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Biochemical and phytochemical studies on *Balanities aegyptiaca* fruits

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ABSTRACT

Objectives: Phytochemical screeninig of Balanities aegyptiaca (B.a.) extract and *invitro* determination of some biochemical effects. **Methods:** preparation of defatted Balanities aegyptiaca total extract, it's characterization was done throughout Firstly, quantitative and qualitative determination of active phenolic components by HPLC and spectrophotometer analysis. Secondly, identification of different function groups by IR analysis and surface elements by electronic microscope, then estimation for saponins content by spectrophotometer, After that estimation of total flavonoids, carbohydrates and proteins. Finally, determination of B. a. extract antioxidant activity by using DPPH, anti-inflammatory activity and antitumor activity using (MCF-7) human breast adenocarcinoma cell line. **Results:** This study revealed that total extract of Balanities fruits showed high levels of antioxidants as each 1 gram contained 10 mg of ascorbic acid and anti-inflammatory activities as it inhibited NO production by 60% compared to positive control group. Also, it revealed high antiproliferation activity IC50=21.2 ug/ml correlated with its phytochemical screening which showed the presence of high levels of phenolics, flavnoids, minerals (Zn, Mg, Ca, K, Na, Fe, Cl, Al, Si, O and Carbon) and Saponins. **Conclusion:** Balanities aegyptiaca extract plays an important role in improving oxidative stress resulted in high antiproliferation activity against (MCF-7) and posses a strong anti-inflammatory agent, Furthermore purification and isolation of active compounds will help in recommendation of new antinflammatory and antitumor herbal treatments.

INTRODUCTION

Medicinal plants contain various phytochemicals that are used for treatment of various diseases. Anti-oxidants present in the plants play an essential role in protecting the cells and tissues against damage caused by oxidative stress [(1).]

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Oxidative stress is the imbalance between excess generation of free radicals and the inability of the body's antioxidant defense system to eliminate the radicals [(2).] Reactive oxygen species (ROS) such as superoxide anion (O_2^-), superoxide hydroxyl (OH^-), peroxyl radical ($RO2^-$) and hydrogen peroxide (H_2O_2) are just some of the major free radical species that are implicated in the pathogenesis of various diseases [(3).] Excessive generation of ROS can have detrimental effects, including damage to lipids, deoxyribonucleic acid DNA or proteins [(4).]

Balanites aegyptiaca, a forest species of socio-economic interest for rural people. The dietary intake of this fruit for local people is very valuable especially in terms of nutrition [(5),] It is known as 'desert date' is a spiny shrub or tree up to l0 meter tall, widely distributed in dry land areas of Africa and South Asia. It is traditionally used in treatment of various ailments such as jaundice, intestinal worm infection, wounds, malaria and epilepsy, insanity, yellow fever, syphilis, helminthiasis, cough, and constipation [(6)]. It contains protein, lipid, carbohydrate, alkaloid, saponin, flavonoid, and organic acid [(7).]

The presence of different phyto-chemicals such as cardiac glycosides, flavornoids and polyphenols, has provi-ded some biochemical basis for ethno pharmacological uses of the (B. a.) parts in the treatment and prevention of various diseases and disorders [(8)]. The mesocarp of (B. a.) fruit basically contains 1.2 to 1.5% proteins, 35 to 37% sugars and 15% organic acids. Chemical analysis of (B. a.) revealed other compounds such as 3-rutinoside and 3-rhamnogalactoside, diosgenin and a mixture of 22R and 22S epimers of 26-(O-β-D-lucopyranosyl)-3-β-[4-O-(β-D-glucopyranosyl)-2-O-(α-L-rhamnopy-ranosyl)-β-glucopyranosyloxy]-22,26-dihydroxyfurost-5-en [(9,10).] Observed antioxidant, xanthine oxidase and acety-lcholinesterase inhibitory activity of leaves. Also, Saponins are well known as protective agents. Saponin comp-ounds are also considered responsible for numerous pharmacological properties including anticarcinogenic activity [(11)]**.** Saponins of several herbs are known to induce apoptosis in some cancer cells and are proposed to be promising modulators of drug resistance [(13).]

Our present study was undertaken to identify bioactive compounds in (B. a.) fruit total extraction and aimed to determine its antioxidant and anti-inflammatory activities.

