Indian Journal of Natural Products and Resources
Vol. 17(2), June 2026, pp. 256-265
DOI: 10.56042/ijnpr.v17i2.17022

Extraction of *T. cordifolia* (Thunb.) by multiple solvents to investigate phytochemical recovery by LC-MS and greenness evaluation by AGREEprep and AGREE

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Received 21 May 2025; revised received 05 March 2026; accepted 15 April 2026

Drug development from plant extracts using solvents of variable polarities has gained immense popularity. The selection of the solvent results in variable phytochemical profiles in the extracts. Solvent selection is primarily based on the targeted phytocompounds. The stem of *Tinospora cordifolia* is used to treat multiple ailments. In the present study, we have explored the extractability of organic solvents of varying polarity and evaluated the greenness of the analysis using various tools. The stem of *T. cordifolia* was extracted by traditional methods in accordance with the Pharmacopoeial guidelines, and phytochemical identification was performed by LC-MS. The methods of phytochemical identification were evaluated for their greenness indexation by using AGREEprep and AGREE tools. The experiment revealed that water was the best solvent for extraction (yield 14.25%) and that the LC-MS phytochemical profiles showed the highest number of phytochemicals (27). Water was the best option (AGREEprep score 0.80) for eco-friendly extraction. The study concludes that during drug development from plant extracts, we must adopt methods and solvents that support sustainable growth, which are expected to be perfectly eco-tuned.

Keywords: AGREE, AGREEprep, Extraction, LC-MS, Solvents, *T. cordifolia*

IPC code; Int. cl. (2021.01)– A61K 36/00, A61K 135/00, B01D 11/00

Introduction

Medicinal plants hold great importance in drug development, either as extracts or as sources of new biomolecules. Many modern drugs have their roots in medicinal plants. The importance of extracts is majorly celebrated in traditional drugs and phyto-pharmaceuticals. The principles of traditional medicine hold that an extract with a greater number of phytochemicals possesses greater therapeutic potential than an extract with a lesser number. The reason behind this school of thought is that phytochemicals exhibit their efficacy through synergism[1]. Sometimes it has also been claimed that some phytochemicals in the plant act to diminish the side effects caused by other phytochemicals[2]. Another important fact relevant to the importance of extracts is that extracts have a better shelf life and are easier to store and handle[3]. Moreover, an extract can be easily formulated into different dosage forms, such as syrup, ointments, tablets, capsules, *vatis*, *gutikas*, etc.[4]. According to traditional medicine, the goal is to obtain the maximum number of phytocompounds in the extract, and this is possible when we rationally select the extraction method and media.

Tinospora cordifolia, commonly known as *guduci* in Sanskrit, is a plant with immense potential for phytocompounds of different classes of secondary metabolites[5-7]. Among the several phytocompounds, the alkaloids and glycosides are the major class of secondary metabolites[8-9]. The stem of *T. cordifolia* has been studied for its numerous biological activities[10]. Its diverse groups of secondary metabolites make this a promising plant for treating human ailments[11,12]. The traditional text is often called *Amrita* (nectar)[13]. Traditional medicines, especially Ayurveda, advocate for the aqueous and hydroalcoholic extracts for drug development. In comparing its phytochemical constituents, a group of researchers has shown that solvent-specific extraction with aqueous, ethanolic, and hydroethanolic media yields varying amounts of secondary metabolites, mainly from these two classes, such as alkaloids and

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Supplementary tables are available online only.

glycosides[14,15]. These studies are limited to the identification and quantification of one or two bioactive markers, and to the exploration of hydro-ethanolic solvents of different composition. Besides these two solvents, there are few other solvents those are unexplored for extraction purposes. The exploration of extractability with other organic solvents, such as hexane, ethyl acetate, chloroform, and methanol, is the need of the day for the development of phyto-pharmaceuticals. The extraction capabilities of individual solvents need to be checked, as no two solvents yield extracts with the same phytochemical makeup[16]. Therefore, the uniqueness of extracts in terms of their phytocompounds is the key to developing a drug from them. Thus, the exploration of phytochemicals in extracts prepared with different organic solvents is the need of the hour. A complete profiling of an extract can be most efficiently performed using LC-MS analysis. A detailed phytochemical profiling of the stem obtained through LC-MS analysis is reported for male and female plants, but the variability in extracts remains unexplored[17]. Therefore, recognising the need for knowledge of phyto-rich extracts, the present study aimed to establish a comprehensive phytochemical profile by LC/MS analysis of solvent-specific extracts.

Another equally important aspect of phytochemical investigation is eco-tuned analysis for sustainable growth with respect to health and the environment. For instance, the random use of solvents, especially hazardous ones, is not only harmful to researchers and workers involved in phytochemical studies but also disrupts environmental balance. With this logic, the present phytochemical investigations evaluated the greenness index using the AGREEprep (Analytical Greenness Evaluation in sample preparation) and AGREE (Analytical Evaluation of Greenness) tools. Thus, the present study, by combining LC-MS analysis and greenness evaluation, provides a comprehensive profiling of phytochemicals and ensures a green approach to this chemical analysis. The significance of the present study lies not only in identifying the optimal extraction media for *T. cordifolia* stems but also in advocating green practices and green chemistry.

