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Pharmacognostical and Phytochemical Analysis of Dry and Wet *Guduchi* (Tinospora cordifolia Willd. & Miers. ex Hook. F. & Thoms.)

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ABSTRACT

Ayurveda is complete science of life practised with an aim of preservation & promotion of health. *Acharya Charaka* has emphasised that any material on earth is valuable in the war against diseases when used with preparation and for a particular reason. *Guduchi* (*Amrita, Giloy*) is the best natural antioxidant which is copiously available all over India. All of its parts are used in traditional medicine system, stem leaves and roots are most important medicinally used parts. It is a versatile resource for all forms of life. It belongs to family *Menispermaceae*. It contains various chemicals that affect the body. Some of these chemicals have an antioxidant impact. While other might increases the ability of body's immune system and some chemicals have activity against cancer cells also. The '*Rasayana'* accords longevity, enhance the memory, improve the health, bestows youth, speech, energy and lustre the skin. So it is one of the most effective *Rasayana* and rejuvenative.

Key Words: *Guduchi, Tinospora cordifolia, Dry and Wet Guduchi*

INTRODUCTION

Guduchi [*Tinospora cordifolia* (Willd.) Miers ex Hook. F. &Thoms.] is one of the important medicinal plant in *Ayurveda*. It belongs to *Menispermaceae* family. *Guduchi* is a large spreading, smooth, perennial, deciduous, climbing shrub scattered throughout India and South Asia. It is also known as *Giloy, Gulancha, Gulbel* in *Hindi, seendal* in Tamil, *amrutaballi* in *Kannad, Golancha* in Bengal, *Amritu* in *Malyalam*[1]. *Guduchi* is a *Sanskrit* word which implies 'it protects body from diseases'[2]. Other synonyms of *Guduchi* are *Cinnodbhava* which means this can be propagated through stem cutting, *Amrita* means one who does not perish easily, *Jwaranashini* means which have antipyretic property[3]. It has many medicinal properties such as anti-inflammatory, anti-diabetic, anti-arthritic, antioxidant, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, anti-allergic and immuno-modulatory activities[4]. It contains mainly Berberin, Giloin, and Tinosporine etc.

TAXONOMICAL CLASSIFICATION OF*GUDUCHI* [*Tinospora cordifolia* (Willd.) Miers Ex Hook. F. &Thoms]

Kingdom - Plantae

Sub kingdom - Tracheobionta
Division - Spermatophyta
Sub division - Angiospermae
Class - Dicotylodeneae
Subclass - Rosideae
Family - Menispermaceae
Genus - *Tinospora*
Species - *cordifolia*

BOTANICAL DISTRIBUTION[5]

Habitat - Throughout tropical regions of India.

Habit - Climbing Shrub

Leaves - Broadly ovate, cordate and long petiolate.

Flowers - Axillary and terminal racemes or racemose panicles; male flowers clustered, females usually solitary

Fruit - Drupes ovoid or subglobose, glossy, red, pea-sized

Seed - White, bean shaped, warty

MATERIALS AND METHODS

Test sample-

Guduchi Tinospora cordifolia (Willd. Miers) was collected from National Institute of Ayurveda, Jaipur, India in the prescribed month for collection of drug before the commencement of rainy season. The *Guduchi* plant was taxonomically specified and authenticated by Botany Department, University of Rajasthan, with reference number RUBL211729.

PHARMACOGNOSTICAL STUDY:

- **Macroscopic study-**

Macroscopic study was carried on the basis of Morphological characters such as colour, odour, taste, size fracture and findings were recorded.

- **Powder microscopy[6]-**

Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials. For examining the characters of the powder take sufficient amount of powder sample in different chemical reagents on a slide and warm over a low flame for a short time. Put drop of glycerine on the slide, cover it with the cover slip and observe under the microscope.

PHYSICO-CHEMICAL PARAMETERS:

DETERMINATION OF MOISTURE CONTENT[7]

The moisture content was measured by putting weighed powder sample of 5gm of drug in oven at 105^{o}C for 5 hours, and weight estimation of sample per every 30 minute, until the weight of the samples came out to be constant, no variation of weight was recorded. This sample was allowed to cool in desiccators at room temperature for 1 hour prior to weighing.