Material and methods:

Chemicals:

Methanol, hexane and ethyle acetate (plant extraction solvents) were purchased from TEDIA Company, USA. Human breast adinocarcinoma cell line (MCF-7) was purchased from ATCC, USA. Biochemical kits were purchased from SIGMA-ALDRICH Company, EGYPT and all other chemicals were of the highest grade commercially available.

Collection and Preparation of fruits:

Balanites aegyptiaca was was purchased from Aswan (halaib date), Egypt. The fruits of (B. a.) were kindly identified and proved by Herbarium of Botany department, faculty of Science, Zagazig University. The fruits were dehydrated and ground into uniform powder using milling machine and keep in dark to prevent the loss of active components until extraction and analysis [(14).]

Extraction of Plant:

Collected fruits were properly washed in water, rinsed using distilled water. The outer cover (The epicarp) was removed by using sterile sharp surgical blade and the fruit pulps/ mesocarps were scarped manually then air dried under shade for about four months then the dried specimens were manually ground into powder using pestle and mortar [(15)]. Exposure to sunlight was avoided to prevent the loss of active components [(14).]

One thousand gram of fruits mesocarp powder was defatted with 2000 ml of n-hexane. The filtrate was extracted by soaking in 2000 ml

methanol for up to 72 h. and, filtered twice with filter paper (Whatman No. 1). Then, the remaining was rextracted with 2000 ml ethyl acetate. The filtrate (Methanol and ethyl acetate extracts) were combined and evaporated using a rotary evaporator (at 40°C) to give semi-solid residues and transferred to freeze dryer to dry. This method was described as **(1)** with slight modification.

First part: **Characterization of *Balanites aegyptiaca* extract:**

1. a. Determination of phenolic comp-ounds in *Balanites aegyptiaca* extract by High Performance Liquid Chro-matography (HPLC):

Identification and determination of the phenolic compounds present in Balanities aegyptiaca were performed using HPLC system was a HP 1100 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler; quaternary pump and a diode array detector were used. The quantitation was integrated by Chemst-ation chromatographic software inter-faced to a personal computer. The analy-tical column was ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 µm, USA). The mobile phase consists of: mobile phase A, methanol; mobile phase B, 2% acetic acid; flow, 1mL min; fixed wavelength, 280 nm, with gradient elution program, A (%)/B (%): 0 min 5/95; 5 min 25/75; 10 min 30/70 20 min 35/65; 30 min 100/0; and back to initial ratio in 10 min. Identification of phenol compounds was performed by compare-son with the retention times of standard substances [16].

1. b. Determination of total phenolic compounds in *Balanites aegyptiaca* extract by Spectrophotometer:

The total phenolic content of fruits was determined by using the Folin-Ciocalteu assay [17]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was added to 25 ml volumetric flask, containing 9 ml of deionized water. A reagent blank using H_2O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 ml) with H_2O and mixed. After incubation for 90 min at ambient temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectro-photometer Lambda 5. Total phenolic content of fruits was expressed as mg gallic acid equivalents (GAE/100 g fresh weight). All samples were analyzed in triplicates.

2. Extraction and Determination of total saponins in *Balanites aegyptiaca* extract by Spectrophotometer:

Take 25g defatted sample in 500 ml conical flask and add 250 ml absolute methanol (99.9%) in ratio of dry weight of the sample to methanol as 1:10. Flask was tightly sealed and kept in a shaker at 25 C and 120 rpm for 24 hour, followed by centrifuging the contents at 3500 rpm for 20 min. After centrifugation, methanol extract was filtered using Whatman filter paper No.1. The result methanolic extracts were evaporated to dryness in vacuum condi-tion using a rotaevaporator. After evaporation dried plant extract dissolve in minimum amount of distilled water (10 ml), transferred into a separating funnel and extracted with equal volume of n-butanol (3 times).

Estimation of total saponins:

Total saponins contents were estimated by prescribed colorimetric methods [18]. 500 µl of solution was taken in different test tubes to which 0.25 ml of vanillin reagent (8%, w/v in 99.9% ethanol) was added. Test tubes were placed in ice-cold water bath and 2.5 ml of 72% (v/v) sulphuric acid was added slowly on the inner side of the wall. After mixing the content in each tube, these were left as such for 3 min. warmed the tubes to 60C for 10 min using a water bath and then cooled them in ice-cold water bath. Absorbance was measured at 544 nm using spectropho-tometer. Quillaja saponin (Sigma-Aldrich) was used as a reference standard and the

content of total saponins was expressed as Quillaja saponin equivalents (QS µg/mg extract).

