ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF BALANITES AEGYPTIACA

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

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"Dissemination of Education for Knowledge, Science and culture"

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VIVEKANAND COLLEGE, KOLHAPUR

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PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that Sanika Gorakhnath Chavan studying in M.Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled "ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF BALANITES AEGYPTIACA " during academic year 2024-2025

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INTRODUCTION

1.0 INTRODUCTION

Balanites aegyptiaca Del, also known as "Desert date' in English, a member of the family Zygophyllaceae, is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia. This tree is native to much of Africa and parts of the Middle East. In India, it is particularly found in Rajasthan, Gujarat, Madhya Pradesh, and This is one of the most common trees in Senegal. It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay, and climatic moisture levels.

Classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Sapundales

Family : Zygophyllaceae

Genus :Balanites Delile

Species : Balanites aegyptiaca (L.) Delile

Figure 1Balanite aegyptiaca tree

Synonyms: Ximenia aegyptiaca L. (excl. Balanites nexburghii Planch), Agialida senegalensis van Tiegh, Agialilida barteri van Tiegh, Agialida tombuctensis van Tiegh, Balanites zizipboides Milhr. Et Schlechter, Balanites latifolia(van Tiegh) Chiov.

Vernacular name:

Ayurvedic : Ingudi, Angaar Vruksha, Taapasadrum, Dirghkantaka

Unani : Hingan, Hanguul

Siddha : Hingan, Hanguul

Folk : Hingol, Hingota, Hingothaa

English : Desert date, Soapberry tree, Thorn tree, Egyptian balsam

It is multi branched, spiny shrubs or tree up to 10 metre tall. Crown spherical, in one or several distinct 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 8 cm long Leaves with two separate leaflets; leaflets are obovate, asymmetric, 2.5 to 6 cm long, bright green, leathery, with fine hairs when young, Flowers in fascicles in the leaf axils, and are fragrant, yellowish-green. Fruit is a rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. Pulp is bitter-sweet and edible is the pyrene (stone), 1.5 to 3 cm long, light brown, fibrous, and extremely hard. It makes up 50 to 60% of the fruit. There arc 500 to 1500 dry, clean seeds per kg.

Flowers are small, inconspicuous, hermaphroditic, and pollinated by insects. Seeds are dispersed by ingestion by birds and animals. The tree begins to flower and fruit at 5 to 7 years of age and maximum seed production is when the trees are 15 to 25 years old. Natural distribution is obscured by cultivation and naturalization. It is believed indigenous to all dry lands south of the Sahara, extending southward to Malawi in the Rift Valley, and to the Arabian Peninsula, introduced into cultivation in Latin America and India. It has wide ecological distribution, but is mainly found on level alluvial sites with deep sandy loam and free access to water. After the seedling stage, it is intolerant to shade and prefers open woodland or savannah for natural regeneration. It is a lowland species, growing up to 1000 m altitude in arc with mean annual temperature of 20 to 30°C and mean annual rainfall of 250 to 400 mm.

Used in food preparations and herbal medicine, especially in Africa and some developing countries. The fresh leaf of the plant acupla in pounded with small amount of root of R. agyptiaca and Cins quadrangularis, and then soaked in water for an hour or two. It is decanted and administered intranasally and orally. Latex of the plant is used in epilepsy, administered through intranasal route in Used as tooth brush. Fruits are used to treat dysentery and constipation. The seed oil is used to treat tumors and wounds. Used as laxative, also used in treatment of hermorrhoid, stomach aches, jaundice, yellow fever, syphilis, and epilepsy A fruit is used to treat liver disease and as a purgative, and sucked by school children as a confectionary in some countries. The bark is used in the treatment of syphilis, round worm infections, and as a fish poison. The aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity Seeds are used as anthelmintic and purgative. Ground seeds are given to camels to cure impaction and cobe In Chifra District, the root of plant is used for the treatment of render pest and antiras. In East Africa, it is widely used as anti-helminthic Root is used in various folk medicines for the treatment of abdominal pain and as purgative, while the bark is employed as a fish poison and also as a remedy for malaria and syphilis The root, bark, kernel, and fruit have been shown to be lethal to mollusks In Sudanese folk medicine, it is used to treat jaundice. Its antimalarial and molluscidal activity is well studied, la ribe anti-plasmodial test of the dichloromethane and methanol (ME) extract of stem bark of the plant showed anti-malarial activity.

In Senegal, Nigeria, Morocco, and Ethiopia, R. aegyptiaar is taken a purgative for colic and stomachache. In Chad, fresh twigs are put on the fire in order to keep insects away. For intestinal worm, the fruits are dried and mashed in millet porridge and eaten, In Libya and Kritrea, the leaves are used for cleaning infected wounds. In Sudan and chadthe bar, B. aegyptia is component of soap. The use of the kernel oil for treatment of wounds has been reported from Nigeria. For contraception, in Nigeria, a mixture of dried leaves powder of A. apie and Rains in water and in Somalia, the bark of mot is crushed and mixed with two glasses of water, which is then filtered. This preparation is repeated for three days and one glass is drunk three times daily for three days.

The crude aqueous extract of root bark of *B. aegyptiaca* was showed a dose-dependent inhibition of spontaneous motility (paralysis) in adult earthworms and also possesses vermicidal activity. It is reported that stem bark water extract (9 g/kg body weight) of Albizia anti-helmintica and fruit mesocarp water extract (9 g/kg body weight) of *B. aegyptiaca* shows significant anthelminthic activity compared with albendazole (20 mg/kg body weight) against Fasciola gigantica adult worm and a single dose of 200 mg/kg body weight of *B. aegyptiaca* fruit mesocarp also showed activity against Schistosoma mansoni in infected mice when compared with praziquantel. Balanitin-7 is isolated from aqueous extract of *B. aegyptiaca* seed and reported as anthelmintic agent when tested by in vitro means of an original anthelmintic assay, using Caenorhabditis elegans as a biological model. The methanolic extract of *B. aegyptiaca* fruits is reported to have anti-helminthic action against different stages of Trichinella spiralis in rats compared with anthelmintic drug albendazole. The aqueous extract of *B. aegyptiaca* also has molluscicidal agent to juvenile and adult *Bulinus globosus* and *Bulinus truncatus*.