DETERMINATION OF PH[8]

The aqueous liquid's pH value can be defined as the usual reciprocal hydrogen ion concentration expressed in gram per litre. It means in fact, quantitative indicator of a solution's acidic or basic essence.

DETERMINATION OF EXTRACTIVE VALUES

Determination of Alcohol Soluble Extractive

A coarsely powdered medicine of 5 gm was macerated with 100 ml of alcohol of the specified

strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and permitted to stand for eighteen hours. The content was filtered using filter paper. The filtrate was moved to a pre-weighed flat bottomed flask and evaporated to dryness on a water bath. Then the dish was kept in oven at 105ºC, to constantly. In reference to the product being dried in air, the proportion of alcohol-soluble extractive was determined.

Determination of Water Soluble Extractive:

A coarsely powdered medicine of 5 gm was macerated with 100 ml of water of the specified strength in a closed flask for twenty-four hours. Continuously stir for six hours using rotary shaker and permitted to stand for eighteen hours. The content is filtered using filter paper. The filtrate is moved to a flat bottomed dish that is pre-weighed and evaporated to dryness on a water bath. Then the dish is kept in oven at 105ºC, to constant weight and weigh. The proportion of water-soluble extractive was determined in reference to the substance being dried in air.

DETERMINATION OF ASH VALUE

Determination of Total Ash[9]

Weighed accurately 5 gm of powdered sample in the silica crucible. The drug was spread evenly in to a thin layer. This crucible was put in a muffle furnace and burned for around 6 hrs or more at a temperature of 450°C, until the ash was fully Carbon free. It was allowed to be cool the ash containing crucible in desiccators and eventually weighed to a constant weight.

Determination of Acid Insoluble Ash

Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the complete ash for 5 minutes with 25 ml of 2M hydrochloric acid, gathered the insoluble ash in a Gooch's crucible or on an ash less filter paper, cleaned with hot water, burned, cool in desiccators and weighed. Calculate the acid - insoluble ash ratio in regard to the drug being air dried.

Determination of Water soluble Ash

Water soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the complete ash for 5 minutes with 25 ml of water; gathered the insoluble substance in a Gooch's Crucible or on an ash less filter paper, cleaned with hot water and burnt at a temperature not exceeding 450^0C for 15 minutes. Subtract the weight of insoluble matter from weight of the ash; the weight differential reflected the water soluble ash. Calculate the water soluble ash percentage in regard to the drug being air dried.

PRELIMINARY PHYTOCHEMICAL ANALYSIS[10]:

The phytochemical analysis of this plant was performed for the detection of active constituents i.e. carbohydrates, amino acid, steroids, alkaloids, protein, saponin, tannin and glycosides.

Tests for Carbohydrates

- ❖ Molisch test
- ❖ Benedict test
- ❖ Fehling solution test

- **Molisch Test:**

2 ml of test Solution was placed in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then around 1ml. of conc.

H_2SO4 is poured from the sides of the test tube and permitted to stand for one minute. The existence of Carbohydrates is indicated by a Purple colour ring was developed at the junction of two layers.

- **Benedict test:**

This is a test for reducing sugars and is composed mainly of $CuSO_4$ and NaOH. Added 1 ml of Benedict's solution to the 4 ml of aqueous drug solution, then heated almost to simmer. Formation of yellow, orange, green and red/brown colour due to the cuprous oxide formation in order of increase the consolidation of simple sugar in the test solution.

- **Fehling solution test:**

In general, it is used for reducing sugars and consists of two solutions that are mixed in situ. Fehling solution A consists of 0.5% of $CuSO_4$ while Fehling solution B consists of Sodium Potassium Tartarate.

Equal amounts of the solutions Fehling A and Fehling B were mixed and 2 ml of aqueous solution of drug was added and then boiled for 5-10 minutes in water bath.

