

***PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY OF INGUDI (BALANITES AEGYPTIACA LINN. DELILE) SEEDS***

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**ABSTRACT**

Ayurveda, the most ancient science has served the humanity since the beginning of life on the earth, whether it is related to lifestyle, ailments or general wellbeing. This ancient science of life helps in maintaining the health by use of herbs. *Ingudi (Balanitesaegyptiaca*Linn.Delile) commonly known as *Hingot* in Hindi, is commonly described in the Ayurvedic texts. This herb is well known for its wound healing property. The drug is found all over India especially in Marupradesh (arid, semi-arid, to sub humid), tropical and hot dry area. The drug possesses Hepato-protective, Cardio-protective, antioxidant, anti-diabetic, anthelmintic, anti-bacterial, anti-inflammatory and analgesic activity. Saponin extract and water extract from fruit kernel of *B. aegyptiacawas* investigated as a mosquito larvicide. Seeds of *B. aegyptiaca* have potent wound-healing activity, as evident from the wound contraction. So the correct identification of this plant is quite necessary. A gross study with its microscopic; external and internal morphological features is merely possible by the study of Pharmacognosy. Transverse section of the seed kernel shows outer 4 to 5 layers of thick walled tegmen made up of sclereids. The sample was subjected to qualitative examination to find out the presence of micro-nutrients &heavy metal constituents. It was found that calcium, iron, phosphorus and sulphur, Manganese, Potassium were absent in the sample. However there is absence of heavy metals in plant which is suggestive of their safety profile. Qualitative analysis reveals presence of certain chemicals like alkaloid, protein, tannins, Glycosides, phenol etc. in seed oil sample. Amongst these phytochemicals, glycosides may be helpful in wound healing action of the drug.

**Keywords:** *Ingudi*, *Balanitesaegyptiaca* Linn. Delile, Pharmacognosy, Phytochemicals

## INTRODUCTION

*Ingudi* has been used in indigenous system of medicine since long time. The authentic source of drug is *Balanitesaegyptiaca* Linn. Delile which belongs to family *Balanitaceae*.<sup>[1]</sup> *Balanitesaegyptiaca* Linn. Delile is a spiny tree with bifoliolate leaves, greyish bark and woody, solitary seeds.<sup>[2]</sup> The tree is commonly found in drier parts of Peninsular India, Rajasthan and from South East Punjab to West Bengal and Sikkim. It is distributed in drier parts of Arabia, Pakistan, Egypt, Tropical Africa and Burma. After the seedling stage, it is intolerant to shade and prefers open woodland for natural regeneration. It is a lowland species, growing up to 1000 m altitude in areas with mean annual temperature of 20 to 30°C and mean annual rainfall of 250 to 400 mm.<sup>[3]</sup> Fruits possess Balanitisines A, B, C, D and E while seeds contain Balanitisine. Leaves of *Balanitesaegyptiaca* Linn. Delile contain nitrogen and diosgenin.<sup>[4]</sup> The kernels also contain 45.0 to 46.1% oil and protein (32.4%), oil contains mainly palmitic, stearic, oleic, and linoleic acids which are the main fatty acids. The oil exhibited anticancer activity against lung, liver, and brain human carcinoma cell lines.<sup>[5-7]</sup> The unripe fruits, seeds and bark are anthelmintic and purgative. The fruit pulp is useful in whooping cough, intrinsic haemorrhage and pulp mixed with goat milk is rubbed on chest to cure pneumonia. It is also used in rat bite.<sup>[8]</sup> The boiled root of the plants can be used as a soup against stomach pain, anthrax. The infusion of root bark also acts as an antidote to snake bites and used in diarrhea. The