A mixture of steroidal saponins: balanitin-6 (28%) and balanitin-7 (72%), isolated from *B. aegyptiaca* kernels, demonstrated appreciable anticancer effects in human cancer cell lines in vitro by using against A549 non–small-cell lung cancer (IC50, 0.3 μM) and U373 glioblastoma (IC50, 0.5 μM) cell lines. Bal6/7 displayed higher antiproliferative activity than etoposide and oxaliplatin, markedly less active than taxol. It indicated that balanitin 6/7 mixture is more a cytotoxic compound than a cytostatic one. In vitro anticancer activities are due to partly depletion of [ATP] leading in turn to major disorganization of actin and it does not induce an increase in intracellular reactive oxygen species. In vivo, bal 6/7 increased the survival time of mice bearing murine L1210 leukemia grafts to the same extent reported for vincristine. The ethanol and petroleum ether extracts of aerial parts of *B.aegyptiaca* have been reported to have significant anti-inflammatory action an on carrageenan-induced hind paw edema in rats, the paw volume was measured plethysmometrically at 0 and 3 hours after injection and analgesic activity by using Eddy's hot plate method and tail-flick method in albino rats. The ethanol and petroleum ether extracts showed a greater anti-inflammatory and analgesic effects comparative with the standard drugs, indomethacin and diclofenac sodium, respectively. It also indicated that the ethanolic extract of *B. aegyptiaca* exhibited more significant activity than petroleum ether in the treatment of pain and inflammation.

AIM
AND OBJECTIVES

AIM AND OBJECTIVE

Aim –

To study the antimicrobial activity and phytochemical analysis from *Balanite aegyptiaca.*

- Following objectives under taken for present study-

1) Determination of the antimicrobial activity of *Balanite aegyptiaca.*

2) Phytochemical analysis of *Balanite aegyptiaca.*

   - Quantitative analysis
   - Qualitative analysis

3) Antifungal activity of *Balanite aegyptiaca.*

REVIEW OF LITERATURE

2.0 Review of Literature

Medicinal plants are playing major role in the treatment of many disease the extract of this plants can act as anti-inflammatory, antioxidant, anti allergic, anti cancerous, analgesic and antidiabetic due to this medicinal properties this plants have been used since century to cure and prevention of different kinds of disease. Derivatives from the medicinal plants and extracts are effective in small amounts, economical and safe to use, with negligible side effects morever medicinal plants are easily accessible and have better compatibility *Balanites* aegyptiaca, also known as the Desert Date, is a species of tree that has been extensively studied for its medicinal, nutritional, and economic importance. (Abu-Al-Futuh, IM. 1983)

Balanites aegyptiaca is an important food source in many African countries, providing fruit, oil, and other edible products. The tree's wood is valued for its durability and resistance to termite damage, making it a valuable resource for timber products. The tree's extracts are used in traditional medicine to treat various ailments, including fever, rheumatism, and skin conditions. *Balanites aegyptiaca* is listed as "Least Concern" on the IUCN Red List, but its populations are declining due to over-exploitation for timber, fuelwood, and other products.

The oil remains stable when heated and has a high smoking point, and therefore its free fatty acid content is low. Its scent and taste are good. The leaves are eaten raw or cooked, the oily seed is boiled to make it less bitter and eaten mixed with sorghum, and the flowers can be eaten. The fruit can be fermented for alcoholic beverages. The seed contains seed oil used as cooking oil. The seed cake remaining after the oil is extracted is commonly used as animal fodder. 7. Medicinal Application of *B. aegyptiaca*. All the parts of *Balanites aegyptiaca* are traditionally used in several folk medicines across the globe. (Casamatta D. A. 2013)

This plant has got tremendous importance and being used in treatment of several diseases and disorders. According Wilson et al, the fruit used as oral hypoglycemic drug in the Sahara region of Africa. Furthermore, Hall and Walker reported that the fruits are also commonly used as purgative, antiparasitic and schistosomicide. According to Chapagain and Wiesman, reported that, the stem, root and leaf extracts of *B.aegyptiaca* have commonly been used as various traditional folk medicines especially in the treatment of parasites, sore throat, constipation and eye irritation as reported by (Gaur et al).

Studies have shown that extracts from *Balanites aegyptiaca* possess antimicrobial activity against various bacteria, fungi, and viruses. The tree's extracts have been found to exhibit anti-inflammatory properties, making it a potential treatment for inflammatory diseases. *Balanites aegyptiaca* extracts have been shown to possess antioxidant activity, which can help protect against oxidative stress and related diseases. The fruit of *Balanites aegyptiaca* is rich in nutrients, including proteins, fibers, and minerals like potassium, magnesium, and iron. Oil nutritional content: The tree's oil is rich in unsaturated fatty acids, particularly oleic acid, which has been linked to several health benefits. (Casamatta D. A. 2013)

Earlier studies on therapeutic values of *B.aegyptiaca* shown anthelminthic, anticancer, antioxidant, mosquito larvicidal Chapagain and Wiesman, anti-inflammatory, anti-diabetic Motaal et al, wound healing, hepatoprotective, hypocholesterolemic, diuretic contraceptive and antiviral activities in various parts of Balanites extracts Gaur et al. Aqueous extract of fruits showed spermicidal activity as reported by Saperoni et al, without local vaginal irritation in human being antidiabetic, treatment of jaundice. Seed is used as expectorant, antibacterial, antifungal, febrifuge, anthelmintic and purgative as indicated by Mitra et al; Chothani and Vaghasiya. Fruit is used in whooping cough, also in leucoderma and other skin diseases. Bark is used as spasmolytic. The seed oil is used to treat tumors and wounds used as laxative, also used in treatment of hemorrhoid, stomach aches, jaundice, yellow fever, syphilis, and epilepsy as explained by Chapagain and Wiesman, the bark of this plant is used in the treatment of syphilis, round worm infections, and as a fish poison.

Further studies are needed to fully characterize the phytochemical composition of *Balanites aegyptiaca* extract. Clinical trials are necessary to evaluate the safety and efficacy of *Balanites aegyptiaca* extracts for various medicinal applications. Research is needed to develop sustainable management practices for *Balanites aegyptiaca* populations to ensure their long-term conservative. Nutritionally, Balanites leaves, flowers and fruit pulp are good sources of protein, K, Fe, Mn, Zn and Cu.

Future Directions:

- Synthesis of Silver Nanoparticles from *Balanites aegyptiaca* peels extract.
- Antimicrobial testing of silver nanoparticles synthesized from *Balanites aegyptiaca* peels extract,
- Applications of Silver Nanoparticles from *Balanites aegyptiaca* peels in Pharmaceutical and Cosmetics products.