Tests for Alkaloids

- ❖ Dragendorff 's reagent test
- ❖ Wagner Test
- ❖ Hager Test

- **Dragendorff's reagent test:**

2 ml of test Solution was added in a test tube in which 2 ml of the Dragondorff's reagent was added. An orange precipitate if formed indicated presence of Alkaloids.

- **Wagner Test:**

Drug solution +few drops of Wagner's reagent (dilute Iodine solution), formation of reddish-brown precipitate.

- **Hager Test:**

For this test a saturated picric acid aqueous solution was used. When this reagent was combined with the test filtrate, an orange yellow precipitate was obtained that suggests the existence of alkaloids.

Test for Amino acids

- **Ninhydrin Test:**

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. When heated with ninhydrin molecules, the protein solution produces a distinctive deep blue or pale yellow colour due to the formation of complex between two free amino acid ninhydrin molecule and nitrogen.

Tests for Proteins

- ❖ Biuret test
- ❖ Xanthoprotic test
- ❖ Millons test

- **Biuret Test:**

In water, a few mg of the residue was taken and 1 ml of 4% sodium hydroxide solution was added to it, accompanied by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

- **Xanthoprotic test:**

A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour shows the presence of proteins.

- **Millons test:**

A small quantity of test sample was taken and 2 to 3 ml of millons reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

Test for Saponin

- **Foam test:**

A small volume of the test sample was taken and shook into a test tube strongly with a small volume of water and sodium bicarbonate. A stable, characteristic honeycomb like froth shows the presence of saponins.

Test for glycosides

- **Borntragor's Test:**

In the ethanolic extract, 1 ml of benzene and 0.5 ml of dilute ammonia solution was applied and a reddish pink colour was observed.

Test for Phenolic Compound

- **Phenolic test:**

The extract was taken in water and warmed; 2 ml of ferric chloride solution was added to the extract, and observed green and blue colour formation.

Test for Flavonoids

- **Shinoda test:**

A small volume of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with several drops of concentrated HCl and 0.5 gm of Mg metal. Appearance or two indicates. The presence of flavonoids suggests the emergence of pink, magenta or crimson colour within a minute or two.

Test for Steroids

- **Salkoweski reaction test:**

In 2 ml of chloroform, a few mg of extract was taken and 2 ml of concentrated H_2SO_4 was added from the side of test tube. Stirred the test tube for few minutes. Red colour appearance shows the presence of steroids.

Test for Tannins

- ❖ Ferric chloride solution
- ❖ Lead acetate
- ❖ Pot. Dichromate

- **Ferric chloride solution:**

A 5 percent ferric chloride solution in 90 % alcohol was prepared. Few drops have been added to a bit of the above filtrate from this solution. The emergence of dark green or deep blue colour shades shows the presence of tannins.

- **Lead acetate:**

The filtrate was combined with 10% w/v solution of basic lead acetate in sterile water. Development of precipitate shows the presence of tannins.

- **Pot. Dichromate:**

Potassium dichromate solution was mixed to the filtrate. Appearance of dark colour suggests tannins are present.

Thin Layer Chromatography[11]

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator, (Mercks) were used. Each plate having dimension 10 cm long and 2 cm width.

Activation of pre-coated Silica gel 60 F_{256} –

Plates were dry over one and half hours in hot oven at 1050 C.

Mobile solution preparation

Toluene: Ethyl Acetate (9: 1)

RESULTS

In the present study of *Tinospora cordifolia* (Willd. Miers) were evaluated for its physico-chemical and phytochemical aspects.