Materials and Methods

Chemicals and reagents

All solvents used in the experiments were HPLC grade and purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Plant material collection, authentication, and processing

Fresh mature portions of *T. cordifolia* stem were collected from the medicinal plant garden of Narendrapur Ramakrishna Mission (Latitude: 19° 19' 60" N, Longitude: 84° 51' 59" E), Kolkata, West Bengal, approved by the State Medicinal Plant Board, in late August 2022, with field book No. 13762 and accession number 5427 in the herbarium of NRKM. Dr S. K. Gupta authenticated the plant material. Pieces of stem (2-5 cm) were taken for study. Stems were washed with running water and then dried in the sunlight. A preliminary pharmacognostical study was performed to characterise the stem (results not shown). Dried stems were coarsely ground for extraction purposes[18].

Extraction in multiple solvents

The present study aims to determine the optimal extraction solvents based on phytochemical content in the extracts. Hence, solvents of varying polarity were used for extraction. Hexane was used as a low-polar solvent, chloroform and ethyl acetate as medium-polar solvents, and methanol and water as high-polar solvents. The standard method of extraction was followed to obtain extracts with these solvents, and extractive values were calculated[19]. For extraction, the plant-to-solvent ratio was maintained at 1:25, and the temperature was set to the solvent's boiling point for three hours. Each extract was filtered through Whatman filter paper number 41, and the filtrates obtained were evaporated to dryness, and the yields were calculated. Dried extracts were refrigerated for further LC-MS analysis.

Qualitative identification of Phytocompounds by LC-MS/MS

Among the five extracts, the hexane extract was the lowest and very insignificant compared with the others. Hence, the phytochemical identifications of the chloroform, ethyl acetate, methanol, and water extracts were investigated through Liquid Chromatography tandem Mass Spectrometry LC-MS/MS.

For the LC-MS study, the dried extracts were partitioned for reconstitution in water, which was considered the best-suited solvent for LC analysis. During sample preparation, it was observed that a very small portion of the solidified extract remained unattended during redissolution. Ultrasonication was performed for 15 minutes at a controlled temperature below 40°C to obtain the insoluble fraction in water. Finally, the filtrates were filtered and analysed by LC-MS/MS.

Instrumentation

Q-Exactive plus Biopharma from Thermo Scientific was utilised. Xcalibur (Version 4.2.28.14), a data-acquisition software from Thermo Scientific, was used. The data processing software employed was Compound Discoverer 3.2 SP1. The column used was Hypersil GOLD 150 x 2.1 mm, 1.9 microns, Thermo Scientific.

Mass spectrometry was performed using a QSTAR Elite LC-MS/MS system from Applied Biosystems MDS Sciex (Concord, ON, Canada) equipped with an ESI ion source.

Chromatographic condition

For chromatographic separation, keeping the column temperature constant at 40°C was found to be most suitable in generating a resolute chromatogram. A four steps gradient program was adopted with mobile phase consisting of 0.1% formic acid in Milli-Q water in channel A and in channel B methanol (5%) for 20 minutes, 0.1% formic acid in Milli-Q water (100%) for 5 minutes, methanol (5%) for next 5 minutes and in last step of gradient the mobile phase consisting of 0.1% formic acid in Milli-Q water in channel A and in channel B methanol (5%) was used with constant flow rate of 0.3 mL/min by keeping the runtime 35 minutes.

Mass Spectrometry condition

For mass spectrometric analysis, the instrumental parameters are set up to maximise the identification of phytocompounds[20]. Nitrogen was used in all cases for 35 minutes of operation. The source parameters were optimised with ESI voltage of 5500 and 4500V for positive and negative ionisation, respectively. Variable gas flow pressure and temperature were maintained between 35-60 psi and 450°C. To minimise the formation of ion clusters, the declustering potential at both ends was fixed at 60V.

MS absorbance threshold and MS/MS absorbance threshold were set to 200 and 5, respectively. The samples were analysed in auto-acquisition mode, set to the m/z range of 70 to 1000 to automatically select candidate ions for MS/MS analysis. Accurate mass measurements of each peak from the total ion chromatograms (TICs) were obtained using the dynamic auto-calibration method, which allows real-time internal calibration during data acquisition[21].

Greenness analysis using AGREEprep and AGREE tools

Analytical methods involving chemicals are generally questionable in terms of their greenness. Though the analysis is in favour of mankind, it often generates waste that is detrimental to the environment. Hence, it becomes essential to evaluate the greenness of the analytical study to support sustainable environmental practices. To evaluate the greenness of the analysis in the present study, the complete analysis was divided into two steps: extraction and identification. The extraction step was referred to as sample preparation, and greenness analysis was evaluated using the AGREEprep tools[22]. The greenness index of the extraction method (sample preparation) was evaluated for every individual extraction using four different solvents. The identification step was considered an analytical step, and its greenness was assessed using the AGREE tools[23]. The greenness index of the identification method was evaluated for a single instance, as the samples (extracts) obtained were subsequently identified using the same method.

Evaluation using the AGREEprep tool comprises 10 components: sample preparation site, renewability of materials, quantity of hazardous waste, operator exposure to hazard, etc.

