

A Comparative phytochemical study of cold water extract, hot water extract and starch form of *Tinospora cordifolia* (Thunb.) Miers. stems

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ABSTRACT

Tinospora cordifolia (Thunb.) Miers. (Guduchi), belonging to family: Menispermaceae hold significant importance in Ayurveda as a medicinal plant and referred to as "Amurta" in Sanskrit. It is renowned for its advantages in strengthening the body's immune system and maintaining harmonious functions across various organs. *T. cordifolia* is characterized by its bitter, pungent, and astringent taste (Rasa), sweet post-digestive effect (Vipaka), and hot potency. Each part of the *T. cordifolia*, including its stems, and leaves, possesses medicinal properties. The objective of this study is to evaluate and compare the phytochemical differences between cold-water extract, hot-water extract (decoction) and starch form of *T. cordifolia* stem. Phytochemical differences were evaluated for three preparations of *T. cordifolia* by (a) screening the phytochemical classes and (b) developing Thin Layer Chromatography (TLC) fingerprints. Among the tested phytochemical classes, terpenoids and steroids were absent in the cold water extract, hot water extract (decoction) and starch form prepared from stems of *T. cordifolia*. According to TLC fingerprint profiles and their Rf values, both cold water extract and hot water extract (decoction) have similarities whereas the starch form of *T. cordifolia* differs from cold water extract and hot water extract (decoction). In conclusion, the presence study revealed the phytochemical differences between cold water extract, hot water extract (decoction) and starch of *T. cordifolia* stem for the first time.

Keywords: *Tinospora cordifolia*, Menispermaceae, Guduchi, Phytochemical classes, Thin Layer Chromatography

1. Introduction

Tinospora cordifolia (Thunb.) Miers is a climbing shrub with a succulent stem and leaves measuring 10-20 cm in diameter. Belonging to the Menispermaceae family and is widely distributed in the Indian subcontinent and China. *T. cordifolia*, known as *Guduchi* and *Amurta* in Sanskrit, means "protects the body from diseases" [1]. The roots, stems, and leaves are integral components of the plant, possessing medicinal and nutritional values. *T. cordifolia* is an important medicinal plant in the Ayurveda system of medicine used in different Ayurveda formulations to treat various ailments such as colds, fever, headache, jaundice, and digestive disorders [2],[3],[4]. Different classes of phytochemical compounds, such as alkaloids, glycosides, aliphatic compounds, diterpenoids, sesquiterpenoids, phenolic compounds, steroids, and polysaccharides, are found in *T. cordifolia* [5],[6]. *T. cordifolia* is extremely useful in strengthening the immune system and keeping the functions of its various organs in harmony [5],[7]. According to the Ayurveda medicinal system, its *Rasa* is *Tikta* (bitter taste), *Veerya* is *Ushna* (hot potency) and *Vipaka* is *Madhura* (post-digestive effect is sweet), *Guna* includes *Laghu* and *Snigdha* (properties are light and oily). It balances *Tridosha* (three body humors *Vata*, *Pitta* and *Kapha*) in the body [8]. The starch form of *T. cordifolia* is traditionally used as a household remedy for chronic fever to alleviate it, reduce burning sensation, and increase energy and appetite [9]. In addition to the starch form of *T. cordifolia*, cold water and hot water extract (decoction) are used in the Traditional and Ayurveda medicinal systems [8],[10]. Therefore, an attempt was made to compare the phytochemical differences of three types of medicinal

preparations prepared from *T. cordifolia* stems using phytochemical screening and Thin Layer Chromatography (TLC) technique.

2. Materials and Methods

2.1. Collection of *Tinospora cordifolia* Stems

Stems of *T. cordifolia* were collected from home gardens in the Kalutara district between August 2023 to October 2023.

2.2. Different medicinal preparations of *Tinospora cordifolia* stems

2.2.1. Cold water extract: A weight of 60 g of powdered stem was taken, and 480 ml of cold water was added to an earthen pot. The mixture was incubated overnight and filtered through a cotton cloth in the following morning [11].

2.2.2. Hot water decoction: A weight of 60 g of coarsely powdered stem was taken and boiled with 1920 ml of water in an earthen pot over a mild flame till the liquid part was reduced to 240ml [11].

2.2.3. Starch form: The ground coarse powder was taken, and four portions of water were added. The mixture was incubated overnight to settle down. Supernatant water was decanted carefully the following day, without disturbing sediment starch. The starch was washed using a minimum amount of water and kept settling down. The sedimented starch was allowed to air dry [12].

2.3. Phytochemical Screening

The presence or absence of saponins, tannins, phenols, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins and reducing sugars qualitatively detected in cold water extract, hot water extract (decoction) and starch form according to the standard protocols [13], [14].

2.4. Development of Thin Layer Chromatography (TLC) fingerprints

TLC fingerprints were developed for cold water extract, hot water extract (decoction) and starch form of *T. cordifolia* stems. The starch form of *T. cordifolia* was extracted into Dichloromethane. Extracts were concentrated separately using Rotavapor (Buchi, B-480). A volume of 10 μ l from each extract was applied as separate spots on TLC plates. Cyclohexane, Dichloromethane, Ethyl acetate and Methanol were used in a ratio of 6: 4: 1: 0.1 (v/v) as the mobile phase. The TLC fingerprints visualized under UV light (at 254 nm and 366 nm). Subsequently, Vanillin-sulphuric acid was sprayed and heated at 100-110 °C. Finally, R_f values were recorded for each spot before (at 254 nm and 366 nm) and after spraying Vanillin-sulphuric acid.

