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Research Article

STANDARDIZATION AND PHYTOCHEMICAL EVALUATION OF *TINOSPORA CORDIFOLIA* (WILLD.) MIERS. (MENISPERMACEAE)

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ABSTRACT

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. *Tinospora cordifolia* (Willd.) Miers commonly known as Amrita or Guduchi is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial. The drug is used in the treatment of fever, diabetes, jaundice, urinary problems, skin diseases and dysentery. The standardization parameter as per WHO guidelines has been carried out in the present study. The stem of the plant was evaluated for its pharmacognostical parameters including morphological and microscopical parameters along with physico-chemical and toxicological parameters. Ash values, extractive values in different solvents, pH determination, fluorescence analysis and phytochemical screening have been carried out in the study. The study will be useful for the identification of stem of *Tinospora cordifolia* and will prevent its adulteration.

Keywords: *Tinospora cordifolia*, Amrita, Guduchi, Standardization, Phytochemical

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in the world health[1-2]. In view of this, WHO recognized the need for standardization and quality control of herbal medicines [3]. WHO has published guidelines in order to define basic criteria for evaluating the quality, safety and efficacy of herbal medicines aimed at assisting national regulatory authorities, scientific organizations and manufacturers in this particular area [4].

Tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi) is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae[5-7]. It is distributed throughout the tropical Indian subcontinent and China, ascending to an altitude of 300 m. In Hindi, the plant is commonly known as Giloy, which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young[8]. Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties[9]. The plant is known as Amrita and the term is attributed to its ability to impart youthfulness, vitality and longevity to the consumer[10]. In the present study, an effort has been made to establish the Pharmacognostical as well as phytochemical study of *Tinospora cordifolia* stem.

MATERIALS AND METHODS

Collection and Identification of Plant material

The stem of the plant *Tinospora cordifolia* (family: Menispermaceae) was collected from Khari-baoli, local market of New Delhi, identified and authenticated by Dr. H. B. Singh (Taxonomist), National Institute of Science Communication And Information Resources (NISCAIR), Pusa Campus, New Delhi, With Specimen No: NISCAIR/RHMD-2010-11/1505/103.

Morphological Characters

The dried stems of Tinospora cordifolia were taken and observed for the various parameters including shape, size, color, taste, surface characteristic, texture, fracture characteristic and appearance of cut surface.

Microscopical Studies

Section cutting

Sections of the stem were taken and soaked in water overnight. Free hand section of stem were cut, cleared and stained with safranin, mounted on a clean glass slide and covered with cover slip using glycerin. It was then observed under microscope.

Powder characteristics

The powder of stems of *T.cordifolia* was passed through sieve no. 60, mounted on glass slide using water, covered with cover slip and viewed under microscope.

Foreign Matter Analysis

A sample of plant material was weighed. It was spread in a thin layer and foreign matter was sorted into groups either by visual inspection, using a magnifying lens or with the help of a suitable sieve according to the requirements. The remainder of the sample was sifted through a sieve no. 250; dust was regarded as mineral admixture. The portion of this sorted foreign matter was weighed. The content of each group was calculated in grams per 100g of air dried sample [11].

Physical Evaluation

The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in WHO[11] guidelines on quality control methods for medicinal plants materials and Ayurvedic pharmacopeia[12]. Fluorescence analysis was carried out according to the method of Chase and Pratt[13] and Kokoski[14].

pH Determination

pH of 1% and 10% solution of powdered stem of *T.cordifolia* was determined using standardized glass electrode.

Phytochemical Screening

Stem of *T.cordifolia* was coarsely powdered and extracted with different solvents, viz., petroleum ether, chloroform, acetone, methanol, hydro-alcohol and water. The extracts were then subjected to phytochemical screening as per standard methods prescribed in literature[15-16].

Swelling Index and Foaming Index

Swelling Index and Foaming index were determined by the method prescribed in WHO guidelines[11].

Determination of Heavy metals

Weighed amount of sample was placed in silica crucible and heated on burner till organic matter was charred. It was then transferred to muffle furnace and heated at 500°C for 5hours. The material was cooled and added 20ml nitric acid. The mixture was boiled on water

bath for 1hour and filtered. The residue was washed with distilled water. and filtered again. Both filterate and water were mixed and final volume was made upto 100ml with distilled water. It was then subjected to absorption reading using Atomic Absorption Spectrometer and amount of trace matter was then determined.

