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Traditional and chemotaxonomic studies of bio guided active constituents of *Tinospora cordifolia* (Willd.) Hook. F. & Thoms. Root

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Abstract
Ayurveda is one of the oldest systems of medicine in the world. There are almost 1200 plants mentioned in Ayurveda for their medicinal properties. Thus, the Pharma therapeutics of Ayurveda is mainly centered on the use of various plant parts in the management of innumerable diseases. *Guduchi* (*Tinospora cordifolia* (Willd.) Hook. F. & Thoms.) Is one such plant praise widely for its efficacy in treatment of various diseases along with its superior rejuvenation property? Ayurvedic pharmacopoea of India has mentioned quit quality standard parameters for assessment of stem of *Tinospora cordifolia*. From the literature review of ancient Ayurveda classics it can be inferred that *Guduchi moola* (Root of *Tinospora cordifolia*) was also used in management of diseases like Shop ha (Inflammation) and Prameha (Diabetes mellitus and urinary disorders). As, *Guduchi* grows by vegetative propagation of stem, inclusion of root will not hamper the reproduction of it. Till date, no work is published on the standardization parameters of Root of *Tinospora cordifolia*. This study aims at generation of data regarding physicochemical and phytochemical parameters of *Guduchi* root using Ash value, loss on drying, extractive value, and qualitative analysis of phytoconstituent along with sophisticated analysis like HPTLC, FTIR and UV spectrophotometry. This article may prove beneficial for further studies on Root of *Tinospora cordifolia*.

Keywords: Ayurveda, Berberine, Dravyaguna, *Guduchi*, HPTLC, *Tinospora cordifolia* root

Introduction
Traditional and complementary medicine (T&CM) is an important and often underestimated health resource with many applications. [1] According to WHO 2002 report, 80% of the world population is still dependent on herbal remedies to treat illness. [2] The Ayurvedic system of medicines is now gaining attention globally due to its efficacy. The standardization of drugs includes: Drug authentication, physical parameters, chemical parameters, chromatographic analysis, and microbiological parameters [3].

Pharmaco-therapeutics in the field of Ayurveda, mainly centers on the use of various mono herbal as well as polyherbal formulations for treatment of various diseases. Specified use of various herbs for numerous ailments can be observed since Vedic period. Charaka Samhita, one of the two foundational texts of Ayurveda, has enlisted numerous plants, herbs, shrubs and their useful parts that can be used medicinally. The properties of various parts of a single plant can behave differently individually and differently with the combination of some other plant or with some other part of same or different plant. So as to predict the therapeutic and clinical efficacy of any plant or any part, it is necessary to analyze and evaluate the whole plant according to its chemical properties and its traditional use (if any).

Guduchi, botanically known as *Tinospora cordifolia* Willd Hook Miers, is one of the most widely used herb in the field of alternative as well as allopathic medicines. It possesses many medicinal and therapeutic properties such as- anti-diabetic, antipyretic, antispasmodic, anti-inflammatory, anti-arthritic, antioxidant, anti-allergic, anti-stress, anti- leprotic, antimalarial, hepatic-protective, immune-modulatory and anti-neoplastic activities. In ancient and modern Ayurvedic literatures *Tinospora cordifolia* (*Guduchi*) has been used for the management of diseases like *Kamala* (hepatitis), Kushtha (skin diseases), *Jwara* (pyrexia), *Prameha* (Diabetes and urinary tract disorders), *Pandu* (anemia), *Krimi* (intestinal worms) and much more. Because of such wide and effective therapeutic window, *T. cordifolia* is widely used in various AYUSH formulations.

Charak Samhita, has also explained use of *Guduchi* root as Medhya rasayana (Nootropic enhancing agent). Though whole plant is of medicinal use but stems are leaves are prominently studied and are praised for their therapeutic efficacy in ancient literature also, a large number of studies are available which prove its wide therapeutic efficacy clinically as well as experimentally.

