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Pharmacognostic and Phytochemical Studies of Leaf Gall of *Terminalia chebula* Retz. Used as Karkatashringi in South Indian Markets

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ABSTRACT

Karkatashringi is an important crude drug employed in various indigenous systems of medicine against several diseases, and the drug has diverse medicinal properties. The present study provides a detailed account of the pharmacognostic investigation carried out on leaf galls of *Terminalia chebula* used as karkatashringi. The study includes macro- and micromorphological characters of gall, fluorescence study of powder, physicochemical studies and preliminary phytochemical screening. In physicochemical studies the moisture, total ash, acid insoluble ash, alcohol soluble, water soluble, petroleum ether extractive and tannin content was found to be 10.06, 6.18, 1.13, 27.36, 31.01, 1.33 and 17.4 percentage respectively. The results of the study help to establish the authenticity of the drug, differentiate the drug from other species and drawing the pharmacopoeial standards for this species.

Keywords: crude drug, microscopical studies, pharmacognosy, pharmacopoeia, physicochemical analysis, substitute

INTRODUCTION

Terminalia chebula Retz. (Combretaceae), commonly known as black myrobalan and haritaki, is an important plant used in indigenous systems of medicine as remedy for fever, cough, diarrhoea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections (Kirtikar and Basu 1987; Anonymous 1999). *T. chebula* has been reported to possess anti-oxidant (Cheng *et al*. 2003), anti-diabetic (Sabu *et al*. 2002), anti-cancer (Saleem *et al*. 2002), anti-mutagenic (Kaur *et al*. 2002) anti-viral (Ahn *et al*. 2002) anti-bacterial (Kim *et al*. 2006; Chattopadhyay *et al*. 2007; Bag *et al*. 2009) and radioprotective (Gandhi and Nayar 2005) activity.

The leaf galls of *T. chebula* are used as a substitute for Karkatshringi, which is widely used in Ayurvedic and other traditional systems of medication. Karkatshringi is the main component of Karkatadi churna, Balabhadra churna, Sringadi churna which are used in treatment of asthma, tuberculosis, indigestion, heart diseases, fevers and liver disorders. Similarly, in the Siddha system of medication, they are used in the preparation of Karisalai lehyam, Venpocesunai nei, Gana thailum for treating diseases like cough, bronchial asthma, diarrhea, dysentery (Shantha *et al*. 1991).

The main hindrance in its use is the non availability of an official monograph and incomplete validation of the galls of *T. chebula*. This encourages traders to adulterate authentic sample with inferior plant products. The process of standardization is achieved by step wise pharmacognostic studies (Ozarkar 2005) which in turn helps us to identify and authenticate the plant material.

In view of its diverse medicinal applications and in order to ensure the quality of its supply, especially at a time in which adulteration and substitution prevail on the crude drug markets of India, the present communication deals with a detailed pharmacognostic evaluation of leaf galls of *T. chebula*.

MATERIALS AND METHODS

T. chebula leaf galls were collected from a local market of Bangalore, Karnataka (Southern India) in May 2010 and authenticated by Dr. S. Sundara Rajan and voucher specimens (JU-RUV-52) were deposited at the Research Unit in Vrkshayurveda, Jain University, Bangalore.

In macroscopical studies the following characters were noted: shape, size, surface, colour, odour, taste, fracture, nature of powder as per standard method of Mukherjee (2002) and Johanson (1940).

The selected samples were stored in a solution containing formalin (5 ml), acetic acid (5 ml) and 70% v/v ethyl alcohol (FAA) (90 ml). After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per Sass (1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (50-60°C m.p.) until tertiary-butyl alcohol solution attained supersaturation. The specimens were cast into paraffin blocks. The paraffin-embedded specimens were sectioned with the help of Senior Rotary Microtome, RMT-30 (Radical Instruments, India). The thickness of the sections was kept between 10 and 12 µm. The dewaxing of the sections was carried out as per the procedure described by Johanson (1940). The section was stained, mounted and observed under a compound microscope.

The colour changes of the powdered sample with respect to different chemical reagents on the basis of different chemical constituents was observed in daylight and ultraviolet light as per the methods described by Chase and Pratt (1949) and Kokoshi *et al*. (1958).

The percentage of physicochemical values viz., moisture content, total ash, acid insoluble ash, Petroleum ether and water-alcohol-soluble extractives were calculated according to the methods described in the Indian Pharmacoepia (Anonymous 1966). The percentage of tannin was also determined using a Jenway-6035 spectrophotometer (Anonymous 1984).