In the subsequent step of identification, greenness evaluation was performed using the AGREE tool. Unlike AGREEprep, the AGREE tool has 12 greenness checkpoints, primarily based on the reagents used, the method type, reagent toxicity, and the power consumption.

Results and Discussion

Optimisation of extracting solvents

The standard extraction method for optimising the best solvent revealed that water was the best solvent among those tested for *T. cordifolia* stems. The extractive values increase with solvent polarity. The order of extractive yields is hexane < chloroform < ethyl acetate < methanol < water, and the extractive values (in %) are 0.87 < 6.35 < 7.15 < 12.37 < 14.25, respectively. The water-soluble extractive value attended the permissible limit set by the Ayurvedic Pharmacopoeia of India[24].

Qualitative identification of secondary metabolites

Though LC-MS/MS QTOF may not detect all compounds, it is probably the most suitable method for detecting phytocompounds in the extract because it is rapid, effective, and highly selective and sensitive. To ensure maximum identification, the appropriate ionisation mode (positive and negative) was selected. To screen and qualitatively identify

secondary metabolites in different solvent extracts, we compared the reported data using m/z cloud software. The images of the positive and negative ESI chromatograms for all four extracts are shown in Figs. 1 to 4.

The presence of the phytocompounds, the molecular peak, and the fragmentation pattern were considered and matched to the library data. Merely matching was not the final consideration; theoretical fragmentations, source annotations, and confidence levels were also taken into account for profiling. Primarily, we compared the obtained m/z values with the theoretical m/z values, then checked the source annotation score or confidence level. A confidence score of 75 or above was considered as a true check. With this logic, we also noted some other secondary metabolites. In the manuscript, we mentioned only the major phytocompounds, whose biological activities have been established in several studies by different researchers. Molecular structures of major bioactive secondary metabolites are represented in Fig. 5.

Let us have a look at the experimental LC-MS data presented in Table 1. Most of the major phytocompounds of the *T. cordifolia* stems are polar in nature. Probably because hexane, being the least polar solvent, produced a low extractive yield, we did not carry out LC-MS identification of phytocompounds in the hexane extract. The list of phytocompounds in Table 1 shows that the extraction

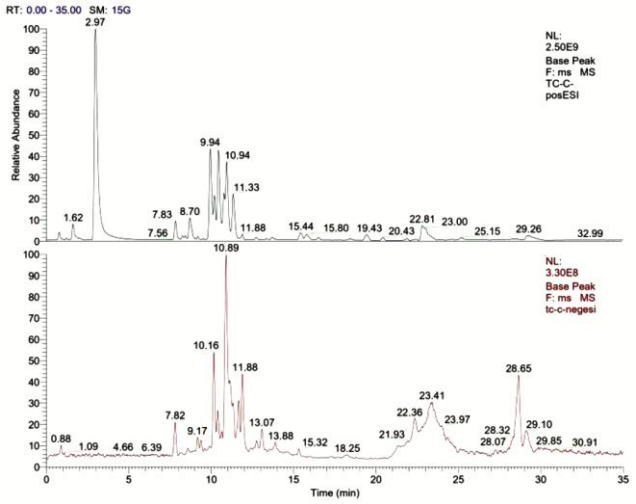

Fig. 1 — LC-MS chromatograms of chloroform extract (positive and negative ionisation).

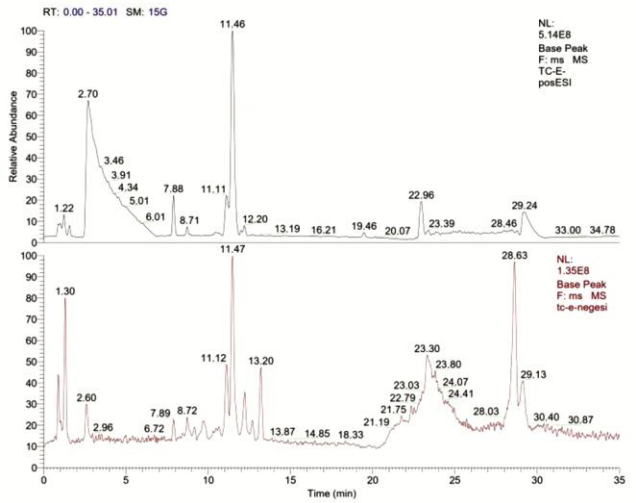

Fig. 2 — LC-MS chromatograms of ethyl acetate extract (positive and negative ionisation).

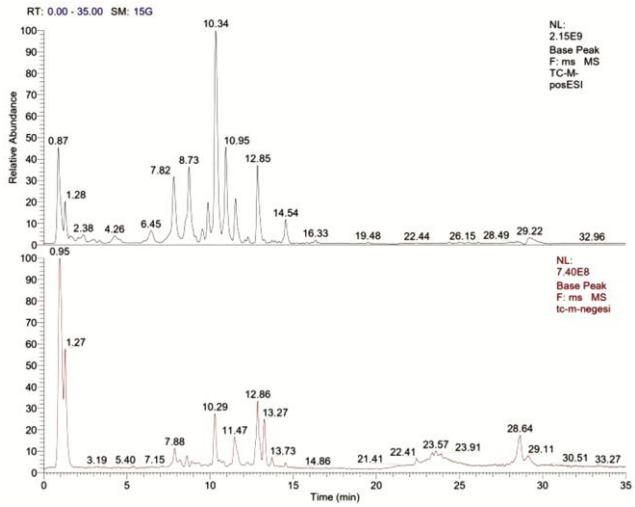

Fig. 3 — LC-MS chromatograms of methanol extract (positive and negative ionisation).

