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Comparative GC-MS analysis on phytochemical constituents in *Tinospora cordifolia* stem (TCS) aqueous extract by different extraction techniques

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Abstract
The present study was designed to identify the phytochemical profile and yield of *Tinospora cordifolia* stem aqueous extracts obtained by Soxhlet extraction and Boiling methods. The Soxhlet extraction had a higher percentage yield (14.89%), when compared to the Boiling method (6.7%). Further, GC-MS analysis of the Soxhlet extract identified 19 phytoconstituents *viz*., alcohols, esters, alkaloids, fatty acids and phenolic compounds. The major compounds were Glycerin (25.08%), Inositol, 1-deoxy-(15.81%) and 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-(9.79%), along with other potent bioactive compounds such as Desulphosinigrin and Columbin. These metabolites have been reported to exhibit diverse pharmacological activities such as antioxidant, anti-inflammatory, anti-diabetic, anti-hypertensive and anti-cancer effects. On contrary, the extract obtained by Boiling method contained 13 compounds, with Inositol, 1-deoxy-(75.30%) being the major compound followed by other polar carbohydrate derivatives. In conclusion, the Soxhlet extraction method not only enhanced the yield but could also extract a diverse spectrum of therapeutically significant phytochemicals. The presence of compounds such as columbin, desulphosinigrin and ferulic acid derivatives deciphers the potential of *T. cordifolia* stem as a promising source in the treatment and management of oxidative stress, metabolic disorders, inflammation and cancer.

Keywords: *Tinospora cordifolia* stem extract, GC-MS, Soxhlet extraction, phytoconstituents

Introduction
Medicinal plants and herbal therapeutic practices have long been used in the Indian medicine to manage a wide range of health conditions, including diabetes. The key advantage of using medicinal plants is their easy availability, minimal side effects and low cost. Numerous studies are currently being conducted to decipher the mechanism of action and efficacy of these medicinal plants [1, 2]. Identification of plant bioactive compounds is crucial for understanding their therapeutic effects in the management of any disease condition. These naturally occurring phytochemicals serve as a valuable lead for developing new pharmaceutical drugs. A significant proportion of FDA-approved medications for treatment of various human diseases have been derived from such natural products [3].

Tinospora cordifolia (Wild.) Miers ex Hook. F. & Thoms., of Menispermaceae family, commonly known as “Amrita” or “Guduchi,” holds significant importance in the Indian Systems of Medicine (ISM) and has been used since ancient times in traditional practices. Notably, three specific medicinal plant species of *Tinospora* namely *cordifolia*, *crispa* and s*inensis* are found exclusively in India [4], emphasizing their importance as native sources rich in bioactive compounds with therapeutic potential. Renowned as a traditional Indian bitter, *T. cordifolia* has been one of the most widely investigated and broadly used medicinal plant in the treatment of various ailments like heart disease, diabetes, leprosy, rheumatoid arthritis, dyspepsia, jaundice, urinary disorders, skin conditions and allergy [5, 6]. *T. cordifolia* has also been reported to reverse diabetic dyslipidemia and oxidative stress [7].

The pharmacological properties of *T. cordifolia* are attributed to the bioactive compounds found in its root, stem and entire plant material *viz*., alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids, steroids, aliphatic compounds, phenolics, essential oils, polysaccharides and a blend of fatty acids [8, 9]. Extraction of these phytoconstituents is very crucial as these compounds are found at a lower concentration in plants.

Various studies have reported that biological activity of *T. cordifolia* polysaccharides were found to be influenced by the extraction method. For instance, *T. cordifolia* methanolic stem extract showed greater antioxidant activity, while methanolic leaf extract showed greater anti-radical activity and the ethyl acetate stem extract showed good anti-radical activity [10, 11]. Thus, the selection of an appropriate solvent system is crucial for maximizing the extraction of phytoconstituents and elucidating their biological activity. Conventional methods like Soxhlet extraction and maceration have been widely used to extract bioactive compounds from medicinal plants. However, usage of modern techniques such as Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE) and Supercritical Fluid Extraction (SFE) have become faster, more efficient and cost-effective alternatives for extraction of phytoconstituents [12].

Identification and quantification of bioactive compounds in plant extract and knowledge on the functional groups or chemical classes of the phytoconstituents is essential to evaluate their therapeutic potential. Analytical techniques such as and Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) spectroscopy are widely used for the identification of functional groups within these bioactive compounds. GC-MS is recognized for its speed, accuracy, and efficiency in detecting a broad spectrum of chemical classes such as alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids requiring only a small quantity of plant extract for analysis [13].

