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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *HARITAKI* (*TERMINALIACHEBULA* RETZ.) FRUIT PULP

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ABSTRACT

Medicinal plants are natural source of medicines, now the world is moving towards the plant based medicines or phytomedicines that strengthening the body system. The plant is found in all over the India especially in deciduous forest and in areas of light rainfall. Among the medicinal plants Haritaki is the most useful plant in Ayurvedic system of medicines. The fruit pulp of Haritaki have high medicinal value. The extract of Haritaki fruit pulp contains substances which has highly medicinal values and used in the treatment of many diseases like fungal infections, leucorrhoea, chronic ulcer, pyorrhea, inhibit bacterial growth etc. The fruit pulp of Haritaki has high medicinal value. The present study deals with the pharmacognostical and preliminary phytochemical studies on the fruit pulp of Haritaki (*Terminalia chebula* Retz.). Pharmacognostical parameters for the fruit pulp were studied with the aim of drawing the pharmacopoeial standards for this species. Macroscopical and Microscopical Characters, physico-chemical constants, quantitative microscopy parameters, extractive values with different solvents, fluorescence analysis of dry powder and its reaction after treatment with chemical reagents. The determination of these characters will help future researchers in their Phytochemical as well as Pharmacological analyses of this species.

Keywords: Haritaki, Ayurveda, Pharmacognostical and Phytochemicals.

INTRODUCTION

Since ages, plants have remained important sources of medicines in our country, which is evidenced through their uses in traditional system of medicine i.e. Ayurveda, Siddha, Unani, Homeopathy and Chinese. The earliest documentation about the usage of plant remedies comes from India as evident from Rigaveda (4000-6000 BC), where Aushadhisukta includes a good number of plants for various ailments. Realizing the importance of medicinal plants as a natural source of newer medicines, now the world is moving towards the plant based medicine or phytomedicines that repair and strengthens body systems (especially the immune system, which can then properly fight foreign invaders) and help to destroy offending pathogens without toxic side effects.[1] The major limitation of modern medicine, being its adverse effects has opened new doors to Ayurveda, where physicians of the traditional systems of medicine are using plants from many years. In developed countries too, people are seeking alternative to modern therapies. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety and efficacy. Keeping this fact in to consideration, the attempts were made to establish physicochemical standards of the plant Haritaki (*Terminaliachebula* Retz.).[2]Haritaki is widely used medicinal plant in Ayurvedic medicine. Haritaki is commonly used in traditional system of medicine due to their wide spectrum of pharmacological activities.[3] The name of Haritaki in Sanskrit is yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and it cures (harayet) all the diseases. Haritaki is a tree which is found in deciduous forest and areas of light rain fall throughout India. Flowers appeared in April to August and fruit ripened in October to January, fruit is drupe like (2-4.5 cm long and 1.2-2.5 cm broad, blackish with longitudinal ridges.[4]

Taxonomical Description[4]

Botanical Name : *Terminaliachebula*Retz.
Kingdom : Plantae
Division : Phanerogams
Class : Monocotyledons
Order : Scytaminiales
Family : Combretaceae
Genus : Terminalia
Species : chebula

Vernacular Names

English – Chebulikmyrobalan, Hindi –Harara, Harad, Gujrat – Hardo, Punjab – Har, Halela, Hurh, Harrar, Tamil – Katukkay, Arab – Halilaj, Assam – Silikha, Hilikha, Urdu – Haejarad.

Classical synonyms

Haritaki, Abhaya, Pathya, Kayastha, Putana, Haimavati, Avyatha, chetaki, Putana, Shiva, Vayastha, Rohini.

Pharmacological properties of Haritaki[5]

The chemical constituents present in Haritaki is the key source of several pharmacological investigations in vivo and in vito reported in table no 1 in summarized form.

Table 1 : Pharmacological properties of haritaki

S.No	Pharmacological activity	Extract type	Organism
1.	Antibacterial	Ethanol extract	Salmonella typhi, Staphylococcus aureus, Bacillus subtilis etc. Helicobacter pylori
2.	Anticancer	Methanol	Human (MCF-7), mouse (S115) breast cancer cell lines
3.	Anticaries	Aqueous	Streptococcus mutans
4.	Anticonvulsnt	Ethanolic, chloroform, petroleum ether, aqueous	Rats
5.	Antidiabetic	Ethanol extract	Rats
6.	Antifungal	Aqueous, alcoholic, ethyl acetate	Aspergillusniger, Aspergillusflavus, Alternariaaltmat
7.	Antimutagenic	Chloroform, aqueous	Salmonella typhimurium
8.	Antioxidant	Ethanolic	Wistar albino male rats
9.	Antiulcer	Methanolic	Wistar albino male rats
10.	Antiviral	Acetone extract	Swine influenza A virus
11.	Cardioprotective	Ethanolic	Adult albino male rats
12.	Cytotoxic	Aacetone extract	Male Wistar rats
13.	Immunomodulatory	Alcoholic	Rats
14.	Radioprotective	Aqueous	Rats
15.	Wound healing	Hydroalcoholic	Diabetic rats