Methods and materials

4.0 Methods and materials

4.1 Materials

Reagents :

1. Mayer's reagent

Composition: Mercuric chloride - 1.36 gm
Potassium iodine - 5 gm
D/W - 100ml

2. Benedict's reagent

Composition:

Copper sulfate pentahydrate (CuSO4.5H2O) - 17.3 gm
Sodium carbonate (Na2CO3) - 100 gm
Sodium citrate. - 173 gm
Distilled water - 1000 ml

3. Biuret reagent

Composition: Copper sulphate - 1.5 gm
Potassium sodium tartrate - 6 gm
2M sodium hydroxide. - 375ml
Potassium iodine - 1 gm
D / W. - 500ml

4.2 Media

4. Nutrient agar

Composition :

Beef extract - 1 gm
Yeast extract - 2gm
Peptone - 5 gm

Sodium chloride - 5 gm
(Nacl)
Agar - 15 gm
D/W - 100 ml

5. Potato Dexrose agar

Composition :

Potato(infusion form) – 20 gm
Dextrose - 2 gm
Agar-Agar - 1.5 gm
D/W - 100 ml

4.3 Methods:

Collection of Plant material

Extraction of *Balanites aegyptiaca* .

Extraction is a common technique used in organic chemistry to isolate a target compound from biological samples such as cells, tissues, or fluids. In the two main types of extraction, which are liquid-liquid extraction and liquid-solid extraction, the separation is based on solubility. After extraction is complete the solvent can be removed and the desired product collected. The process of separating or extracting particular compounds or components, is referred to as extraction. Steps involved in extraction process

1) Find the source-

E.g. Plant (*Balanites aegyptiaca*)

2) Selection of plant part-

E.g. Fruit Coat, Fruit Peel, Fruit Leaf, Fruit Pulp.

3) Collection -

Collect *Balanites aegyptiaca* fruit dry coat, Peel, Leaf, Pulp

4) Washing-

Tap water is used for washing.

5) Cutting of fruit dry Coat, Fruit Leaf.

6) Drying-

In Hot Air Oven at 40°c. For 3-4 days.

7) Powdering-

Dry peels can be powdered by use of mortar.

8) Methods of extraction-

1. Maceration.

It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay, and climatic moisture levels, from arid to subhumid. It is relatively tolerant of flooding, livestock activity and wildfire. The plants were collected from their natural habitat, form different parts of Solapur District.

Chemicals: The entire chemicals used in the present study are of analytical grade.

Figure 2 Balanite aegyptiaca of fruit, pulp, leaf, peel

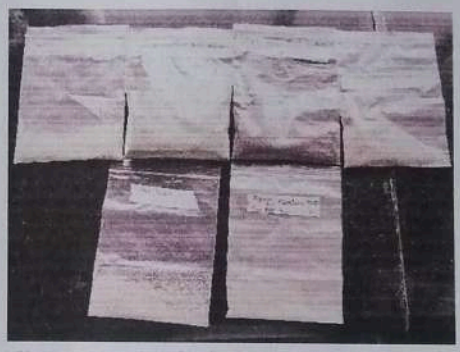


Figure 3 Poweder of Balanite aegyptiaca fruit part

Preparation of plant (fruit peel) extract

The collected plant material was carefully washed under running tap water followed by sterilized distilled water, and air dried at room temperature in laboratory for 3-5 days. These dried plant materials were then homogenized to a fine coarse powder using a mortar pestle and then stored in air tight containers until further use. Various organic solvents viz. water and ethanol were used for extractions.

Extraction methods: Maceration Method

A biological process in which the phytoconstituents are highly concentrated with gradual shaking is referred as maceration. Maceration process is influenced by numerous factors such as temperature, duration, solvent used, etc. It is an easy and popular technique that uses the concepts of diffusional solubility to extract active ingredients. Maceration is a method of extraction where plant material is soaked in a solvent, typically a liquid like distilled water , ethanol and methanol to extract desired compounds. The principle involves allowing the solvent to penetrate the plant material, facilitating the dissolution of soluble compounds. This process occurs over an extended period, allowing for a comprehensive extraction. After maceration, the solvent is separated, leaving behind the extracted substances, often in the form of a liquid extract

For Maceration Method we used Shaker or Shaker Incubator.

Procedure:

3-4 g of *Balanite aegyptiaca* powder was weighed and dissolved in 30-40 ml of distilled water, Ethanol and Mathanol. The flask was covered with cotton and aluminium foil to prevent contamination. Efforts were made to ensure the powder was completely dissolved in the water. The flask was then incubated at room temperature for 2-3 days in a shaker.

After two days, when a separated layer between the extract and distilled water was observed, the mixture was filtered using filter paper or muslin cloth. The filtrate was collected and evaporated using a boiling water bath. Once evaporation was complete, the extract was transferred to a closed container and stored at 4°C for further use.

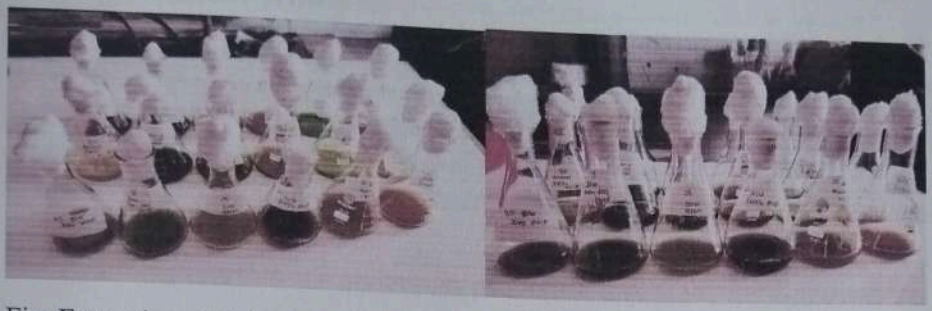


Fig. Extraction of *Balanite aegyptiaca* by Maceration Method using VDRL shaker.

Extraction of plant *Balanite aegyptiaca* by maceration was performed by using 5gm powder *Balanite aegyptiaca* of given plant sample dissolved it in 30 ml distilled water, Ethanol and Methanol then covered it in the flask by using cotton or aluminium foil. After preparing the flask, the Flask were placed on VDRL shaker for 2 to 3 days to seperate out two layers real extract and crude extract by using Filteration.

In Maceration method, shaking and its duration time had a positive effect on yield of the extract. As compared to other method maceration method is very simple.

Phytochemical Analysis

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. The name comes from Greek φυτόν (phyton) 'plant'. Some phytochemicals have been used as poisons and others as traditional medicine. As a term, phytochemicals is generally used to describe plant compounds that are under research with unestablished effects on health, and are not essential nutrients.

