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Tinospora cordifolia (Wild) Hook.f. (Thomas) grown in Sri Lanka: Pharmacognostical, physico- chemical and phytochemical analysis of the stem

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ABSTRACT

Tinospora cordifolia (wild) Hook.f. (Thomas) is an important medicinal plant distributed throughout in Sri Lanka and commonly known as Rasakinda in Sinhala and Giloy in English. It is widely used in Sri Lankan traditional medical system and Ayurveda for the treatment of diabetes mellitus, fever, arthritis, skin diseases and for Rasayana (rejuvenating) therapies due to its anti-inflammatory, hypoglyceamic, immunomodulatory, antioxident, anti-allergy, antipyretic, antiarthritic and various other medicinal properties. Imported *T. cordifolia* stems and Sri Lankan grown *T. cordifolia* stems can be found in Sri Lankan herbal market. Moreover, different varieties of Tinospora species known as Tikthakinda, Bukinda and Gatakinda are available in the market under the name of Rasakinda which leads to adulteration. Hence, in this research an attempt was made to develop standards for genuine *T. cordifolia* grown in Sri Lanka. Stems of *T. cordifolia* evaluated for macroscopical, microscopical, physico-chemical, phytochemical constituents, TLC and HPTLC fingerprint patterns. Microscopical examinations of *T. cordifolia* stem exhibited the wheel shaped appearance at the transverse cut surface, which is a main characteristic feature of the family Menespermaceae. Stem also showed the mucilage cells and abundant starch granules. Percentages of total ash, water soluble ash and acid insoluble ash, were 9.1%+0.1, 2.31%+0.1, <0.1% respectively. Among the percentages of extractable matter of *T. cordifolia*, highest amount was shown in hot water extract (16.2 %+0.3). Heavy metals (Hg, As, Cd, Pb) were within the limits given in WHO guidelines. Phytochemical screening revealed the presence of phenols, saponins, tannins, steroids, flavonoids, terpenoids and cardiac glycosides. TLC fingerprint of *T. cordifolia* was developed using butanol: ethyl acetate: acetic acid: water in a ratio of 5: 8: 6:2 v/v and compared with one of its marker compound, Berberine. The HPTLC fingerprint patt ern of *T. cordifolia* showed a spot bearing the same Rf value corresponds to Berberine, at wavelength 254 nm. In conclusion, the results obtained from this study can be used as a standard reference for Sri Lankan grown *T. cordifolia* stems.

Keywords: *Tinospora cordifolia*, Rasakinda, Pharmacognosy, Phytochemicals.

INTRODUCTION

Tinospora cordifolia (Wild) Hook.f. (Thomas) is an important medicinal plant which is extensively used in Sri Lankan traditional system of medicine and Ayurveda. This plant is known as *Rasakinda* in Sinhala and Giloy in English. *T. cordifolia* is a large perennial climbing plant belonging to family Menispermaceae. It is a glabrous, succulent, woody climbing shrub grown in India, Sri Lanka and Burma. It grows well in the tropical region, often attains a great height and climbs up the trunks of large trees. The stem is gray or creamy white, deeply cleft spirally and longitudinally, with the space between spotted with large rosette-like lenticels. The wood is white, soft and the freshly cut surface quickly assumes a yellow tint when exposed to air. Leaves are simple, alternate, long petiolate, chordate in shape showing multicoated reticulate venation. Long threads like aerial roots come up from the branches. Flowers are small and Unisexual. Male flowers are in clusters and female flower are solitary. Aggregate fruit is red, fleshy, with many drupelets on thick stalk. (Jayaweera, 1982) [8].