Susshrut Samhita, an ancient Ayurvedic Sanskrit text on medicine and surgery, explained a group of herbs called as *Valli panchamula*, Valli meaning climber, panch means 5 and Mula means root. Thus, Valli panchamula describes a class of five climbers whose root is to be used for medicinal purpose. These 5 plants are grouped together as they possess the similar properties and may depicts similar chemical composition w.r.t Root. This *Valli panchamula*, comprises of Vidari (*Ipomoea digitata* L.), *Sariva* (*Hemidesmus indicus* R. Br), Rajani (*Rubia cordifolia* L.), *Guduchi* (*Tinospora cordifolia* Willd Hook Miers) and *Ajashrungi* (*Gymnema sylvestre* L.). The group of these 5 plants can be used in effective treatment of Raktapitta (Haematological disorders), Shopha (Inflammations), Prameha (Urinary disorders and Diabetes mellitus) and Shukradosh (Disorders of semen). Ancient literature of Ayurveda holds the proof of medicinal and therapeutic efficacy of Giloe roots, but conventional researche lacks the clinical as well as experimental evidence depicting the use of *Guduchi* root, thus, here is an attempt to standardize the root of *Tinospora cordifolia* so that it can be effectively used for treatment of various diseases.

Materials and methodology

Experimental analysis can be helpful in assuring the quality, purity and authenticity of the given drug. World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles [4].

Analytical analysis of drug including Physics-chemical, Phytochemical, HPTLC, FTIR, UV-Visible parameters was performed in All India Institute of Ayurveda, Delhi (RRDR laboratory).

Physicochemical study

1. Loss on Drying (LOD)

Around 2 g of air-dried raw drug was kept in a well-ventilated oven for 3 h at 105 ºC. After removal the drug from the oven it was allowed to cool to room temperature by keeping the dried raw drug in a desiccator for 20 min. Then it was again weighed. The loss on drying (moisture content) in percentage was calculated as follows:

Weight of Empty the Petri dish = W_{empty}

Weight of the Petri dish with air-dried raw drug = W+ wet

Weight of the air-dried raw drug = $W_{+ wet} - Empty = W_{wet}$

Weight of the oven-dried (105 ˚C) raw drug = $W+_{dry} - Empty = W_{dry}$

% LOD =

2. Extractive values

a) In ethanol extract

Around 2.0 g of raw drug was weighed (moisture content was considered). 50 mL of Et OH was added into raw drug and kept for 24h at room temperature. After filtering it, 10 mL was taken and evaporated on a water bath. Thereafter, it was heated at 105 OC in a well-ventilated oven till constant weight was achieved. The above experiment was performed twice, and an average value was reported. The ethanol extract value was calculated as follows:

Drug weight = (moisture content was subtracted to calculate the drug weight)

Empty Beaker weight = empty

Beaker +dried Extract weight = w + drug

% ETOH extract = =%w/

In aqueous extract

In a closely related experiment, water extract value was determined using 50 mL of water

instead of ethanol.

Empty Beaker weight = wempty

Beaker +dried Extract weight = w+drug

% H2O extract = =%w/w

3. Ash values

a. Determination of total cash value

Around 2.0 g of raw drug was weighed (moisture content was considered) in a thermally resistant previously weighed crucible. The crucible along with its content is heated to 450 ºC for 30 min. The crucible was cooled in a desiccator for 15 min and weighed.

Repeated this procedure till constant weight was obtained.

Then the percentage of total ash was calculated with reference to the drug weight.

Empty Crucible weight= w empty

Drug weight==

Crucible +ash weight = w + ash

Total ash=

% Total ash =%w/

b. Determination of acid insoluble ash value

The ash obtained (as directed under total cash value) was boiled with 25 ml of 10% HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited for 15 minutes at a temperature about 450 °C. The crucible was cooled in a desiccator for 15min and weighed. The heating was repeated till a constant weight was reached. The above experiment was performed twice, and an average value was reported.