Two types of phytochemical analysis are performed.

1. Qualitative analysis

Qualitative analysis is performed to know presence or absence of phyto constituents.

2. Quantitative analysis

Quantitative analysis is used to know quantity of the phytoconstituents present in extract.

Qualitative analysis:

1. **Test for Protein**

   Ninhydrin Test

   Test solution treated with ninhydrin reagent gives blue colour.

2. **Test for Carbohydrates**

   Molisch's Test

   Test solution with few drops of Molisch's reagent and 2ml concentrated sulphuric acid added slowly from side of test tube purple ring junction of liquid.

3. **Test for Oxylate**

   4ml extract was mixed with 2ml of Acetic acid, 1 drop of Fecl3 and 2ml concentrated H2SO4 brown ring indicated positive test for oxylate.

4. **Test for Carboxylic acid**

   Extract was mixed with sodium bicarbonate solution and chaked for effervescence.

5. **Test for Quinons**

Extract was treated with NaoH solution and then violet colour was observed.

6. **Test for Tannins**

$K_2Cr_2O_7$ solution with hydrochloric acid in a test tube. Add tube 2-3 ml of plant extract in that test tube. Tannins are present a greenish-yellow colour appears within 5-10 min.

7. **Test for Resins**

Extract was treated with a 3-4 ml of CuSO4 solution after proper mixing for 1-2 min and green precipitation proves resins.

8. **Test for Steroids**

To 1 ml extract, added 10 ml Chloroform and equal volume of concentrated H_2SO_4 layer showed yellow colour with green fluorescence.

9. **Test for Terpenoids**

Salkowaski's Test

When few drops of concentrated sulphuric acids are added to the test solution, shaken ald allowed to stand, lower turn, red indicating the presence of Terpenoids.

10. **Test for Flavonoids**

Zinc-Hydrochloric acid-reduction test-

Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

11. **Test for Alkaloids**

Mayer's test

Test solution treated with mayer's reagent (iodine n potassium iodide) gives brown precipitate.

12. **Test for Saponins:**

Foam test: Sample when mixed with water & shaken show the formation of foam which is stable.

Quantitative Test

Anti-Inflammatory assay by protein denaturation method using Plant *Balanite aegyptiaca*

Aim:

To evaluate the anti-inflammatory activity of the given extract by protein denaturation method.

Principle:

The protein denaturation method is often used to assay anti-inflammatory activity. In this assay, the principle revolves around the prevention of protein denaturation, which is a key step in the inflammatory process. Proteins denature during inflammation, leading to structural changes. In the anti-inflammatory assay, a sample is tested to determine its ability to inhibit the denaturation of a protein, often using a standard protein like albumin. The degree of protein denaturation is assessed by measuring changes in turbidity or absorbance. A lower absorbance or turbidity in the presence of the sample indicates its potential anti-inflammatory activity, as it suggests protection against protein denaturation that occurs during inflammation.

Reagents preparation:

1) Egg albumin & PBS (Phosphate buffer saline)

2) Standard- Diclofenac sodium

3) Plant Extract

Procedure:

1) The reaction Mixture contains 0.4 ml of egg albumin (collected from fresh hen's egg), 5.6% phosphate-buffered saline (PBS).

2) Further add 1ml plant extract& Standard-Diclofenac sodium.

3) Incubate the reaction mixture for 30mins at 70°C

4) After successful incubation measure the absorbance of reaction mixture at 510nm

Formula: % OF INHIBITION O.D.= OF CONTROL-O.D. OF TEST/O.D. OF CONTROL* 100

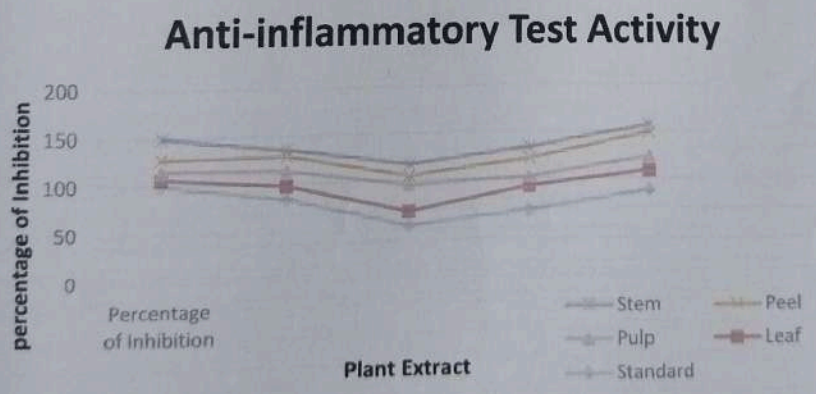
Table 1 Optical Density at 530 nm by Anti-inflammatory test

Plant Extract	Optical Density at 530 nm				
	200	400	600	800	1000
Standard	0.00	0.19	0.58	0.40	0.15
Leaf	1.33	1.41	1.43	1.55	1.50
Pulp	1.34	1.43	1.58	1.36	1.43
Peel	1.40	1.44	1.36	1.49	1.55
Stem	1.50	1.32	1.09	1.10	1.34

Table 2 Percentage of Inhibition of Anti-inflammatory test

Plant Extract	Percentage of Inhibition				
	200	400	600	800	1000
Standard	100	84.67	53.22	67.74	87.90
Leaf	7.25	13.70	15.32	25.00	20.96
Pulp	8.06	15.32	27.42	9.67	15.32
Peel	12.90	16.12	9.67	20.16	25.00
Stem	20.96	6.45	12.09	11.29	8.06

Figure 6 The Graph of Anti-inflammatory test Activity



Result:

Estimation of anti-inflammatory activity of *Balanite aegyptiaca* by protein denaturation method was performed using 0.4 ml of fresh egg albumin and 5.6 ml PBS (Phosphate Buffer Saline) and 1 ml plant extract was added and incubated it for 30 minutes at 70°Celsius in water bath. After incubation, the absorbance of reaction mixture was measured at 530 nm and percentage of inhibition was calculated.

Conclusion:

The ability of flavonoid-rich extract of *Balanite aegyptica* shows anti-inflammatory activity and can be used in formulations against inflammation.