In Sanskrit, this plant is commonly known as Amruta and Guduchi, which refers to the 'heavenly elixir' and 'protect from the diseases' respectively. In Ayurveda it is designated as Rasayana (rejuvenating) drug recommended to enhance general body resistance and promote longevity (Joshi and Kaur, 2016) [9]. This plant is well known for its adaptogenic and immunomodulatory activities. (Kalikar *et al.,* 2008) [10]. It is mainly used for the treatment of diabetes mellitus, fever, rheumatism, skin diseases and for rejuvenating therapies in Sri Lankan traditional medical system and Ayurveda, (Sharma, 1998; Compendium of Medicinal Plants, 2004) [17, 2]. Modern scientific findings revealed that *T. cordifolia* possess anti-inflammatory, hypoglyceamic, immunomodulatory, antioxidant, anti-allergy, anti-pyretic, anti-arthritic,

anticancer, anti-tumor, antimicrobial anti-HIV and various other medicinal properties (Sharma *et al.*, 2019) [16].

Authentic Ayurveda text books have mentioned three varieties of Tinospora which can identified in Sri Lanka as well as in India. The other three varieties of *Tinospora* known as Tiktakinda (*T. crispa*), Bukinda (*T. malabarica*) and Gatakinda (*T.sinensis*) belong to family Menespermaceae (Jayaweera, 1982) [8]. These three varieties of *Tinospora* are available in the local market under the common name of Rasakinda. Using different varieties may be affect the efficacy of the drug and be a problem in treatment. Hence, developing quality parameters for the identification of genuine raw material is essential. Therefore, the main objective of this study was to characterize pharmacognostical, physicochemical and phytochemical parameters along with Thin Layer Chromatography (TLC) fingerprinting for *T. cordifolia* which is grown in Sri Lanka.

METHODOLOGY

Collection and identification of raw materials

Fresh parts of *T. cordifolia* stem were collected from Himbutana area of Colombo district, Sri Lanka. Stems were authenticated by the Curator of National Herbarium of Peradeniya, Sri Lanka and Department of DravyagunaVignana, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Macroscopical and microscopical examinations

Stem of *T.cordifolia* was macroscopically and microscopically identified according to characters mentioned in Ayurveda Pharmacopeia of India (1989).

Physico-chemical studies

Physicochemical studies of the dried stem were done according to the WHO (2011) herbal drug standardization guidelines. Hot and cold water extractable matter, hot and cold methanol extractable matter, moisture content, total ash, acid insoluble ash and water soluble ash and heavy metal residues were determined.

Preparation of extracts

Water extract: powder sample (10 g) of *T. cordifolia* stem was added to a round bottom containing 50 ml of water and refluxed for 4 h. Then filtered and filtrated was concentrated using a rotary evaporator up to 15 ml.

Methanol extract: powder sample (10 g) of *T. cordifolia* stem was added to a round bottom containing 50 ml of methanol and refluxed for 4 h. Then filtered and filtrated was concentrated using a rotary evaporator up to 15 ml.

Qualitative phytochemical analysis

Phytochemical analysis for hot water extract and hot methanol extract of *T. cordifolia* stems was done to detect presence or absence of alkaloids, phenols, terpinoids, flavonoids, steroids, saponins, cardiac glycosides using standard procedures described by Goveas (Goveas and Abraham, 2014) [6] and Dahanayake and co-workers (2019a) with some modifications.

Development of Thin Layer Chromatography fingerprint

TLC fingerprint of methanolic extract of *T. cordifolia* was developed using butanol: ethyl acetate: acetic acid: water in a ratio of 5: 8: 6:2 v/v and compared with one of its marker compound, Berberine. In addition, HPTLC fingerprint patterns for methanolic extract of *T. cordifolia* and Berberine were obtained.

Statistical analysis

All data were expressed as Mean ± SEM.