PHARMACOGNOSTICAL STUDY:

- **Colour**- Yellowish Light brown
- **Odour**- Odour Less
- **Taste**- Bitter

Powder Microscopy (Figure 1)

THIN LAYER CHROMATOGRAPHY:

Thin layer Chromatography is a tool for separation and identification of chemical constituent present in herb with mobile solution- Toluene: Ethyl Acetate (9: 1)

R_f VALUE OF SAMPLES:

Dry sample of *Guduchi*

Alcoholic extract -0.96, 0.91, 0.87, 0.82, 0.79, 0.34, 0.12

Aqueous Extract -0.93, 0.86, 0.74, 0.55, 0.25, 0.16, 0.11

Wet sample of *Guduchi*

Alcoholic extract - 0.98, 0.91, 0.87, 0.83, 0.82, 0.79, 0.75, 0.48, 0.129

Aqueous Extract - 0.77, 0.69, 0.56, 0.32, 0.25, 0.09

Guduchi

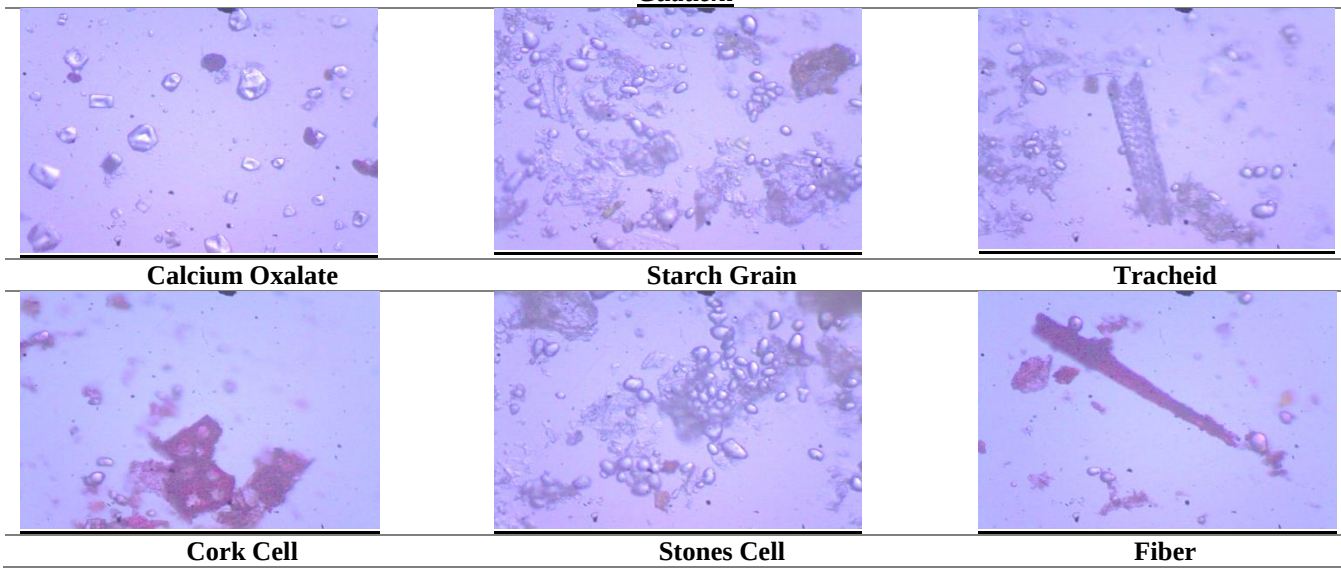

Figure 1 Powder Microscopy of stem of *Tinospora cordifolia* (Willd & Miers.)

DISCUSSION

Pharmacognostical study shows yellowish light brown colour, odour less and bitter taste. Calcium oxalate crystals, tracheid, starch grains, cork cells, stone cells and fibres were found in powder microscopy (as mentioned in fig.1). The outcome of physicochemical analysis is as follows: moisture content of *Tinospora cordifolia* (Willd. Miers) stem, dry sample is 4.3% and wet sample is 5.0%, dry drug's extractive value of water soluble extract is 16.63% and wet sample is 11.9%, dry drug's extractive value of alcohol soluble extract is 5.95% and wet sample is 7.25%, total ash of dry sample is 12.56% and wet sample is 8.21%, acid insoluble ash of dry drug is 9.23% and wet drug is 5.67%, water soluble ash for dry drug is 2.94% and wet drug is 3.12% (as mentioned in table no.1).