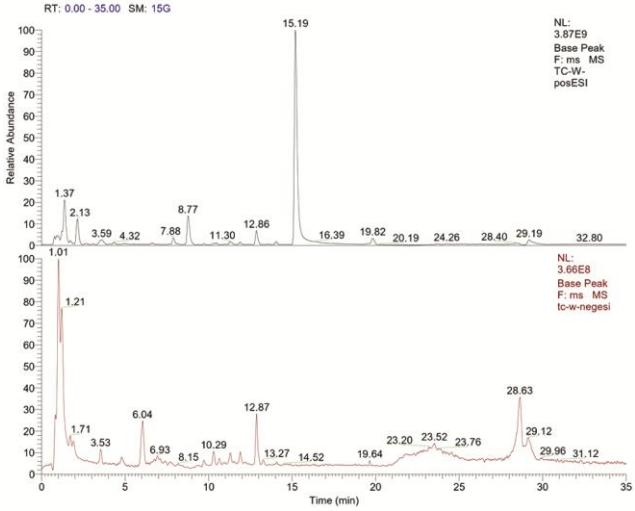

Fig. 4 — LC-MS chromatograms of water extract (positive and negative ionisation).

Table 1 — Lists of secondary metabolites identified in different solvent extracts of *T. cordifolia* stem

Class of phytocompounds	Name of phytocompound	Available in extracts[#]
Alkaloids	Tinosporine	C, E, M, W
	Magnoflorine	C, E, M, W
	Berberine	C, E, M, W
	Choline	C, E,
	Jatrorrhizine	C, M, W
	Palmatine	E, W
	Tembeterine	C, E
	Berbemine	C, E, M, W
	Berbine	C, M, W
Glycosides	Tinosporaside	C, E, M, W
	Cordifolioside A	M, W
	Cordifolioside B	M, W
	Cordifolioside C	W
	Cordifolioside D	W
	Cordifolioside E	W
	Cordioside	E, M, W
	Tinocordioside	M, W
	Tinocordifolioside	C, M, W
	Palmatoside	E, W
	Tinosporide	C, E, M
Terpenoids	Furanolactone	M
	Clerodane	E, M, W
	Tinocordifolin	C, M
	Ecdysterone	E, M
	Makisterone	W
	Giloinsterol	W
Phytosterols	Beta-sitosterol	C, E, M, W
	Stigmasterol	M, W
	Kaempferol	E, M
	Ecdyson	C, E, M, W
	Giloin	W
	Heptacosanol	M
	Octacosanol	E, M
Other unclassified derivatives	Sinapic acid	C, E, W
	Tinosporan acetate	E, W
	Tinosporal acetate	E, W
	Tinosporidine	C, M
	Tinosponone	E, M

[#] C= chloroform, E= ethyl acetate, M= methanol, W= water

efficiency increases with solvent polarity. This trend remains linear up to water.

A preliminary observation from the LC-MS dataset is that solvents with the lowest extractive yields contain the fewest phytocompounds, whereas those with the highest yields contain the most. This fact is supported by data showing that the chloroform extract contains only 16 phytocompounds, whereas the water extract contains 27. Though this statement is merely an empirical one, the facts still support it. A linear relationship between the extractive yields of solvents and the number of phytocompounds identified in corresponding extracts is observed. For instance, extractive yields and numbers of phytocompounds in chloroform are 7.35% and 16, respectively; in ethyl acetate, 8.15% and 21; in methanol, 12.37% and 25; and in water, 14.25% and 27, respectively.

A very remarkable observation is that water extract is a rich source of glycosides because all the glycosides (10) identified from the stem are present in