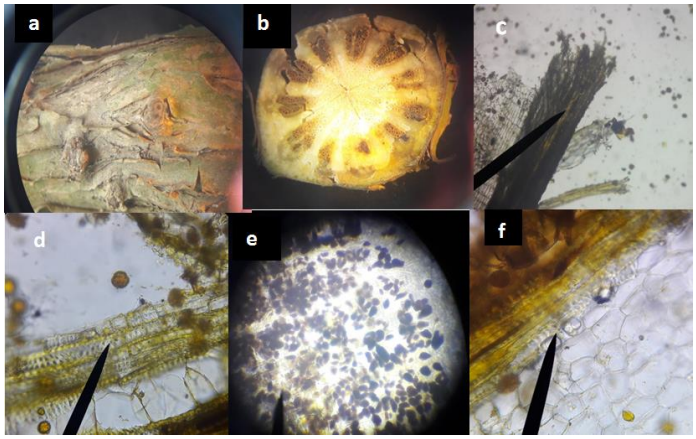
RESULTS AND DISCUSSION

Identification of the genuine plant is an important step of herbal drug standardization. It is necessary to obtain quality and effective drug. Bioassays related to herbal drugs plays a significant role in the process of herbal drug standardization. Pharmacognostical studies, detection of various physico-chemical parameters such as ash values, extractive values in different solvents and phytochemical parameters are being used to standardize the herbal drugs (Folashade *et al.,* 2012) [5]. Therefore, pharmacognostical studies are more important at the beginning of the manufacturing process of the herbal drugs for the identification of genuine plant materials. Currently, herbal materials are easily adulterated with plants of same species and also with low quality plant materials due to the high demand of natural medicines. This condition also worsens by the incorrect identification of plant materials due to lack of knowledge about the medicinal plants. Therefore, an examination to determine macroscopic and microscopic characteristics of the usage parts of the plant is the first step of establishing the identity and purity of plant materials and it should be carried out before any tests are undertaken (WHO, 2011). Macroscopic examination of *T. cordifolia*: The pieces of stems are cylindrical and varying thickness ranging from 0.7-5cm. Fresh stems covered with ash-green colour papery bark which is easily peeling off. When removing the bark mature fresh stems have light brown surfaces with warty protuberances due to circular lenticels. Transverse section of the stem shows radial structure with visible medullary rays traversing porous tissues. Microscopic examination of *T. cordifolia***:** Transverse section of the stem shows 2-3 layers of cork cells and cork broken at some places due to opening of lenticels. Cortex is wide and contains mucilage cells. Cork cells are polygonal and thick walled filled with plenty of starch grains. Starch grains are simple or ovoid in shape. Vascular zone composed of more wedge shaped strips of xylum. Pitted trachidal vessel can be seen in xylum region (Figure 1). Therefore, anatomical features of *T. cordifolia* may help to establish the botanical identity. Identification of vegetable materials can be done accurately by using comprehensive histology (Metcalfe and Chalk, 1979) [12]. This applies in identification of adulterants and substitutes of the raw materials and help to detect genuine raw materials. *T. cordifolia* is an important medicinal plant in Ayurveda and Sri Lankan traditional system of medicine. Therefore, correct pharmacognostical identification is need of time. This pharmacognostical study will provide reference for identification of *T. cordifolia* which is grown in Sri Lanka. According to the anatomical features mentioned by Meena and co-workers (2010) [11], revealed that the Indian verity of *T. cordifolia* show simple to 3-compound starch grains and prismatic crystals of calcium oxalate in the stem (Meena *et al*., 2010) [11]. Is it same in Sri Lankan variety of *Tinospora cordifolia*.