Anti-diabetic Assay by Alpha- Amylase Method using Plant *Balanite aegyptica*

An antidiabetic assay using the alpha-amylase method is designed to evaluate the potential of substances to inhibit the enzyme alpha-amylase, crucial in carbohydrate digestion. Inhibition of alpha-amylase can help regulate glucose levels, making this assay valuable in identifying compounds with anti-diabetic properties. The assessment often involves measuring the inhibition's impact on the breakdown of starch, providing insights into a substance's ability to modulate postprandial glucose levels, a key aspect in diabetes management.

Aim:

To perform anti- diabetic activity by alpha amylase method.

Principle:

In the alpha-amylase inhibition assay, the principle lies in assessing the ability of a substance to inhibit the activity of the enzyme alpha-amylase. Alpha-amylase is involved in the breakdown of complex carbohydrates into simpler sugars, including glucose. Inhibition of this enzyme can help regulate postprandial glucose levels, making it relevant to anti-diabetic research. The degree of inhibition is often measured by assessing the reduced formation of reducing sugars, typically through colorimetric or spectrophotometric methods. A lower absorbance or color development indicates a higher level of inhibition, suggesting potential anti-diabetic properties by regulating carbohydrate digestion.

Reagent preparation:

1) Plant extract

2) DNSA

3) Test sample 0.5% Alpha amylase

4) 1% starch.

Procedure:

1) The reaction mixture contains 0.5ml of Plant/std- Acarbose. & 0.5 ml of a-amylase (0.5%)

2) further add 1% starch in and above reaction mixture.

3) Incubate the reaction mixture at RT. for 30 min.

4) Further add reaction 2 ml DNSA in an above reaction mixture.

5) Incubate the reaction mixture for 15 min in boiling water bath, 100°C

6) After successful Incubation Measure the absorbance of the reaction mixture at 620 nm.

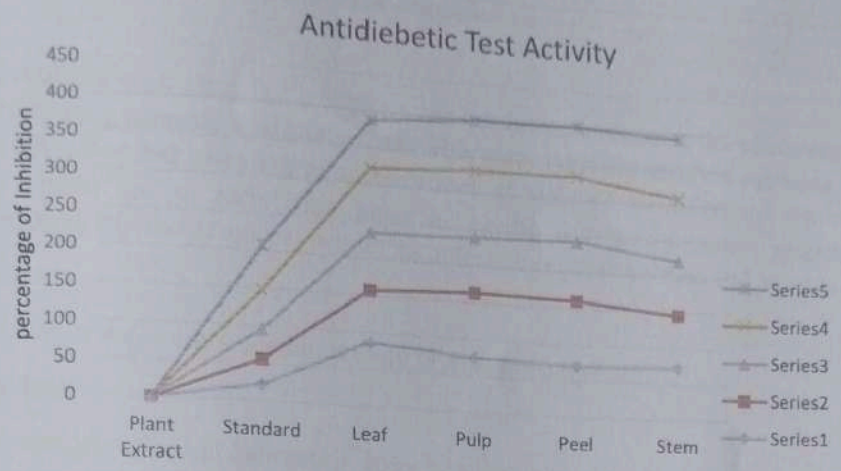
Table 5 Optical Density at 530 nm by Anti-diebetic test

Plant Extract	Optical Density at 620 nm				
	200	400	600	800	1000
Standard	0.49	0.51	0.54	0.65	0.75
Leaf	0.20	0.46	0.34	0.19	0.54
Pulp	0.36	0.18	0.39	0.12	0.47
Peel	0.44	0.16	0.28	0.16	0.46
Stem	0.42	0.41	0.37	0.20	0.25

Table 5 Percentage of Inhibition of Anti-diebetic test

Plant Extract	Percentage of Inhibition				
	200	400	600	800	1000
Standard	22.10	34.15	39.45	52.30	59.00
Leaf	87.01	70.12	77.92	87.66	64.93
Pulp	75.62	88.31	74.67	92.20	69.48
Peel	71.42	89.61	81.81	89.61	70.12
Stem	72.72	73.37	75.97	87.01	83.76

Figure 8 The Graph of Antidiabetic assay of Plant Extract



Result:

Estimation of anti-diabetic activity of_*Balanite aegyptica* by alpha amylase method was performed using 0.5 ml of plant extract added in 0.5 ml 0.5% of alpha amylase further 0.5ml 1% starch was added and incubated it for 30 minutes at room temperature.

After incubation 2ml DNSA was added in reaction mixture and again incubated for 15 minutes in a boiling water bath and then measured the absorbance of reaction mixture at 530 nm and also percentage of inhibition was calculated.

Conclusion:

The anti-diabetic assay using the alpha amylase method is a valuable technique for evaluating the potential of various compounds or extracts in managing diabetes. This method measures the enzymatic activity of alpha amylase, which plays a crucial role in the breakdown of carbohydrates, and its inhibition is known to have a positive impact on blood glucose levels.

Anti-Helmenthic Assay of *Balanite aegyptica*

An antihelminthic assay is a laboratory test conducted to evaluate the effectiveness of substances in combating parasitic worm infections. This assay typically involves exposing the worms to the test substance and assessing parameters such as viability. mortility, and reproductive capacity. The results aid in identifying potential agents with anti-parasitic properties, crucial for developing treatments against helminthic infections in both animals and humans.

Aim:

To estimate the anti-helmenthic activity of *Balanite aegyptica*.

Principle:

The principle of an antihelminthic assay involves evaluating the ability of a substance to inhibit the survival, growth, or reproduction of parasitic worms, known as helminths. This assay is designed to identify potential agents with anti-parasitic properties. Typically, the process includes exposing the worms to the test substance and assessing parameters like mortality, motility, and reproductive capabilities. The degree of inhibition or damage to the helminths provides valuable insights into the efficacy of the tested compound as a potential treatment for parasitic worm infections.

Reagents:

1) Plant extract

2) Standard- Albendazole

Procedure:

1) Take 2 petri plates. Clean the petri plates and air dry.

2) Collect the earthworm and wash them with the help of water to remove the unwanted soil dust.

3) Take 5 earthworms in each petri plates and close with the help of petri lid.

4) Load drug (Albendazole) and sample in different petri plates and note the time of death.

Observation Table:

Sr.No	Plant Exctarct	Death Time Period
1	Peel	1min 17 Sec
2	Pulp	1min 16 Sec
3	Stem	10Sec
4	Leaf	20 Sec
5	Standard(Albendazole)	12 Min

Result:

Estimation of anti helminthic activity of plant *Balanite aegyptiaca* was performed by using 2 petri plates, cleaned and dried it. After selecting the earthworms clean them, then earthworms were distributed in each petri plate. In one petri plate sample 5 ml was added and in another one standard (Albendazole) 10 ml was added. Then time of death of Earthworms was measured.

