



Analytical Profile of an Unparalleled Drug *Tinospora crispa* W.R.T Guduchi

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Guduchi is a highly potential drug which is mentioned in Ayurveda classics. The clinical utility of this drug is widely known. The *Tinospora* species are wrapped under the broad spectrum Guduchi in the Ayurveda classics. *Tinospora* is one of the important genera of the family, consisting of about 15 species. Some medicinally important species includes *T. cordifolia*, *T. malabarica*, *T. tomentosa*, *T. crispa*, *T. uliginosa*, *T. crispa* etc. In the current article the drug *Tinospora crispa* is considered in detail. *Tinospora crispa* is used irrationally by the society owing to its various therapeutic benefits. *Tinospora crispa* (L.) Hook. f. & Thomson (Menispermaceae), found in the rainforests or mixed deciduous forests in Asia and Africa, is used in traditional medicines to treat numerous health conditions.

Materials & Methods: A detailed review of the drug has been carried out through the available literature. After considering the review the analytical profiling of the drug has been done. The phytochemical analyses review of *T. crispa* revealed the presence of alkaloids, flavonoids, and flavone glycosides, triterpenes, diterpenes and diterpene glycosides, cis clerodane-type furanoditerpenoids, lactones, sterols, lignans, and nucleosides. The literary review showed that the crude extracts and isolated compounds of *T. crispa* possessed a broad range of pharmacological

activities such as anti-inflammatory, antioxidant, immunomodulatory, cytotoxic, antimalarial, cardioprotective, and anti-diabetic activities. So, in the current research study a sincere attempt has been made to study regarding the drug *Tinospora crispa* through its analytical profile.

Results: Through this research article able to make out the available literature and the analytical profile of the drug *Tinospora crispa*.

Discussion: The current analytical profile reveals the presence of alkaloids, tannins, flavonoids, saponins etc which directly connects to the various activities like Anti-pyretic, Anti-inflammatory, Antioxidant etc of the drug *Tinospora crispa*.

Conclusion: The current research study points towards the review of the drug and the analytical profile which is beneficial to carry out the next phase of research studies in connection with this drug *Tinospora crispa*.

Keywords: *Tinospora crispa*; guduchi; fever; menispermaceae.

1. INTRODUCTION

Herbs are the sources of crude drugs that are used to treat pathologic conditions, often chronic in nature, or to achieve or retain a state of improved health. Several cultures have distinct uses of plants for the treatment of various diseases (Wyk and Wink, 2004). This traditional knowledge has been vocally passed on through a number of generations; therefore, these traditional remedies are still in practice.

Guduchi has been acclaimed as a highly potential drug in Ayurveda, for preservation of health, prevention of disease and curative measures. It is attributed with a wide range of pharmacological activities. It is the most commonly used non-controversial drug. Its antecedency dates back to Vedic period. During the dreadful war between Rama and Ravana several vanaras (monkey soldiers) were killed. After the victory, vanaras (monkey soldiers) who had died were given a new life by Lord Indra through sprinkling of nectar. During this act when the drops of nectar (Amruta) fell on the ground, Guduchi plant originated.

The *Tinospora* is an important genus belong to the family Menispermaceae in the major group of Angiosperms. This genus consists of approximately 30 species and a few species are potential medicinal importance. They are generally climbing or twining shrubs distributed throughout the tropical and subtropical regions of Africa, Asia, Australia and the Pacific [1]. As a therapeutic agent, *Tinospora* species have been used by various cultures as traditional medicine for antipyretic, anti-inflammation, antimicrobial, antimalarial and antihyperglycemia properties [2,3].

Tinospora crispa (L.) Hook. f. & Thomson is a medicinal plant belongs to the genus *Tinospora* of Menispermaceae family. It is prevalent in primary rainforests or mixed deciduous forests of South East Asia and Africa including Thailand, Malaysia, and Indonesia (Pathak et al., 1995). It has been used in conventional medicine to treat numerous pathologies in Malaysia (Najib Nik a Rahman et al., 1999), Indonesia (Dweck and Cavin, 2006), Thailand (Kongsaktrakoon et al., 1984), and the Philippines (Quisumbing, 1951). There was a previous review of the secondary metabolites and biological activities of *T. crispa* (Koay and Amir, 2013), however, critical assessment of the present knowledge is needed to provide the perspectives and directions for future research and potential applications. The purpose of this review is to provide an updated and complete overview of the botany, phytochemistry, traditional uses, and pharmacological activities of *T. crispa*. Moreover, the present knowledge obtained through review of literature mainly from experimental studies was critically assessed to provide evidences and justifications for local and traditional uses of *T. crispa* and to propose future research prospects and potential therapeutic uses for this plant.

