Nile College

pharmacy program

Batch (2)

Phytochemical Screening, Aroximate analysis, and Thin layer chromatography for *Balanites aegyptica*

A Thesis Submitted to the Faculty of Pharmacy of Nile College in Partial Fulfillment of the Requirements of the Degree of Bachelor of pharmacy

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DEDICATION

To
Our parents
Whom give love kindness and strong force to go in our life
And everybody help us to complete this research
Our family and our best friends
Acknowledgment

Foremost, we are deepest, grateful to Allah, for the good health and wellbeing that were necessary to complete this thesis.

We place on record, our sincere gratitude to doctor Asim Halfawi for his supervision and guidance in this thesis.

We are also grateful to Dr. Najla Abdelmonem and are extremely thankful and indebted to her for sharing her research expertise and valuable guidance.

We wish to express our thanks to faculty of Pharmacy, Nile college and all teachers and staff for supporting and helping us broaden our knowledge.

And our deepest gratitude to parents and friends whom supporting us.

We take this opportunity to express our sense of gratitude to one and all, who directly or indirectly, have lent their hands in this venture.
Abstract

The desert date (Balanites aegyptica) is an evergreen tree belonging to the *Balanitace* family. It is mainly grown in the dried regions of Africa, the Middle East, and South Asia. In Sudan, the Balanites is found growing neutrally in Kordofan. Part of this plant has been used for many folk medicines in Africa and Asia. Balanites oil is considered to be used by ancient Egyptian good source of cosmetic. For this study, fruits were collected from Khartoum market, the extraction using Soxhlet procedure. The phytochemical screening of several lab experiments. The percentage of proximate analysis was found to be (6.05%) Ash content, lipid content (0.11%), fiber content (1.33%), moisture content (13.69%), protein content (7.014%), and carbohydrate content (71.68%), and thin layer chromatography. Balanites aegyptica was indicated for use as blood lowering sugar and as antibacterial.

Thin layer chromatography showed that the presence of aglycone.

**Key words**: Balanites aegyptica, Phytochemical screening, thin layer chromatography and proximate analysis.
## List of content