3. Determination of antioxidant cont-ent of *Balanites aegyptiaca* extract by 2, 2-diphenyl-1-picrylhydrazy(DPPH):

DPPH assay was measured in methanolic extract. An aliquot of methanolic extract (50 µl) was mixed with the ethanol DPPH solution (0.5 mM, 0.25 ml) and the acetate buffer (100 mM, pH 5.5, 0.5 ml). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. The results are expressed as an IC50 value that represents the amount of fruit powder (in mg) providing 50% inhibition of 2,2-diphenyl-1-picrylhy-drazylradical (DPPH) [(19).]

4. Determination of total flavnoides in *Balanites aegyptiaca* extract:

Total flavonoids content was measured in methanolic extract by the aluminum chloride colorimetric assay [(20)]. An aliquot (1 ml) of extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg/l) was added to 10 ml volumetric flask containing 4 ml of dd H_2O. To the flask was added 0.3 ml 5% $NaNO_2$. After 5 min. 0.3 ml 10% AlCl, was added. After 6 min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of fruits was expressed as mg catechin equivalents (CE)/100 g fresh weight. Samples were analyzed in triplicates.

5. Determination of total proteins in *Balanites aegyptiaca* extract:

Digestion was done according to [(21).]

1 g ground sample was weighed into digestion flask, recording weight (W) to nearest 0.1 mg. To the flask 16.7 g K2SO4, 0.01 g anhydrous copper sulfate, and 0.6 g TiO2 was added. Then 20 ml sulfuric acid was added. Flask placed on preheated burner (adjusted to bring 250 ml water at 25°C to rolling boil in 5 min) and heated until white fumes clear bulb of flask, swirled gently (heating continued for 4 h).

Distillation:

Titration flask was prepared by adding 25ml of 4% boric acid, 3 to 4 drops kjldahl indicator was added, then the condenser tip was immersed inside boric solution slowly down side of flask, sufficient 40% sodium hydroxide solution (approximately 80 ml) was added to make mixture strongly alkali. Immediately flask connected to dist-illation apparatus and distilled at about 7.5 boil rate (temperature set to bring 250 ml water at 25°C to boil in 7.5 min) until at least 150 ml distillate is collected in titrating flask. Finally, digestion flask and titrating flask were removed from unit**.**

Titration:

Standard HCL 0.1N was titrated to pink endpoint (color changed from green to pink) and volume was recorded to nearest 0.01 ml.

Calculation: Percent Nitrogen (N)
%N =VHCL x NHCL x 0.014 x 100/ W

- VHCL = ml standard HCL needed to titrate sample
- NHCL = Normality of HCL
- W = sample weight

Percent Crude Protein (CP)
CP = % N X F
F = 6.25 for all forages and feeds except wheat grains

6. Determination of total carbohydrates in *Balanites aegyptiaca* extract:

The phenol sulphuric acid method [(22)] was used to estimate carbohydrates

Materials: Phenol 5% redistilled (reagent grade) phenol (50g) dissolved in water and diluted to one liter, Sulphuric acid 96% reagent grade, Standard glucose: stock -100 mg in 100

ml of water. Working standard – 10 ml of stock diluted to 100 ml with distilled water.

7. Determination of surface elements in *Balanites aegyptiaca* extract by electron microscope:

A part of *balanites aegyptiaca* were coated with a thin film of gold-palladium on the observed surface under high vacuum conditions and the internal structure was examined by a **JEOL** scanning electron microscope type JXA 840A, Japan. Scanning electro micro-graphs of these samples were taken at magnifications of 500X.

Second Part: **Chemical studies on *Balanites aegyptiaca* extraction *in vitro*:-**

1. Determination of anti-inflammatory activity of *Balanites aegyptiaca* extract:

Raw murine macrophages (RAW 264.7) were seeded in 96-well plates at 0.5×10^5 cells / well for 2 hours in RPMI without phenol red. The cells were stimulated with lip polysaccharides (LPS) with final concentrations of 100 μgmL^{-1}. Stimulated cells after two extra hours were either treated with serial concentrations of the tested samples, Dexamethasone (50 ng/ml) as a potent anti-inflammatory, left with the (LPS) alone, or left untreated at all as a negative control. After total 24 hours time interval the supernatants were removed and assessed for Nitric oxide (NO). Nitrite accumulation was used as an indicator of NO production using a microplate assay based on the Griess reaction. This method was done according to [(23)] by using spectrophoto-meter at 540 nm.