MATERIALS AND METHODS

SAMPLE COLLECTION AND AUTHENTICATION

Drug was collected from the vicinity of Varanasi (MohanlalRajnish shop, Goladinanath, Varanasi). And authenticated by Prof. K.N. Dwivedi, Professor and Dr. B. Ram, Associate professor, department of Dravyaguna, faculty of Ayurveda, IMS, BHU. Sample of collected raw drug were kept in the museum of the department of Dravyaguna, faculty of Ayurveda, IMS, BHU, with specimen accession number DG/17/135. Macroscopic and microscopic evaluation was carried out with Fruit pulp. Fruit pulp 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 PROPERTIES OF FRUIT PULP[6]

5 gm coarse powder of sample was taken in a petri dish and examined for organoleptic properties like shape, size, smell, taste, texture etc.

MICROSCOPIC STUDY OF FRUIT PULP[7]

5 gm powder of fruit pericarp of harÍtakÍ was boiled with chloral hydrate solution in small quantity. Cleaved powder was removed in three separate watch glasses respectively and stained with phloroglucinol, dil sulphuric acid, sudan red III, alcoholic picric acid and iodine to find

out the presence of lignified tissues, calcium oxalate crystal, oil globules, and starch etc.

Total ash value
About 2gm of powdered drug was weighed accurately into a silica crucible and incinerated at 450^{0}C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air-dried substance.

Acid in-soluble ash value
Ash obtained from total ash was boiled with 25 ml of 2N HCl for few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 450^{0}C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance.

Water soluble ash
Ash obtained from total ash was boiled with 25 ml of distilled water for few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 450^{0}C in until free from carbon. The crucible was cooled and weighed. Percentage of water-soluble ash was calculated with reference to air-dried substance.

Water soluble extractive value
5g of the crude powder was taken into a conical flask and 100 ml of water was added to it. This mixture was stirred gently and warmed in a water bath for 30 minutes. The solution was shaken gently at intervals. Then the solution was taken from the water bath and cooled and filtered through a cotton plug, 25 ml of the filtrate was taken and evaporated to dryness. The residue was weighed.

Loss on drying
The percentages of active chemical constituents in crude drugs are given in terms of air-dried drugs. Hence the moisture content of drug was determined. 2 gm of powdered drug was transferred into a petridish and the contents were distributed evenly to a depth not exceeding 10 mm. The loaded petridish was heated at 105^{0}C in hot air oven and weighed at different time intervals until a constant weight was obtained. The difference in weight after drying and initial weight is the moisture content. Respective moisture content (%) for both the samples was calculated.

PRELIMINARY SCREENING OF PHYTOCHEMICALS[5]
The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them.

Test for Alkaloids

- **Dragendorff'stest**
  Dissolve a few mg of hydro-alcoholic until an acid reaction occurs, then add 1 ml of Dragendorff's reagent, an orange or orange-red precipitate is produced immediately.
- **Hager's test**
  1 ml of Hydroalcoholic extract of the drug was taken in a test tube, adding a few drops of Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids.
- **Wagner's test**
  Acidifying 1 ml of hydro-alcoholic extract of the drug with 1.5% w/v of hydrochloric acid and adding a few drops of Wagner's reagent. A yellow or brown precipitate is formed.
- **Mayer's test**
  Adding a few drops of Mayer's reagent to 1 ml of hydro-alcoholic extract of the drug. White or pale yellow precipitate is formed.

Carbohydrates

- **Anthrone test**
  Take 2 ml of Anthrone test solution, adding 0.5 ml of hydro-alcoholic extract of the drug. A green or blue colour indicates the presence of carbohydrates.
- **Benedict's test**
  Take 0.5 ml of hydro-alcoholic extract of the drug adding 5 ml of Benedict's solution and boiling for 5 minutes. Formation of a brick red coloured precipitate is due to the presence of carbohydrates.

- **Fehling's test**
  Take 2 ml of hydro-alcoholic extract of the drug adding 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boiling the contents of the test tube for few

minutes. A red or brick red precipitate is formed.