infusion of root bark acts as a fish poison. The paste of root bark is used in Herpes zoster.<sup>[9]</sup> Saponin extract and water extract from fruit kernel of *B. aegyptiaca* was investigated as a mosquito larvicide. Both extracts were tested against second and fourth instar larvae of the three mosquito species namely *Anopheles arabiensis*, *Culexquinquefasciatus*, and *A. aegypti*. The saponin was found more active than the water extract. The plant possesses many biological activities such as antimicrobial, antioxidant, antidiabetic, antiasthmatic, etc. They were found to be toxic to pests, molluscs and larvae. Even though they are said to be toxic, their minimum level of consumption do not cause any serious effects.<sup>[10,11]</sup> As the seeds of the plant are highly effective in curing different disorders, there is a need for the proper standardization of the drug. This study has been taken to investigate the organoleptic characters, microscopic study, physicochemical, phytochemical analysis, heavy metal determination and chromatographic study of *Ingudi* seeds.

## MATERIALS AND METHODS:

### Seeds collection and Authentication:

Specimen of *B.aegyptiaca* Linn. Delile along with fresh fruits was collected from Ramgad area, Jaipur in the month of March. The Botanical Authentication was carried out by Botany Department of Rajasthan University Jaipur (Rajasthan), Approval No.RUBL211356. Seeds were then taken out from the fruit and kept for further study.

### **Pharmacognostical Studies:**

#### **Macroscopic evaluation or Organoleptic Study:**

Collected and authenticated seeds of *Balanites aegyptiaca* were dried and various organoleptic characters viz. colour, odour, taste, texture and shape were studied. Oil extracted from seeds of Ingudi was also observed for colour, consistency, odour and taste.<sup>[12-15]</sup>

#### **Microscopic evaluation:**

Transverse section of kernel of seed was done and findings were recorded.

#### **Phytochemical Evaluation:**

Some common parameters which are normally used for the analysis of oils are specific gravity, refractive index, acid value, saponification value and iodine value. Hence the Ingudi oil sample was analysed for these parameters, by employing routine procedure.

#### **Qualitative examination of inorganic matters:**

5ml of sample was taken in a beaker and mixed with 50ml petroleum ether for proper dilution and different chemicals are used for detecting the presence of mineral elements like Calcium, Iron, Manganese, Potassium and Sulphur.

#### **Qualitative examination of organic matters:**

Sample was tested for the presence of various active phyto-compounds like phenols, tannin, flavonoid, protein, reducing sugar, carbohydrates, lipids, saponin, triterpenoid alkaloid, resins, volatile oils, anthraquinone and Quinine.

### **Determination of heavy metals:**

5ml of sample was taken in a beaker and treated with different chemicals for detecting the presence of different Heavy metals like Cobalt, Copper, Mercury Compounds, Nickel compounds, Barium compounds, Lead and Arsenic.

#### **Chromatographic study:**

Thin layer chromatography (TLC) was performed in which seed oil of *Ingudi* is used as prepared sample. Solvent system was prepared by taking Chloroform: n-hexane: Toluene: Ethyl acetate: Glacial acetic acid in a ratio of 4: 1.3: 1: 0.9: 0.1. The spots obtained from both the extracts were examined under on exposure to Iodine vapours and Vanillin Sulphuric acid reagent.<sup>[16]</sup>

### **RESULTS:**

#### **Macroscopic Study**

The seeds of *Ingudi* are very hard and strong, oval to elliptic in shape, thick at the middle, narrow and pointed towards both the base and apex. They are 1.5 to 3.5 cm in length and 1 to 2 cm in broadness; light brown, fibrous and extremely hard. The seed is endospermic and on breaking the hard testa, the kernel cannot be separated easily. The kernel of the seed is in the size of 1 to 2.5 cm long and 0.6 to 1.5 cm thick oval to elliptic in shape and light yellowish in colour. Externally 7 to 10 dichotomously branched and somewhat broad and distinct lines are also running from the base to the apex of the kernel. The kernel is the actual useful part of the seed. The odour of the kernel is indistinct and the taste is bitter. Organoleptic characters of seeds and oil are given in table 1 and figure 1.