Determination of Pesticidal Residue

Coarsely powdered sample (10g) was mixed with 120ml of mixture of acetonitrile & water in a beaker. It was kept overnight and filtered using non-absorbent cotton pad premixed with Acetonitrile. Filterate was transferred to a separating funnel and shaken with 120ml of 5% NaCl solution. It was extracted thrice with 50ml of n-hexane; the organic layer was combined and dried over anhydrous sodium sulphate. It was concentrated to 5ml on water bath. The extract was further cleaned by adding 20-25g preactivated florosil (preactivation temperature- 500-55°C) and 5g of anhydrous sodium sulphate previously rinsed with petroleum ether. The mixture was eluted very slowly with 150ml of solvent containing n-hexane (141ml) and diethyl ether (9ml), keeping drop rate of 1drop/second. The extract was concentrated on water bath close to dryness and the volume was made upto 1ml with n-hexane. It was then subjected to GC-MS analysis for estimation of residual organic chlorine pesticides (viz. α and β HCH, δ-HCH, DDT and metabolites).

Determination of Micro-organisms

Determination of micro-organisms was done to ensure that the drug was free from any harmful microbes for its use in herbal formulations and was done as per WHO guidelines[11].

Thin Layer Chromatography Profile

TLC of different extracts was carried out to determine the number and nature of components present in it and was performed as per WHO guidelines[11].

RESULTS AND DISCUSSION

Morphological Parameters

Morphological characteristics of stem of T.cordifolia have been described in Table 1 and fig 1.

Table 1: **Observation of Organoleptic Characters of *Tinospora cordifolia* stem**

S. No.	Parameters	Stem
1.	Colour	Light brown
2.	Odour	Odourless
3.	Taste	Bitter and astringent
4.	Size	0.6 to 5 cm in diameter
5.	Shape	Cylindrical
6.	Touch	Smooth and splintery
7.	Fracture	Fibrous

Microscopical Characteristics

T.S of T.cordifolia stem shows cork, cortex and vasculature. The cork comprises of an outer zone of thick-walled brownish compressed cells and an inner zone of thin walled colorless, tangentially arranged cells. The cork tissue is broken at some places due to lenticels. Vascular zone is composed of discrete vascular strands with 10 to 12 or more wedge shaped strips of xylem, externally surrounded by semi-circular of phloem, alternating, with wide medullary rays; phloem consist of the usual elements. Some of the cells of phloem parenchyma contain calcium oxalate crystals; xylem consists of vessel elements, tracheids, parenchyma and fibres. Microscopical details are shown in fig 2.

Powder microscopy shows starch grains that are simple, ovoid or ovoid elliptical. Powder of stem of T.cordifolia was light to dark brown in color. Pericyclic fibres lignified and are associated with a large number of crystal fibres containing a single prism in each chamber. Bordered pitted vessel, cork cells and part of the xylem associated with medullary rays was also seen in the stem powder. Results are shown in fig 3-8.