<table>
<thead>
<tr>
<th>TITLE</th>
<th>Page NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>I</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>II</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>III</td>
</tr>
<tr>
<td>LIST OF CONTENT</td>
<td>IV</td>
</tr>
<tr>
<td>LIST OF TABLE</td>
<td>VII</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>VIII</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>1</td>
</tr>
<tr>
<td>Introduction and Literature review</td>
<td>2</td>
</tr>
<tr>
<td>1.1.2 Description of the plant</td>
<td>2</td>
</tr>
<tr>
<td>1.2.3 Distribution of the plant</td>
<td>3</td>
</tr>
<tr>
<td>1.2.4 Proximate analysis</td>
<td>3</td>
</tr>
<tr>
<td>1.2.5 Thin layer chromatograph</td>
<td>3</td>
</tr>
<tr>
<td>1.2.6 Phytochemical screening</td>
<td>4</td>
</tr>
<tr>
<td>1.2 General objective</td>
<td></td>
</tr>
<tr>
<td>1.3 Specific objective</td>
<td>4</td>
</tr>
<tr>
<td>2. Literature Review</td>
<td>5</td>
</tr>
<tr>
<td>1.3 Study Rational</td>
<td>6</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>7</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>7</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>2.1 Study design</td>
<td>8</td>
</tr>
<tr>
<td>2.2 Study area</td>
<td>8</td>
</tr>
<tr>
<td>2.3 Source of fruits</td>
<td>8</td>
</tr>
<tr>
<td>2.4 Equipment</td>
<td>8</td>
</tr>
<tr>
<td>2.4.1 Lab equipment</td>
<td>8</td>
</tr>
<tr>
<td>2.5 plant</td>
<td>8</td>
</tr>
<tr>
<td>2.6 Phytochemical Screening</td>
<td>8</td>
</tr>
<tr>
<td>2.7 Thin chromatography</td>
<td>8</td>
</tr>
<tr>
<td>2.8.1 Ethanol extraction</td>
<td>9</td>
</tr>
<tr>
<td>2.8.2 Ether extraction</td>
<td>9</td>
</tr>
<tr>
<td>2.9 Phytochemical screening</td>
<td>9</td>
</tr>
<tr>
<td>2.9.1 Test of alkaloid</td>
<td>9</td>
</tr>
<tr>
<td>2.9.2 Test for steroids</td>
<td>9</td>
</tr>
<tr>
<td>2.9.4 Test of Reduced sugar test</td>
<td>9</td>
</tr>
<tr>
<td>2.9.5 Test for carbohydrates</td>
<td>10</td>
</tr>
<tr>
<td>2.9.6 Anthracene glycoside</td>
<td>10</td>
</tr>
<tr>
<td>2.9.7 Test for tannins</td>
<td>10</td>
</tr>
<tr>
<td>2.9.8 Test for saponin</td>
<td>10</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.9.8 Test for saponin</td>
<td>10</td>
</tr>
<tr>
<td><strong>2.10 Approximate analysis</strong></td>
<td>10</td>
</tr>
<tr>
<td>2.10.1 Determination of moisture content</td>
<td>10</td>
</tr>
<tr>
<td>2.10.2 Determination of ash content</td>
<td>11</td>
</tr>
<tr>
<td>2.10.3 Determination of Protein</td>
<td>11</td>
</tr>
<tr>
<td>2.10.4 Determination of crude fat</td>
<td>12</td>
</tr>
<tr>
<td>2.10.5 Total Carbohydrates</td>
<td>13</td>
</tr>
<tr>
<td>2.3 Thin layer chromatography</td>
<td>13</td>
</tr>
<tr>
<td><strong>Chapter</strong></td>
<td>14</td>
</tr>
<tr>
<td>3.1 Phytochemical screening</td>
<td>15</td>
</tr>
<tr>
<td>3.1 Phytochemical screening</td>
<td>16</td>
</tr>
<tr>
<td>3.3 Thin layer chromatography</td>
<td>16</td>
</tr>
<tr>
<td><strong>3.2 Discussion</strong></td>
<td>17</td>
</tr>
<tr>
<td><strong>Chapter</strong></td>
<td>18</td>
</tr>
<tr>
<td>4.1 Conclusion</td>
<td>19</td>
</tr>
<tr>
<td>4.2 Recommendation</td>
<td>20</td>
</tr>
<tr>
<td>Reference List</td>
<td>21</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>No</th>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phytochemical Screening</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Proximate Analysis</td>
<td>16</td>
</tr>
</tbody>
</table>

List of Figures:

<table>
<thead>
<tr>
<th>No.</th>
<th>Figure</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fruit of Balanites aegyptica</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Africa map of Balanites aegyptica</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>TLC results</td>
<td>13</td>
</tr>
</tbody>
</table>
CHAPTER ONE

Introduction

And

Literature Review
1.1 INTRODUCTION

Medicinal plants have long played important roles in the treatment of the disease in all over the world (Mohamed Rafieddy kopai, 2012). Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons (Sing R, 2015). Plants have been utilized as medicines for thousands of years. These medicines initially took the form of the crude drugs such as teas, tinctures, poultices, powders and other herbal formulations. The specific plants to be used in the methods of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in herbals. In more recent history, the use of plants as medicines has involved the isolation of active compounds (Marcy J Balunas et al., 2010). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, have been widely observed. Furthermore an increasing reliance on the use of medicinal plants in the industrialized in societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Lucy Hoaeay et al., 1999).

1.1.2 Description of the Plant:

*Balanites aegyptica* belong to the family, balanitaceae. It is known by various names, e.g. Arabic name: Heglig (tree), lalob (fruit); trade name: zaccone, zachun, desert date (dried fruit). *Balanites aegyptica* it is multi branched, ever green tea, spiny shrub or tree which grows up to 10 meter in high. The leaves are alternate, two foliate, petioles are 3-6 mm long, leaflets have broadly pointed petioles up to 5 mm long the spines of plant are simple straight, stout, rigid, green, alternate, supra axillaries clusters.

The flowers are small, bisexual greenish, white, few or many in number, the sepals are five in number, ovate and 3 mm long. The petals are five in number (two). The ovary is ovoid, silky. The fruit is an ovoid drupe, 2-5 cm long found in short thick stalk. The ripe fruit is
brown or pale brown with a brittle coat inclosing brown or brown green sticky pulp and hard stone seed. (Ichihara, K and et.al, 2010).