2. Determination of antitumor activity of *Balanites aegyptiaca* extract on breast cell line:

Measurement of potential cytotoxicity by SRB assay:

Potential cytotoxicity of (B. a.) extract was tested using the method of [(24).]

Cells were plated in 96-multiwell plate (10^4cells/well) for 24 hrs before treatment with the compound to allow attachment of cell to the wall of the plate.

Different concentration of (B. a.) extract (0, 5, 12, 25, 50 ug/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose.

Monolayer cells were incubated with (B. a.) extract for 48 hrs at 37ºC and in atmosphere of 5% CO_2.After 48 hrs, Cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.

Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader.

The relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.

Results

The surface element profile of the total extract of Bal. fruit was impressive, and contained some key elements such as, Fe, Zn, Ca, Al, K, Mg, Cl, Si, O and C. It may be concluded that the fruits of Bal. contribute to nutrient intake by the consuming populations in Africa and Middle East.

Results of antitumor activity of Balanites aegyptiaca extract on human breast adinocarcinoma cell line (MCF-7): B. a. extract demonstrated high antiproliferation activity against breast cell line (MCF-7) resulted in IC50= 21.2 ug/ml.

Discussion

By using spectrophotometric assay total polyphenols in *Balanites aegyptiaca* (B. a.) extract which was found to be 77.5 (mgGAE/g). These results are in accordance with **(1)** who reported that the phytochemical analysis for methanol extract of (B. a.) fruit revealed the presence of saponin, terpenoids, phenolic compounds and alkaloids, with

considerable quantities of total phenolics (212 ± 2.6 mg GAE/g).

Also, are in line with [25] reported that phytochemical investigation on leaves of (B. a.) methanolic extract showed the presence of saponins, tanins, phenols and anthraquinones**.** Therefore, in the light of these results (B. a.) showed antioxidant activity. These results also revealed that the methanolic extract of (B. a.) fruit was free radical scavengers, acting possibly as primary antioxidants.

Total phenolic acids also was evaluated by HPLC which revealed that the presence of some compounds such as Catechin 6.78(mg/g) ≈33.9mg/ml , Gallic acid 1.54(mg/g) ≈7.7mg/ml, Chlorogenic 1.45(mg/g) ≈7.25mg/ml , Vanillic 4.34(mg/g) ≈21.7mg/ml, p-Cumaric 2.6(mg/g) ≈13mg/ml , Ferulic 3.34(mg/g) ≈16.7mg/ml and Rutin 3.67(mg/g) ≈18.35mg/ml.

These results are in agreement with [26] who reported the presence of Chlorogenic acid <0.2 , *p*-Coumaric acid 21.26 ± 0.63,Ferulic acid 7.09 ± 0.07and Rutin 425.71 ± 2.15 in HPLC chromatogram of *O.basilicum* sample . Also, [27] stated that Rutin and isorhamnetin were identified by HPLC finger-print chromatograms of active Extracts The concentrations of rutin in *Balanites aegyptiaca* two extracts were found to be 0.239 and 0.031% and those of isorhamnetin were 0.004 and 0.007%, respectively.

Total saponins found in (B. a.) extract was 184.57±2.71 mg/ml ≈ 36.91mg QS/g, this result showed that (B. a.) total extract contained saponins which are in agreement with [8] who reported that (B. a.) leaves and root bark may be used to reduce blood pressure and cholesterol level in blood. This result could be due to the presence of saponins in the plant parts.

Balanites aegyptiaca extract is considered as a potential source of natural antioxidant, and incorporation of these extracts into foods could enhance their nutritional and antioxidant potentials. Our data corroborate those reported by [28].

DPPH radicals are widely used to investigate the scavenging activity of natural compounds. These free radicals are stable in ethanol and show maximum absorbance at 517 nm. When DPPH radicals encounter a protondonating substance such as an antioxidant, the radicals are scavenged and their absorbance reduced [28].

Free radical scavenging effects of total extract of fruit of (B. a.) was measured with ascorbic acid as standard compound by using DPPH method which results in each gram of Bal. dry matter contained 10 mg of ascorbic acid so total extract of *Balanites aegyptiaca* (L.) Delile are more effective as antioxidant 10 mg (ascorbic acid) /g (dry matter).

