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Artigo

Pharmacognostical studies on tubers of *Momordica tuberosa* Cogn., Cucurbitaceae

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RESUMO: "Estudos farmacognósticos de tubérculos de *Momordica tuberosa* Cogn., Cucurbitaceae". Este trabalho teve como objetivo estabelecer um perfil farmacognóstico dos tuvérculos da planta ***Momordica tuberosa*** Cogn., Cucurbitaceae. A morfoanatomia dos tubérculos da espécie foi realizada para estabelecer um perfil completo que possa ajudar na identificação e evitar problemas na sua taxonomia. Os ensaios foram realizados usando microscópio ótico, procedimentos fisico-químicos e fitoquímicos estabelecidos pela OMS. Os parâmetros apresentados podem ser usados para apresetar a autenticidade dos tubérculos desta espécie, uma vez que esta parte da droga é utilizada tradicionalmente na Índia e, também, para diferenciá-la de outras espécies de *Momordica*.

Unitermos: anatomia, seção transversal, fisicoquímica, farmacognosia, tubérculos.

ABSTRACT: The study was aimed at establishing pharmacognostical profile for the tubers of plant *Momordica tuberosa* Cogn., Cucurbitaceae. Morphoanatomy of tubers of this plant were studied in order to establish its complete profile to aid in its identification and avoid confusion in its taxanomic species. These were established using light microscopy, WHO recommended physicochemical and phytochemical procedures. The parameters presented here may be used to establish the authenticity of tubers of this plant as this part has been used traditionally in India and also to differentiate between closely related *Momordica* species.

Keywords: anatomy, transverse section, physicochemical, pharmacognosy, tubers.

INTRODUCTION

The plant *Momordica tuberosa* Cogn., Cucurbitaceae, originating in tropical regions of India and South East Asia. M. tuberosa Cogn. is commonly known as karchikai (Kannada) or athalkai (Tamil) and has been traditionally used as abortifacient in India (Kirtikar & Basu, 1991). The plant is a climbing annual or perennial herb with slender, scandent, branched, striate stem. The leaves are orbicular-reniform in outline, deeply cordate at the base, obtusely lobed with five-seven lobes. Fruits are pyriform or broadly fusiform, fleshy dark green and eight-ribbed, sparsely hairy. The roots are woody, tuberous and perennial (Nadakarni, 1976).There are reports suggesting its antihyperglycemic (Kameshwar Rao et al., 2003), anti-implantation and antiovulatory (Koneri et al., 2006), antidiarrhoeal (Vrushabendra Swamy et al., 2008) and anticonvulsant activities (Srinivas Murthy et al., 2007). We have earlier reported (Pramod Kumar et al., 2008) the in vitro and in vivo antioxidant activity of its tubers which was used as a basis to evaluate its hepatoprotective property in CCl4 model. Cucurbitacins, bitter substances, have been reported from the fruits of this plant (Bharathi Dhasan et al., 2008). Fruits are reported to contain citric acid, maleic acid and vitamin C (Parvati & Kumar, 2002).

However, there are no reports on the pharmacognostical features of the plant. Hence, the present investigation is an attempt in this direction and includes morphological and anatomical evaluation, determination of physico-chemical constants and preliminary phytochemical screening of different extracts of *M. tuberosa.*

MATERIAL AND METHODS

Plant material

The tubers of *Momordica tuberosa* Cogn., Cucurbitaceae, were collected from the suburban fields of Raichur during January and were identified and authenticated by Prof. Srivatsa, retired Professor of Botany, L.V.D. College, Raichur. A Herbarium specimen (VLCP-02/05) was deposited in the Department of

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Chemicals and instruments

Rotary microtome was used to take sections of the tubers. Compound microscope, glass slides, cover slips, watch glass and other common glassware were used in this experiment. Photographs were taken with using Nikon Labphot 2 Microscopic Unit. Various solvents used mainly ethanol (95%), petroleum ether, chloroform and reagents used for staining different sections like toluidine blue, safranin, fast-green and iodine in KI were procured from S D Fine chemicals, Mumbai, India.

