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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *BALANITES AEGYPTIACA* LINN. DELILE. STEM BARK

Gupta Satish Chand*, Shenoy Sumanth, Kotecha Mita
Department of Dravyaguna, National Institute of Ayurveda, Jaipur, Rajasthan, India

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*E-mail: dr.sgupta1982@gmail.com.

ABSTRACT
Various pharmacognostical parameters including macroscopy, microscopy, Physiochemical and behavior of powdered drug on treatment with different chemical reagents were studied on the stem bark of *Balanites aegyptiaca* Linn. Delile. (Family- Balanitaceae).The successive extraction of plant bark was undertaken by using various solvents of increasing polarity and the extracts thus obtained were subjected for phytochemical analysis. The phytochemical investigation revealed the presence of alkaloids, glycosides, flavonoids, and phenolic compounds mainly. These preliminary data may be helpful in developing the standardization parameters of *Balanites aegyptiaca* Linn. Delile stem bark.
Keywords : Microscopy, Physiochemical, Phytochemistry, *Balanites aegyptiaca*

INTRODUCTION

Balanites aegyptiaca Linn. Delile. belongs to the family Balanitaceae. It is one of the most common plant species of the dry land areas of Africa, Middle East & South Asia[1].In India, it is particularly found in Rajasthan, Gujarat, Madhyapradesh.The plant is well described in Ayurvedic classics with the name 'Ingudi'.In Hindi, it is called as 'Hingot' and in English as Desert Date. The tree can grow to 6-10 meters in height, is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal moisture.

The word balanites alludes to the fruit which somewhat resembles to acorn & aegyptiaca means, this plant is extensively found in Egypt. The stem bark of plant is useful in curing mental diseases, epilepsy, yellow fever, jaundice and syphilis and can also act as a fumigant to heal circumcision wounds[2]. The methanolic extract, butanolic extract, and the saponins isolated from the stem bark of *B. aegyptiaca* exhibited anti-inflammatory, antinociceptive and antioxidant activity[3].In Indian traditional and folklore system of medicine, the stem bark of *B. aegyptiaca* is used in treatment of epilepsy. The stem bark contains a sesquiterpene, Balanitol and the saponins, deltonin and protodeltonin[4]. The chloroform extract is reported to contain furanocoumarin, bergapten and a dihydrofuranocoumarin, marmesin[5]. Dicholoromethane extract has yielded two types of alkaloids N-*trans*-feruloyltyramine and N-*cis*-feruloyltyramine and other metabolites like vanillic acid, syringic acid and 3-hydroxy-1-(4-hydroxy-3 methoxyphenyl)-1-propanone[6].Ethanolic extract of *B. aegyptiaca* possesses biologically active compounds that have anxiolytic and sedative properties[7], supporting the ethnomedicinal use of the plant as antipsychotic and antiepileptic agents.

MATERIAL AND METHODS

Plant Material

The stem bark of *Balanites aegyptiaca* Linn.Delile. was collected by self, in the month of September, from Shivdaaspura, Jaipur. The plant material was authenticated at B.S.I.(Botanical Survey of India) Jodhpur, Rajasthan, by P.M. Padhey and was given a voucher number BSI/AZC/ 2011/Tech /407. Organoleptic evaluation was carried out with intact bark. Bark was pulverized in the mechanical grinder to a moderate fine powder to carry out microscopic studies and was stored in a well closed airtight vessel for further analysis. All reagent and chemicals used for the study were of analytical grade.

Organoleptic, Macroscopial And Microscopical Evaluation

The freshly collected bark of the plant were evenly spread on a clean dry plastic sheet and investigated for different organoleptic features by repeated observations by conventional methods and were recorded. Macroscopy Characters of stem bark was documented. For microscopical evaluation fine sections of the bark were taken with the help of microtome, stained and temporarily mounted following usual micro techniques and representative diagrams were taken with the help of inverted microscope for photo documentation. The different powder characteristics were studied according to standard methods[8].Schulze's Maceration fluid ($Kclo_3$ + 50% HNo_3) is used to disintegrate hard woody substances such as sclereids, xylem elements etc. This fluid is a powerful oxidizing agent and it rapidly oxidizes and removes lignin from vegetable tissues[9].

Staining Characteristic of Bark Powder

Each time a small amount of powdered bark was treated with different types of chemical reagents as mentioned in Table-2 and the color characteristics were observed in day light.

Behavior of Bark Powder towards Some Chemical Reagents

The pale yellowish powder of *Balanites aegyptiaca* bark was treated with different chemical reagents. The mixture of the powdered drug and chemicals were allowed to warm and cool down for two hours. Changed color of powdered drug was noted.