This result could be due to that the fruit mesocarp contains a large variety of phytochemicals amongst which are the pregnane glycosides, coumarins, flavor-noids, 6-methyl-diosgenin and furostanol saponins [53] *and* [54] who reported that (B. a.) fruit extract could be electron donors, and hence can react with free radicals to convert them to more stable products and terminate the radical chain reaction.

Also, [52] suggested that it may also be possible that the combined effect (synergistic or antagonistic) of saponins with other phytochemicals may responsible for observed antioxidant activity where earlier studies attributed the antioxidant activity of plants to the presence of these secondary metabolites [51].

Moreover, **Kumawat *et al.*, (2012b)** [31] reported that alcoholic extract of (B. a.) could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

Flavnoids of Total extract of *balanities aegyptiaca* fruit was found to be (9.5mg CAT/g dry matter) in which catchine was used as standard compound. These results are in accordance with [28] who showed that aqueous extracts of (B. a.) fruit showed the presence of flavnoids (3.21±0.59 %) fresh extract and for boiled extract was (3.80±0.49%) and [29] who

reported that the plants investigated are rich in alkaloids, flavonoids, steroids, terpenoids. which are also in analogy with previous reports of [6].

Percentage of crude protein of (B. a.) extract was found to be 3.15 ±0.17%. These results are in agreement with [5] who revealed that a protein content of (B. a.) fruit pulps was found to be 9.06 g 100 g-1

There were 92.97 mg glucose/g dry of total carbohydrates which present in (B. a.) extract. These results are in agreement with [30] who reported that phytoconstituents were isolated from plant showed the presence of fluorescence compounds, carbohydrates, protein & amino acid, glycosides, saponin, tannins and flavonoids. As several factors may explain these variations such as the impact of soil and climatic conditions, the maturity of analyzed fruits, or changes in analysis methods used reported by [5].

The surface element profile of the (B. a.) fruit total extract was impressive, and contained some key elements such as, Fe, Zn, Ca, Al, K, Mg, Cl, Si, O and C. It may be concluded that the fruits of (B. a.) contribute to nutrient intake by the consuming populations in Africa and Middle East**,** who revealed that Phytochemical screening indicated the presence of saponin, flavonoids, steroids, alkaloids and cardiac glycosides in the fruit mesocarp of (B. a.).

These results are in agreement with [32] who reported that Seven of the most important medicinal plants in the literature on Egyptian traditional and popular medicine are 'Halfa barr' (Cympobogon proximus), 'Salam' (Acacia ehrenbergiana), 'Kharaaz' (Acacia albida), 'Ghalqa' (Pergularia tomentosa), 'Argel' (Solenostemma arghel), 'Hegleeg' (Balanites aegyptiaca) and 'Handal' (Citrullus colocynthis). Contained minor elements (Zn, Cu, Se, Mn and Fe) and major elements (K, Na, Ca, Mg. The highest concentration was that of zinc, ranging from 15.4 to 73.7 (mg kg_1). Although some plants were found to accumulate elements, their contents are still below the international safety limits for both human and animal consumption.

Potassium weight was 1.53%, it helps to maintain body weight and regulate water and electrolyte balance in the blood and tissues [33].

The calcium weight was determined to be 6.41%. Calcium helps in the regulation of muscle contraction require-ed by children, infants and foetuses for bones and teeth development [34], the zinc weight was given as 0.31%. Zinc is said to be an essential trace element for protein and nucleic acid synthesis and normal body development [35]. Zinc also stimulates the activity of vitamins, and the formation of red and white blood cells [36]. Zinc plays a role in improving male fertility, The iron weight was given as 1.06%, and compares favorably with other vegetables. Iron is said to be an important element in the diet of pregnant women, nursing mothers, infants, convalescing patients and the elderly to prevent anemia and other related diseases [37].

The magnesium weight was found to be 0.49%. Magnesium plays fund-amental roles in most reactions involving phosphate transfer. It is believed to be essential in the structural stability of nucleic acids. It plays a significant role in the intestinal absorption of electrolyte in the body. Its deficiency in man includes severe diarrhea and persistent migraines [38].