1.2.3 Distribution of The plant

The Balanites aegyptica has long history is an economical and medicinal plant and commonly used in many regions of Africa. It is grows in north Zimbabwe and through the sahle. Balanites aegyptica is wide spread in north kordofan through the central Sudan. (J. P. Yadav, et al. 2013).

It is an ever green tree grown in dry savannah areas of africa and south asia. (A. M. MOHAMED, et al, 2013).

Fig.(2) Africa map of Balanites aegyptica
1.2.4 Aproximate analysis

This refers to determination of the major constituent of the feed and is used to assess if a feed is within its normal compositional parameters or somehow been adulterated. This method potentiate nutrients in feed into component water ash crude protein, ether extract and crude fiber. Other use this technique as tool thermally characterized fuel and ashes, by studying it is melting behavior or structural changes. (Consuedo pizarro, an et.al, 2013).

1.2.5 Thin layer Chromatograph

Is the chromatography technique used to separate volatile mixtures. Performed on sheet of glass, plastic or aluminum foil, which is coated within a thin layer of adsorbent material usually silica gel, aluminum oxide or cellulose.

The use of TLC simplifies the technique, material and time necessary for analysis. (JOSEPHL STANAECK, et.al, 2011).

1.2.6 Phytochemical Screening

It refers to the extraction, screening and identification of the medicinally active substances found in plants. (J. P. Yadav, etal, 2013).
2. Literature Review

In Nigeria (Aguzue, Onyine et.al 2010) The study indicated the presence of the saponin, flavonoides, Alkaloids and antheraquinone.

In India (J.P.Yadavand and et.al, 2010) The result of study showed that the presence of Saponin, flavonoid, steroidal and Alkaloid.

In locally in Sudan (Ahmed S. Kabbashi, 2015) The result of study was indicated the presence of Alkaloid, steroidal, Saponin, flavonoid.

In USA (Sadig Ismaaila, 2013) The result of the proximate analysis showed that the presence moisture content, protein, fiber, Ash, lipid and carbohydrate within the range.

Regionally in Nigeria (C.EROMOSELE, et.al, 1993) also the result of the study showed the proximate chemical comsition of the fruit of Balanities eagyptica.

In Saudi a Arabia (Emad M.Aballa et.al, 2012) The result indicated the presence of Alkaloids, Saponin and steroid.

In Nigeria (Ann Lohlum, 2012) The result of study found the chemical composition of proximate analysis Ash, lipid, fiber, protein, carbohydrate and moisture.


In Sudan (I.C.EROMSELE, et.al 2013) The proximate compositions of Balanites aegyptica is lipid, ash, protein, moisture, carbohydrate and fiber.
1.3 Study Rational

The medicinal plant have a traditional role in the cure, treatment and prevention of number of disease. They are considered safer than synthetics drugs, because they are naturally derived and are often less toxic and rarely of any physiological significant to the patient. The Balanities aegyptica is one of the plants that is used in traditional medicine in India and other parts of Asia and Africa.

In this study we hope to identify the phytochemical screening and thin layer.
Objectives

1.2 General Objective

To Investigate phytochemical screening, thin layer chromatography and proximate analysis

1.3 Specific Objectives

To Extract the plant by different methods.

To Study presence or absence of phytochemical screening using *Balanites aegyptica*.

To Confirm phytochemical screening by using TLC.

To Study proximate analysis of plant extract.
Chapter Two

Materials and Methods
2.1 Study Design

This study is a laboratory basic descriptive study.

2.2 Study Area

This study was conducted in Nile college laboratory, Khartoum and the laboratory and agricultural analysis.

2.3 Source of Fruits

The fruit for this study were obtained from the market of Khartoum.

2.4 Equipments

2.4.1 Lab Equipments

Round bottom, conical flask, pipette, automatic pipette, burette, graduated cylinder, test tubes, separating funnel, capillary tubes, Petri dish.

2.4.2 Instruments

Soxlet extractor, sensitive balance,.

2.5 Plant

Petroleum ether, n-hexane, ether.