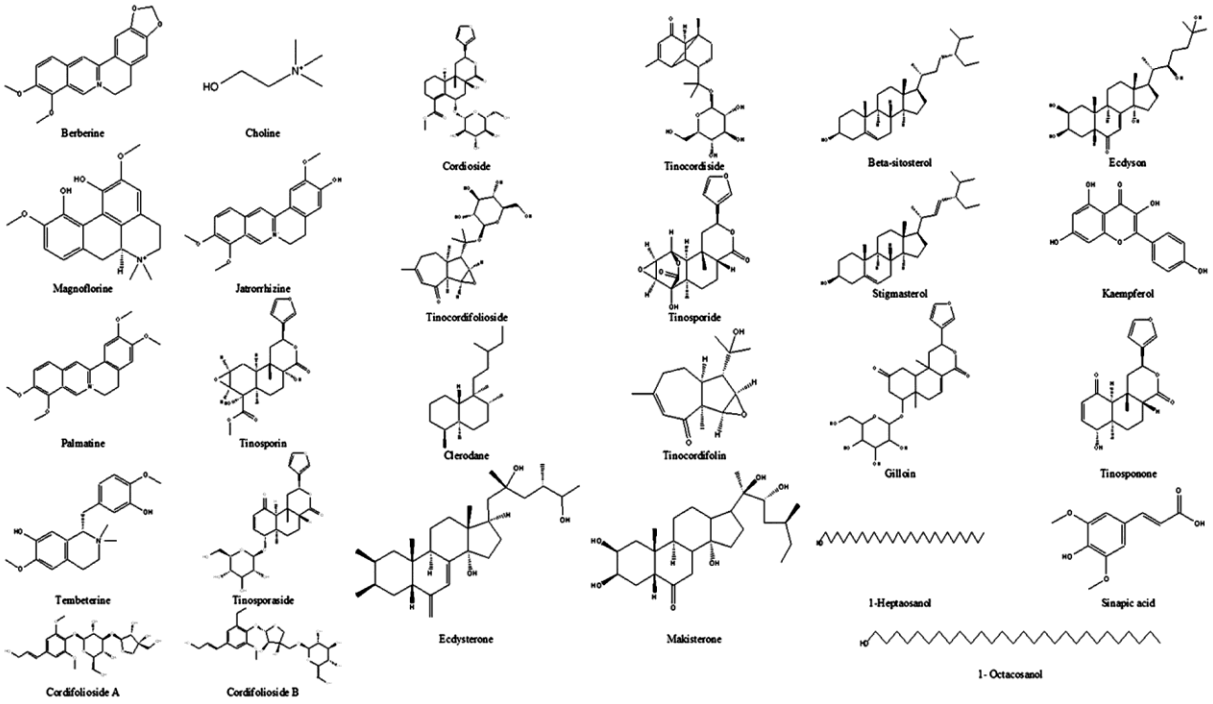

Fig. 5 — Molecular structures of major bioactive secondary metabolites.

the water extract. Still, the other solvents have glycoside content far lower than that of water. For example, chloroform contains 2 glycosides, ethyl acetate contains 3, and methanol contains 5. The molecular structure of glycosides can explain how solvent influences glycoside extraction. A glycoside molecule consists of a glycone and an aglycone part. Between these two, the glycone moiety is a sugar with greater water solubility. Possibly due to the nature of the sugar moiety, the glycoside has a greater affinity for water, as a general principle of like dissolves like holds.

While comparing the extractive yields and the numbers of phytocompounds between methanol and water extracts, it has been observed that both solvents contain almost the same number of phytocompounds (marginally higher in water). Still, the extractive yields of these two solvents are not very close. A possible explanation in support of this fact is that, generally, glycosides are higher molecular weight compounds than those of alkaloids, terpenoids, etc. Hence, the greater number of glycosides in the water extract ultimately contributes to a higher extractive yield.

Another noteworthy observation is that alkaloids are present in all the extracts at very high levels. All the solvent extracts contain 6 to 7 alkaloids. The alkaloids present in the stem of *T. cordifolia*, magnoflorine, berberine, tembetarine, choline, jattrorhizine, and palmitin possess a benzylisoquinoline skeleton. These alkaloids exist as quaternary alkaloidal salts with an iminium moiety in a hydrophobic framework. Hence, owing to this structural feature of bearing both hydrophobic and hydrophilic parts within the same molecule, these alkaloids tend to dissolve in both organic and aqueous media. This may be the reason behind the presence of alkaloids in all the organic and aqueous solvents. List of secondary metabolites along with their class and m/z precursors detected by LC-MS/MS in different extracts of *T. cordifolia* stem are given in the Supplementary file. In the supplementary file, Tables S1, S2, S3 and S4 list the secondary metabolites detected by LC-MS/MS in chloroform, ethyl acetate, methanol, and water extracts, respectively.

The comprehensive list of phytocompounds mentioned in Table 1, reveals a fact of extracting efficiency that the extractability of the solvents goes up with their polarity. This trend remains linear up to water. The phytocompounds present in the root are rarely non-polar, possibly because hexane is unable to extract them efficiently, whereas chloroform is a better-extracting medium. Water has efficiently extracted a range of glycosides, including phenolic

and flavonoid glycosides. Therefore, it may be stated that the polarity of solvents may have a critical role in extracting the phytochemicals from the plant[24].

Another factor is the boiling point of the solvents. It is thought that the higher the boiling temperature, the greater the exposure of phytocompounds to solvents, because at higher temperatures the cell wall ruptures, allowing the phytochemicals to come out of the plant matrix[25]. This logic supports the presence of water extraction, but it does not fit while comparing the boiling temperatures and phytochemical profiles of ethyl while acetate and methanol. For instance, ethyl acetate has a higher boiling point than methanol, but its extractability of secondary metabolites or phytocompounds does not depend on their boiling points.