Percentages of total ash, water soluble ash, acid insoluble ash, water extractable matter and ethanol extractable matter were summarized in Table 1. Among the percentages of extractable matter of *T. cordifolia*, highest amount was shown in hot water extract (16.2 %±0.3) followed by cold water extract (7.0% ±0.1). Extractable matter of cold methanol extract (3.7% ±0.7) was almost similar to that of hot methanol (4.0% ±0.3). When preparing decoction from *T. cordifolia*, hot water extraction technique is used. Hence, these values proved that decoction contain more extractable matter. These amounts are also comparable with the information mentioned in Ayurveda Pharmacopia of India (1989) pertaining dry stems of *T. cordifolia*. According to API, Alcohol soluble extractives should not be less than 3 % and water soluble extractives should not be less than 11 % (Ayurveda Pharmacopia of India, 1989). The total ash usually consists of both physiologic ash and non-physiologic ash (eg. carbonates, phosphates, silicates and silica). The water soluble ash indicates the amount of inorganic elements in the sample and acid insoluble ash indicates the presence of silica in the plant materials. The high values of acid insoluble ash point toward the high contaminants with soil materials. The total ash, water soluble and acid insoluble ash contents of *T.cordifolia* (Table 1) are comparable with the reference values mentioned in Ayurveda Pharmacopia of India (1989). The Inductively Couple Plasma - Mass Spectrometry (ICP-MS) was used to analyze the heavy metals (Pb, Cd, As, Hg) in *T. cordifolia* stem powder. The levels of heavy metals were summarized in Table 2. Medicinal plants can present health risks due to the presence of toxic metals such as Pb, Cd, As and Hg which are hazardous to humans. According to the WHO guidelines, heavy metals in herbs or herbal medicines should be within the limits mentioned by WHO. Heavy metals are stored in different parts of the plant which enter through the biological cycle of the plant. The concentrations of Pb, Cd, As and Hg analysis in the stem powder of the *T. cordifolia* plant using ICP-MS were found to be within the acceptable limits.

T. cordifolia is often cultivated for their stems and it is rich in bioactive compounds. Bioactive compounds such as alkaloids, saponins, tannins, phenols, cardiac glycosides, flavanoids, and terpenoids have been widely reported to be present in stems (Mgbeahuruike *et al.,* 2017) [13]. The pharmacological activities of these phytochemicals comprise health benefits which help to preventing from diseases and cure the diseases (Zhang *et al.,* 2015) [19]. The qualitative analysis of the phytochemicals in hot water and hot methanol extracts of *T. cordifolia* stems showed the presence of the alkaloids, saponins, tannins, flavanoids, terpinoids, phenols, steroids and cardiac glycosides (Table 3). Phytochemical analysis is very important to determine the quality of drug and to detect therapeutic efficacy. In contrast, according to Grover and co-workers (2013) [7] flavonoids were not detected (by alkaline reagent test, lead acetate test and shinoda test) in petroleum ether and water extracts of *T. cordifolia* stems.

At present, TLC and HPTLC techniques are commonly used for quality assessment in herbal preparations. These methods widely employed in pharmaceutical industry in process of identification, development and in quality control of herbal products (Soni and Navad, 2010; Dahanayake *et al.,* 2019a; 2019b) [18, 3, 4] . However, HPTLC is a modern adaptation of TLC with better and advance separation efficiency and detection limit (Shivatare *et al.,* 2013) [15]. TLC and HPTLC fingerprint patterns of methanolic extract of *T. cordifolia* stem showed the presence of various chemical compounds and compared with the marker compound Berberine, a well known alkaloid (Figure 2 and 3).



(a) bark with warty prominences (b) wheel shape appearance (c) cork cells (d) pitted fibers (e) simple starch grains (f) secretory cells

Figure 1: Anatomical features of *Tinospora cordifolia* stem

Table 1: Physico-chemical parameters and extractable matter of *Tinospora cordifolia* stems

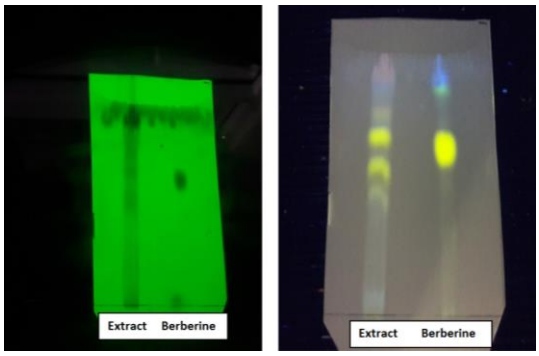
Parameters	Percentages
Total ash content	9.1 % ± 0.1
Water soluble ash content	2.3 % ± 0.1
Acid insoluble ash content	< 0.1 %
Cold water soluble extractable matters	7.0 % ± 0.1
Hot water soluble extractable matters	16.2 % ± 0.3
Cold methanol soluble extractable matters	3.7 % ± 0.7
Hot methanol soluble extractable matters	4.0 % ± 0.3
pH	