2.6 Phytochemical Screening

Fehling, ethanol,
either gelicatial acetic acid, ammonia, ferric chloride, diluted HCL, H₂SO₄, alpha naphtha, ethanol, ether.

2.7 Thin Layer Chromatography

Di ethyl either, plant, NH₃
2.8.1 Ethanolic Extraction

30 grams of the sample was weighted and placed inside soxhlet apparatus. A condenser and round bottom flask were filtered the extractor. 250 ml of ether was placed in the extractor and the temperature was placed to 70°C that is boiling point of ether. The sample was then removed from the extractor the allowed the extracting solvent to be recovering.

The extract was evaporate and then kept in bottle in refrigerator. (WHO, 1997).

2.8.2 Ether Extraction

30 grams of the sample was weighted and placed inside soxhlet apparatus. A condenser and round bottom flask were filtered the extractor. 250 ml of ether was placed in the extractor and the temperature was placed to 65°C that is boiling point of ether. The sample was then removed from the extractor the allowed the extracting solvent to be recovering.

The extract was evaporate and then kept in bottle in refrigerator. (WHO, 1997).

Water extraction

30 grams of the sample was immersed in the water for 24 hours. the extract was evaporate and then kept and put in the bottle.

2.9 Phytochemical Screening

2.9.1 Test Of Alkaloid

1 ml of diluted HCL was added to 1 ml of extraction, then few drops of wanger or dragendroff where added to the mixture and the result was observed. (WHO, 1997).

2.9.2 Test for Steroids

1 ml of chloroform, 1 ml of glacial acetic acid were added to 1 ml of extraction, then 1 ml concentrated sulfuric acid was added gradually on the wall of the tube and the result was observed. (WHO, 1997).
2.9.4 Test for Reduced Sugar Test

Few drops of ammonium chloride (NH4CL%) were added to 1 ml of extraction and the result was observed.

2.9.5 Test for Carbohydrates

1 ml of alpha naphthol was added to 1 ml of extraction, then 1 ml of concentrated sulfuric acid was added gradually on the wall of the tube and the result was observed.

2.9.6 Anthracene Glycoside

1 ml of fehling solution was added to 1 ml of extraction and the result was observed.

2.9.7 Test for Tannins

Few drops of ferric chloride were added to 1 ml of extraction and the result was observed.

2.9.8 Test for Saponin

1 ml of water was added to 1 ml of extraction, then mixture was shaking for seconds and the result was observed.

2.10 Approximate Analysis

Methods:-

Chemical composition determined according to A.O.A.C (2010)

2.10.1 Determination of Moisture Content:

Moisture was determined by over drying method. 2g from wet, dry matter of will-mixed sample was accurately weighed in clean, dried crucible (W1). The crucible was allowed in an oven at 100-105°C for 3h until a constant weight was obtained. Then the crucible was placed in the desiccators for 30 min to cool. After cooling it was weighed again (W2). The percent moisture was calculated by following formula. (Maluventhan ,2010).

\[
\% \text{ Moisture} = \frac{W_1 - W_2 \times 100}{w.t \text{ of sample}}
\]

Where:

W1 = Initial weight of crucible + sample

W2 = Final weight of crucible + sample
2.10.2 Determination of Ash Content:

Weight of clean empty crucible was noted (W1). 2 gran of each of sample was taken in crucible (W2). The crucible was placed in muffle furnace at 550°C for 3h the appearances of gray white ash indicate oxidation of all organic matter in the sample. After ashing furnace was switch off. The crucible as cooled and weighted (W3). Percent ash was calculated by following formula. (Maluventhan, 2010).

\[
\text{Ash \%} = \frac{\text{Difference in wt. of Ash} \times 100}{\text{Wt. of sample}}
\]

\[
\text{Difference in wt. of Ash} = W3 - W1
\]

2.10.3 Determination of Protein:

Principle: protein in the sample was determination by macro Kjeldahl method. The samples ware digested by heating with concentrated sulphuric acid (H2SO4) in the presence of digestion mixture. The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 2% boric was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated

Reagents:
- 0.1N HCL (standart)
- Concentrated sulphuric acid
- Sodium hydroxide solution 40% W/W
- Digestion mixture: Potassium sulphate (K2SO4).
- Boric acid: Dissolved 40g of boric acid in sufficient distilled water and made in volume up to 100 ml.
- Indicator: Methyl red