Macroscopic and microscopic analysis

The macroscopic and microscopic features of the tubers were studied according to Easu (1964) and Brain & Turner (1975). For microscopic studies cross section were prepared according to procedure of Johansen (1940). The sections were stained with toluidine blue as per the method published by O'Brien et al. (1964). Where ever necessary sections were also stained with safranin and Fast-green and IKI (for starch). Glycerin mounted temporary preparations were made for macerated/cleared material. Powdered materials of tubers were cleared with NaOH, stained with phloroglucinol and conc. HCl and mounted in glycerin medium. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard books (Easu, 1964).

Physico-chemical analysis

The ash values of tubers were determined as per the Indian Pharmacopoeia (1996) methods and crude fiber content by Dutch process (Wallis, 1989). The behavior of the powdered tubers with different chemical reagents was studied. Size of starch grains determined was according to Kokate (1996) and angle of repose for tuber powder (Funnel method) were also determined. Foaming index was as per WHO guidelines on quality control methods for medicinal plant materials (WHO/PHARM, 1992). For the determination of bulk density Tapping method was followed (Sourabh, 2004).

Preliminary phytochemical screening

This was carried out by using methods of Kokate (1996) and Khandelwal (2005).

RESULTS AND DISCUSSION

Macroscopic characters

The roots are woody, tuberous, and perennial, about 4 to 8 cm in diameter, light brownish yellow in color with characteristic odor and highly bitter taste. Fractured surface is fibrous. Inner tuber is whitish cream colored. The tubers show many rootlets and some black patches here and there.

Microscopic characters

Transverse section of tubers

The root tuber is napi form, soft and smooth. It consists of a periderm which is seen in deeper part of cortex. The cortex outer to the periderm has fissured forming deep, narrow fissures (Figure 1). The periderm consists of wide and distinct phellem and equally developed phelloderm (Figure 1). The phellem is 300 µm wide and has thin walled suberised tabular cells; the phelloderm has radial files of large, squarish or rectangular thin walled cells (Figure 2). The phelloderm is 500 µm wide. The inner boundary of the phelloderm is marked by a thin layer of branchy sclerids (Figure 1, 2).

The portion inner to the periderm consists of radially aligned parallel rows of parenchyma cells which are densely loaded with starch grains (Figure 1). The starch grains are highly variable in size; but mostly they are circular concentric type with "+" shaped dark band when viewed under polarized light microscope (Figure 3). The middle portion of the tuber has small nests of vascular strands. The strand has one or two wide xylem elements and a cluster of small xylem elements. Phloem occurs in the outer portion of xylem strand (Figure 2).

The central core of the tuber consists of several radial, triangular, larger vascular strands having a few wide xylem elements associated with the xylem fibres. Phloem occurs in the thick block, outer to the xylem. The inner ground tissue is also parenchymatous, with dense load of starch grains.

Powder characters

Powder of tubers show presence of cork cells, xylem vessels with pitting and prisms of calcium oxalates of different sizes (Figure 4).

Figure 1. Transeverse section of tubers (**Fi**-Fissures; **GP**-Ground parenchyma; **Pd**-Phelloderm; **Pm**-Phellem; **VB**-vascular bundle).

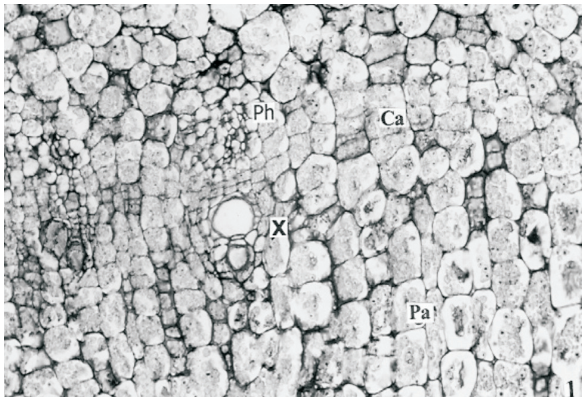


Figure 2. Transeverse section of tubers; Vascular bundle enlarged (**Ca**-Cambium; **Pa**- Parenchyma cells; **Ph**-Phloem; **X**-Xylem).

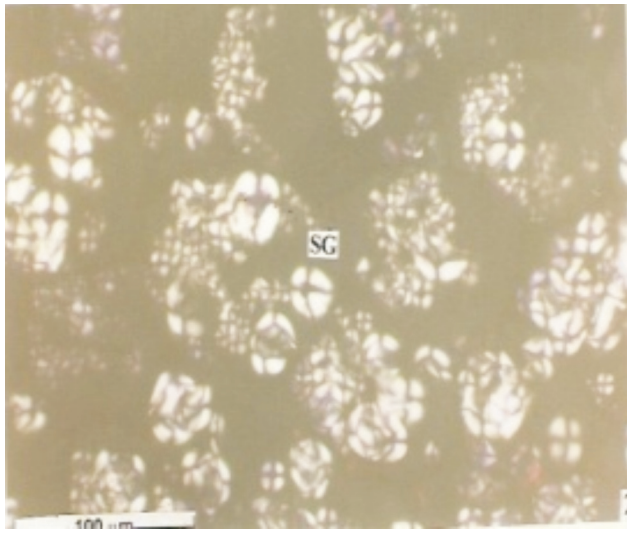
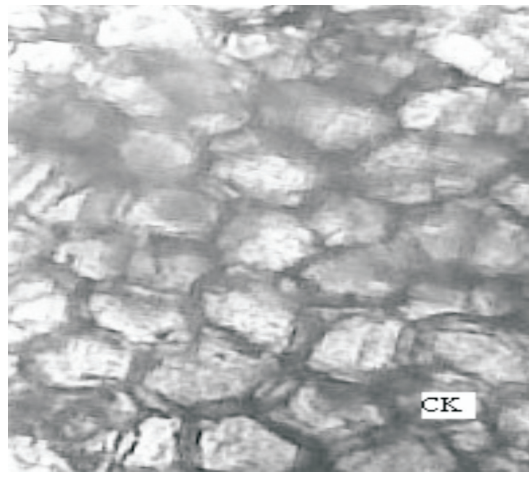
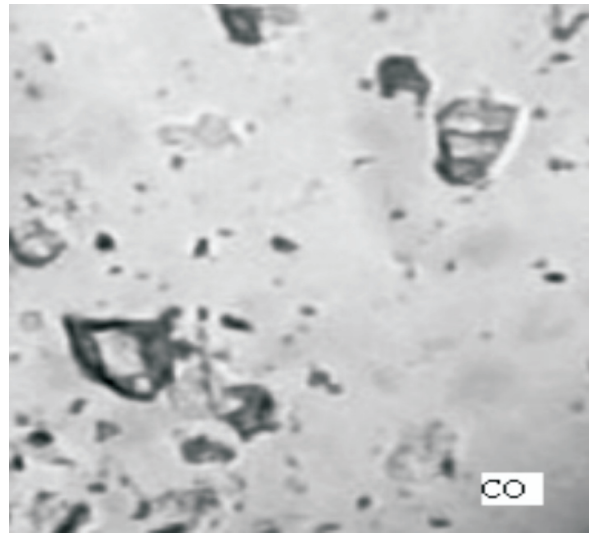