Therefore, having stated the above facts, it may be stated that none of the individual factors is responsible for extracting the phytocompounds. Thus, the extractions of phytocompounds collectively depend on the solubility and polarity of the phytomolecules and, to some extent, the boiling nature of the solvent used for extractions. The results of qualitative identification indicate that, empirically, the ascending order of solvents for extracting secondary metabolites is chloroform, ethyl acetate, methanol, and water. This order is based on the number of phytocompounds and classes of secondary metabolites detected in that particular solvent extract. Each solvent has its own extractability for pulling secondary metabolites from plant roots. These observations are a guiding parameter in drug development. In a nutshell, it may be stated that phytomolecule-driven drug development should have a clue for solvent selection, and this piece of work can well serve that criterion. It is essential to optimise the extracting solvent for target-oriented drug development from medicinal plants. Therefore, the prudent selection of solvents for drug discovery from green sources is highly essential. Choosing the wrong solvent not only underutilises natural resources but also imbalances the ecosystem by releasing solvents into the environment. Another important point to mention is that the extractive yield alone is not responsible for the bioactivity, which truly depends on the phytochemicals, especially bioactive phytomolecules. Hence, according to that logic, water is best among all the solvents tested, not because it yields the highest extractive yields, but because it contains majorly bioactive phytomolecules, namely alkaloids and glycosides.

Statistical analysis

The content of phytochemicals (number of phytocompounds in different classes) was compared using XLSTAT to analyse the percentiles of the classes of phytocompounds corresponding to the solvent used for extraction. Pie diagrams representing the area under consideration for depiction of the results are given in Fig. 6a-b. The numbers given in every sector of the pie diagram indicate the percentage contributions. From the given diagram, the quantitative presence can be estimated empirically. For example, in Fig. 6a, the ethyl acetate extract is composed of 33, 14, 10, 19, and 24% of alkaloids, glycosides, terpenoids, phyto steroids, and other compounds, respectively. Similarly, water contributes 26, 37, 4, 19, and 15% to alkaloids, glycosides, terpenoids, phyto steroids, and other compounds respectively. Looking at Fig. 6b, it is clear that alkaloids are highest in chloroform extracts, glycosides and terpenoids are highest in water and methanol extracts, respectively. In contrast, phytosterols are equally extracted by both methanol and water. Thus, by critically comparing these

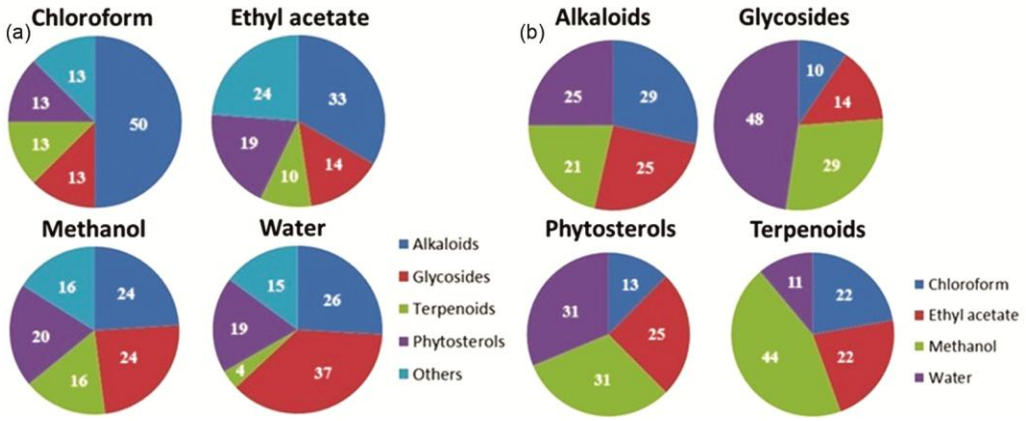

Fig. 6 — Quantitative pie diagram based on (a) solvent extractability, and (b) phytochemical make up.

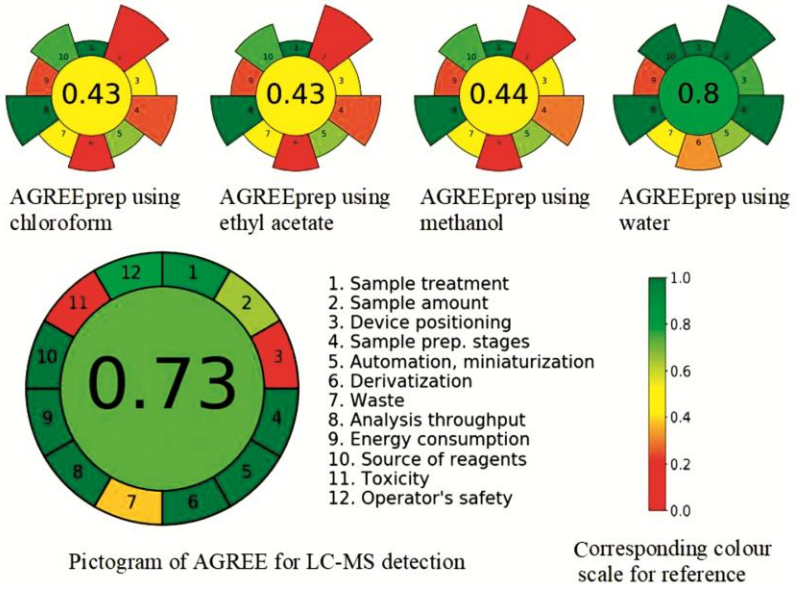

Fig. 7 — Scintometric scores by greenness analysis through AGREEprep and AGREE tools.

extraction patterns, it is somewhat clear that water is the most efficient among the five solvents used here.