Procedure: Protein in the sample was determined by Kjeldhal method. 2g of dried samples was taken in 100 ml digestion flask. Add 25 ml of concentrated H2SO4 and 0.4g of digestion mixture i.e K2SO4 CuSO4 (8:1). The flask was heated in electrical heater of 2hrs. The sample was cooled and diluted with distilled water. And then placed in the Distillation apparatus. A 20 ml of 40% NaOH where added and the Distillation was applied for atb least 10 min. and NH3 produced was collected as NH OH4 in inaconical flask containing 20ml of 4% poric acid solution. During distillation yellowish color appears due to NH OH4. Theb distillate was then titrated against standart 0.02 N HCL solution using universal indicator (Broo cresol green and methyl red alcohol) till the appearance of pink color. Percent crude protein content of the sample was calculated by using the following formula. (Maluventhan, 2010).
%N = \frac{\text{Volume of HCL} \times 0.02 \times 14 \times 100}{\text{Wt of sample} \times 1000}

% Cured protein = 6.25 \times % N (*. Correction factor)

Where

0.02 = Normality of HCL

14 = Milli equivalent weight of Nitrogen

2.10.4 Determination of Crude Fat:

Dry extraction method for fat determination was implied it consisted of extracting dry sample with some organic solvent, since all the fat materials e.g. fats phospho lipid, sterols, fatty acid, carotenoids, Pigments, chlorophyll etc. are extraction therefore, the result are frequently to as cruse fat. Fats were determent by intermittent soxhlet extraction apparatus. Crude fat was determined by ether extract method using soxhelt apparatus. Approximately 2g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Wieghed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into the apparatus. Turned on water and heater to start extraction. After 2h siphoning allow ether to evaporate and disconnect beaker before last siphoning transferred extract into clean glass dish with ether washing and evaporate ether on water bath. Then placed the dish in an oven at 105°C for 2 hours and cooled it in a desiccators.

A moisture free and ether extracted sample of crude fiber made of cellulose was first digested with dilute H2SO4 and then with dilute KOH solution. The undigested residue collected after digestion was ignited and loss in weight after ignition.

Reagents:

Solution of sulphuric acid (0.128) 7.1ml 96% per 1000ml of distilled water, Potassium hydroxide (0.223M) 12.5 g per 1000 ml of distilled water acetone (foam suppresser)

Procedure: using the fiber system. 2g sample of sample of deffated sample (W0). 150 ml of preheated H2SO4 solution added and then heated to boiling for 30 min. and then filtered the residue was washed three time with hot. (Maluventhan, 2010).

2.10.5 Total Carbohydrates:

Will be calculated by substracting the sum of total fat, protein, ash and fiber content on a dry matter basis. (Ramasawmy and et.al, 1999).
2.3 Thin Layer Chromatography:

2 grams of mesocarp were extracted with methanol on water bath under reflux for 30 minutes. The extract was filtered and the solvent removed.

Then 50 ml of 2 N HCL were added to the residue and heated on heating mantle for 1 hour, after cooling.

The mixture was extracted with diethyl ether, the diethyl ether was extract on chromatography on silica gel on TLC plate using hexane _ ethyl acetate (9:1) as solvent and vanillin-Sulfuric acid used as spray reagent.
Chapter Three

Results and Discussion
3.1 Phytochemical Screening

Table No (1) contain

Phytochemical Screening of *Balanites* aegyptica Fruits Phytochemical

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Ethanol extraction</th>
<th>Ether extraction</th>
<th>Water extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>+V</td>
<td>+V</td>
<td>+V</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+V</td>
<td>+V</td>
<td>-V</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+V</td>
<td>+V</td>
<td>+V</td>
</tr>
<tr>
<td>Antheracen Glycosides</td>
<td>-V</td>
<td>-V</td>
<td>-V</td>
</tr>
<tr>
<td>Tanin</td>
<td>-V</td>
<td>-V</td>
<td>+V</td>
</tr>
<tr>
<td>Saponin</td>
<td>+V</td>
<td>+V</td>
<td>+V</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+V</td>
<td>-V</td>
<td>+V</td>
</tr>
<tr>
<td>Reduced sugar</td>
<td>+V</td>
<td>-V</td>
<td>+V</td>
</tr>
</tbody>
</table>