2. REVIEW OF LITERATURE

2.1 Varieties

Samhitas have not mentioned the varieties of Guduchi, while Mahendra Bhogika of Dhanawantari Nighantu [4] has identified two varieties of Guduchi, viz,

1. Guduchi-*Tinospora cordifolia* (wild) Miers ex. Hook and Thomas.

2. Kandodbhava Guduchi: -*Tinospora sinensis* (lour) or *Tinospora malabarica*. In kaiyyadeva Nighantu [5] classification of Guduchi is not mentioned. But Acharya P V Sharma mentioned in the classification regarding Kanda yukta Guduchi as *Tinospora Malabarica*. In Shaligrama Nighantu [6] explained about the mythological origin of Guduchi. Also, it explains about the Kanda Guduchi with its synonyms as Kandotbhava, Kandamruta, Pindaguduchika, Bahuchinna, Bahuruha and Kandarohini. In Nighantu Adarsha [7] Guduchi classification is not mentioned. The therapeutic benefits have been highlighted more. In Vanaushadhi Sangraha [8] also mentioned regarding the Guduchi, but as such types are not mentioned. It can be observed that one more species was added by Dr Kirtikar and Dr Basu in their book of Indian Medicinal plants. Totally three varieties are identified botanically:

1. *Tinospora cordifolia* Miers.
2. *Tinospora crispa* Miers.
3. *Tinospora malabarica* Miers (*T. Sinensis*) [Often substituted]

2.2 Vernacular Names

T. crispa [9], is known as "Patawali," "Akar Patawali," "Seruntum," or "Akar Seruntum" in Malaysia (Noor et al., 1989), "Brotawali," "Antawali," and "Andawali" in Indonesia (Roosita et al., 2008; Koay and Amir, 2013), "Makabuhay" (meaning "You may live") in Philippines, (Quisumbing, 1951), "Boraphet" in Thailand, "Da ye ruanjinteng" in China (Li et al., 2006), "Banndol Pech" in Cambodia (Hout et al., 2006) "Guloncho-ban" or "Golonchi" in Bangladesh (Rahmatullah et al., 2011), and "Lyann span Zeb kayenn" in Martinique island (Longuefosse and Nossin, 1996).

2.3 Plant Description

1. Botanical name: *Tinospora crispa* L. Hook

Synonyms: *Menispermum crispum* Linn,
Menispermum rimosum Blanco,
Cocculus cordifolius Walp, *Cocculus villosus* DC,
Tinospora tuberculata, *Tinospora rumphii*,
Cocculus crispum, *Menispermum tuberculatum*,

Menispermum verrucosum *Tinospora crispa* (L.) Miers ex Hook. f. & Thoms. [10].

2. Taxonomy Kingdom:

Plantae Division: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Menispermaceae

Genus: *Tinospora*

Species: *crispa*

Habit: small herb which widely grows in temperate and tropical parts [11]. **Habitat:** Tropical and subtropical Philippines, Indonesia, Malaysia, Thailand, India, China & Vietnam [11]. **Stem:** Old stems fleshy, with very prominent blunt tubercles. Younger stems slightly fleshy, epidermis thin, membranous, brownish, glabrous. [12]. **Leaf:** lenticels large and prominently raised. Petiole 5–15 cm, glabrous; leaf blade broadly ovate to orbicular, slightly fleshy, very thinly papery when dried, both surfaces glabrous, base deeply to shallowly cordate, lobes rounded, margin entire, apex acuminate, palmately 5–7 veined, abaxial basal vein axils with shallow glabrous pockets [12]. **Flower:** flowers 2 or 3 fascicled. Male inflorescences are very slender, 5–10 cm or longer. Male flower has sepals 6 in 2 whorls, green, glabrous, outer 3 ovate, ca. 1 mm, inner 3 obovate, 2.5–3 mm; petals 3–6, yellow, obovate-spatulate, 1.6–2.5 mm; stamens 6, as long as petals. Female inflorescences 2–6 cm, flowers mostly 1 per node. Female flower has sepals and petals as in male; staminodes 6 to 1 mm; carpels 3, ca. 2 mm, stigma lobes very short. Fruiting peduncle 15–20 mm; carpophores 2–3 mm [12]. **Drupes:** orange, sub globose, to 2 cm when fresh; endocarp semi ovoid, with conspicuous ridge abaxially, surface finely rugulose to almost smooth, adaxial aperture elliptic, small [12].