The different *in vitro* chemical activities of (B. a.) extract were done by first determination of the anti-inflamma-tory activity for (B. a.) extract (activity on NO production) **fig (5).**

Total (B. a.) extract showed strong anti-inflammatory effect at all used concentration range from 500-62.5 µg/ml which inhibited nitric oxide to levels more than that of Dexamethasone (strong anti-inflammatory drug) especi-ally at range 250-62.5 µg/ml as (B. a.) extract at concentration 62.5 µg/ml showed inhibition of NO production by 60% compared to LPS (lip poly-saccharides which induced inflamma-tion) group as positive control group thus the extract was considered as a potent anti-inflammatory agent. These results could be due to the presence of saponins which has anti-inflammatory properties. This is consistent with

the report of [39] who showed that (B. a.) fruit is used in the treatment of inflammation.

Furthermore, the presence of stero-ids on the leaves and root bark of (B. a.) fruit suggest that the plant part may be used as anti-inflammatory and analgesic agents reported by [40].

These results are in agreement with [8] who revealed that the leaves and root bark of (B. a.) fruit can be used for anti-inflammatory treatment.

Seconed determination of the antitumor activity for (B. a.) extract against human breast cancer cell line (MCF-7) which illustrated as shown in **fig (6)** cleared that (B. a.) extract demonstrated high antiproliferation activity against breast cancer cell line (MCF-7) resulted in IC50= 21.2µg/ml.

These results could be due to the saponins of ethanol extract of *Balanites aegyptiaca* which was able to stabilize reactive oxygen species by reacting with them and oxidizes subsequently to more stable and less reactive radicals. In this respect saponins in (B. a.) play an important role as antioxidant for prevention of oxidative damage [41].

Rejinold *et al*., (2011) [42] showed that saponins are well known for their potential anticancer activity. However, the reported value for saponin is 50 g/ml to show toxicity against cancer cells

The selective inhibition of the growth of tumor has been observed by triterpenoid saponins (avicins from Acacia victoriae) by cell cycle arrest in human breast cancer cell line and apoptosis in leukaemia cell line [43].

Saponin (triterpenoidor steroid)-induced apoptosis is primarily caused by stimulating the cytochrome c–caspase 9–caspase 3 pathway in the human cancer and other cell lines [44, 45, 46].

Davis and Matthew *et al*., (2000) [47], explained the antitumor activity of (B. a.) extract was to the flavonoids found in the extraction which are known to inhibit production of heat shock proteins in several malignant cell lines, including breast cancer, leukemia, and colon cancer

These results are in agreement with [48] who reported that the compoundsSAP-1016(3b-O-b-D-xylopyranosyl-(1-3)-b-D-glucopyranosyl-(1-4)-[a-L- rhamnopyranosyl-(1-2)]-b-D-glucopyranoside) exhibited potent antiproliferative activity against MCF-7 human breast cancer cells and HT-29 human colon cancer cells, with IC50 values of 2.4±0.35 and 3.3±0.19 mmol/l, respectively, compared with dioscin, one of the most potent cytotoxic spirostane saponins, with IC50 values of 3.1±0.39 and 4.9±0.32 mmol/l, respectively.

It is reviewed that fixed oil of Balanites aegyptiaca exhibited anticancer activity against lung, liver and brain human carcinoma cell lines. It also had antimutagenic activity against *Fasciola gigantica* induced mutagenicity. [12].

Elie *et al*., (2010) [11]. Showed that saponins extracted from *Balanites aegyptiaca* have potent anti cancer activity against MCF-7 human breast cancer cells and HT-29 human colon cancer cells.

For diosgenyl saponins the presence of an-l-Rhap-(1 → 2)-β-d-Glcp sequence at C-3 was considered beneficial for cytotoxicity activity [49]. In addition, [50] reported that balanitoside, extracted from *Balanites aegyptiaca*, posses an α-l-Rha*p*-(1 → 2)-*β*-d-Glc*p* sequence at C-3 which mean that balanitoside have cytotoxicity properties that were in line with the current results.

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Table (1): Phenolic acids of *Balanites aegyptiaca* total extract by HPLC.

phenolic compound	conc (mg/g)
Catechin	6.78
Gallic acid	1.54
Chlorogenic	1.45
Vanillic	4.34
p-Cumaric	2.6
Ferulic	3.34
Rutin	3.67

Table (2): Results of antioxidant activity.

Content	Amount
1. Total Phenolics	77.5 (mg GAE matter) ≈ 387.5 (mg GAE / ml)
2. Total Saponins	184.57 ± 2.71 (mg Qs / ml) ≈ 36.91 (mg Qs/ g)
3. (DPPH) assay	10 mg (ascorbic acid) / g (dry matter)
4. Flavnoids	9.5mg CAT/g dry matter ≈ 47.5mgCAT/ml

Table (3): Results of nutritional value.

