at frequency 2897.18, 2889.46 and 3093.93$cm^{-1}$ for C-H (alkane). The summary of the GC-MS analysis of the Dark gummy residue, fraction I and fraction II were given at table 7, 8 and 9. By comparing the GC-MS results with NMR it is observe that; three compounds were identified which are Estrogen, Estra-1-3-5-(10)-trien-3-ol and Ethinyl estradiol.

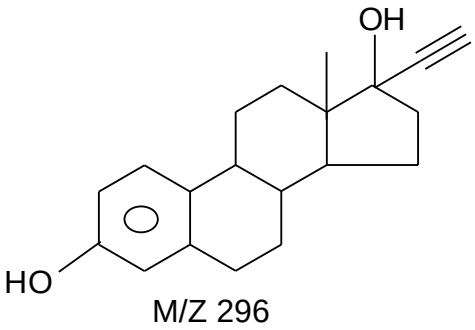



**Structure 1: Estrogen**

Estrogen is relevant to health and disease state because of it is converted to estrone sulfate. It is the predominant estrogen in postmenopausal women (Helen *et al 2004*). Esterone and estropipate are used to treat abnormalities related to gonadotropin hormone dysfunction, vasomotor symptoms, atrophic vaginitis, and vulvar atrophy associated with menopause, and also for the prevention of osteoporosis due to estrogen deficiency.




**Structure 2: Estra-1, 3, 5-(10)-trien-3-Ol**




**Structure 3: Ethinyl Estradiol**

Ethinyl Estradiol and Estra-1, 3, 5-(10)-trien-3-Ol was known to have been use for combined oral contraceptive pills (Meher *et al*, 2013), (Meher, *et al,* 2013). The pharmacological studies of *B. aegyptiaca* was reported by JP Yadav and Manju Panghal 2010 to have demonstrated insecticidal, antimicrobial, antibacterial, antifungal, hepatoprotective, anticancerous, antioxidant, antihelminthic,

molluscicidal antiparasitic, antidiabetic and anti-inflammatory activities. Research carried out using different *in vitro* and *in vivo* techniques of biological evaluation support most of these claims. B. aegyptiaca may also act as a potential natural larvicidal agent against mosquito larvae, owing to the presence of saponins which are effective in mosquito control, safe to mammals and available in high concentration. So, this plant can be used as a drug in mosquito control.

Analysis was also carried out by Hamid *et al.,* 2001 using extract of the stem bark of B. Aegyptiaca (L.) the extract was prepared by boiling 30 gram of the powdered bark in 100 ml. distilled water for five minutes, and then 100 ml of the filtrate was taken orally for every 8 hours for three months in an attempt to treats HIV/AIDS. He reported that 90% of the patients infected with HIV/AIDS were completely treated. The same dose was also used for one month in also an attempt to treat patients with Leukemia in which he reported all the patients was completely cured without any adverse effect. *Balanites* leaves were also reported by Clement *et al* 2011 to be boiled and mixed with groundnuts and/or sesame and eaten with staple foods such as cassava, potatoes and millet bread as a traditional delicacy.

## CONCLUTION

The steroids contents on the basis of spectroscopic and chemical evidences points to the following compounds, Esterone, Etra-1,3,5-(10)-trien-3-ol, and Ethinyl Estradiol. Presence of these steroidal compounds in the root bark of *Balanite aegyptiaca* may be responsible for its use in traditional medicine for birth control, abortion, treatment of menstrual disorders as well as use in lactation simulation.

## RECOMMENDATIONS

The findings in the research have justified that the plant has the potentiality in ethno-medicine and therefore it can be use as a source of raw material for pharmaceutical industries for new drug formulation. Below are the recommendations made:

- After column chromatography fraction obtained should be methylated for proper separation and characterization.
- To separate individual compounds, purify and characterize each as an addition to the plant chemical compounds.
- Isolated and characterize compounds should be explore for their medicinal application.
- Toxicity test should be carried out on the Isolates.

## REFERENCES

1. Clement A. O, Jacob G. A, James M. K, Refaat A. A. A, Paul O. Joseph O. and Zewge T. (2011) Journal of Biological Sciences vol. 6 (1) Pp.15-24

2. Daya L. C and Vaghasiya H.U (2011). A review on *Balanites aegyptiaca* Del (desert date): Pytochemical constituents. Traditional uses and pharmacological activity. 5(9):55-62

3. Doughari J.M, Pukuma M.S and De N (2007); Antibacterial effects of Balaniteaegyptiaca L. Del. And Moringaoleifera lam on Salmonella typhi. African Sound of Biotechnology vol. 6 pp2212-5

4. El-olemy M.M, Almuhtutadi F.J and AfifiA.A (1994); Experimental phytochemistry, a laboratory manual, King Saud University Press pp 8-9.