As an immunomodulator:

- Methanol, chloroform, n- butanol extracts of *T. crispa* were demonstrated, which has lack of enhancing activity on immune system, still it is used as medicine for other immunological disorders such as autoimmune disease or cancers [13]. *T. crispa* extract could induce cytokine which were likely to be involved in anti-inflammatory activity [14]. It did not

suggest that *T. crispa* could also inhibit other arms of the immune responses because, *T. cordifolia*, a closely-related plant compounds were shown to enhance both the immunity. Whether, *T. crispa* compounds reveals a potent effect on the immune system should be further investigated [15].

As a therapeutic agent:

- Antihyperglycaemic effect of *T. crispa* is physiological suggests that the extract contains compounds which could be purified for use in the treatment of type II diabetes [12]. *Tinospora crispa* is as effective as an appetite enhancer as compared against the reference drug megestrol acetate [12]. The cycloeucaleanol and cycloeucalenone present in the stems produced mild cardiotoxic effects [12]. It had been used as traditional medicine in rural society to treat fever, cholera, snake bites, rheumatism and fever due to malaria. *T. crispa* has shown to have an antihyperglycemia effect by augmenting the release of insulin. Its antimalarial activity, antibacterial, anti-inflammatory and antioxidant properties [12]. *T. crispa* has a dose-dependent antiproliferative activity against many types of cancer cells where the lowest IC50 is found to be present in the methanol extract on breast cancer cells [16]. *Tinospora crispa* can be used externally for its strong anti-oxidant and antiradical properties. Flavonoids (amongst them apigenin) are best known for their ability to act as powerful anti-oxidants, and also have anti-allergic and antiviral properties [12]. Indonesians use an infusion of the stems to treat fevers and malaria. They can also be used to treat stomach ache and jaundice. The infusion is also useful in fevers caused by smallpox and cholera. Another popular use of this infusion is in a mixture for treating indigestion [17].

Phytochemistry (Active chemical constituents):

- The whole plant contains bitter principle, columbine, 2.22%, trace of alkaloid and a glucoside. Amorphous bitter principle, picroretine & trace of barberine were isolated. Later from root bark a bitter

principles glucoside and some alkaloids were isolated. Picroretine is trace of an alkaloid, & a substance similar to glycyrrhizin. There are two alkaloids tinosporine & tinosporidine also present. Three compounds, identified as N-cis-feruloyltyramine, N-transferuloyltyramine and secoisolariciresinol, exhibiting antioxidant and radical scavenging properties towards β -carotene and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, were isolated from the CH₂Cl₂ extract of stems of *T. crispa*.

2.4 Traditional & Therapeutic Uses

T. crispa [9] is an ingredient in Thai folk remedies. Decoction from the stem of *T. crispa* has been used as an antipyretic, in the treatment of internal inflammations, decreasing thirst, enhancing hunger, cooling down body temperature, and for the maintenance of good health (Kongsaktragoon et al., 1984; Dweck and Cavin, 2006). The cold infusion of the seed has been used to treat intoxication caused by drugs or alcohol. An infusion of its stem is drunk as vermifuge, a decoction of the stem is used to wash aching eyes and syphilitic sores, the crushed leaves are applied on wounds and made into dressing for itch. In Indonesia (Borneo) it has been used for the treatment of diabetes, hypertension, and backache (Dweck and Cavin, 2006). *T. crispa* has been used conventionally against a wide variety of health ailments by Yao communities of China. They used it to treat bruises, septicaemia, fever, fracture, scabies, and other tropical ulcer-related disorders (Li et al. 2006). In Malaysia, *T. crispa* is used traditionally for numerous therapeutic purposes like diabetes, hypertension, stimulation of appetite, and protection from mosquito bites (Gimlette and Burkill, 1930). The infusion from the stems is used as a vermifuge. Personal communications with local traditional medicine practitioners highlighted its popular use as a general tonic. Moreover, it is used as an anti-parasitic agent in both humans and domestic animals (Noor et al. 1989). In Bangladesh, the juice of stem is used in the treatment of intestinal disorders, jaundice, rheumatism, body pain, paralysis, skin disease, and leprosy. The aqueous leaf extract is used to treat flatulence, dyspepsia, diarrhoea, and rheumatism by traditional therapists in the Philippines. It is also used to prepare a poultice with coconut oil to treat arthritis.