Content	Amount
Total Proteins	3.15 ± 0.17% in ml ≈ 0.63% in g dry matter
Total Carbohydrates	464.87 ± 0.89 mg (glucose) / ml ≈ 92.97 mg (glucose) / g (dry matter)

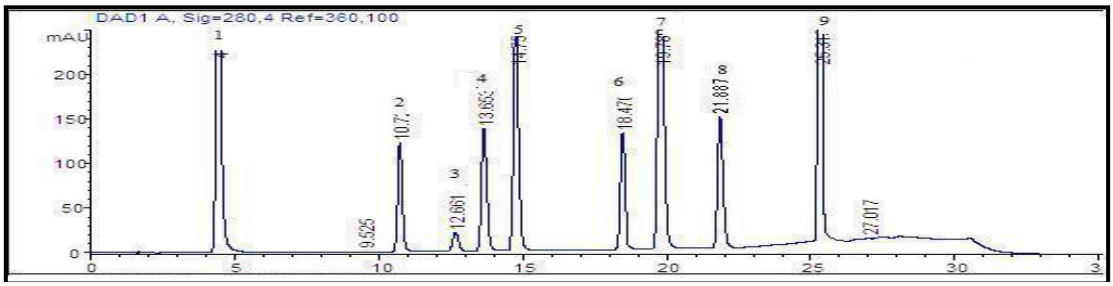

Fig. (1): Typical HPLC chromatogram of phenolic acids; (1) catachin, (2) gallic acid, (3) chlorogenic acid, (4) caffic acid, (5) vanillic acid, (6) p-cumaric acid, (7) ferulic acid, (8) rutin, (9) cinnamic acid.

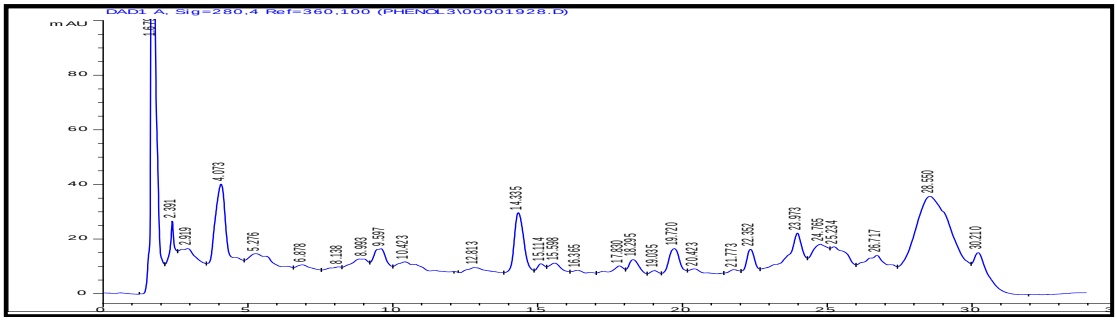

Fig (2): HPLC of phenolic contents in *Balanites aegyptiaca* total extract.

Results of surface elements in *Balanites aegyptiaca* extract by electron microscope:

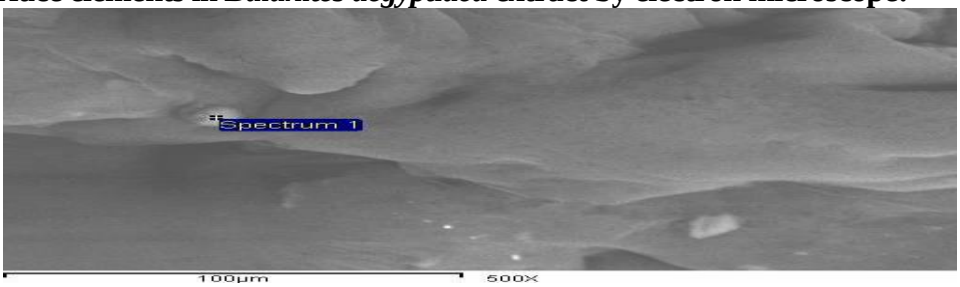

Fig (3): Section in surface of *Balanites aegyptiaca* extract.