Greenness analysis

Analysis using Greenness tools generates a scorecard ranging from 0 to 1. An analysis with a minimum score of 0.5 can be categorised as environmentally friendly, whereas scores between 0.5 and 0.75 are usually considered "moderately green", and any score above 0.75 is considered green. When a method acquires a value below 0.5, it is marked as not green[22].

The sample preparation technique using different extraction solvents was analysed using AGREEprep tools for their greenness index. While comparing the index, it was observed that extraction with chloroform, ethyl acetate, and methanol is marked as not green, with scores of 0.43, 0.43, and 0.44, respectively, whereas extraction with water is definitely in the green category, with a score of 0.8. The scintometric scores of the greenness analysis, represented as a pictogram, are shown in Fig. 7. The identifications of phytochemicals for all four extracts were performed using the same method; hence, the single greenness metrics from the AGREE tool were used to test it. The evaluated greenness score was 0.73 using the AGREE tools. According to the AGREE tool, the method has seven green parts (components 1, 4, 5, 6, 8, 9, and 10), two light green parts (components 2 and 12), one yellow part (component 7), and two red parts (components 3 and 11). These components, when combined, generated a total AGREE score of 0.73, indicating the method is moderately green.

Let us have an in-depth analysis of the results. The use of chloroform, ethyl acetate, and methanol was considered hazardous, and their recovery rates are very low; on the other hand, the quantity of waste is remarkably high. Hence, these factors contribute negatively to a good extent to a score below 0.5. Compared to these solvents, water is the greenest solvent, with far less impact on the environment due to its non-toxicity, non-flammability, and other factors. Thus, in the present analysis, the use of water is highly recommended for green chemistry. In the present analytical work, the indication of red components (such as 3 and 11) is a scope for improving greenness as an integral part of green chemistry.

Conclusion

The study examines solvent-specific extraction and the varying effectiveness of different solvents in obtaining phytochemicals. It notes that solvents with different polarities yield diverse phytochemical profiles. Organic solvents like chloroform and ethyl acetate can extract limited amounts of phytochemicals. Methanol is an effective extraction medium for a wide range of phytochemicals. Remarkably, water is the most efficient solvent in extracting maximum numbers of phytochemicals of different classes of secondary metabolites. Another

positive note about the present study's outcome is that, water is not only the best way to extract but also a sustainable way to explore natural resources without harming the environment, thanks to its greenness. Understanding solvent properties is crucial, as using an unsuitable solvent can diminish potency and waste resources. This research guides the selection of optimal solvents for the development of phytopharmaceuticals from *T. cordifolia*, with significant implications for drug discovery, sustainable processes and resource use. In a broader sense, combining LC-MS profiling with greenness analysis can be considered a novel approach for medicinal plants that contain diversified classes of secondary metabolites for two reasons. Firstly, profiling provides solvent-based extractability, and greenness evaluation indicates the sustainability of chemical analysis for eco-tuned research and development.

Conflict of interest

The authors individually and jointly declare that there are no conflicts of interest associated with this article.

AI use disclosure

During the preparation of this manuscript, the author(s) did not use AI/LLM. Authors ensure accuracy and originality of the content. The author(s) take full responsibility for the integrity and final content of the published article.

Acknowledgement

The authors acknowledge the Director, SAIF-IIT, Mumbai, for providing the infrastructure for spectral analysis. The present work was funded by the Central Council for Research in Ayurvedic Sciences, New Delhi, through the IMR project mode (No. HQ-PROJ011/80/2022-PROJ, vide Office Order No. 700/2022-23, dated 17.10.2022)

Availability of data and materials

Associated data and the materials will be made available on request to the project funding Authority.

References

1 Zhang A, Sun H and Wang X, Potentiating therapeutic effects by enhancing synergism based on active constituents from traditional medicine, *Phytother Res*, 2014, **28**(4), 526-533, doi: 10.1002/ptr.5032.

2 Gurib-Fakim A, Medicinal plants: Traditions of yesterday and drugs of tomorrow, *Mol Asp Med*, 2006, **27**(1), 1-93, doi: 10.1016/j.mam.2005.07.008.

3 Kumadoh D, Kwakye K O, Kuntworbe N, Adi-Dako O and Appenahier J A, Determination of shelf life of four herbal medicinal products using high-performance liquid chromatography analyses of markers and the Systat Sigmaplot software, *J Appl Pharmaceut Sci*, 2020, **10**(6), 72-80, doi: 10.7324/JAPS.2020.10610.

4 Anonymous, Ayurvedic Pharmacopoeia of India. Part III, vol. 3, Min. of Health & Family Welfare, Govt. of India New Delhi, 2008, 242-244.

5 Sharma P, Dwivedee B P, Bisht D, Dash A K and Kumar D, The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*, *Heliyon*, 2019, **5**(9), doi: 10.1016/j.heliyon.2019.e02437.

6 Gandhi Y, Mishra S K, Kumar V, Rawat H, Kumar R, *et al.*, Phytochemical marker-based analysis, antioxidant potential, and shelf-life study of geographically different samples of *Tinospora cordifolia* (Stem), *Curr Indian Sci*, 2024, **2**(1), e2210299X324745, doi: 10.2174/012210299X324745240808062057.