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Research Note

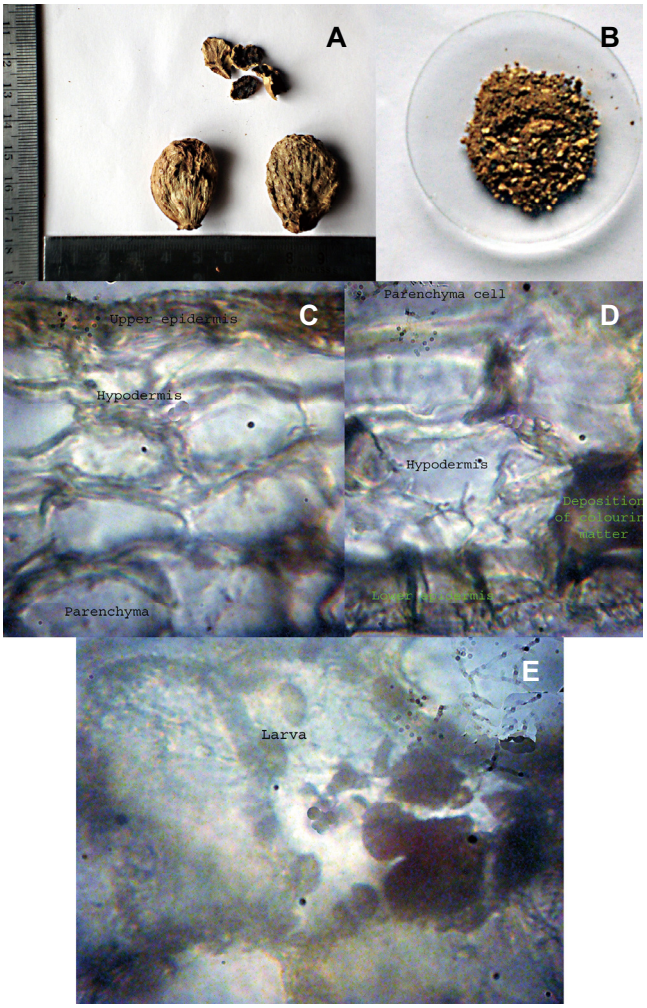

Fig. 1 ***Terminalia chebula*****.** (**A**) Leaf gall; (**B**) leaf gall powder; (**C, D**) cells of the epidermis; (**E**) larva in parenchyma cells.

The phytochemical analysis of petroleum ether, chloroform, ethanol and water extracts was carried out using the methods as described in Harborne (1984), Trease and Evans (1989), Kokate *et al.* (1998), Khandelwal (2005).

RESULTS AND DISCUSSION

Karkatashringi is currently being used in the treatment of various disease conditions and its original constituent is widely substituted by *Pistacia integerrima*, *Rhus succedanea*, *Garuga pinnata* and *Quercus infectoria*. So the standardization of a crude drug is an integral part in establishing correct identity of the plant material. The results of these investigations could therefore, serve as a basis for proper identification, collections and investigations of the plant material.

Macroscopically, the gall (**Fig. 1A**) appears to be ovate to obovate, laterally compressed, flattened, with divergent longitudinal striations from base measuring 2.5-3.5 cm in length and 1.5-2.2 cm broad, outer surface is rough with divergent longitudinal striations interrupted with small nodes on surface. The galls are grey to grayish-yellow externally and buff to blackish-brown internally. The fracture are short granular and brittle in nature. While the powder (**Fig. 1B**) is mixture of coarse and fine particals, Yellowish-brown in colour, feels like rough to smooth with characteristic odour and astringent taste.

In microscopy the outline of transverse section reveals the adhered vascular cells at places. The detailed section shows upper and lower epidermis followed by hypodermis (**Fig. 1C, 1D**). The upper epidermal cells are comparatively larger in size while lower cells are more compact. Deposition of dark brown coloring matter is more concentrated to lower side. Hypodermis in upper zone is followed by large zone parenchyma, outer being smaller than inner thick walled parenchyma. Larva was seen in these thick walled parenchyma cells (**Fig. 1E**). Vascular cambium is not apparent. Phellogen is seen forming the phellem in advanced stages. Vascular bundles composed of xylem and phloem were capped with a layer of sclerenchyma. Sclerenchyma cells were not clearly seen.

Powder when treated with different chemical reagents, showed different colour reactions. The fluorescence behaviour (**Table 1**) helps to identify and decide the authenticity of the crude powdered drug.

The physico-chemical parameters of the plant material, viz., percentage moisture content, total ash, acid insoluble ash, and of the various extractives, i.e., tannins, water-alcohol-petroleum ether solubles, were determined and the results are 10.06, 6.18, 1.13, 17.4, 31.01, 27.36 and 1.33%, respectively. The studies like moisture contents not only indicate the loss of water from drug material but also some chemical constituents which may undergo changes with weight loss due to the temperature. The total ash values also indicate the inorganic radicals like carbonates, phosphates, silicates, silica, etc. The acid insoluble ash value indicates the contamination with siliceous materials like earth and sand. All these parameters are unique to the plant and will help in standardization. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Ozarkar 2005).

A known quantity of dried plant material was extracted in a Soxhlet apparatus with petroleum ether, chloroform, ethanol and then water successfully and tested for different constituent's viz. alkaloids, flavonoids, triterpenoids, steroids, tannins, carbohydrates, saponins and glycosides. The preliminary phytochemical evaluation **Table 2** revealed the presence of several secondary metabolites which are known to possess various pharmacological effects. In last four decades the scientists are keen to evaluate many plant drugs

Table 2 Preliminary phytochemical analysis of crude extracts of *Terminalia chebula* leaf gall.

Chemical constituents	Petroleum ether	Chloroform	Ethanol	Water
Phenols	-	-	+	+
Flavonoids	-	-	+	+
Steroids	+	+	+	-
Triterpenes	+	+	+	+
Tannins	-	-	+	+
Saponins	-	-	+	+
Alkaloids	-	+	+	-
Glycosides	-	-	+	-
Carbohydrates	-	-	-	-

Table 1 Fluorescent studies of *Terminalia chebula* leaf gall.

Treatment	Visible light	Long wavelength UV light
Powder + 1N NaOH (aqueous)	Royal red	Brown
Powder + 1N NaOH (alcoholic)	Light golden yellow	Fluorescent bright yellow
Powder + 1N HCl	Yellow orange	Light brown
Powder + acetic acid	Golden yellow	Fluorescent light green
Powder + ethyl alcohol	Orange yellow	Fluorescent light brown
Powder + H_2SO_4 (50%)	Light brown	Brown
Powder as such	Greyish-yellow	Orange

used in medicinal folklore, due to their specific healing properties, health action and non-toxic effects (Singh *et al.* 2002). This pharmacognostic study of the Karkatashringi will be useful to various pharmaceutical industries as reference parameters for the analysis of their commercial samples.

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