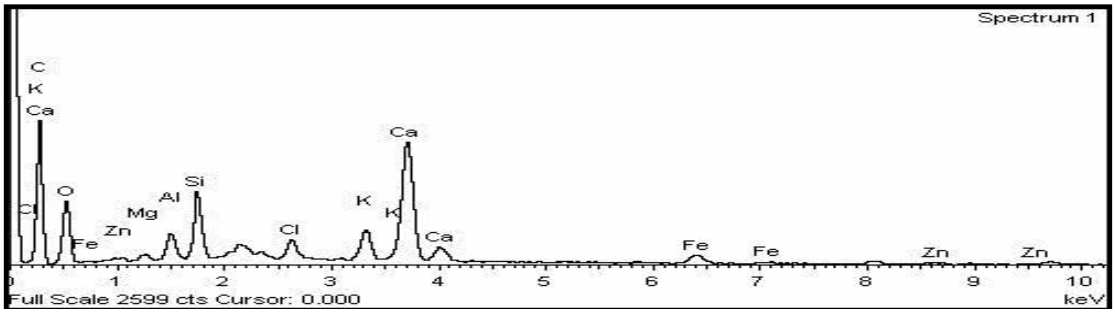

Fig (4): *Balanites aegyptiaca* extract total surface element.

Table (4): The different surface element weight % and atomic% of *Balanites aegyptiaca* extract.

Element	Weight%	Atomic%
C K	48.36	59.81
O K	36.37	33.77
Mg K	0.49	0.30
Al K	1.35	0.74
Si K	3.20	1.69
Cl K	0.92	0.39
K K	1.53	0.58
Ca K	6.41	2.38
Fe K	1.06	0.28
Zn K	0.31	0.07
Totals	100.00	

Results of anti-inflammatory activity of *Balanites aegyptiaca* extract:

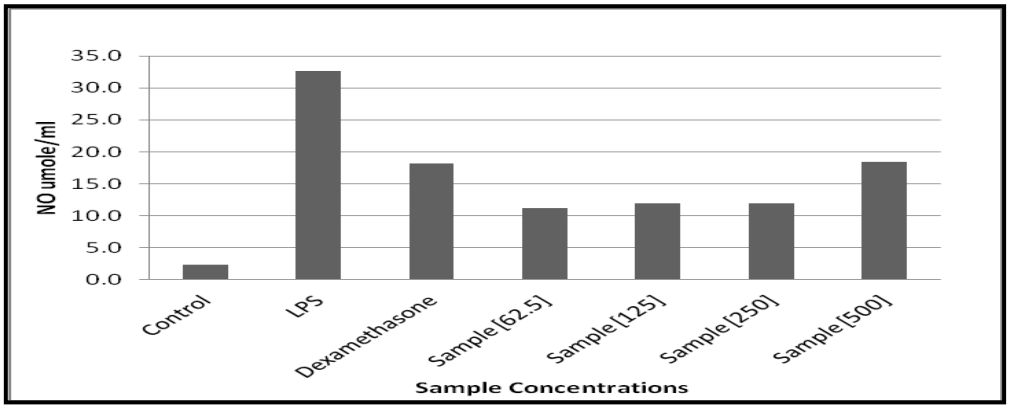

Fig (5): Anti-inflammatory activity of *Balanites aegyptiaca* extraction compared to LPS and Dexamethasone

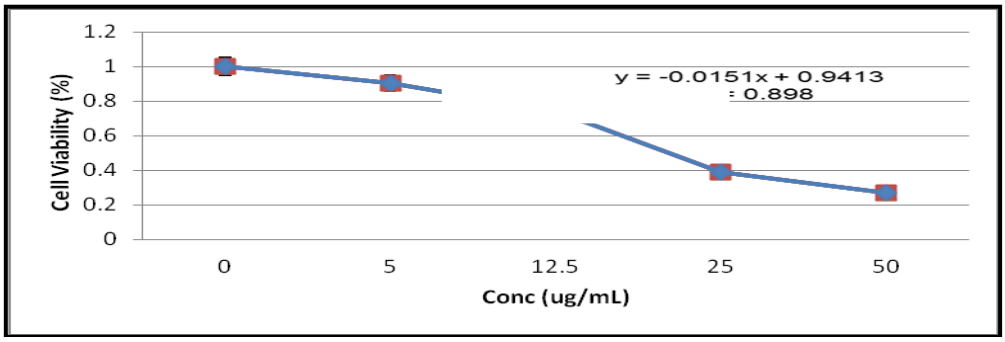

Fig (6): anti tumor activity of *Balanites aegyptiaca* extract on breast cell line (MCF-7).