Results are expressed as mean ± S.E.M., n=3

Table 2: Heavy metal residues of *Tinospora cordifolia* stems

Heavy metals	Concentration (mg/kg)
Pb	0.2 mg/kg
Cd	< 0.05 mg/kg
As	0.05 mg/kg
Hg	0.06 mg/kg

Results are expressed as mean ± S.E.M., n=3



at 254 nm (b) 366 nm

Figure 2: Thin Layer Fingerprint profiles of methanolic extract of *Tinospora cordifolia* stems and Berberine at (a) 254 nm and (b) 366 nm

Table 3: Phytochemicals of hot water extract and hot methanolic extracts of *Tinospora cordifolia* stems

Phyto constituent	Test	Hot water extract of *T. cordifoilia*	Hot methanol extract of *T. cordifoilia*
Saponins	Frothing test	+	+++
Tannins	$Fecl_3$ test	+++ (Black precipitate)	+++ (Black precipitate)
	$Pb(OAC)_2$ test	+++ (Yellow precipitate)	++ (Yellow precipitate)
Alkaloids	Tannic acid test	++ (Yellow crystalline precipitate)	++ (Yellow crystalline precipitate)
	Picric acid test	+++ (Yellow crystalline precipitate)	+ (Yellow precipitate)
	Wagner test	+++ (Red colour)	+++ (Red colour)
Flavonoids	Ammonia solution + conc. H_2SO_4	+++ (Yellow colour)	+++ (Yellow colour)
Phenols	$Pb(OAC)_2$ test	+++ (Yellow precipitate)	++ (Yellow precipitate)
	Folin reagent test	+++ (Blue colour)	+++ (Blue colour)
Terpinoids	Salkowski test	++ (Reddish brown)	++ (Reddish brown)
	Sesquiterpenes test	++ (Brown green colour)	++ (Brown green colour)
Steroids	LibermanBurchard Test	+++ (Blue green colour)	+++ (Blue green colour)
Cardiac glycosides		Reddish brown ring formed	Reddish brown ring formed

+++ high amounts ++ moderate amounts + low amounts

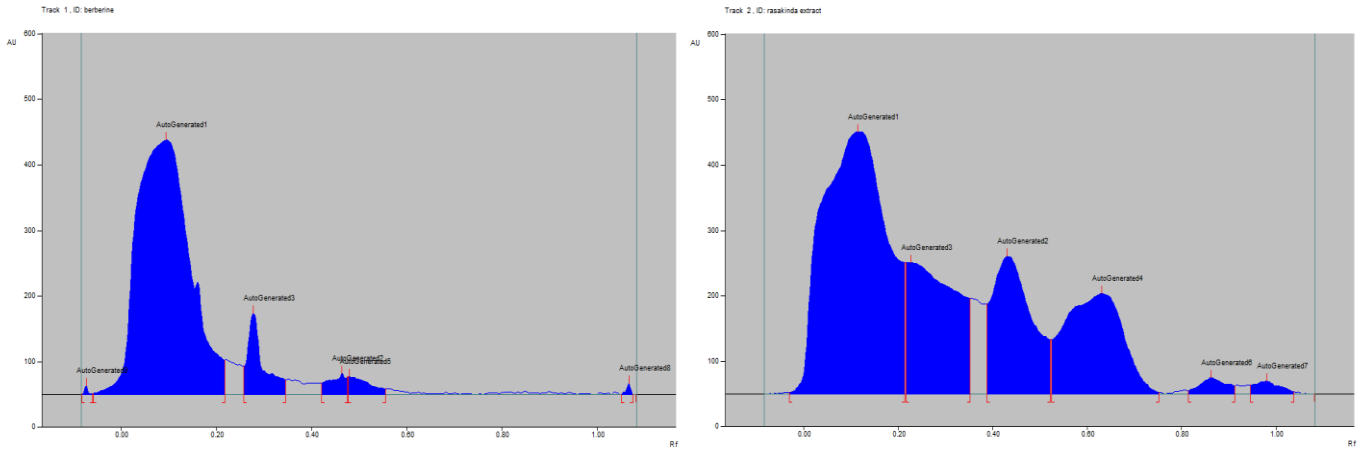


Figure 3: HPTLC Fingerprint profiles of methanolic extract of *Tinospora cordifolia* stems and Berberine