As per time taken by earthworms time for death was pulp-1.16 Sec , Peel- 1.17 Sec, Stem-10Sec, leaf-20Sec and for standard time for paralysis was 10minutes and time for death was 12 minutes.

Conclusion:

Plant extract of *Balanite aegyptiaca* shows effective activity against earthworm. Thus can be used to treat diseases caused by parasitic intestinal.

6.0 Antifungal Test

Anti-Fungal Activity by Well Diffusion Method using Plant *Balanite aegyptiaca*

Antifungal activity refers to the ability of a substance to inhibit or kill the growth of fungi. This activity is of particular interest in various fields, including medicine, agriculture, and industry, where fungal infections or contamination can have significant implications. Antifungal agents can be natural or synthetic compounds that target specific fungal structures or functions.

Aim:

To study the Anti-Fungal activity by well diffusion method.

Principle:

The principle of antifungal activity by the well diffusion method involves ,assisting the ability of a substance to inhibit fungal growth. In this method, the substance is placed in wells on an agar plate inoculated with fungal cultures. As the substance diffuses into the agar, it creates a concentration gradient, leading to inhibition of fungal growth around the well. The size of the resulting zone of inhibition is indicative of the substance's effectiveness against the tested fungi, providing valuable information about its antifungal activity.

Reagents:

1) Plant extract

2) Standard-Fluconazole

Procedure:

1) Prepare the Nutrient Agar and Potato Dextrose Agar media and autoclave it.

2) Pour the media into respective petri dishes and allow to solidify it.

3) Prepare the inoculum and spread it on the top of Nutrient Agar and Potato Dextrose Agar plate media.

4) After preparation of plates make the well with the help of cork borer.

5) Inoculate the sample in particular wells.

6) After the inoculation incubate the plates at room temperature for 24 hrs.

Result:

Estimation of antifungal activity of plant by *Balanite aegyptiaca* using well diffusion method was performed by preparing the sterile Nutrient Agar and Potato Dextrose agar medium and the fungal inoculum. Then inoculum was spread on the plates and after spreading the wells are prepared by using cork borer and in that wells the sample 100 microliter and standard (Fluconazole) 100 microliter were inoculated and incubated the plates at room temperature for the 2-3 days and after the successful incubation, the zone of inhibition was measured.

As per zone of inhibition the zone of sample was 15 mm and zone of inhibition for the standard was 22 mm

Conclusion:

In this study antifungal activities of assessed by *Balanite aegyptiaca* well diffusion method.

RESULT AND DISCUSSION

RESULT AND DISCUSSION

Observation Table

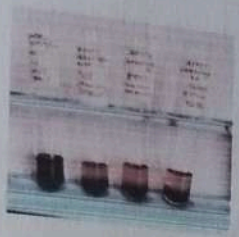
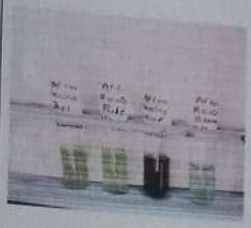
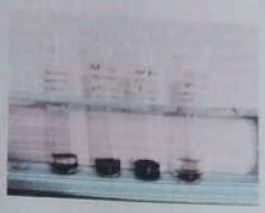
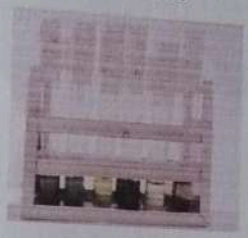
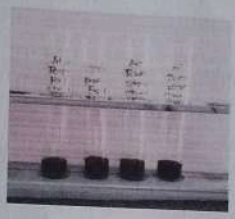
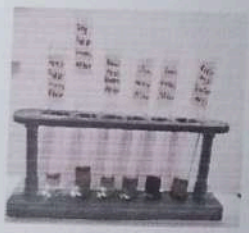
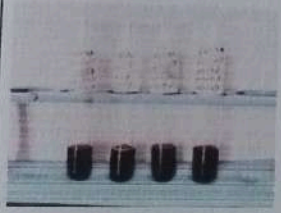
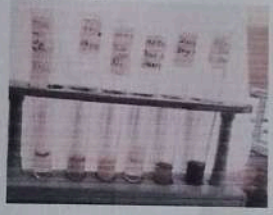
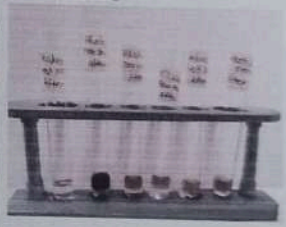
Table 1 : **Phytochemical Analysis of qualitative test by *Balanite aegyptiaca***

Sr. No	Name of Compound	Observation	Plant Extract			
			Pulp	**Leaf**	**Peel**	**Stem**
1	Protein • Ninhydrin Test	Blue Colour Observed	Present	Present	Absent	Present
2	Carbohydrate • Molisch's Test	Purpel ring appeared	Absent	Absent	Present	Absent
3	Oxylate	Brown ring indicated	Present	Present	Present	Present
4	Carboxylic acid	Effervescence Observed	Absent	Present	Absent	Absent
5	Quinones	No Colour formation	Absent	Absent	Absent	Absent
6	Tannins • NaOH Test	Formation of emulsion	Present	Present	Present	Present
7	Resins	Green Precipitate Formed	Present	Present	Present	Present
8	Steroids	Brown ring indicated	Present	Present	Present	Present
9	Terpenoids • Salkowaski's Test	Red Colour indicated	Present	Present	Present	Present
10	Flavonoids	Red Colour observed	Present	Present	Present	Absent
11	Saponins Foam Test	Foam Observed	Present	Absent	Present	Absent
12	Alkanoids • Mayer's Test	Brown Precipitate Formed	Absent	Present	Absent	Absent

Present – Positive Test

Absent – Negative Test

Table 2 ***Phytochemical Analysis of qualitative test by Balanite aegyptiaca***

Protein 	Carbohydrate 	Resins 	Steroids 
Oxylate 	Carboxylic Acid 	Terpenoids 	Flavonoids 
Quinones 	Tannins 	Saponins 	Alkanoids 