PHYSICO-CHEMICAL ANLYSIS OF *GUDUCHI* STEM

Table 1 Physico-chemical analysis of *Tinospora cordifolia* (Willd. Miers.)

S.N.	PARAMETERS	RESULT		Reference (A.P.I.)
		Wet	Dry	

1.	Moisture Content (%)	5.0 %	4.3%	-
2.	Ph	6.1	5.9	-
3.	Water Soluble Extractive (%)	11.9%	16.63%	NLT 11%
4.	Alcohol Soluble Extractive (%)	7.25%	5.95%	NLT 3%
5.	Total Ash (%)	8.21%	12.56%	NMT 16%
6.	Acid Insoluble Ash (%)	5.67%	9.23%	NMT 16%
7.	Water soluble Ash (%)	3.12%	2.94%	-

Numerous phytochemicals have been found in both extracts during the preliminary phytochemical screening. Aqueous extract of *Tinospora cordifolia* (Willd. Miers) stem shows the presence of carbohydrates, alkaloids, amino acids, proteins, saponins, glycosides, flavonoids, steroids and tannins. Alcoholic extract of *Tinospora cordifolia* (Willd. Miers) stem showed the existence of carbohydrates, alkaloids, protein, flavonoids and glycosides in wet sample (as mentioned in table no.2).

PHYTOCHEMICAL ANALYSIS OF *GUDUCHI* STEM:

Table 2 Phytochemical tests of extracts of *Tinospora cordifolia* (Willd. Miers.)

S.N.	NAME OF TEST	DRY		WET	
		Aq. Extract	Alc. Extract	Aq. Extract	Alc. Extract
1.	**CARBOHYDRATE**				
	Molisch test	+ve	+ve	+ve	+ve
	Benedict test	+ve	-ve	-ve	-ve
	Fehling test	+ve	+ve	+ve	+ve
2.	**ALKALOIDS**				
	Dragendorff test	**+ve**	**+ve**	**+ve**	**+ve**
	Wagner's test	**-ve**	**-ve**	**-ve**	**-ve**
	Hager's test	**-ve**	**-ve**	**-ve**	**-ve**
3.	**AMINO ACIDS**				
	Ninhydrine	**+ve**	**-ve**	**+ve**	**-ve**
4.	**PROTEIN**				
	Biuret test	**-ve**	**+ve**	**-ve**	**+ve**
	Xanthoprotictest	**+ve**	**+ve**	**+ve**	**+ve**
	Millon test	**+ve**	**+ve**	**+ve**	**+ve**
5.	**SAPONIN**				
	Foam test	**+ve**	**-ve**	**+ve**	**-ve**
6.	**GLYCOSIDES**				
	Borntrager's test	**+ve**	**-ve**	**+ve**	**+ve**
7.	**PHENOLIC COMPOUND**				
	Phenolic test	**-ve**	**-ve**	**-ve**	**-ve**
8.	**FLAVONOIDS**				
	Shinoda test	**+ve**	**+ve**	**+ve**	**+ve**
10.	**STEROIDS**				
	Salkowaski	**+ve**	**-ve**	**+ve**	**-ve**
11.	**TANNINS**				
	$Fecl_3$	**-ve**	**-ve**	**-ve**	**-ve**
	Lead acetate	**+ve**	**-ve**	**+ve**	**-ve**
	Pot. Dichromate	**-ve**	**-ve**	**-ve**	**-ve**

CONCLUSION

Dry drug's extractive value of water soluble extract is more as compared to wet drug. While wet drug's extractive value of alcohol soluble extract is more as compared to dry sample. Value of total ash and acid insoluble ash is high in dry

drug and water soluble ash value is high in wet sample.

The existence and quantification of bioactive principles will be useful for phytochemical screening and analysis and can contribute to drug discovery and production. Our research indicated the existence of important medicinal components within the studied species. This plant's phytochemical analysis can be useful for developing new, more effective and advanced drugs. *Guduchi* stem extract includes carbohydrates, alkaloids, amino acids, proteins, saponins, glycosides, flavonoids, steroids and tannins.

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