5. Fabricants D.S and Farnsworth N.R (2001); The Value of plants use in Traditional Medicine for drugs discovery. Environmental Health perspect. 109 supply 1 (supply1) pp 69-75 PMC 1240543. PMID 11250806.http://www.ncib.nlm.nih.gov/pmc/articles/pmc1240543/

6. Hamid, Osman A/monieum , A/wahab, Mohy Eldin Hassan , Abdu, Zeidan Zeidan and Idris, Sakina Mohamed(2011.) BALANITES AEGYPTIACA EXTRACTS FOR TREATMENT OF HIV/AIDS AND LEUKEMIA

7. Harbone J.B (1998); Phytochemical methods- A guide to modern Techniques of plant Analysis. Chapman and Hall, London, pp 182-190.

8. Helen V., Jan M. K. and Carolyn L. G (2004). Verney's Midwefry.fourth edition. Jones and Bartlett publishers. Pp 340.

9. Kamel M.S (2007); Afurostanolsaponins from *Balaniteaegyptiaca*phytochemistry, Reproductive Biology in *Balaniteaegyptiaca*(L). A semi-arid Khare Indian Medicinal Plants pp 77-8.

10. Maurice M. (1993). *Handbook of African medicinal plants*. Boca Raton u.a.: CRC Press. p. 129. ISBN 084934266X

11. Meher C.P, sethy S.P and PchoaiahB(2013); Structure and Biological Activities, steroid meities. Research Journal of pharmaceutical, Biological and Chemical Science Vol. 4(1) pp 253-272.

12. Ndoye M, Isma'ila D. and yaye K.G (2004); Reproductive Biology in Balaniteaegyptiaca(L) Del., a semi-arid forest tree.African Journal of Biotechnology.Vol 3(1) pp 30-46.

13. Obidah W, Nadro M.S, Tiyafo G.O andWurochekke A.U (2009); Toxicity of crude Balaniteaegyptiaca seed oil in rats.Journal of American Science, volume 5.Pp 13-6.

14. Ojo O.O, Nadro M.S, Tella I.O (2006); Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced hepatoxicity.African Journal of Biotechnology vol. 5 pp 755-60.

15. Khare C.P (2007); Indian Medicinal Plants, An Illustrated Dictionary, Springer pp 77-8.

16. Pack G.R, Dorbek O.L, Herald D.L (1991); Isolation and structure of cytostatic steroidal saponins from the African Medicinal plant Balaniteaegyptiaca. Journal of Natural Product vol. 54 pp 1491-502.

17. Sofowora A. (1993); Medicinal plants and Traditional Medicine in Africa. Spectrum books Ltd, Ibadan Nigeria pp. 289.

18. Tapsell L.C, Hemphill I, Cobiac L, Path C.S, Sullivan D.R, Fenech M, Roodenrys S, Keogh J.B, Chiftan pm, Williams P.G, Fazio V.A and Inge K.E (2006); Health Benefits of Herbs and Spices; the past, the present, the future med. J Aust vol. 21(4) pp 185.

19. Vijigiri D. and Sharma P.P (2010); Traditional uses of plants in indigenous falklone of Nizamabred District, Andhra pradesh, India. Ethnobotanical leaflets vol. 4 pp 29-45.

20. J.P, Manju P.(2010); *Balanites aegyptiaca* (L.) Del. (Hingot): A review of its traditional uses, phytochemistry and pharmacological properties 4 (3) Pp 140-146

21. Zarrough I.M, Nugud A.D, Bashir A.K and Mageed A. (1990); Balaniteaegyptiacaas a Mosquitolarvicide, pharm Biol.Vol. 18. Pp 267-71.

22. Yves G. and Dechassa L. "Wild-Food Plants in Southern Ethiopia: Reflections on the role of 'famine-foods' at a time of drought" UN-OCHA Report, March 2000 (accessed 15 January 2009)

23. worldagroforestry.org/treedb2/speciesprofile.php?Spid=279

24. WWW.Igeneric drugs.com/%3Fs%3D Esterone

**Reference** to this paper should be made as follows: **M.U. Muhammad *et al* (2015), Isolation and Characterization of Steroids in the Root Back of *Balanite egyptiaca***. *J. of Biological Science and Bioconservation,* Vol. 7, No. 1, Pp. 158 – 174.