- **Molisch's test**
  In a test tube containing 2 ml of hydro-alcoholic extract of the drug adding 2 drops of a freshly prepared 20% alcoholic solution of β- naphthol and mix, pouring 2 ml conc. sulphuric acid so as to from a layer below the mixture. Carbohydrates, if present, produce a red-violet ring, which disappears on the addition of an excess of alkali solution.

Flavonoids

- **Shinoda's test**
  In a test tube containing 0.5 ml of hydro-alcoholic extract of the drug, adding 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

Proteins

- **Biuret's test**
  To 1 ml of hot hydro-alcoholic of the drug adding 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.
- **Millon's test**
  Dissolving a small quantity of hydro-alcoholic of the drug in 1 ml of distilled water and adding 5-6 drops of Millon's reagent. A white precipitate is formed which turns red on heating.

Saponins

In a test tube containing about 5 ml of a hydro-alcoholic of the drug adding a drop of sodium bicarbonate solution, shaking the mixture vigorously and leave for 3 minutes. Honeycomb like froth is formed.

Steroids

- **Liebermann-Burchard's test**
  Adding 2 ml of acetic anhydride solution to 1 ml of hydro-alcoholic extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish colour is developed which turns to blue.
- **Salkowski Reaction**
  Adding 1 ml of conc. sulphuric acid to 2 ml of hydro-alcoholic of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer.

Tannins

To 1–2 ml of plant hydro-alcoholic extract, adding a few drops of 5% $FeCl_3$ solution was added. A green colour indicates the presence of gallotannins while brown colour tannins.

Glycosides

Detection of glycoside on paper spray solution No. 1 (0.5 % aqueous sol. of Sodium metaperiodate) & waiting for 10 minutes after then spraying solution No. 2 [0.5% Benzidine (w/v) in solution of Ethanol–acetic Acid (4:1)], white spot with blue back ground shows presence of glycoside.

RESULTS

Organoleptic properties

The physical properties of haritaki was observed and reported below (Table no 2)(Fig no 1&2). Yellowish fruit is brown, ovoid and 20-35 mm long, 10-25 mm wide, wrinkled and ribbed. Astringent taste and pericarp is 3-5 mm thick and fibrous, non-adherent to the seed. Powder is brownish in color and shows a few fibres, vessels with simple pits and groups of sclereids.

Table 2: Organoleptic properties crude drug powder

S.No.	Parameters	Observation
I	**Physical tests**	
	Nature	Coarse powder
	Colour	Yellowish
	Odour	Characteristic
	Taste	Bitter
II	**Foreign matter**	Nil
III	**Fluorescence**	Dark blue

Fig. 1: Dry crude fruits of haritaki

Fig. 2 : Powder of fruit pulp

Physico-chemical analysis of fruit pulp

The physic-chemical analysis of haritaki fruit pulp was performed. And results are reported below (table no 3&4).

Table 3 : Physico-chemical analysis of fruit pulp

S.No.	Reagents	Observations	Characteristics
1.	Phlorogucinol + con. HCL	Pink	Lignified tissues and vascular bundles
2.	Alcoholic Picric acid	Yellow	Aleurone grains
3.	Sudan Red III	Red	Oil globules and cuticles.