Ayurvedic point of view:**Table 1. Synonyms, Nirukti and its meanings**

Synonyms	Nirukti	Meaning
Amrutha Amruthavalli	Nasthi mrutham maranam yasyaha sa Amrutavath-tasya roghanasakatvaa	That which is imperishable The creeping plant that endows life by destroying disease
Chinnaruha	Chinna api rohati eti	That which grows even after cutting or that which exists.
Chinnodbhava	Chinna chedane api udbhava Utpattiti yasyaha sa	which can be propagated through stem cutting
Chakralakshani	Chakram lakshana yasya eti	wheel like appearance on cross section
Guduchi Jwarari Kundali	Gud rakshane Jwarasya arihi Kundalakarena vardhate	That which protects from diseases Febrifuge, Anti-pyretic Forming a ring, curled and moving in circles.
Mandali Vatsadani Vishalya	Mandalakarena vardhamana Vasairadhyate priyatwaditi Vigatam shalyam tatpraharadi janitam vedanadi nashe yatha	Circular, wheel, anything round That which is eaten by calves That which relieves pain caused by shalya/trauma, free from pain

3. MATERIALS AND METHODS**3.1 Identification & Authentication of the Drug**

The correct identification of herbal plant is the first step in quality control as recommended by WHO. The fresh stems of *Tinospora crispa* for the current study has been collected from Tirumala, Trivandrum, Kerala. The stem along with the leaves was sent to FRLHT, Bangalore for identification and authentication of the drug. It has been authenticated as *Tinospora crispa* by Dr Noorunnisabeegum S, Associate Professor, Centre for Conservation of Natural Resources, TDU.

3.2 Analytical Profile of the Drug

The analytical profile of the raw drug *Tinospora crispa* is done with the reference of PLIM guidelines. The Analytical study has been carried out in Vasu Pharmaceuticals, Vadodara, Gujarat,

India. The following are the analytical profile data:

Physiochemical Studies: Physiochemical parameters like Macroscopic & Microscopic studies, pH, LOD, total ash, acid insoluble ash and water soluble and alcohol soluble extractive values, total alkaloids, Heavy metal detection, Microbial limit tests, HPTLC were determined as per PLIM guidelines

Phytochemical screenings: Preliminary qualitative phytochemical screening was carried out and revealed the presence of a wide range of phytoconstituents Alkaloid, Starch, Carbohydrates, Tannins & Polyphenols, Flavonoids, Saponins & Steroids. The HPTLC Fingerprinting analysis was carried out in crude stem powder of *Tinospora crispa* with solvent system i.e. Chloroform: Methanol(9:1 v/v) using CAMAG HPTLC system consisting of Linomat 5-Applicator. The chromatogram obtained was studied under 254 nm, 366 nm and 540nm after derivatization.

4. RESULTS**Table 2. Analytical profile with anticipatory interpretation**

SI No.	Parameters	Analytical Profile	Anticipatory Interpretation
1	pH	5.03	Quantitative indication of acidic nature of the drug
2	Loss on Drying	9.22%	Indicates the loss of amount of water & other volatile impurities present in the sample
3	Total Ash	5.62%	It indicates the purity of the drug As per API, for <i>Tinospora cordifolia</i> it should not be

SI No.	Parameters	Analytical Profile	Anticipatory Interpretation
4	Acid insoluble Ash	1.08%	more than 16% The inorganic contents like siliceous materials are 1.08% only As per API, for <i>Tinospora cordifolia</i> it should not be more than 3%
5	Water Soluble Extractive	17.22%	Indicates the water-soluble constituents in the trial raw drug As per API, for <i>Tinospora cordifolia</i> it should not be less than 11%
6	Alcohol Soluble Extractive	17.64%	Indicates the Alcohol soluble constituents in the trial raw drug As per API, for <i>Tinospora cordifolia</i> it should not be less than 3%
7	Total Alkaloids by Gravimetry	2.33%	The possible alkaloids are Tinosporin, Magnoflorine, Berberine, Choline, Jatrorrhizine. Article data reveals the berberine can show the antipyretic activity

Note: The aforesaid analytical profile has been compared with the values given for the *Tinospora* species ie *Tinospora cordifolia* in the Ayurveda Pharmacopeia of India.