**Table 1:** Organoleptic characters of seeds and oil of *Balanitesaegyptica*:

Organoleptic observations of seeds		
1	<i>Sparsha</i>	Rough
2	<i>Rupa</i>	Convexwithasuperiorradical.
3	<i>Rasa</i>	Bitter
4	<i>Gandha</i>	Characteristic
Organoleptic characters of Oil		
1	Coarse powder of seeds	2 kg
2	Oil obtained from seeds	600ml
3	Colour	Paleyellow
4	Consistency	Liquid
5	Nature	Non-sticky and oily
6	Odour	Odourless
7	Taste	Pungent and Bitter in Taste
8	Solubility in Water	Insoluble

### Microscopic Study:

#### Microscopic character of Testa and Kernel

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The outer seed coat or testa is very hard, stony and shell like and is made up of highly lignified stone cells. The stones cells are thick walled, with very narrow lumen. The testa of the seed is not useful in medicine. Inside of the testa there is the kernel of the seed. The kernel is about 1cm to 2.5 cm long and 0.6 cm to 1.25 cm thick, obovate to elliptic in shape externally light yellowish in colour with narrow longitudinal impression. T.S. of the kernel shows outer 4 to 5 layers of thick walled Tegmen made up sclereids. Just below there are few layers of thin walled compressed parenchymatous cells containing pigments. Inside of this there are several layers of compressed cells with light brownish colour, containing plenty of oil globules and rosette crystals of calcium oxalate. The inner most region contain an embryo with two thin cotyledons and a small radicle. The embryo is made up of thin walled closely arranged parenchyma and the

cotyledons are protected by a unit layered epidermis. Transverse section of *Ingudi* seeds is shown in Figure 2.

#### Phytochemical Evaluation

The sample (*Ingudi* oil) taken for the present study, was analysed chemically for various parameters, which are commonly employed for the analysis of oil samples. The analytical data has been presented in Table 2.

**Table 2:** Phytochemical analysis of *Ingudi*Oil:

PARAMETER	VALUES
1. Boiling point	195.66 <sup>0</sup> c
1. Specific gravity	0.88
2. Refractive index	1.422
3. Viscosity	0.4889
4. Acid value	3.11
5. Saponification value	354.19
6. Iodine value	13.808
7. Total fatty matter	86.51

#### Qualitative examination of inorganic matters:

Qualitative analysis of inorganic matters is presented in Table 3.

**Table 3:** Observation of Qualitative analysis of inorganic matter:

S. No.	Minerals	Status of Presence
1.	Calcium	-
2.	Iron	-
3.	Sulphur	-
4.	Manganese	-
5.	Potassium	-

**Qualitative examination of organic matters:**

Qualitative analysis of organic matters is presented in Table 4.

**Table 4:** Observation of Qualitative analysis of organic matter:

S.No	Test/Method	Status of Presence
1.	Carbohydrate	+
2.	Alkaloid	+
3.	Proteins	+
4.	Tannin	+
5.	Glycosides	+
6.	Saponin	-
7.	Phenol	+

**Table 6:** Rf. Values of sample

S.No	Samples	On Exposure to Iodine Vapours		Vanillin sulphuric acid reagent	
		Spots	Rf value	Spots	Rf value
01	Seed oil	5	Rf. 0.09, 0.45, 0.50, 0.54, 0.96	5	Rf. 0.09, 0.45, 0.50, 0.54, 0.96

**DISCUSSION**

Standardization is the need of the hour to establish the identity, purity and safety of Ayurveda drugs. Macroscopic, microscopic, physico-chemical, phytochemical, heavy metal analysis and chromatographic evaluation of *Ingudi* seeds has been carried out in this study. As the phytochemical analysis of the *IngudiTaila* could not be compared due to lack of comparative data in the Ayurvedic Pharmacopoeia of

**Determination of heavy metals:** Heavy metal analysis of the sample is shown in Table 5.

**Table 5:** Qualitative Analysis of Heavy metal:

S.No.	Heavy Metals	Status of Presence
1.	Cobalt	-
2.	Copper	-
3.	Mercury	-
4.	Nickel	-
5.	Barium	-
6.	Lead	-
7.	Arsenic	-

**Chromatographic study:**

- Mobile Phase: Chloroform: n-hexane: Toluene: Ethyl acetate: Glacial acetic acid (4: 1.3: 1: • 9: • 1)
- Stationary Phase: Thin layer chromatographic plates (Silica gel, 60 F254) Merck, Germany
- Distance Travelled: 5.5

Rf values of seed oil on exposure to Iodine and Vanillin sulphuric acid reagent are shown in Table 6.