Empty Crucible weight = w_{empty}

Drug weight =

Crucible +acid insoluble ash weight = w+ acid (i) ash

Acid insoluble ash = w+ acid (i) ash – w_{empty}

% acid insoluble ash = %w/w

Phytochemical study

1. Preliminary analysis-

The study drug was subjected to cold extraction in six solvents namely, 50% hydro alcoholic solution (Ethanol: water), N-hexane, Methanol, Ethanol and Chloroform. 5 gm. of powdered drug was added with 50 ml of each of the solvent. The mixture was then subjected to intermittent stirring for next 6 hours and then kept stand still for next 18 hrs. After 24 hrs, the extracts were filtered and filtrates were used for further evaluation of phyto constituents.

A. **Estimation of proteins (Million's test):** 2 ml of extract and few drops of Million's reagent. Appearance white colour precipitate indicates the presence of protein (Rasch and Swift 1960).
B. **Estimation of Alkaloids (Dragendorff's test):** 1 ml extract and few drops of Dragendorff's reagent were added. Formation of yellow precipitate indicates the alkaloids present. (Waldi, 1965)
C. **Estimation of flavonoids (Shinoda test):** Few ml of

extracts and few fragments of magnesium turnings and then drops wise concentrated HCL was added. Pink colour was observed indicates the presence of flavonoids (Mace, 1963).

D. Estimation of saponins (Foam Test): 2 ml Extracts and 4 ml of distilled water was added. Vigorously shaken in a test tube for few minutes. Formation of foam indicates the of saponins (Kokate, 1999).

E. Estimation of Tannins: 1 ml of cool filtrate is taken and distilled upto 5ml (Distilled water), few drops of 10% FeCl3 was added. Bluish black or brownish green precipitates indicates the presence of Tannins.

Analytical analysis

1. HPTLC study

- Standard solution preparation: Standard was prepared in methanol 1:40 mg/ml.
- Sample preparation: 1mg/10ml sample was prepared.
- HPTLC plate images in 254nm and 366 nm is attached.
- From the study of it is found that 4.70µg of standard compound (Berberine) is present in 100mg sample.

2. FTIR study

Fourier transform infrared spectroscopy (FTIR) is a detects the infrared spectrum of absorption, emission, and photoconductivity of solid, liquid, and gas. It`s spectrum is recorded between 4000 and 400 cm^{-1} [5] Each and every functional group has its own disparate vibrational energy, this energy is used to identify a molecule through the combination of all of the functional groups [6]. Sample absorbs infrared rays at characteristic frequencies depending on its bond strength and structure. Transmitted part of beam enters the detector for measurement of interferogram signals, which is finally processed by computers providing the absorption data.

For analysis of fine powder of *Tinospora cordifolia* root, universal attenuated total reflectance (UATR) technique is used, as it is easy and quicker in implementation. FTIR analysis was done using PerkinElmer spectrum.

The interpretation of peaks is summed up in Table 07.

3. UV-Visible analysis

UV-Vis spectroscopy is an analytical technique that measures the number of discrete wavelengths of UV or visible light absorbed or transmitted by a sample compared to a blank or reference sample [7]. This property is influenced by the composition of the sample, which can provide information about what is in the sample and in what concentration. Here, UV spectroscopy of methanolic extract of *Tinospora cordifolia* root is performed. The extract was prepared using cold maceration method in the ratio 1:10 and then subjected to 6 serial dilutions. The extract was analyzed (Analytical Jena specord plus spectrophotometry) and wavelength of maximum absorption was noted and compared with the wavelength of Berberine.

The absorption spectrum is presented in graph 03. From the analysis it is evident that maximum UV absorption was obtained at the wavelength 212 nm.