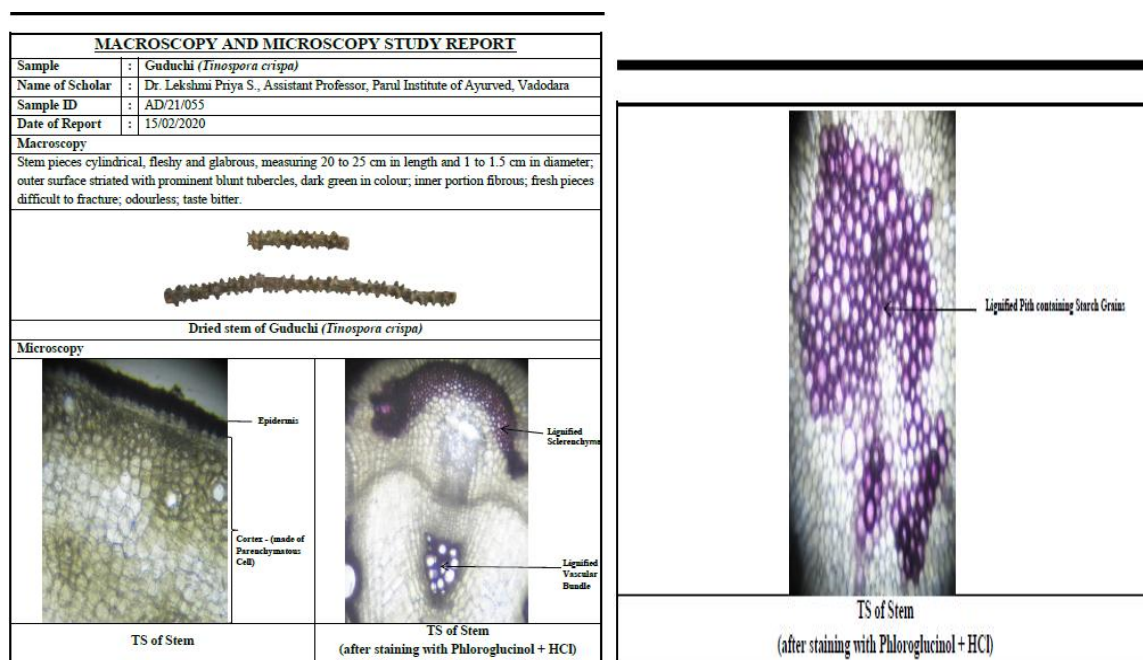


Fig. 1. Macroscopic & Microscopic Raw Drug Study Reports

Table 3. Phytoconstituent assay

1	Alkaloid, Starch, Carbohydrates	+++	Major Phytoconstituents in starch may be Amylose & Amylopectin. Also, may be polysaccharides like 1-4 linked Glycan
2	Tannins & Polyphenols, Flavonoids	++	-
3	Saponins & Steroids	+	-
4	Proteins & Terpenoids	Absent	-

Table 4. Heavy metal Analysis

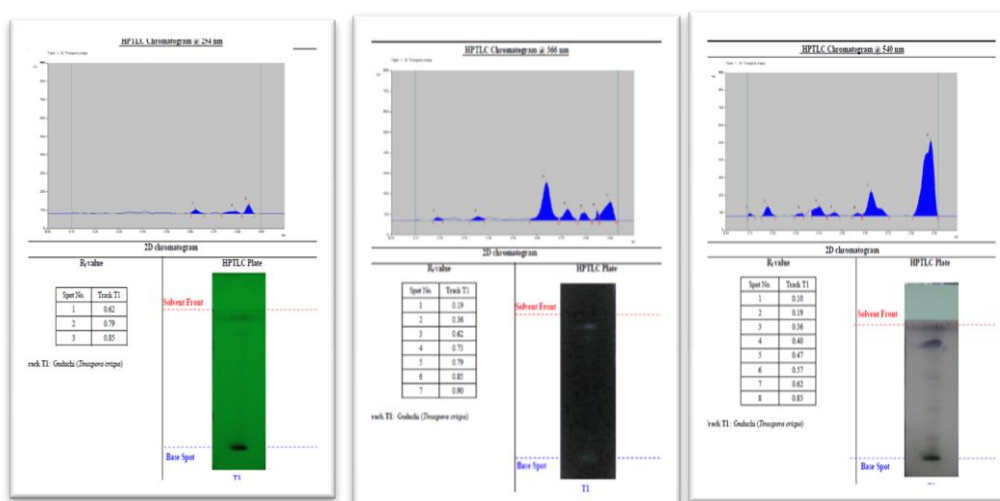
1	Mercury & Arsenic	Not detected	-
2	Lead	7.46ppm	Within Normal Limits
3	Cadmium	0.06ppm	Within Normal Limits