3. Results and Discussion

Different phytochemical classes such as tannins, alkaloids, phenolic compounds, flavonoids, alkaloids, terpenoids, steroids, saponins, carbohydrates, proteins, and reducing sugars present or absent in the cold water extract, hot water extract (decoction) and starch form of *T. cordifolia* stem are listed in Table 1.

Table 1: Phytochemicals present in different medicinal preparations of *Tinospora cordifolia* stem

Type of Phytochemical	Test	Cold water extract	Hot water extract	Starch Form
Tannins	Ferric chloride test	++	+	++
	Lead acetate test	++	+	++
	Vanillin test	-	-	-
Phenols	Lead acetate test	++	++	++
	Vanillin test	-	-	-
Flavonoids	Ammonia with conc. H ₂ SO ₄	+	+	+
	Magnesium with conc. HCl	-	-	-
Alkaloids	Wagner reagent	+	+	++
Terpenoids	Salkowski test	-	-	-
	Sesquiterpenes	-	-	-
Steroids	Acetic anhydride with conc. H ₂ SO ₄	-	-	-
	Lieberman Burchard test	-	-	-
Saponins	Froth test	+	+	++
Carbohydrates	Iodine test	+	+	++
Proteins	Biuret test	+	+	+
Reducing Sugars	Fehling's test	++	+	++

+: Positive response ++: Intensely positive response -: Negative response

Results revealed that among the tested phytochemical classes, terpenoids and steroids were absent in the cold-water extract, hot water extract (decoction) and starch form prepared from stems of *T. cordifolia* grown in Sri Lanka. However, terpenoids such as tinosporide, furano-lactone diterpene, furanolactone clerodane diterpene, furanoid diterpene and steroids such as giloinsterol, β -gitosterol, 20 α -hydroxy ecdysone were reported to be presented in *T. cordifolia* grown in other countries [15]. Moreover, alkaloids, saponins and carbohydrates were prominent in the starch form of *T. cordifolia* stem.

Thin Layer Chromatography (TLC) is a rapid, inexpensive, and simple technique used to identify active ingredients, determine the identity of the medicinal plants or polyherbal preparations and detect adulterants, contamination or substitution [16],[17],[18]. In the present study, TLC fingerprint was examined under UV light at wavelengths of 254 nm and 366 nm and sprayed Vanillin-sulphuric acid to visualize the colors (Figure 1). Moreover, R_f values were recorded for cold water extract, hot water extract (decoction) and starch form prepared from *T. cordifolia* stems (Table 2).

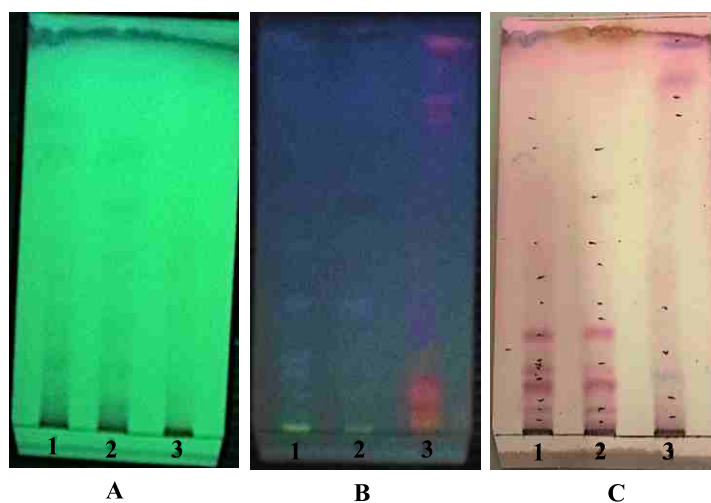


Figure 1: Thin Layer Chromatography fingerprints of cold water extract (1), hot water extract (2) and starch form (3) of *Tinospora cordifolia* stem under 254 nm (A), 366 nm (B) and after spraying Vanillin-sulphuric acid ©

Table 2: *R_f* values cold water extract, hot water extract and starch form of *Tinospora cordifolia* stem under UV light and after spraying Vanillin-sulphuric acid

Observation Criteria	Types of <i>Tinospora cordifolia</i> stem extract	R _f values
Under 254 nm and 366 nm	Cold water extract	0.03, 0.05, 0.07, 0.13, 0.17, 0.22, 0.29, 0.37, 0.44, 0.67
	Hot water extract (decoction)	0.03, 0.05, 0.16, 0.26, 0.29, 0.38, 0.44, 0.56, 0.67
	Starch form	0.03, 0.05, 0.16, 0.26, 0.29, 0.39, 0.59, 0.73, 0.84, 0.94
After spraying Vanillin-sulphuric acid	Cold water extract	0.03, 0.05, 0.07, 0.11, 0.15, 0.18, 0.23, 0.35, 0.44, 0.94, 0.96
	Hot water extract (decoction)	0.03, 0.05, 0.07, 0.11, 0.15, 0.18, 0.23, 0.57, 0.88, 0.94, 0.99
	Starch form	0.06, 0.13, 0.18, 0.24, 0.39, 0.59, 0.73, 0.84, 0.94

As per the TLC fingerprint profiles (Figure 1) and their R_f values, both cold water extract and hot water extract (decoction) have similarities whereas the starch form of *T. cordifolia* differs from cold water extract and hot water extract (decoction). In conclusion, the presence study revealed the phytochemical differences of cold water extract, hot water extract (decoction) and starch form of *T. cordifolia* stem for the first time.

Competing interests: The authors declare no competing interests.

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