Result & Discussion

Image 01: HPTLC plates

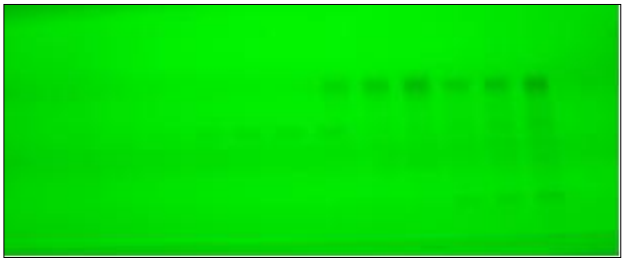


Fig 1: Visualized at wavelength of 254 nm

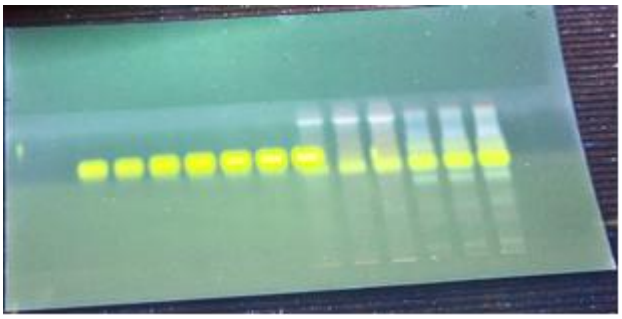


Fig 2: Visualized at wavelength of 366 nm-

Table 1: Physic-chemical analysis

S. No	Parameters	Calculated values
1.	Total Ash	7.75%
2.	Acid- insoluble Ash	0.9%
3.	Alcohol soluble extractive values	6.85%
4.	Water soluble extractive	13.2%
5.	LOD	7.9%

Table 2: Phytochemical analysis

S. No	Tests	Extract	
		Alcoholic (Methanol)	Chloroform-water
1.	Proteins	Present	Absent
2.	Flavonoids	Present	Absent
3.	Tannins	Present	Absent
4.	Saponin	Absent	Present
5.	Alkaloids	Present	Absent

Table 3: HPTLC fingerprint data

Botanical name	*Tinospora cordifolia* Willd Hook Miers
Family	Menispermeaceae
Part studied	Root
Chemical constituents	Berberine, Tinosporide
Mobile phase	Butanol: Ethyl acetate: acetic acid: water
Stationary phase	Merck, TLC Silica gel 60 F_{254} (100mm*100 mm)
Sample applied	2 -6µl of Berberine standard (in gradually increasing dose) 2 µl and 5 µl of sample.
Extract	Methanolic extract (1:10) prepared by cold maceration method.
Detection	254 nm and 366 nm
Derivatization	Not required.

Table 4: Observations of HPTLC study

Track Assignment

Track	Vial ID	Description	Volume	Position	Type
1	1	Berberine	2.0 µl	N/A	Reference
2	1	Berberine	3.0 µl	N/A	Reference
3	1	Berberine	4.0 µl	N/A	Reference
4	1	Berberine	5.0 µl	N/A	Reference
5	1	Berberine	6.0 µl	N/A	Reference
6	2	TCR	2.0 µl	N/A	Sample
7	2	TCR	5.0 µl	N/A	Sample

Table 5: Rf values

Substance Berberine 1 (R_F 0.353 +/- 0.010):

Track	R_F	X (mm)	Y (mm)
1	0.358	15.0	30.2
2	0.358	25.4	30.2
3	0.360	35.8	30.3
4	0.358	46.2	30.2
5	0.353	56.6	29.9
6	0.350	67.0	29.7
7	0.353	77.4	29.9