The present study was carried out to assess the influence of extraction protocol on the phytochemical composition of *T. cordifolia* aqueous stem extracts. Therefore, aqueous stem extract of *T. cordifolia* was prepared by two different extraction methods *viz*., Boiling and Soxhlet protocols, which was followed by quantitative GCMS phytochemical analysis. The study thus aims to document the major bioactive compounds that could serve as leads for the development of valuable therapeutics.

Materials and Methods

Materials used

Fresh *Tinospora cordifolia* stem was collected from natural vegetation found at Egmore, Chennai and was identified at Department of Agronomy, Madras Veterinary College, Chennai. *T. cordifolia* stem (TCS) extract was prepared using Triple distilled water as solvent in both Boiling method and Soxhlet method of extraction. The following instruments were used for carrying out TCS extraction *viz.*, Soxhlet extraction apparatus (Make: Guna), serological water bath, and refrigerated centrifuge (Thermo Scientific Sorvall ST 8R). Phytochemical profiling was performed using a Gas Chromatography System (Agilent 8890) coupled with a Single Quadrupole Mass Spectrometer (5977B MSD) analyzer.

Preparation of *Tinospora cordifolia* stem extract

The stem of *T. cordifolia* was washed, shade dried for 30 days and then were ground into coarse powder.

Extraction by Boiling method

To 10 g of *Tinospora cordifolia* stem powder, 200 ml distilled water was added and boiled for 20 minutes. It was then cooled and filtered using a strainer. The filtrate was then centrifuged at 8000 rpm at 10 °C for 15 minutes [14]. The supernatant was collected and kept in a serological water bath at 60 °C for solvent evaporation and subsequently dried. The final extract of *Tinospora cordifolia* stem (TCS) was stored at-20 °C for future phytochemical analysis.

Extraction by Soxhlet method

The extraction process was carried out in a Soxhlet apparatus using 10 g of TCS powder and 200 ml of distilled water. The extraction was carried out for a period of 15 hours at 75 °C. The extract was then centrifuged at 8000 rpm at 10 °C for 15 minutes [15]. The supernatant was collected and kept at 60 °C for solvent evaporation and subsequently dried. The final extract of *Tinospora cordifolia* stem (TCS) was stored at-20 °C for future phytochemical analysis.

The extraction yield was calculated using the following formula for both the above methods:

$$\text{Yield (\%)} = \frac{\text{Weight of the extract obtained}}{\text{Weight of the TCS powder taken for extraction}} \text{X } 100$$

Quantitative analysis of the phytochemicals by GC-MS

GC-MS analysis of *Tinospora cordifolia* stem extracts were performed using Agilent 8890 at Sophisticated Analytical Instrument Facility (SAIF), IIT Madras, Chennai. For GC-MS detection, a carrier gas (helium) at a constant flow rate of 1.2 ml/min, and an injection volume of 1µl was employed. The ion source temperature was 230 °C and the oven temperature was programmed from 60 °C to 350 °C. The oven temperature was maintained at 50 °C (isothermal) and mass spectra transfer line temperature was 280 °C. The compounds were detected in the range of 50-600 amu by NIST library search.

Results and Discussion

The comparative analysis of *T. cordifolia* aqueous stem extracts prepared by Soxhlet extraction and by the boiling method revealed distinct variations in both the percentage yield and composition of phytochemicals. The percentage yield of Soxhlet extraction (14.89%) was more than twice of the yield obtained by the boiling method (6.7%), indicating that the continuous circulation of solvent in the Soxhlet apparatus enhanced the extraction efficiency by favouring solubilization of bioactive compounds.

Quantitative analysis of phytochemicals by GCMS

Phytochemical screening of the Soxhlet extract of *T. cordifolia* stem using GC-MS (Fig.1) showed a diverse range of 19 phytoconstituents such as alcohols, esters, alkaloids, fatty acids and phenolic compounds. Major compounds identified were Glycerin (25.08%), Inositol, 1-deoxy-(15.81%) and 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-(9.79%) (Table 1). The polyols (such as inositol) might act as modulators of oxidative metabolism which may help to decrease oxidative stress [16].