Table 5. Microbial Limits Tests

1	Total Microbial Plate Count	2723 cfu /gm	Within Normal Limits
2	Total Yeast & Mould Count	2337 cfu/ gm	Within Normal Limits
3	<i>E coli, Salmonella, S. aureus, P. aeruginosa</i>	Absent	-

Table 6. HPTLC fingerprint analysis report

Sample: <i>Tinospora crispa</i> stem powder		
Visualization	No: of spots	Rf value
254nm	3	0.62,0.79,0.85
366nm	7	0.19,0.36,0.62,0.73,0.79,0.85,0.90
540nm	8	0.10,0.19,0.36,0.40,0.47,0.57,0.62,0.85

**Fig. 2. @254nm Visualization Fig. 3. @366nm Visualization Fig. 4. @540nm Visualization**

4.1 HPTLC Fingerprinting Analysis

Fingerprint Analysis of crude powder of *Tinospora crispa* stem at 254nm,366 nm and 540nm: The Rf value of different compounds at254 nm,366nm and 540nm are shown in Table No:06. Derivatization was done by Anisaldehyde Sulphuric acid reagent.

5. DISCUSSION

Herein the current research article the Ayurvedic and modern point of view regarding Guduchi and *Tinospora crispa* has been highlighted. The analytical studies conducted on this drug also prove the features of *Tinospora crispa*.We tried to document the existing phytochemistry, pharmacological properties, and application researches on *T. crispa*. Also, the analytical profile of *Tinospora crispa* has also been reflected. The macroscopic studies of the collected study drug show the evident features of *Tinospora crispa* like outer surface striated with prominent blunt tubercles, inner fibrous portion

with bitter taste. The microscopic studies of *Tinospora crispa* shows the presence of lignified sclerenchyma, lignified vascular bundle which is in consonance with the drug.The analytical parameters result with its anticipatory interpretations have been already mentioned in Table No:02.The qualitative phytoconstituent assay shows the presence of Alkaloid, Starch, Carbohydrates, Tannins & Polyphenols, Flavonoids, Saponins & Steroids which reveal the Antioxidant, Antipyretic, Antidiabetic activity of *Tinospora crispa*.The HPTLC fingerprinting screening also shows the presence of spots at different visualization and Rf values. As the calibration curved is not carried out in the current study,it can be assessed from HPTLC report that the secondary metabolites are present in the *Tinospora crispa*. Also, through the literary review it was evident that *T. crispa* has the potential multiple pharmacological and therapeutic activities in the management of hypertension, lumbago, postpartum remedy, tuberculosis, haemorrhoids, wound healing,

itching, muscle pain, etc, which can be explained by the presence of various terpenoids, alkaloids, lignans and nucleosides in the herb. The biological activities and chemical nature of the bioactive compounds must be of great attention for the researchers. Diterpenoid glycosides from *T. crispa* have shown promising antidiabetic activity. However, future studies are necessary to address issues regarding composition of the extract, explicability of preclinical experiments, and lack of transformation of the preclinical results to clinical efficacy.

6. CONCLUSION

Further systematic studies are necessary to evaluate the efficacy using standardized extracts of *T. crispa*, and to identify the bioactive molecules responsible for the biological activities so that cost-effective, potential medicinal drug and health products can be developed at a large scale. Also, attempts should be made to conduct serious randomized human trials and determine modes or mechanisms of action, bioavailability, pharmacokinetics, and physiological pathways for specific bioactive of *T. crispa* which might be responsible behind the protective effects offered by extracts rich in flavonoids and terpenoids in many pharmacological studies. In the current research works through the phytoconstituent assay also it reveals the presence of secondary metabolites which may be responsible for the desired activities of the drug *T. crispa*. The Ayurvedic classical text books there is the mentioning of only two types of Guduchi. Based on this *Tinospora crispa* cannot be correlated. By compiling from other articles and through the analytical studies performed also the current research work provides a vivid picture regarding the drug *Tinospora crispa*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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SIGNIFICANCE OF STUDY

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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