Physico-Chemical Evaluations

Physico-chemical parameters such as the total ash, acid insoluble ash, acid soluble ash, water insoluble ash, water soluble ash were determined. Considering the diversity of chemical nature and properties of contents of drugs, different solvents benzene, petroleum ether, chloroform, methanol (95%), chloroform water of extractive values were determined as per reported methods[10-12]. All determinations were performed thrice and the results were presented as mean.

Phytochemical Screening

Qualitative examination of inorganic matters & determination of heavy metals was done as per reported methods. The dried powdered bark was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents. The dried powdered bark (100 g) was extracted successively hexane, petroleum ether, benzene, chloroform, acetone, methanol, water in a Soxhlet Extractor by continuous hot percolation. Each time before extracting with the next solvent of higher polarity the powdered material (marc) was dried in a hot air oven below 50°C for 10 minutes. Each extract was concentrated in vacuum on a Rota Evaporator (Buchi type) and finally dried in hot air oven. The dried extracts were dissolved in respective solvents, with which it was extracted, and were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material[13].

RESULTS

Organoleptic Evaluation

The Organoleptic characters found are discussed in Table 1.

Macroscopial Evaluation

In Pharmacognosy the term "bark" is used to describe all the tissues found external to the cambium in the branch, stem, or root. Barks will consist of all or some of the following tissues: Rhytidoma (dead tissues), Cork, Phellogen (meristematic), Phelloderm, Cortex and Secondary Phloem.

Shape & Size: Curved or channeled very hard, varies in length, 5 -8 cm in width and 3 to 5 mm in thickness.

Outer Surface: Very rough, longitudinally irregularly ridged, furrowed, shows well developed circular lenticels and scales of rhytidoma at places, grayish dirty brown in color.

Inner Surface: Rough, fibrous, shows rows of longitudinal running pits on the smoothly cut surface, creamish yellow in color.

Fracture: Hard, outer is granular, inner is splintery.

Taste : Bitter.

Odor : Slightly sternulatory.

Microscopical Evaluation

Diagrammatic T.S of the bark shows outer wide zone of cork consisting of lignified and non lignified zone and at places the patches of rhytidoma occasionally penetrate inside the phloem tissue. A very wide zone of phloem consistency of multiseriate medullary rays, groups of fibers and sclereids and stone cells alternating with sieve tissue and ceretenchyma are found.

Detailed transverse section shows an outer wide zone of cork consisting of lignified, and suberised cell bands penetrating towards the inner tissue in irregular forms, constituting the pieces of rhytidoma which at times enter inside the outer phloem tissue. The cells of rhytidoma are dark brown, thick walled, impregnated with tannins. Their nature differ accordingly to the area of their penetration e.g. in the cortex, the cells are embedded with rows at groups of the sclereids, stone cells or occasionally isolated fibers also. If rhytidoma penetrates deep inside upto the outer tissue of the phloem then it exhibits rows of medullary rays. Sclereids, fibers, parenchyma embedded with plenty of prismatic crystals of calcium oxalate and starch grains. The inner phloem where rhytidoma has not penetrated is a wide zone consisting of multiseriate 10 to 25 rows medullary rays running straight, parallel or in sinous manner. At many places the cells of the medullary rays becomes sclerosed and contain giant prismatic crystals of calcium oxalate, alternating with the groups of thick & thin walled fibers, running in tangential bands, and surrounded by idioblast containing prismatic crystals of calcium oxalate. Phloem tissue alternating with groups of parenchyma and sieve tubes gets obliterated at places exhibiting caretenchyma bands throughout. Prismatic crystals of calcium oxalate and simple starch grains are embedded in the parenchymatous tissue throughout the section.

Powder Microscopy

Fibrous, Coarse, pale yellow colored powder with bitter taste. Odor is irritating to the mucous membrane of the nostrils when fine particles enter in it, the effect known as sternulatory.

The diagnostic characters of the powder are fragments of cork in surface view and in transversely cut view, the cells being lignified and occasionally suberised at places, fragments of dark brown colored cells embedded with tannins of rhytidoma, abundant prismatic crystals of calcium oxalate scattered as such throughout or embedded in the parenchymatous cells of cortex, phloem of medullary rays of various size, few embedded in the phloem are of very giant sized, radially cut medullary rays crossing the phloem fibers, tangentially cut medullary rays, seen as broad oval pits associated with groups of fibers. Starch grains irregular shape & size, traversed as such throughout or embedded in the parenchymatous cells. Isolated or groups of phloem fibers with characteristic bent at places, large number of sclereids of various size and shapes, stone cells and crystal fibers are found.

Staining Characteristic of Bark Powder

The observations of color reactions are reported in Table 2.