The other major phytoconstituents identified were Desulphosinigrin (a glucosinolate derivative), Decanoic acid, 3-hydroxy-, methyl ester and Columbin (a diterpenoid furan compound). Desulphosinigrin was reported to possess an anti-asthmatic effect [17] and Columbin was found to exhibit anti-diabetic [18, 19], anti-cancerous [20], anti-venom [21] and anti-trypanosomal properties [22]. The presence of Decanoic acid, 3-hydroxy-, methyl ester and other fatty acid derivatives indicates the extraction of medium-chain fatty esters, which have been reported as potent anti-mycotic agents [23].

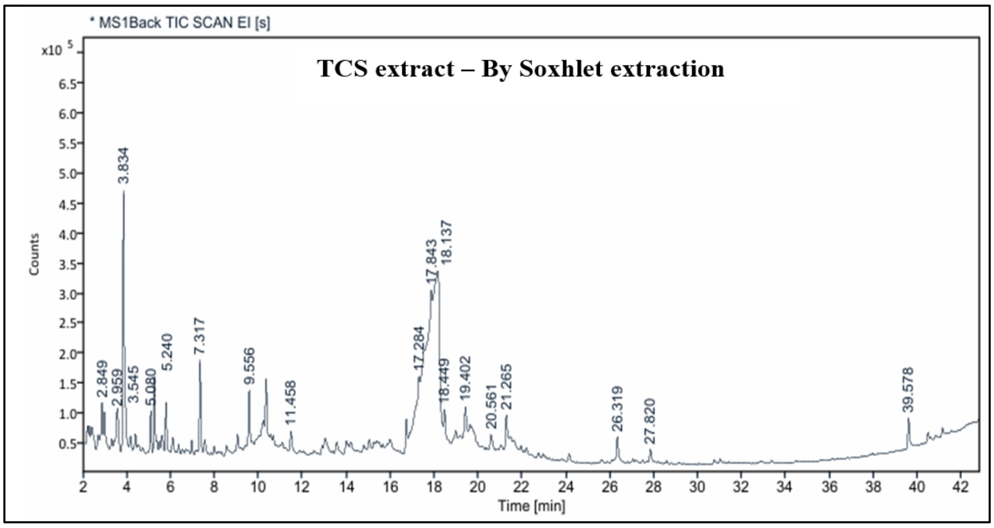

Fig. 1: GC-MS chromatogram of *T. cordifolia* stem extract by Soxhlet extraction

On contrary, the extract of *T. cordifolia* stem obtained by boiling method revealed a less diverse profile with 13 phytoconstituents only (Fig. 2). The predominant compound was Inositol, 1-deoxy-(75.30%), followed by Glyceraldehyde (5.83%), Glycerin (4.66%), Dihydroxyacetone (3.35%) and 5-Keto-D-Fructose (2.44%) (Table 2). The 5-Keto-D-Fructose (5-KF), was reported to possess anti-bacterial activity against *Bacillus subtilis* and *Escherichia coli* and exerts pronounced cytotoxic effect against colon cancer cells [24]**.** A minor trisaccharide Melezitose (1.15%) was also identified and was found to have anti-cancer effect against lung cancer [25]. The predominance of carbohydrate and polyol derivatives suggests that direct boiling favoured the extraction of highly polar, water-soluble primary metabolites. However, prolonged heating at high temperature by this Boiling method of extraction might have caused degradation of thermolabile phytochemicals, accounting for the reduced diversity and lower yield.

The presence of common metabolites such as Inositol, 1-deoxy-, Glycerin, Dihydroxyacetone, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-and (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol in both extracts indicates that these are the main hydrophilic constituents of *T. cordifolia* stem which can be extracted by both mild and exhaustive aqueous methods. (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (ferulic acid derivative) possesses a wide range of physiological properties such as antioxidant, anti-inflammatory, antibacterial, anti-cancer, and antithrombotic activity [26]. It is also a potent vasodilator [27, 28] and has been reported to arrest rapid cell division in cholangiocarcinoma [29].