5.2 Biomedical application

1.1 Anti-inflammatory aasay by protein denaturation method using *Balanite aegyptiaca* leaf, pulp, stem, peel extract

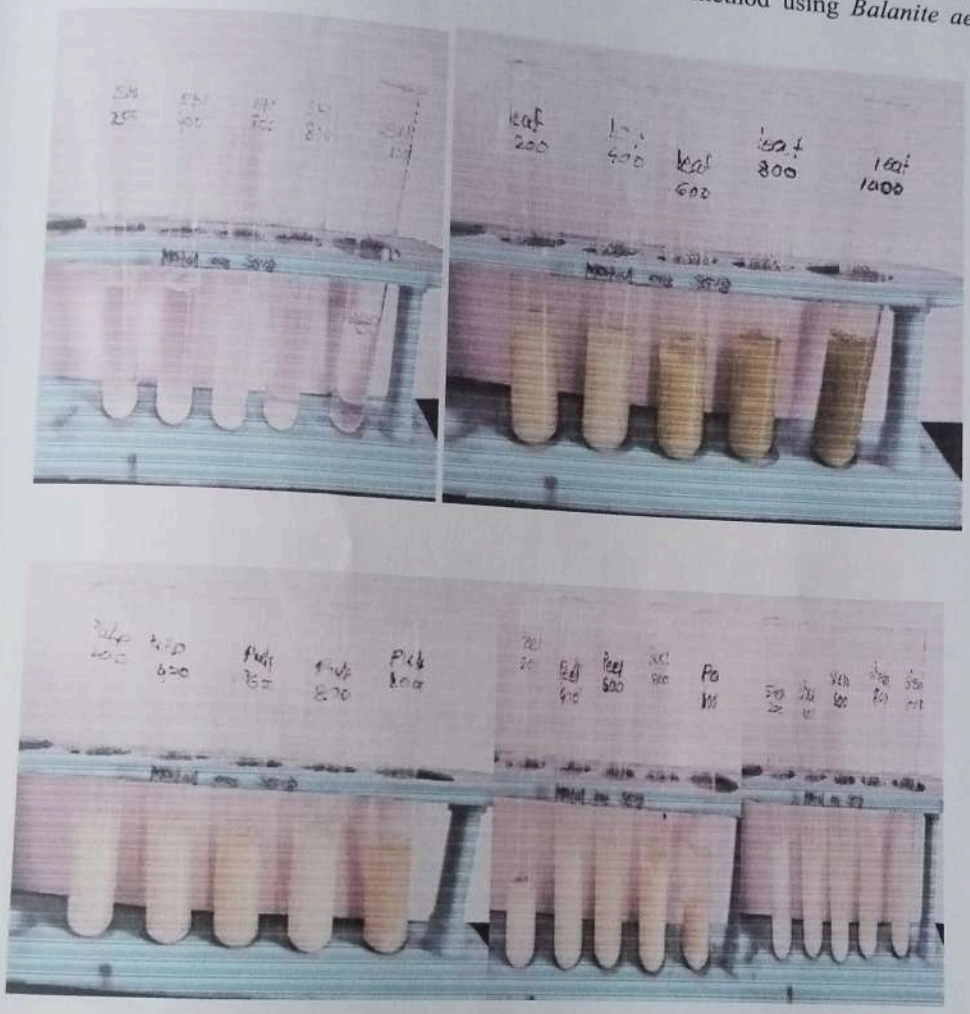


Figure 1.1 Anti-inflammatory Assay by protein denaturation method using Plant *Balanite aegyptiaca* Test samp

1.2 Anti-diebetic Assay by Alpha-Amylas Method using Plant *Balanite aegyptiaca* leaf, pulp, stem, peel extract

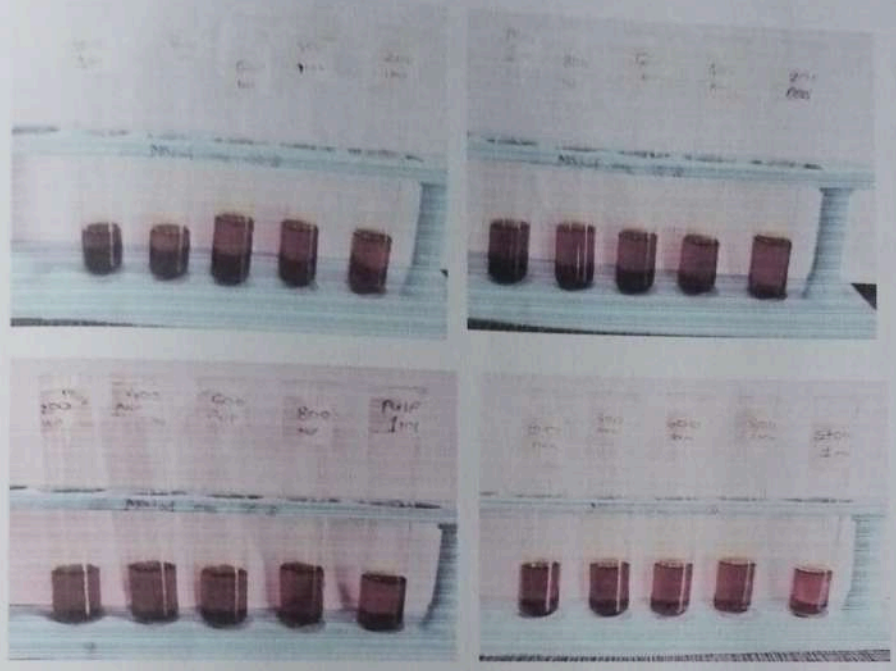


Figure 1.2 Anti-diebetic Assay by Alpha-Amylas Method using Plant Balanite aegyptiaca Test sample

1.3 Anti-helminthic Assay by using Plant *Balanite aegyptiaca* leaf, pulp, stem, peel extract

Figure 1.3 Anti-helminthic Assay by using Plant Balanite aegyptiaca Test sample

1.4 Anti-fungal activity by well diffusion method using Balanite aegyptiaca peels, pulp, leaf, Stem extract.

Result:

Antifungal activities by well difusion method was assesed using Balanite aegyptiaca peels, pulp, leaf, Stem extract.