India but obtained data from the various test like Refractive index, Specific gravity, Acid value, Iodine value and Saponification value of *IngudiTaila* would be helpful to analyse and to standardize the drug for the next study. The sample was subjected to qualitative examination to find out the presence of micro-nutrients & heavy metal constituents. It was found that calcium, iron, phosphorus & Sulphur, Manganese, Potassium were absent in the sample.

However there is absence of heavy metals in plant which is suggestive of their safety profile. Qualitative analysis reveals presence of certain chemicals like alkaloid, protein, tannins, Glycosides, phenol etc. in seed oil sample. Amongst these tannins, glycosides may be helpful in wound healing action of the drug. Tannins may provide benefit by reducing colonisation of *S. aureus* with better quality of healing and at the same time not increasing toxicity. Tannins are known as anti-oxidants and blood purifiers with anti-inflammatory actions. As the oxidation process hampers the wound healing, antioxidants protect the tissue from the oxidative damage. Since antioxidants are recorded to be helpful to control oxidative damage but it may not be claimed that they prevent it, but they might be considered up to some extent of assistance. Glycosides accelerate the healing process and reduce the scarring as the mechanism of action proposed which here involves the regeneration of skin through stimulation of stem cells that allow healing without substantial scar formation.

## CONCLUSION

*Ingudi* (*Balanitesaegyptiaca*Linn.Delile) commonly known as *Hingot* in Hindi, is commonly described in the Ayurvedic texts. Seeds of *Balanitesaegyptica* are well known for their wound healing property. In this paper, more emphasis was given on the pharmacognostical and phytochemical study so as to achieve lead molecules in search of novel herbal drug. This Preliminary phytochemical study of *Balanitesaegyptica* revealed certain chemicals like alkaloid, protein, tannins, Glycosides, phenol etc. in seed oil sample. Among these, tannins and

glycosides are highly responsible for the wound healing activity of the seeds. Further, experimental and clinical studies must be done to make the drug a standard compound for wound healing.

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**Figure 1:** Photograph of *Ingudi* (Tree with unripe –ripe fruit, seed, seed oil)



**Unripe fruit of Ingudi**



**Ripe fruit of Ingudi**



**Single fruit of Ingudi**



**Fruit with seed**

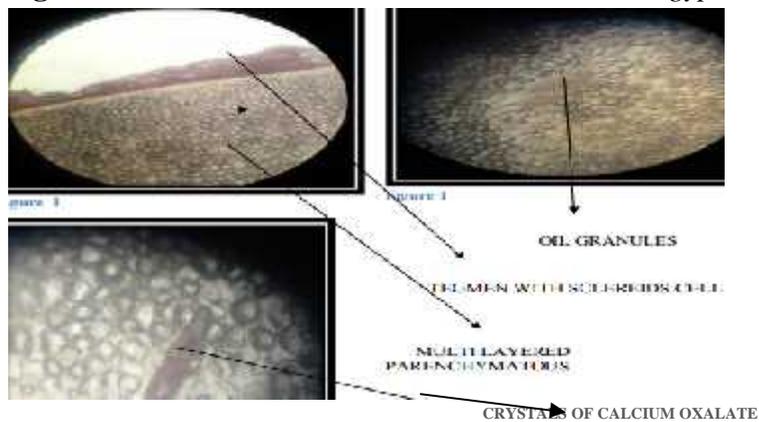


**Seeds of Ingudi**



**Ingudi seed oil**

**Figure 2: Transverse Section of Seed of *Balanites aegyptica***



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