Behavior of Bark Powder towards Some Chemical Reagents

The observations are reported in Table 3.

Physico-Chemical Evaluation

The physicochemical studies and Successive extractive values of stem bark of *Balanites aegyptiaca* Linn. Delile. are summarized in Table 4 and Table 5.

Phytochemical Screening

Inorganic substances (Ca,Fe,P,S,Mn,K) were present. Heavy metals (Co,Cu,Hg,Ni,Zn,Ag,Bi,Pb,Ba) were absent. The results demonstrated presence of carbohydrate, saponin, flavanoids, tannins, alkaloids mainly in the stem bark of *Balanites aegyptiaca.* The presences of various phytoconstitutes in various extracts are summarized in Table 6.

DISCUSSION AND CONCLUSION

Pharmacognosy enfolds the knowledge of history, distribution, cultivation, collection, processing for market and preservation, the study of organoleptic, physical, chemical and the uses of crude drugs. The objective of Pharmacognosy is to contribute towards establishment of rational relationship between the chemical moieties of naturally occurring drugs and their biological and therapeutic effects, which ultimately helps in the standardization of the plant[14].

Morphological and microscopical characteristics of a plant are of great importance and used for the standardization studies of plant. The microscopical study of stem bark showed the presence of rhytidoma, phellogen, phelloderm, medullary rays, phloem fibres, sclereids, calcium oxalate crystals and sieve tubes. Powder Microscopy showed that the starch grains and prismatic calcium oxalate crystals. Reticulate fibers were found, that are specific identification of Balanitaceae family.

The physical evaluation furnished different ash values, extractive values in different solvents. Total ash, acid insoluble ash and water soluble ash values were also determined. The phytochemical investigation revealed the

presence of carbohydrate, saponin, flavanoids, tannins, alkaloids and phenolic compounds mainly in the stem bark of *Balanites aegyptiaca*. Thus a vast array of compounds was found to be present in the plant under present study. Thus a variety of standardization parameters viz. morphological, microscopical, physico-chemical, phytochemical were studied and data was generated for the assessment of quality of plant material, and also to check the adulteration and substitution etc., which may be helpful for future reference.

After present investigation it can be concluded that the pharmacognostic study of stem bark of *Balanites aegyptiaca* have furnished a set of qualitative and quantitative parameters that can serve as an important source of information which may substantiate the existing pharmacopoeial data to ascertain the identity and to determine and track the quality and purity of the plant material in future studies.

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Table 1 Organoleptic Characteristic of Stem Bark of *Balanites aegyptiaca* Linn. Delile.

Parameters	
Sparsha (Touch)	Rukṣha (Dryness)
Rupa (Colour)	Medium yellowish
Rasa(Taste)	Tikta (Bitter)
Gandha(Odour)	Slight Tikṣna (Slightly Sternutatory)

Table 2 Staining Characteristic of Bark Powder

Stains	Observations
A. Iodine	Bluish black starch grains observed
B. Chloral hydrate	Calcium Oxalate Crystal present
C. Phloroglucinol & dilute HCl.	Pink colored lignified cells observed.

Table 3: Reactions of the Stem bark Powder of *Balanites aegyptiaca* with different Chemical Reagents

Chemical Reagent	Observation
Conc.Sulphuric Acid	Dark Black
Conc.Hydrochloric Acid	Light Brown
Con.Nitric Acid	Reddish yellow
Picric Acid	Chocolate color
Glacial Acetic Acid	Light grey
Iodine Solution	Light yellow
Sodium hydroxide solution (aq.5%)	Cream color
Potassium hydroxide solution (aq.5%)	Yellowish white
Ferric chloride solution (aq.)	Dark brown
Powder as such	Light yellowish

Table 4 Physico –Chemical Properties of *Balanites aegyptiaca* Linn. Delile. Stem Bark

Quantitative Standards	% w/w
1.Total Ash Value	9.460
2. Acid Insoluble Ash	2.30
3. Water Soluble Ash	7.51
4. Loss of weight on drying 105^{o}C	55
5. Alcohol soluble extractive value	8.18
6. Water soluble extractive value	7.70
7. Ph Value	
A. 1% Aqueous Solution	5.30
B. 10% Aqueous Solution	4.90

Table 5. Successive Extractive Values of the Stem Bark of *Balanites aegyptiaca*

Serial Number	Solvent	Weight of Drug	Average Extractive value (%w/w)
1.	Benzene	5 gm	3.01
2.	Petrolium ether	5 gm	3.60
3.	Chloroform	5 gm	1.60
4.	Methanol 95%	5 gm	5.45
5.	Chloroform Water	5gm	4.80