7 Sultana S, Ali M and Jameel M, Phytochemical investigation and isolation of new compounds from the stems of *Tinospora cordifolia* Miers., *Trends Phytochem Res*, 2017, **1**(2), 83-92.

8 Reddy N M and Reddy R N, *Tinospora cordifolia* chemical constituents and medicinal properties: a review, *Sch Acad J Pharm*, 2015, **4**(8), 364-369.

9 Chaudhary A, Das R, Mehta K and Mehta D K, Indian herb *Tinospora cordifolia* and Tinospora species: Phytochemical and therapeutic application, *Heliyon*, 2024, **10**(10), e31229, doi: 10.1016/j.heliyon.2024.e31229.

10 Singh N and Yadav S S, Anti-dengue therapeutic potential of *Tinospora cordifolia* and its bioactives, *J Ethnopharmacol*, 2024, 118242, doi: 10.1016/j.jep.2024.118242.

11 Bisht A, Tewari D, Kumar S and Chandra S, Network pharmacology, molecular docking, and molecular dynamics simulation to elucidate the mechanism of anti-aging action of *Tinospora cordifolia*, *Mol Divers*, 2024, **28**(3), 1743-1763, doi: 10.1007/s11030-023-10684-w.

12 Rai P, Garain T and Gupta D, Phytochemical analysis of *Tinospora cordifolia* and *Withania somnifera* and their therapeutic activities with special reference to COVID-19, *World J Exp Med*, 2024, **14**(3), 95512, doi: 10.5493/wjem.v14.i3.95512.

13 Saha S and Ghosh S, *Tinospora cordifolia*: One plant, many roles, *Anc Sci Life*, 2012, **31**(4), 151-159, doi: 10.4103/0257-7941.107344.

14 Hazra K, Mitra A, Singh R and Hazra J, Rationalisation of extractive protocol by high-performance thin-layer chromatographic–densitometric quantification of berberine in multiple hydroalcoholic extract of *Tinospora cordifolia* stem, *J Planar Chromatogr*, 2021, **34**(2), 157–163, doi: 10.1007/s00764-021-00098-5.

15 Hazra K, Kumar D, Debnath S, Mondal S, Batule M, *et al.*, Dynamicity and extractability of hydroalcoholic solvents for *Tinospora cordifolia* stem: An investigation for target-oriented traditional drug discovery based on biologically active phytocompounds, *Vegetos*, 2024, **37**(2), 1-11, doi: 10.1007/s42535-024-00835-1.

16 Hazra K, Kumar D, Mitra A, Dutta S, Sarkar S, *et al.*, Phytopharmacognostic profiling of *Prunus cerasoides* Buch.-Ham. ex D. Don, heartwood, *Indian J Nat Prod Resour*, 2024, **15**(1), 1-12.

17 Bajpai V, Singh A, Chandra P, Negi M P, Kumar N, *et al.*, Analysis of phytochemical variations in dioecious *Tinospora cordifolia* stems using HPLC/QTOF MS/MS and UPLC/QqQLIT-MS/MS, *Phytochem Anal*, 2016, **27**(2), 92-99, doi: 10.1002/pca.2601.

18 Anonymous, Quality Control Methods for Medicinal Plant Materials, World Health Organisation, Geneva, 2011, 3-5.

19 Anonymous, Ayurvedic Pharmacopoeia of India, Part I, vol. 4, Ministry of Health & Family Welfare, Govt. of India New Delhi, 2004, 242-244.

20 Hazra K, Kumar D, Dutta S, Dighe D, Saha S, *et al.*, Endurance of phytocompounds in classical preparation from the root of *Rauvolfia serpentina*: pharmaceutical dosage validation by HPTLC, HPLC–UV, and LC–MS/MS, *J Anal Sci Technol*, 2024, **15**, 57, doi: 10.1186/s40543-024-00472-y.

21 Kumar D, Hazra K, Mondal S, Arya D, Bhradwaj Y, *et al.*, The diversity of the phytochemical array in unexplored high altitude medicinal plant *Gonostegia hirta* (Blume. ex Hassk.) Miq., *Indian J Nat Prod Resour*, 2025, **16**(1), 113-122, doi: 10.56042/ijnpr.v16i1.4187.

22 Wojnowski W, Tobiszewski M, Pena-Pereira F and Psillakis E, AGREEprep–analytical greenness metric for sample preparation, *TrAC Trends Anal Chem*, 2022, **149**, 116553, doi: 10.1016/j.trac.2022.116553.

23 Pena-Pereira F, Wojnowski W and Tobiszewski M, AGREE—Analytical GREEnness metric approach and software, *Anal Chem*, 2020, **92**(14), 10076-10082, doi: 10.1021/acs.analchem.0c01887.

24 Liegeois J F, Hayen J L and Taouba H, Solubility and extractability in the Pharmaceutical Sciences: A demonstration to address these essential concepts, *J Chem Ed*, 2024, **101**(12), 5547-5555, doi: 10.1021/acs.jchemed. 4c00568.

25 Jagdale S K and Nawale R B, Experimental measurement–correlation of solubility and dissolution thermodynamics study of itraconazole in pure monosolvents at various temperatures, *Drug Dev Ind Pharm*, 2021, **47**(7), 1038-1051, doi: 10.1080/03639045.2021.1879841.