CONCLUSION

Results of macroscopic and microscopic observations, physico-chemical parameters, phytochemical, analysis can be used to identification of genuine drug, assess the quality and detection of any adulteration for *T. cordifolia*. Further, these values can be used as a reference for setting limits for the quality assurance of Sri Lankan grown *T. cordifolia* stems.

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REFERENCES

1. Ayurveda Pharmacopiea of India. Publication of India, 1989.
2. Compendium of Medicinal Plants: A Sri Lankan study. Department of Ayurveda, Sri Lanka, 2004.
3. Dahanayake JM, Perera PK, Galappatty PG, Fernando P, Arawwawala M. Pharmacognostical, physico-chemical and phytochemical evaluation for standardization of three *Piper* species used in Ayurvedic medicine. Asian Journal of Pharmacognosy. 2019a; 3(2):18-28.
4. Dahanayake JM, Perera PK, Galappatty PG, Perera HDSM, Arawwawala LDAM. Comparative Phytochemical analysis and antioxidant activities of Tamalakyadi decoction with its modified dosage forms. Evidence Based Complementary and Alternative Medicine. 2019b, Article ID 6037137.
5. Folashade KO, Omoregie EH, Ochogu AP. Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation. 2012; 4(3):101-112.
6. Goveas SW, Abraham A. Extraction and secondary metabolite analysis of *Coscinium fenestratum* (Gaertn.) Colebr: an Important medicinal plant of Western Ghats. International Journal of Pharmaceutical Sciences and Research. 2014; 5(8):3484-3489.
7. Grover D, Dutta S, Farswan AS. *Tinospora cordifolia*: Pharmacognostical and Phytochemical Screening. Journal of Pharmacy and Research. 2013; 1(1):13-17.
8. Jayaweera DMA. Medicinal plants (Indigenous and exotic) used in Sri Lanka, Part IV. Publication of National Science Council of Sri Lanka. 1982; p.81.
9. Joshi G, Kaur R. *Tinospora cordifolia:* a phyto-pharmacological review. International Journal of Pharmaceutical Sciences and Research. 2016; 7(3):890-897.
10. Kalikar MV, Thawani VR, Varadpande UK, Sontakke SD, Singh RP, Khiyani RK. Immunomodulatory effect of *Tinospora cordifolia* extract in human immuno-deficiency virus positive patients. Indian Journal of Pharmacology. 2008; 40(3):107–110.

11. Meena AK, Singh A, Panda P, Mishra S, Rao MM. *Tinospora cordifolia*: Its bioactivities and evaluation of physicochemical properties. International Journal of Pharmacognosy and Phytochemical Research. 2010; 2(2):50-55.
12. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons. Oxford Clarendon Press. 1979; pp.1–275.
13. Mgbeahuruike EE, Yrjönen T, Vuorela H, Holm Y. Bioactive compounds from medicinal plants. Focus on Piper species. South African Journal of Botany. 2017; 112:54–69.
14. Quality control methods for herbal materials. Publication of World Health Organization, 2011.
15. Shivatare RS, Nagore DH, Nipanikar SU. 'HPTLC' an important tool in standardization of herbal medical product: A review Journal of Scientific and Innovative Research. 2013; 2(6):1086-1096.
16. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon. 2019; 5:e02437.
17. Sharma PV. Dravyaguna Vignana, Volume II, Chaukambha Bharati Academy, 1998.
18. Soni K, Navad T. HPTLC- its application in herbal drug industry. The Pharm Review. 2010; 4:112-117.
19. Zhang YJ, Gan RYG, Li S, Zhou Y, Li A, Xu DP, Li HB. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015; 20(12):21138-21156.

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