Table 6. Observation of Quantitative analysis of organic matter of Balanites aegyptiaca Stem bark :-

*+ present, - absent,

S.N	Test for Organic matter	Hexane	Pet.Ether	Benzene	chloroform	Acetone	Methanol	Water
1.	**Carbohydrate**	+	+	-	+	+	+	+
2.	**Tannin**	+	+	+	_	+	+	_
3.	**Alkaloid**	+	_	+	+	+	+	+
4.	**Saponin**	_	+	_	+	+	_	+
5.	**Sterols**	+	_	+	+	_	+	+
6.	**Proteins**	+	+	+	+	_	+	+
7.	**Flavonoids**	+	_	_	_	+	+	+
8.	**Resins**	_	_	_	_	_	_	_
9.	**Glycosides**	+	+	_	+	+	+	+

Figure 1- Ingudi (Balanites aegyptiaca Linn.Delile)

Figure 2- Outer Surface of Stem Bark

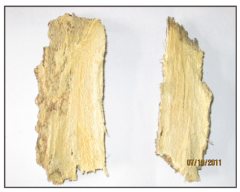

Figure 3- Inner Surface of Stem Bark

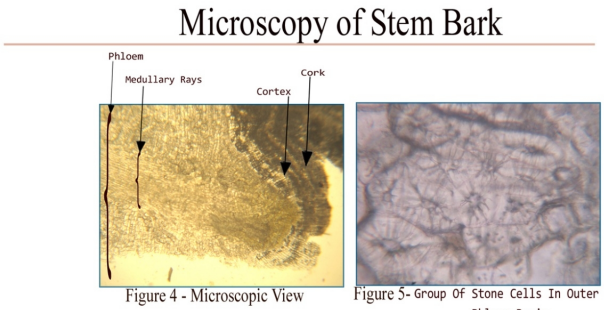

Figure 4 - Microscopic View

Figure 5- Group Of Stone Cells In Outer Phloem Regin

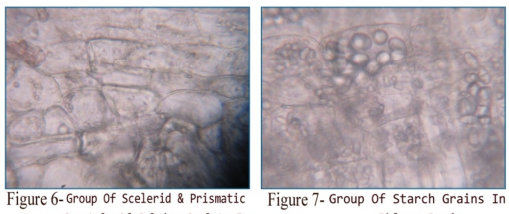

Figure 6- Group Of Scelerid & Prismatic Crystals Of Calcium Oxalate In Phloem Regin

Figure 7- Group Of Starch Grains In Phloem Regin

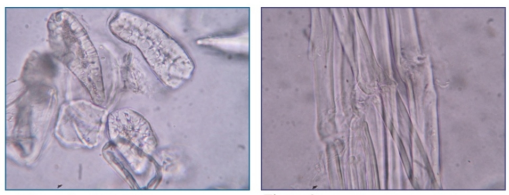

Figure 8 - Sclerids

Figure 9 - Reticulate Fibers In Powder Microscopy

Powder Microscopy of Stem Bark

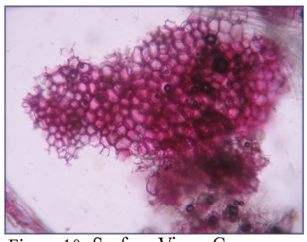

Figure 10 -Surface View -Group of Cork Cells

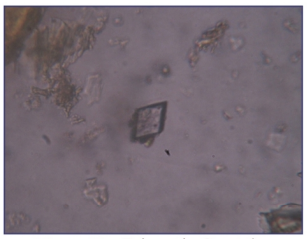

Figure 11 - Prismatic Crystals of Calcium Oxalate

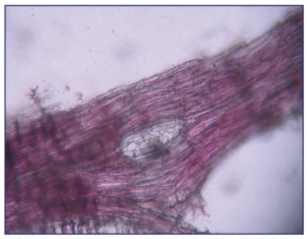

Figure 12 -Tangentially Cut Medullary Rays Adjecent To Fibers

Figure 13 - Cork In Transverse Section View

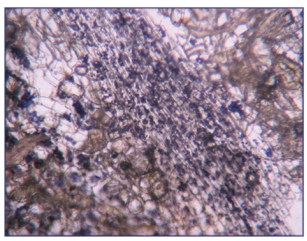

Figure 14 -Starch Grains In Medullary Rays

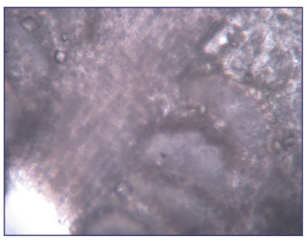

Figure 15 - Group of Fiber At Inner Phloem Region Adjecent to Medullary Rays

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