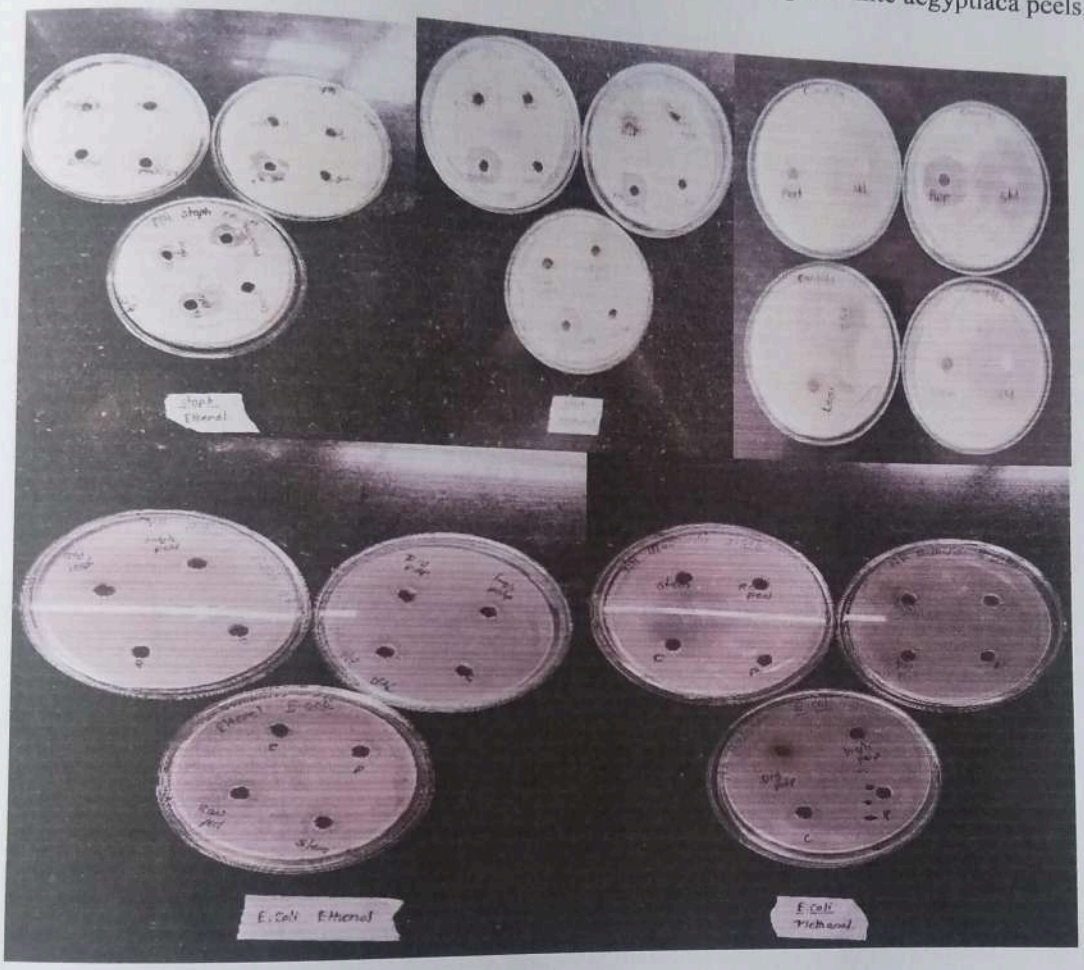


Figure 2 Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* peels, pulp, leaf, Stem extract.

Observation Table:

- Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* peels, pulp, leaf, Stem extract in ethanol (*E.coli*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	Plant Extract
1	Leaf	23 mm	1 mm	14 mm
2	Stem	18 mm	15 mm	18 mm
3	Pulp	23 mm	21 mm	21mm
4	Peel	18 mm	15 mm	11 mm

- Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* peels, pulp, leaf, Stem extract in methanol *(E.coli)*

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Methanol)	Plant Extract
1	Leaf	-	23 mm	14 mm
2	Stem	15 mm	13 mm	-
3	Pulp	1 mm	23 mm	23 mm
4	Peel	15mm	18 mm	18 mm

- Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* peels, pulp, leaf, Stem extract in ethanol (*Staphylococcus aureus*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (ethanol)	Plant Extract
1	Leaf	29 mm	1 mm	-
2	Stem	25 mm	-	5 mm
3	Pulp	21 mm	1 mm	15 mm
4	Peel	25 mm	1 mm	6 mm

- Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* peels, pulp, leaf, Stem extract in methanol (*Staphylococcus aureus*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (methanol)	Plant Extract
1	Leaf	3 mm	3 mm	14 mm
2	Stem	33 mm	1 mm	11 mm
3	Pulp	3 mm	3 mm	25 mm
4	Peel	33 mm	1 mm	11 mm

- Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* peels, pulp, leaf, Stem extract in methanol (*Candida albicans*)

Sr. no	**Plant Extract Sample**	**Zone of Inhibition (diameter in mm)**	
		Standard(Fluconazole)	**Sample**
1	Leaf	7 mm	-
2	Stem	8 mm	-
3	Pulp	9 mm	25 mm
4	Peel	11mm	11 mm

CONCLUSIONS

6.0 CONCLUSIONS

Balanites aegyptiaca, commonly known as Desert Date, is a versatile and valuable plant species with rich phytochemical constituents (saponins, flavonoids, alkaloids, glycosides, and terpenoids). Multiple traditional uses (medicinal, food, timber, and fuel).Scientifically-backed health benefits (antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and anticancer properties). Potential applications in pharmaceuticals, cosmetics, and food industries. *Balanites aegyptiaca* is a valuable plant species with significant potential for medicinal, economic, and environmental benefits. Further research, sustainable practices, and community engagement are necessary to unlock its full potential

Extensive literature survey revealed that 'desert date' has a long history of traditional uses for wide ranges of disease. It has been experimentally proved that *B. aegyptiaca* Del possess antioxidant, antimicrobial, anticancer, diuretic, hypocholesterolemic, wound-healing, antiviral, anti-diebetic, hepatoprotective, mosquito larvicidal, anti-inflammatory and analgesic, antivenin, anthelmintic, cardioprotective cum antioxidant activity, and antinociceptive properties. Bark, fruits, seeds, seed oil, and leaves of this plant are widely used in folk medicine. In recent years, emphasis of research has been on utilizing traditional medicines that have long and proven history of treating various ailments. So, further studies need to be carried out to explore *B. aegyptiaca* Del for its potential in curing and treating disease.

In conclusion, the phytochemical analysis of *Balanites aegyptiaca* revealed a rich diversity of bioactive compounds with potential therapeutic applications. The antioxidant, cytotoxic, and antimicrobial activities support traditional uses in folk medicine. These findings suggest potential applications in medicine, agriculture, and industry.

Future Directions:

- Synthesis of Silver Nanoparticles from *Balanites aegyptiaca* peels extract.
- Antimicrobial testing of silver nanoparticles synthesized from *Balanites aegyptiaca* peels extract,
- Applications of silver Nanoparticles from *Balanites aegyptiaca* peels in Pharmaceutical and Cosmetics products.

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6.0 BIBLIOGRAPHY

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