(J.P. Yadavand *et al.*, 2012) the result of study showed that the presence of Alkaloid, and saponin, steroidal, flavanoid and carbohydrate. (Aguzue, Onyine *et al.*, 2012) The study indicated that the presence of saponin, flavonid, Alkaloid, antheracen, reduced sugar and carbohydrate.
3.2A Proximate Analysis of *Balanites aegyptica*

Table No (2):- Ash Content, Fatty Acid Content, Fiber Content, Moisture Content, Protein Content, Starch Content of *Balanites aegyptica*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ash content</th>
<th>Fatty acid content</th>
<th>Fiber content</th>
<th>Moisture content</th>
<th>Protein content</th>
<th>Starch content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value(%)</td>
<td>71.68</td>
<td>7.14</td>
<td>13.69</td>
<td>1.33</td>
<td>0.11</td>
<td>6.05</td>
</tr>
</tbody>
</table>

In this study the result of proximate analysis for *Balanites aegyptica* was found the presence of Ash content 6.05%, lipid content 0.11%, fiber 1.33%, moisture content 13.69%, protein content 7.14% and carbohydrate content 71.68% in comparison with:

(Sadig ismaaila, 2013) The result of the proximate analysis show that the presence of protein 7.30%, lipid 0.13%, ash 6.40%, fiber 1.33% carbohydrate 70.18% and moisture 13.80%.

3.3 Thin layer chromatography

![Fig. (3) TLC result](image-url)
3.2 Discussion

In this study the results in phytochemical screening for Balanites aegyptica was found the presence of sterol, Alkaloid, flavonoid, reduced sugar, anthracen, saponin, reduced sugar and carbohydrate in comparison with:

(Aguzue, Onyine et al., 2012) The study indicated that the presence of saponin, flavonoid, Alkaloid, anthracen, reduced sugar and carbohydrate.

(J.P. Yadavand et al., 2012) The result of study showed that the presence of Alkaloid, and saponin, steroidal, flavanoid and carbohydrate.

(Ahmed S. Kabbashi, 2015) The result of study indicated the presence of Alkaloid, saponin, flavanoid and carbohydrate.

Also in this study the result of proximate analysis for Balanites aegyptica was found the presence of Ash content 6.05%, lipid content 0.11%, fiber 1.33%, moisture content 13.69%, protein content 7.14% and carbohydrate content 71.68% in comparison with:

(Sadig Ismaaila, 2013) The result of the proximate analysis show that the presence of protein 7.30%, lipid 0.13%, ash 6.40%, fiber 1.30% carbohydrate 70.18% and moisture 13.80%.

(C. Eromosele et al. 1993) also the result of the study showed the proximate chemical composition of Balanites aegyptica.

(I.I. Nakamnia, S.A et al., 2010) The study result of presence of lipid, fiber, ash, protein, moisture and carbohydrate.


(S. M. Cook and Awmok D. A, 2008) The result showed that proximate analysis contain lipid, protein, carbohydrate, ash and moisture content.

Also in this study the result of thin layer chromatography showed that the Balanites aegyptica fruits contain a glycone.
The result of the study showed that the presence of a glycone.

The result of study showed that the presence of saponin, carbohydrate, tannin, alkaloid, and flavonid.

The result of study showed that the presence of alkaloid, saponin, tannin, and carbohydrate.

The result of study showed there is no reduced sugar and anthraquinone.

The result of study showed that there is no reduced sugar and anthraquinone.
Conclusion

The study of Balanites aegyptica fruit found that they contain steroid, flavanoid, alkaloid, saponin, carbohydrate and reduced sugar.

Proximate analysis showed that Balanites aegyptica fruits contain Ash content 6.05%, lipid 0.11%, fiber 1.33%, moisture 13.69%, protein 7.14%, carbohydrate 71.68%.

Conducted thin layer chromatography found that Balanites aegyptica fruits contain Aglycon.
Recommendations

The Balanites aegyptica fruits obtained from phytochemical screening indicate possible use as lowering blood glucose level, antibacterial and can be uses in other source. Further research is required to determine which purpose(s) of the fruits is most likely to be used.
Reference:


