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Research Article

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ASSESSMENT OF QUALITY OF *Tinospora cordifolia* (WILLD.) MIERS. (MENISPERMACEAE): PHARMACOGNOSTICAL AND PHYTO - PHYSICOCHEMICAL PROFILE

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ABSTRACT

Pharmacognostical standardization of dried, matured pieces of stem of *Tinospora cordifolia* (Willd.) Miers., (Menispermaceae) has been carried out in the present study. The study includes macroscopical and microscopical evaluation along with estimation of its physicochemical parameters such as ash and extractive values and preliminary phytochemical screening. It also includes quantification of some of the active constituents like terpenoids and alkaloids. The present study reveals standardization profile for drug like *Tinospora cordifolia* (Willd.), which would be of immense value in botanical identification and authentication of plant drug and may help us in preventing its adulteration.

Keywords: *Tinospora cordifolia:* Standardization, Pharmacognosy, Physicochemical standards.

INTRODUCTION

The therapeutic use of herbal medicine is gaining considerable momentum in the world during the past decade. Hence, quality control standards for various medicinal plant used in indigenous system of medicine are becoming more relevant, unlike in the past when traditional doctors would themselves dispense the medicines. The varied geographical conditions, coupled with problem of different vernacular names these plants are known by, a great deal of adulteration is encountered in the commercial markets. Therefore, reproducible standards of each plant are necessary for effective quality control. An important factor, which contributes the consistent quality of herbal products, is to have adequate control on the quality of medicinal plants.

Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect.[1] Standardization serves number of purposes including: Batch to batch consistency, Confirmation of correct amount of dosage or extract per dosage unit, positive control to indicate possible loss or degradation during manufacturing.

Thus, the present study deals with standardization of medicinal plant i.e. *Tinospora cordifolia* (Willd.) Miers (Menispermaceae),[2,3] distributed in tropical and subtropical India up to 1200m elevation. It shows the presence of terpenoids, alkaloids, lignan, carbohydrates, bitters, steroids and glycosides.[4, 5] The drug has shown to have anti – cancer[6], anti-inflammatory[7], analgesic, CNS depressant[9], cardio tonic activities[8], anti –oxidant, antipyretic, hepatoprotective, diuretic, anti-stress, antihyperglycemic[10,11], anti- tuberculotic[12], hypolipidemic. It is also used in constipation, diabetes, fevers, skin diseases, vomiting and vaginal discharges. Due to its wide therapeutic importance it is worthwhile to obtain various qualitative and quantitative standards of drug to prevent its adulteration.

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MATERIALS AND METHODS

Plant Material Authentication

The identity of the plant was confirmed by Dr P Jayaraman, Botanist, PARC, Chennai, PARC/2010/656. The plant was compared with voucher specimen in the institute. For further confirmation, the microscopic characteristics of this plant were studied and compared with available literature. The fresh plant material collected was thoroughly cleaned and air-dried. It was then homogenized to fine powder and stored in air-tight bottles for further studies.

Foreign Matter

The term "Foreign Matter" is used to designate any matter, which does not form a part of the drug as defined in the monograph.100g of the powdered drug is taken and spread out in a thin layer. Plant material collected should be free from foreign matters like soil, insect parts or animal excreta. They are separated and weighed and the percentage was calculated.

Pharmacognostic Studies

Macroscopic study: Macroscopic observation of *Tinospora cordifolia* stem was done. It comprised of shape, size, surface characteristics, texture, color, consistency, odour, taste, etc.

Microscopic study: Transverse sections of *Tinospora cordifolia* stem were taken by using a microtome.

Permanent mount of stem was prepared using saffranin fast green stain by double staining technique.[13]

Physicochemical Studies

Physicochemical parameters were determined as per guidelines of WHO. Total ash value, loss on drying, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value were determined.[14,15]

Preliminary Phytochemical Studies

The stem of *Tinospora cordifolia*. (Willd.) Miers were coarsely powdered and extracted with methanol and water (6:4) using cold maceration technique. The extract is filtered, concentrated and dried in a Rota evaporator initially followed by vacuum desiccation. Preliminary phytochemical screening of extract was done for the presence of various phytoconstituents by using standard procedure.[16]

Pesticide Residue[17]

Pesticide residue is done by TLC by using Benzene: methanol as mobile phase and Precoated silica gel 60 F254 TLC plate of 0.2mm thickness. Detection by UV from 200 to 300nm.

Microbial Contamination[18]

For the safe use of the plant drug, microbial count was done and checked whether total aerobic count, total yeast and mould count are within the prescribed WHO limits.

Quantitative Estimations

Total terpenoid content was estimated Gas Liquid chromatography as described by Harborne JB.[16] Total alkaloid content was estimated according to the method described by Edeoga.[19]

RESULTS

Foreign Matter

Foreign matter was found to be 0.2%. The permissible limit as per standards in not more than 2%.

Pharmacognostic Studies

The pharmacognostic study is the major and reliable criteria for identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The detailed and systematic pharmacognostic evaluation gave valuable information for future studies.

Macroscopic Studies

Macroscopically the stems are succulent, soft, possessing long, filiform, aerial root arising from branches. Bark warty, creamish white or grey brown; wood soft, perforated. Dried sample consists of 5 to 10cm long conical pieces, light in weight; bark light and papery, brittle, dark brown; wood with longitudinal surface ridges, and radially divided into wedge shaped pieces in cross-sections (figure **1**). Pieces difficult to fracture when fully dried and can be torn only by twisting; odourless; taste bitter.

Figure 1. Macroscopic characteristics of stem of *Tinospora cordifolia* (WILLD.) MIERS

Figure 1a. Fresh stem

Figure 1b.Dried Stem

Microscopic studies

The microscopic studies of stem showed following tissue systems:

Cork: The cork comprises of an outer zone of thick walled brownish compressed cells and an inner zone of thin walled colourless, tangentially arranged cells. The cork tissue is broken at some places due to lenticels.

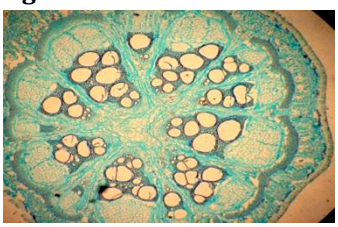
Cortex: Cortex is wide. The outer zone of cortex consists of 3 to 5 rows of irregularly arranged tangentially elongated chlorenchymatous cells and the cells situated towards the inner side are polygonal in shape filled with abundant starch grains. The starch grains are simple, ovoid; several secretory cells found scattered in the cortex. Pericyclic fibres lignified are associated with a large number of crystal fibres containing a single prism in each chamber.

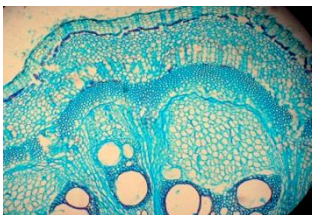
Vascular bundle: Vascular zone is composed of discrete vascular strands with 10 to 12 or more wedge shaped strips of xylem, externally surrounded by semi circular strips of phloem alternating with wide medullary rays; phloem parenchyma contain calcium oxalate crystals; cambium is of 1-2 layers; xylem consists of vessel elements, tracheids, parenchyma and fibres. Vessel elements cylindrical in shape bearing bordered pits. Medullary rays 15 to 20 cells wide. Pith mostly made up of large thin walled cells containing starch grains.

The presence of discrete vascular strands in the mature stem of *Tinospora cordifolia* is one of the anomalous secondary structures found in Menispermaceae.

Photomicrographs of the microscopic characteristic of stem of *Tinospora cordifolia* (WILLD.) MIERS in different views

Figure 2a. TS of stem of Tinospora cordifolia





Enlarged view of characters

Figure 2b. Cork

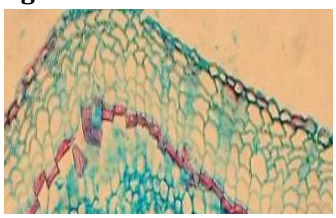


Figure 2c. cortex

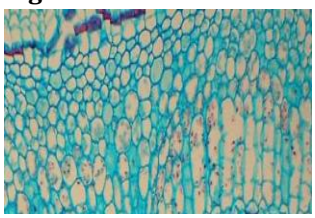


Figure 2d. pericycle

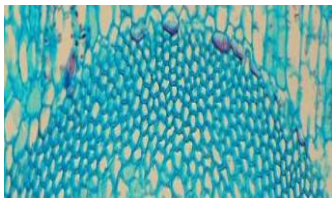


Figure 2e. Phloem

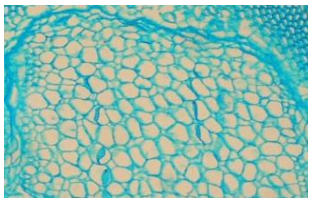


Figure 2f. Xylem

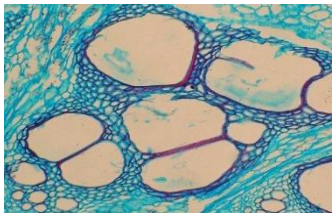
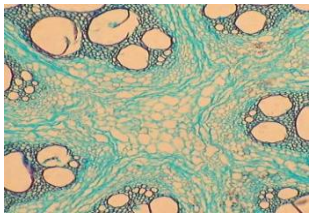


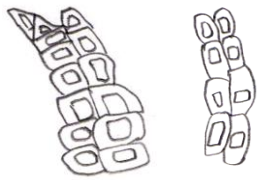
Figure 2g. Pith



Powder Characteristics

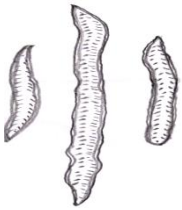
Creamish brown, starch grains, tracheids, fibres, crystal fibres containing prisms of calcium and vessel elements with bordered pits. The crude powder of *Tinospora cordifolia* was creamish brown in color, odourless and slightly bitter in taste. Microscopy study of powder showed the presence of fibres which are lignified, long with blunt ends. Tracheids with bordered pits and horizontal perforations. Pericylic fibres are longer than tracheids. Xylem vessels cylindrical and bear bordered pits. Starch grains are present in parenchymatous cells. (Figure **3**).

Figure 3. Photomicrographs of the specific characteristics determined from the powder study of *Tinospora cordifolia* stem

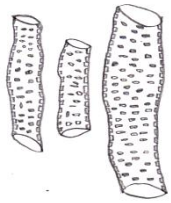


Crytal fibres, 50µ

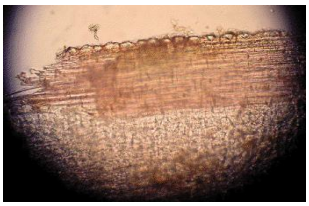
Starch grains, 50µ

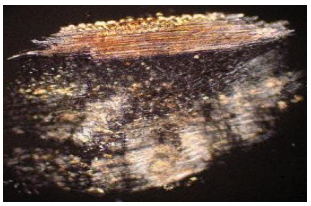


Tracheids, 50µ



Vessel elements, 50µ





Photomicrographs of crystal fibres, against light and dark background

Physicochemical Studies

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.