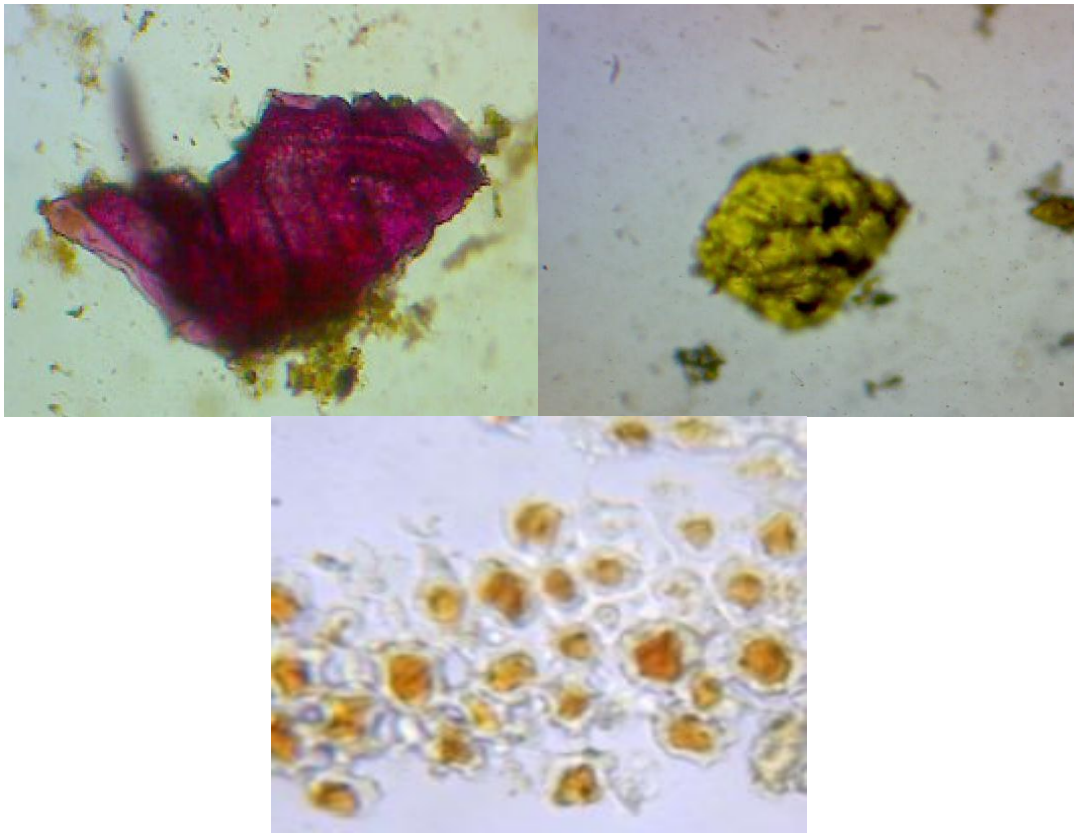

Fig. 3: Lignified Tissues
Fig. 4: Aleurone grains
Fig. 5 : Oil globules

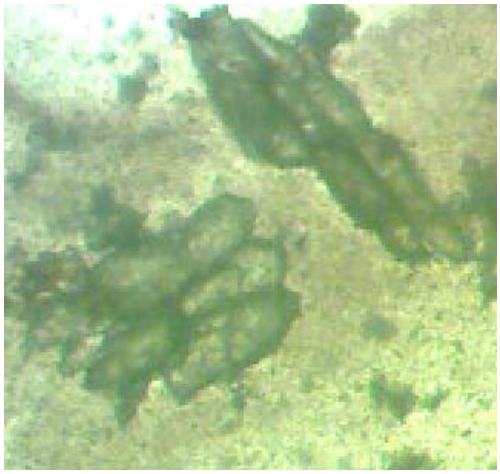

Fig. 6: Hemicellulose-Endospermic wall

Table 4 : Physico-chemical analysis of fruit pulp

S.NO.	Observed value in percent		Reported value
1.	Total ash	5.9 %	Not more than 5.5 %
2.	Acid insoluble ash	0.40 %	Not more than 0.5 %
3.	Water soluble ash	42.8 %	Not less than 40 %
4.	Water soluble extractive value	60.0 %	Not less than 56 %
5	Alcohol soluble extractive value	49.22 %	Not less than 40 %
6.	Loss on drying	10.0 %	Not less than 9.0 % at 105^{o}C

Phytochemical screening

The phytochemical analysis of fruit pulp extract was performed and found that protein, amino acid, glycosides, flavonoids, vitamins, alkaloids, tannin and phenolic compounds are present while carbohydrates, and steroids are absent (table no 5).

Table 5 : Phytochemical analysis of fruit pulp extract

S.No.	Chemical tests	Observations
1.	Carbohydrates	-
2.	Protiens	+
3.	Amino acids	+
4.	Glycosides	+
5.	Flavanoids	+
6.	Alkaloids	+
7.	Tannins and Phenolic Compounds	+
8.	Steroids	-
9.	Vitamins	+

DISCUSSION

'Pharmacognosy' is meant by identification of drugs by its every aspect, habit, cultivation, procurement, micro and macroscopic characters, physical and chemical properties etc. In present study pharmacognostical standards have been established with regards to seed of Ingudi (BalanitesaegyptiacaLinn.Delile, Local name Hingot). Powder microscopy of seeds showed the presence of trichomes and endosperms containing polygonal cellulosic cells (Figure 5) and xylem containing Lignified cells (Figure 3) with oil globules, and aleurones grains, Calcium oxalate crystals. 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 shows the presence of Saponin glycosides, Volatile oils, Proteins, Amino Acid, Fat & Oils, Steroids, Flavanoids, Tannins and Phenolic Compounds in the seeds of Balanitesaegyptiaca. Study was carried out in order to assess the quality of seeds of Balanitesaegyptiaca Linn. Delile and also to detect the adulteration and substitution etc., which may be helpful to researchers in future.

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