Figure 3. SG-Starch grains in the tubers





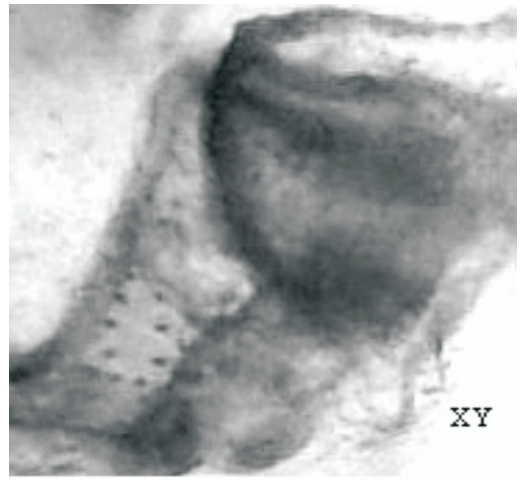


Figure 4. Powder characters; CK-cork cells, CO-prisms of calcium oxalate (Under polarized light microscope) and XY-xylem Vessel.

Preliminary phytochemical screening

The screening for phytochemicals revealed the presence of sterols, saponins, triterpenoids cardiac glycosides, bitters and carbohydrates (Table 1).

Physico-chemical constants

Ash values signify the amount of inorganic impurities, resistant materials like sand, soil and stone particles in crude drugs. The percentages of total ash, acid insoluble ash, water soluble ash and sulphated ash are given in Table 2 and extractive values in different solvents are given in Table 3. Results of behavior of the powder with different chemical reagents in visible light are given in Table 4. The physical properties and nature of different extracts prepared by successive extraction method are given in Table 5. To determine powder characters of 40 mesh size, the angle of repose and bulk density were also calculated. To determine powder characters of 40 mesh size, the angle of repose and bulk density were also calculated. The drug showed an angle of repose of 40°10" and 0.65 g/c.c. bulk density. Size of the starch grain was 9.9 to 33 µ, with average size being 19.07 µ. The foaming index was 265±13.23.

Table 1. Preliminary phytochemical screening of tubers of *M. tuberosa.*

Tests	Pet ether	Chloroform	Ethanol	Water
Alkaloids	----	----	----	----
Sterols	+	+	----	----
Triterpenes	+	+	+	----
Saponins	----	----	+	+
Flavonoids	----	----	----	----
Tannins	----	----	----	----
Fixed oils & Fats	----	----	----	----
Gums & Mucilages	----	----	----	----
Carbohydrates	----	----	+	+
Cardiac Glycosides	----	----	+	+
Proteins & Amino acids	----	----	----	----

+ Indicates respective constituent present

Table 2. Ash values of tubers of *M.tuberosa*

Parameters	Values {% (w/w) ± SD}
Total ash	3.86±0.004
Acid insoluble ash	1.26±0.01
Water soluble ash	2.90±0.002
Sulphated ash	1.26±0.014

Table 3. Extractive Values of tubers of *M.tuberosa*.

Solvents	Values {% (w/w) ± SD}
Pet Ether (60 - 80 °C)	0.603±0.08
Chloroform	01.13±0.152
Ethanol (95%)	14.40±0.20
Water	17.86±0.611

Table 4. Behaviour of powdered tubers on treatment with different chemical reagents.

Reagents	Colour developed in day light
Powder as such	Cream
1N NaOH (aq)	Brown
Picric acid	Yellow
Glacial acetic acid	Dark yellow
1N HCl	Yellow
1N HNO_3	Cream
5% Iodine	Blue
40% NaOH + few drops of 10% lead acetate	Brown
Ammonia	Yellow
Con H_2SO_4	Purplish brown
5% $Fecl_3$	Light brown
10% sodium hydroxide +copper sulphate	Greenish blue

Table 5. Colour and consistency of tuber extracts of M. tuberosa

Extracts	Colour	Consistency
Petroleum ether (60 - 80°C)	Pale yellowish	Waxy
Chloroform	Light Brown	Sticky
Ethanol (95%)	Dark brown	Semi solid
Water	Dark brown	Sticky paste

CONCLUSION

The present study of pharmacognostical evaluation of tubers of *Momordica tuberosa* Cogn., Cucurbitaceae will provide most needed information on its identification. To authenticate and substantiate the tubers, morphological and microscopical studies will be of great help which could even be used to distinguish it from other species of *Momordica.*

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