The results of physicochemical parameter analysis of crude powder of *Tinospora cordifolia* stem were shown in Table **1**. The average values are expressed as percentage

Table1. Determination of proximate parameters of crude powder of *Tinospora cordifolia* stem

S.No	Physicochemical standards	Results % w/w	Standard value % w/w
1	Total Ash	7.5	NMT 10 %
2	Acid Insoluble ash	1.16	NMT 3%
3	Water soluble extractive value	12.05	NLT 9%
4	Alcohol soluble extractive value	7.27	NLT 1.5%
5	Loss on drying	2.31	NMT 10%

of air-dried material. The drug showed 2.31% of loss on drying. It contained 7.5% of total ash and about 1.16% of acid insoluble ash. The percentage extractive yield of crude powder extracted in water and alcohol were 12.04 and 7.27 respectively.

The crude stem powder of *Tinospora cordifolia* and was analyzed for the presence of heavy metals.[20] The results (Table **2**) showed that arsenic, mercury and cadmium were not present in any of the samples; The presence of lead was found only to be 5 ppm. Although, there was minor presence of some heavy metals but the sample did not exceed the limit given according to the WHO guidelines. Therefore, the samples investigated were free from heavy metal contamination.

Table 2. Determination of heavy metals

S.No.	Heavy metal	Result (PPM)
1	Arsenic	Nil
2	Lead	Less than 5ppm
3	Cadmium	Nil
4	Mercury	Nil

Preliminary Phytochemical Studies

Preliminary phytochemical screening showed the presence of terpenoids, alkaloids, lignans, carbohydrates, glycosides, bitters, proteins, tannins, steroids, etc.(Table **3**)

Table 3. Phytochemical studies

S.No.	Constituents	Results
1	Terpenoids	+
2	Flavones	-
3	Lignans	+
4	Alkaloids	+
5	Carbohydrates	+
6	Glycosides	+
7	Bitters	+
8	Phenols	-
9	Proteins	+
10	Resins	-
11	Saponins	-
12	Tannins	+
13	Steroids	+
14	Anthrquinones	-
15	Gums	-

+ = present, - = absent

Pesticide Residue

From the results it can be concluded that the plant material is totally safe and there is no traceable limit of pesticide in them.(Table **4**)

Table 4. Test for pesticide residue

S.NO	PESTICIDE RESIDUE	EXTRACT
1	DDT	ND
2	Benzene Hexachloride	ND
3	Aldrin	ND
4	Dieldrin	ND
5	Lindane	ND
6	Chloropyrophos	ND
7	Enosulphan	ND

ND – not detected (Concentration less than the minimum detection limit even in ng/l units).

Microbiological Studies

As per the WHO standards, the plant material is free from microbial load and safe for further use in formulation.(Table **5**)

Table 5. Test for microbial contamination

S.No	Parameter	Values obtained	WHO limit
1	Total bacterial count	5 cfu/gm	NMT 1000cfu/g
2	Yeast and moulds	Nil	NMT 100cfu/g
3	*Escheria coli*	Negative	Absent
4	Salmonella	Negative	Absent
5	S.aureus	Negative	Absent

Quantification

The total terpenoid content was found to be 0.185mg/100g of sample and the total alkaloid content was found to be 0.2998mg/100gm of the sample.

DISCUSSION

The primary steps for establishing the quality control profile of any plant drug is the macroscopic and microscopic evaluation and according to WHO, botanical standards should be proposed as a protocol for the diagnosis of the herbal drug. The histochemical studies give a preliminary idea about the type of compounds and their accumulation in the plant tissues. This is of great interest for quality control in basic research and drug production, especially for imported items and for raw material sold by traditional herbalists.

Physicochemical standards such as total ash value helps us in determining both physiological ash (plant tissue) and non-physiological ash (extraneous matter like sand and soil), whereas acid insoluble ash gives an idea about the amount of silica present, especially as sand and siliceous earth. Extractive values help us in determining the amount of active constituents and is done on plant materials for which as yet no suitable chemical or biological assay exists. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like terpenoids acts as anti-bacterial and antineoplastic and alkaloids possess a good analgesic, anti-inflammatory and anti-oxidants activity. The quantified values of the above phytoconstituents can be used as a major tool for obtaining a quality control profile for a drug.

CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in Ayurvedic system of medicine.

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