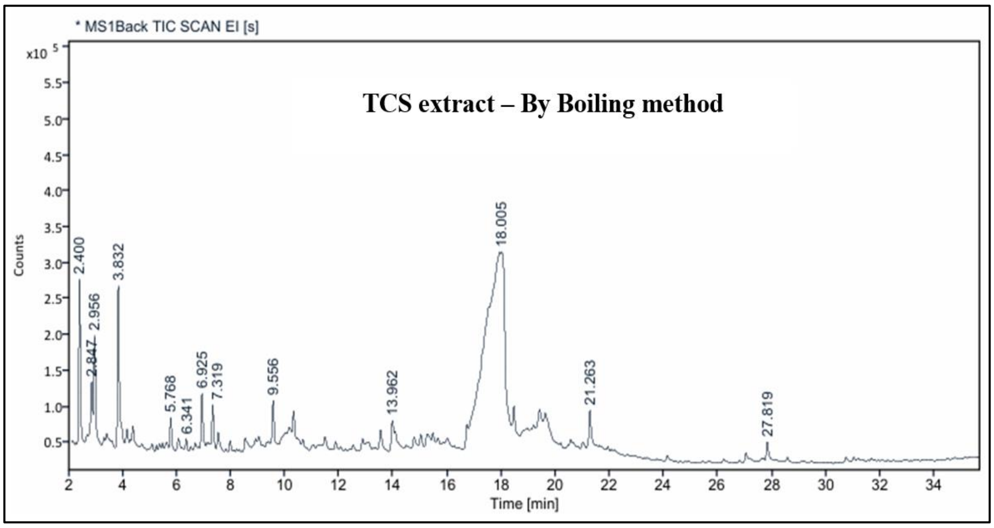

Fig 2: GC-MS chromatogram of *T. cordifolia* stem extract by Boiling method

Table 1: Phytochemicals detected in Soxhlet extraction of *T. cordifolia* stem extract

S. No.	Compound Name	RT (min)	Area %
1	Glycerin	3.834	25.08
2	Inositol, 1-deoxy-	18.137	15.81
3	4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6- methyl-	7.317	9.79
4	Desulphosinigrin	17.284	7.79
5	1-Butanamine, 3-methyl- N-(3-methylbutylidene)-	5.240	6.09
6	Decanoic acid, 3- hydroxy-, methyl ester	9.556	5.84
7	Columbin	39.578	4.59
8	1-(2- Tetrahydro furyl methyl) piperidine	3.545	4.00
9	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	5.080	2.64
10	1-Cyclohexene-1-propanol, α,2,6,6-tetramethyl-	19.402	2.49
11	Inositol, 1-deoxy-	17.843	2.45
12	Cyclohexanone, 2-(2-butynyl)-	11.458	2.35
13	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d] pyrazine	26.319	2.34
14	(E)-4-(3-Hydroxyprop-1- en-1-yl)-2-methoxyphenol	21.265	2.19
15	l-Alanine, N-methoxycarbonyl, butyl ester	2.849	1.86
16	Dihydroxyacetone	2.959	1.43
17	3,5-Dimethoxy-4- hydroxybenzyl alcohol	18.449	1.17
18	trans-Sinapyl alcohol	27.820	1.05
19	Uric acid	20.561	1.04

Table 2: Phytochemicals detected in Boiling method extraction of *T. cordifolia* stem extract

S. No.	Compound Name	RT (min)	Area %
1	Inositol, 1-deoxy-	18.005	75.30
2	Glyceraldehyde	2.400	5.83
3	Glycerin	3.832	4.66
4	Dihydroxyacetone	2.956	3.35
5	5-Keto-D-fructose	6.925	2.44
6	Acetic acid, 2,2'- [oxy bis(2,1-ethanediyloxy)] bis-	9.556	1.77
7	4H-Pyran-4-one, 2,3- dihydro-3,5-dihydroxy-6- methyl-	7.319	1.34
8	(E)-4-(3-Hydroxyprop-1- en-1-yl)-2-methoxyphenol	21.263	1.31
9	Melezitose	13.962	1.15
10	l-Alanine, N methoxycarbonyl, butyl ester	2.847	0.97
11	Acetic acid,6-morpholin-4-yl-9-oxo bicyclo [3.3.1] non-3-yle	5.768	0.89
12	1-Propyl-3,6- diazahomoadamantan-9- ol	27.819	0.69
13	Tetradecane, 2,6,10- trimethyl-	6.341	0.30

Conclusion

The present study highlights the significance of extraction methodology in determining the phytochemical composition and yield of *Tinospora cordifolia* stem extract. The Soxhlet extraction method proved to be superior to boiling process, yielding a higher percentage of extract enriched with diverse bioactive compounds, including alkaloids, terpenoids, phenolics and fatty acid esters. The metabolites identified in the study have been attributed with antioxidant, anti-inflammatory, anti-hypertensive, anti-cancerous, anti-bacterial, anti-diabetic and lipid-regulating properties, suggesting their potential role in mitigating various disease conditions. Thus, the study not only establishes the Soxhlet method as an effective approach for maximizing phytochemical recovery but also documents the key bioactive compounds found in *T. cordifolia* stem that may be further explored for developing effective therapeutic agents.

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