Fig. 1: Stem of *T.cordifolia*

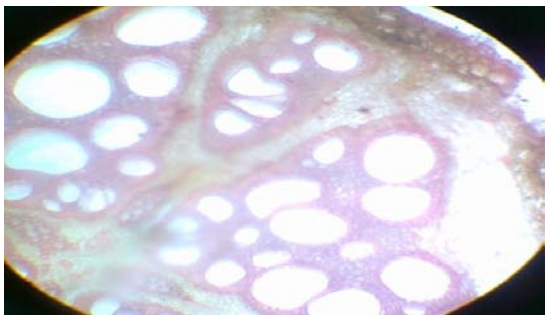

Fig. 2: T.S. of stem of *T.cordifolia*

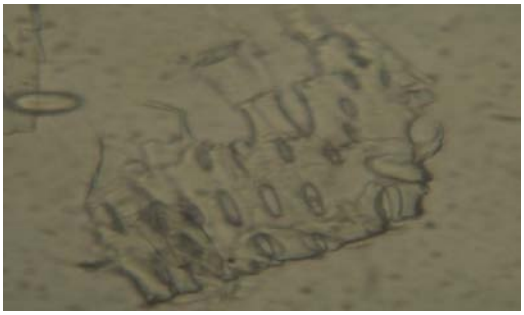

Fig. 3: Bordered pitted vessel

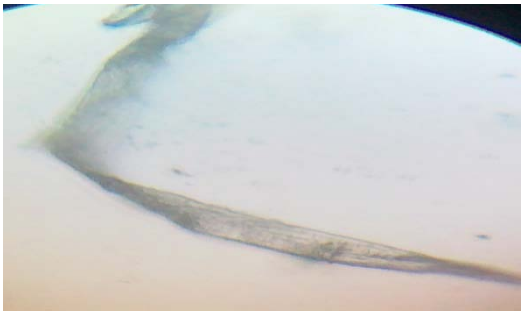

Fig. 4: Fibres

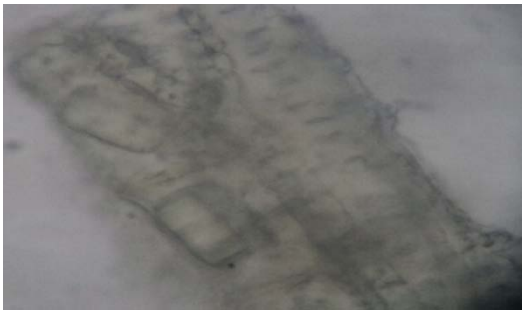

Fig. 5: Uniseriate medullary rays

Fig. 6: Part of the xylem associated with medullary rays

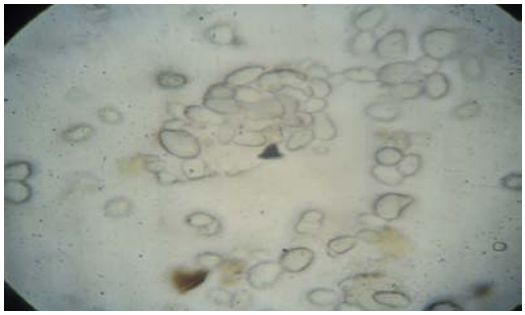

Fig. 7: Starch granules

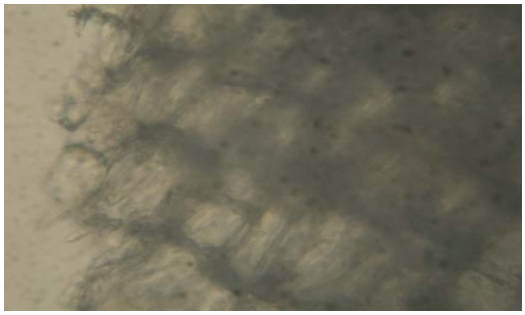

Fig. 8: Cork cells

Phytochemical Screening

In the preliminary phytochemical screening, the various extracts of drugs were tested to detect the presence of any particular type of constituent. The tests were found to be positive for alkaloid, glycosides, sterols and carbohydrates. Results of phytochemical screening are listed in Table 3.

Fluorescence Analysis

The Fluorescence analysis of the drug sample before and after treatment with various reagents and their observation in day light, short and long wavelength UV light was carried out. These characters can be used for the identification of drugs to find out any kind of adulterations. Results of fluorescence analysis are given in Table 4.

Heavy Metal Analysis

Heavy metal analysis was carried out for determination of Arsenic, Lead, Mercury and Cadmium by atomic absorption spectrometry method as mentioned in WHO guidelines, are found to be within the prescribed limit and are reported in Table 5.

Physical Parameters

Results of foreign matter analysis, loss on drying, ash values, extractive values in different solvents, pH determination, swelling index and foaming index are given in Table 2.

Pesticidal Residue

Pesticidal residue was determined by the method of GC-MS and all observations were found to be within the prescribed limits. Results are reported in Table 6.

Microbial Determination

Microbial determination was carried out for Total viable aerobic count and Total aerobic count by serial dilution method. Results are reported in Table 7.

Thin Layer Chromatography Profile

Petroleum ether extract, chloroform extract and methanolic extract were subjected to TLC analysis using solvents of different polarity. The R_f values of the resolved components were determined and detailed results of number of components present are given in Table 7.

Table 2: Physical Parameters

S. No.	Parameters	Values		
1.	Foreign matter analysis	1.20% w/w		
2.	Loss on drying	4.17% w/w		
3.	Ash Values			
	A. Total ash	5.94% w/w		
	B. Acid insoluble ash	1.49% w/w		
	C. Water soluble ash	0.78% w/w		
4.	Extractive Values	Cold Maceration (w/w)	Hot Extraction (w/w)	Successive Extraction (w/w)
	A. Petroleum ether	1.76%	3.8%	5.73%
	B. Chloroform	2.63%	6.96%	10.13%
	C. Acetone	3.0%	9.16%	11.40%
	D. Methanol	10.93%	12.73%	15.76%
	E. Hydro-alcohol	25.43%	18.33%	22.03%
	F. Aqueous	32.76%	27.93%	28.76%
5.	pH Determination			
	A. 1% Solution	7.62		
	B. 10% Solution	7.08		
6.	Swelling Index	Absent		
7.	Foaming Index	Less than 100		