Table 6: Result of HPTLC with beriberi's ne as marker compound

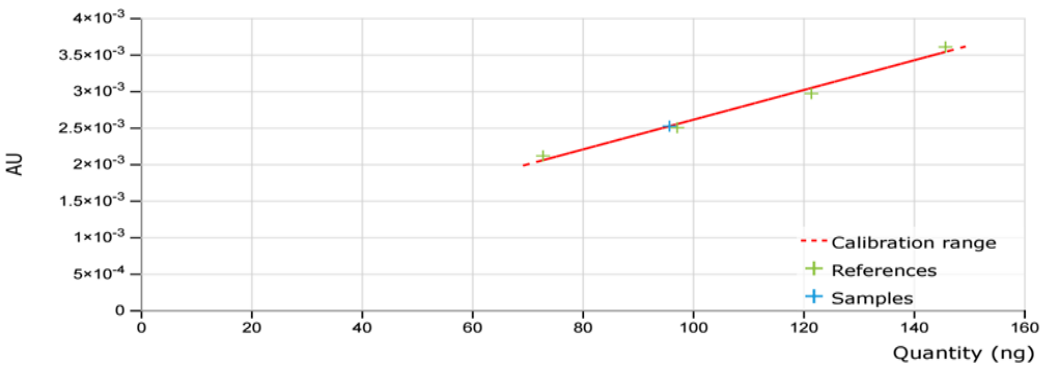
	Rf	Colour of band	Quantity
Berber in	0.35	Yellow	24.30 ng/ml
T. Cordifolia	0.35	Yellow	47.90 mcg/100 mg

Table 7: Interpretation of FTIR analysis [8]

Peak wavelength	Appearance	Group	Compound class
3330.47	Medium	N-H stretching	Secondary amine
1624.21	Medium	C=C stretching	Conjugated alkene
1317.44	Strong	C-F stretching	Fluorine compound
1030.95	Medium	C-N stretching	Amine

Calibration results:

Area calibration for substance Berberine 2 @ 254 nm:

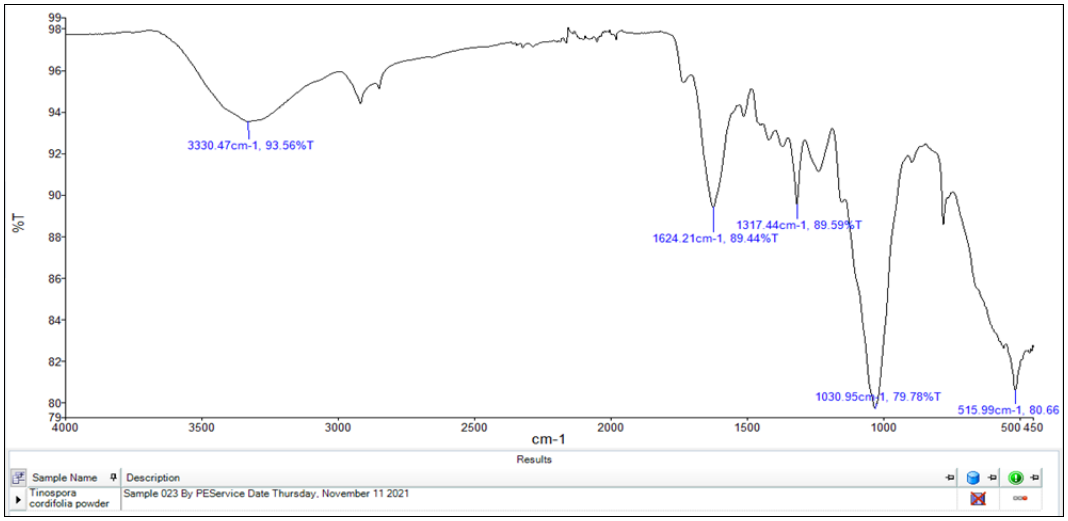


Regression mode	Linear-2
Range deviation	5.00 %
Related substances	Default
Number of references	4
Calibration function	$y=2.034\times10^{-8}x+5.698\times10^{-4}$
Coefficient of variation	CV 2.28 %
Correlation coefficient	R=0.993430

Results:

Berberine 2	(1 sample assignments) @ 254 nm		
Sample '2'	47.90 µg/ml	(CV unavailable)	(1 applications)
			47.90 µg in 100.00 mg
Volume: 2.0 µl	47.90 µg/ml	(CV unavailable)	(1 replicas)
Track 6	47.90 µg/ml	95.79 ng	

Graph 1: Calibration curve



Graph 2: FTIR analysis

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