Table 3: Observations of Phytochemical Screening of *T.cordifolia*

Phytochemical Investigation of *T.Cordifolia*								
Extract constituents			**P E**	**$CHCl_3$**	**Acetone**	**MeOH**	**Hydro-alcohol**	**Aq**
Alkaloids	Dragendroff's Test		-	+	+	+	+	+
	Wagner Test		-	+	+	+	+	+
	Mayer Test		-	-	-	-	-	-
	Hagers Test		-	-	+	+	+	+
	Molisch Test		-	-	-	+	+	+
Carbohydrates	Fehlings Test		-	-	-	+	+	+
	Benedicts Test		-	-	-	+	+	+
Glycosides		Keller killiani Test	-	+	-	-	+	+
	Cardiac Glycoside	Libermann Test	-	-	-	-	-	-
	Anthraquinone Glycoside	Borntragers Test	-	-	-	-	-	-
	Saponin Glycoside		-	-	-	-	-	-
Tannins	5% $FeCl_3$		-	-	-	-	-	-
&	Gelatin Test		-	-	-	-	-	-
Phenols	Precipitation Test	Lead acetate	-	-	+	+	+	+
		Acetic acid	-	-	-	+	+	+
		Dil.HNO_3	-	-	-	+	+	+
		Pot. dichromate	-	-	-	+	+	+
	Zinc chloride Test		-	-	+	+	+	+
Flavonoids	Lead acetate Test		-	-	+	+	+	+
	Ammonia Test		-	-	+	+	+	+
Proteins	Biuret Test		-	-	-	-	-	-
	Xanthoproteic Test		-	-	-	-	-	-
	Millon's Test		-	-	-	-	-	-
Steroids	Liberman Burchard Test		-	-	-	-	-	-
	Salkowaski Test		+	+	+	+	-	-
	Liberman Test		-	-	-	-	-	-

(+) Present; (-) Absent

Table 4: Observations of Fluorescence Analysis

S.No.	**Materials / Treatments**	**Observations Under UV Cabinet**		
		Day Light	**At Short wavelength(254nm)**	**At Long Wavelength (366nm)**
1.	Drug powder as such	Light brown	Dark brown	Creamish brown
2.	Drug + Conc. H_2SO_4	Dark brown	Light brown	Light brown
3	Drug + Acetic acid	Light brown	Light brown	Light brown
4.	Drug + Acetic acid + Conc. H_2SO_4	Dark brown	Blackish brown	Greenish brown
5.	Drug + I_2 soln.	Reddish brown	Reddish brown	Dark brown
6.	Drug + 5% $FeCl_3$	Yellow	Light cream	Dark brown
7.	Drug + Conc. HNO_3	Reddish brown	Yellowish brown	Dark brown
8.	Drug + few drops of NH_4OH	Creamish Brown	Light brown	Dark brown
9.	Drug + 10% NaOH	Creamish brown	Brownish cream	Brown
10.	Drug + 10% NaOH + a drop of $CuSO_4$	Brown	Cream	Brown
11.	Drug + 10% NaOH + a drop of lead acetate	Creamish brown	brown	brown
12.	Drug + conc. HNO_3 + excess of ammonia	Yellowish brown	Creamish brown	Dark brown
13.	Drug + a drop of $FeCl_3$ + Conc. H_2SO_4	green	Yellowish brown	Dark green

Table 5: Estimation of Heavy Metals of *T.cordifolia*

S. No.	**Type of Heavy Metal**	**Max. Residual Limits (ppm)**	**T.cordifolia (ppm)**
1.	Arsenic	3.000	0.226
2.	Lead	10.000	1.8018
3.	Mercury	1.000	0.01598
4.	Cadmium	0.300	0.01452

Table 6: Estimation of Pesticide Residue of *T.cordifolia*

Observations of Pesticide Residues in *T.cordifolia*		
S. No.	**Type of Pesticide**	***T.cordifolia (ppb)***
1.	α HCH	0.025
2.	β HCH	0.065
3.	γ HCH	0.236
4.	P-P'DDE	0.034
5.	P-P' DDT	0.601

Table 7: Determination of Aerobic Count and Microbial Load of *T.cordifolia*

S. No.	Microbiological examination	UOM	Result	Specifications
1	Total aerobic count	CFU per g	870000	1250000
2	*Enterobacteriaceae*	per g	NAD	1000
3	*E. coli*	per g	NAD	10
4	*Salmonella sp.*	per 25g	Absent	Absent
5	*Staphyloccous aureus*	per g	NAD	100
6	Yeasts	per g	NAD	100
7	Moulds	per g	6000	10000
8	*Bacillus cereus*	per g	NAD	1000
9	*Pseudomonas aeruginosa*	per g	NAD	100

NAD = Not Adequately Detected

Table 7: TLC Profile of different extracts of *T.cordifolia*

S. No.	Extracts	Solvent System	No. of Spots Observed		R_f Values
			In U. V. (366nm)	In I_2	
1.	Pet. Ether	$CHCl_3$:EtOAc:Benzene3:1.5:0.5	0	2	0.41, 0.79
2.	Chloroform	$CHCl_3$:EtOAc:MeOH3:1:1	1	6	0.14, 0.32, 0.55, 0.65, 0.71, 0.79
3.	Methanol	$CHCl_3$:MeOH3:2	1	7	0.10, 0.16, 0.26, 0.32, 0.56, 0.66, 0.72

CONCLUSION

The results of the present study have established the specifications of the quality profile of the drug *Tinospora cordifolia* stem. The drug should be standardized before any research and the results should be within specifications.

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