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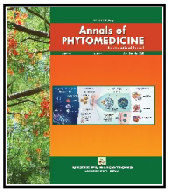

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Phytochemical and qualitative analysis of bioactive compounds in the stem of *Tinospora cordifolia* (Thunb.) Miers

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Abstract

The current study examines the qualitative and phytochemical composition of *Tinospora cordifolia* (Thunb.) Miers stem, focusing on macro- and micro-mineral composition, total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC). Macro- and micro-mineral analysis exhibited a high potassium concentration, followed by calcium, magnesium, and iron, with cadmium present in small levels. The methanolic extract had the highest TPC (2.90 ± 0.36 mg GAE g^{-1}), TFC (3.79 ± 0.48 mg QE g^{-1}), and TAC (0.0938 ± 0.0045 mg B g^{-1}), suggesting *T. cordifolia* as a rich source of antioxidant phytochemicals. These results are consistent with current research that contrasts phenolics, flavonoids, and alkaloids in terms of their antioxidant, antibacterial, and anti-inflammatory properties. This compositional profile supports *T. cordifolia's* potential use in functional foods and phytopharmaceutical preparations.

1. Introduction

Traditional knowledge of using plants and herbs for treatment has been passed down for generations. However, plants have been essential to life since the beginning of human history by providing food, raw materials for medicine, and a host of other necessities. According to the World Health Organisation (WHO), 80% of the global population relies on traditional medicines that use plant-based extracts or active ingredients (Kumar *et al.,* 2025). Due to their easy accessibility, purported safety, and ease of preparation, herbal medicines have gained a reputation as "the people's medicines". In recent years, there has been a noticeable shift towards a more holistic approach to human health, emphasising the need to combine necessary nutrients with traditional natural remedies (Goyal and Chauhan, 2024). Many bioactive secondary metabolites found in medicinal plants and herbs can activate the body's natural immune system and are good for human health with no negative side effects.

Plant-derived compounds have gained popularity for their diverse applications. Plant-based phytopharmaceuticals, such as anthocyanidins, carotenoids, lycopenes, flavonoids, glucosinolates, isoflavonoids, limonoids, polyphenols, omega-3 fatty acids, and phytoestrogens, can have pharmacological effects on humans. Plant-derived bioactive chemicals and minerals are essential for improving human health and preventing chronic diseases (Nakadate *et al.,* 2025).

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Nutritional profiling, which includes both macronutrients (carbohydrates, proteins, and lipids) and micronutrients (minerals and trace elements), provides insights into the dietary relevance of medicinal plants (Jyothsna *et al.,* 2020). Similarly, phytochemical measurement of phenolics, flavonoids, and alkaloids provides useful information about their medicinal potential (Sipoloni *et al.,* 2025).

T. cordifolia (Menispermaceae), often known as Giloy, is a popular medicinal plant in Ayurvedic and traditional medicine due to its immunomodulatory, antioxidant, anti-inflammatory, and antidiabetic effects (Kabilan *et al.,* 2025, Singh *et al.,* 2025). The plant's leaves, stems, fruits, and roots all contain remarkably high amounts of nutrients, minerals, and phytochemicals, as do fat, protein, dietary fibres, and additional nutrients (Kumar *et al.,* 2025). Numerous chemical constituents found in the plant, such as phenolics, glycosides, alkaloids, polysaccharides, lactones, aliphatic compounds, diterpenoid steroids, and sesquiterpenoids, have been investigated for their potential medicinal uses. The alkaloid demonstrated a strong antioxidant characteristic, while berberine exhibits a strong antiviral effect (Jatav, 2023). Its phytochemical components, particularly phenolic compounds, flavonoids, and alkaloids, which have powerful antioxidant and free radical scavenging properties, are primarily responsible for the therapeutic potential (Sharma *et al.,* 2024).

Bioactive compounds found in functional foods and drugs can help promote wellness by reducing inflammation, eradicating fungal infection, and protecting cells from damage (Vasanthkumar *et al.,* 2024). *T. cordifolia* is rich in bioactive chemicals, including glycosides, terpenoid steroids, phenolics, polysaccharides, aliphatic compounds, and alkaloids (Patil *et al.,* 2021). Specifically, the stem extract is rich in bioactive chemicals, particularly phenolics, followed by

flavonoids, alkaloids, and terpenoids (Annisa *et al.,* 2025). These chemicals exhibit anticancer efficacy through multiple apoptotic pathways, such as enhancing ROS, inhibiting cell cycle, activating caspase pathways, and inhibiting cell proliferation (Anjum *et al.,* 2023). Phenolic chemicals, defined by hydroxylated aromatic rings, help to reduce oxidative stress by giving hydrogen atoms or electrons to reactive oxygen species (ROS) (Yadav *et al.,* 2025). Flavonoids, a type of polyphenol, are known for their anti-inflammatory, antibacterial, and cardioprotective properties (Rodríguez-Negrete *et al.,* 2024). Alkaloids are nitrogen-containing secondary metabolites with a variety of pharmacological properties, including analgesic, anticancer, and antibacterial activities. It indicates that *T. cordifolia* therapy may be an essential defence against cisplatin's side effects.

Medicinal plant research aims to discover new therapeutic chemicals and molecules for medication development. Despite numerous pharmacological studies, there is little systematic information on *T. cordifolia's* combined nutritional and phytochemical makeup. This work aims to bridge the gap by analysing its macro- and micro-mineral composition, along with a quantitative assessment of TPC, TFC, and TAC, thereby providing an integrated profile of its nutritional and functional value.

2. Materials and Methods

2.1 Procurement of sample and authentication

The sample was procured from Herbal Garden, School of Organic Farming, Punjab Agricultural University (PAU), Ludhiana, Punjab, India. The department provided the stems of *T. cordifolia* (latitude: 30.900965, longitude: 75.857277; GPS coordinates: 30°542 3.47403 N, 75°512 26.19723 E). The plant material was taxonomically identified and authenticated by Dr. Avneetpal Singh, Department of Botany, Punjab University, Patiala, Punjab, India. The Herbarium Number is 878.

2.2 Preparation of plant powder

The authenticated stems were shade-dried for three weeks (Figure 1) and then ground into a fine powder using an HG1100 mortar grinder. The powdered substance was kept in airtight containers until further use.

Figure 1: Preparation of plant material.

2.3 Mineral analysis

A 250 ml conical flask containing about 1 g of dried, powdered plant material was filled with diluted nitric acid (Figure 2) (HNO_3: distilled water = 2:1). The plant material was thoroughly digested by boiling the mixture strongly, after which it was allowed to cool to normal temperature and dried by evaporating it. Following the addition of double-distilled water, the liquid was filtered into a 250 ml volumetric flask. Dilute nitric acid was used to repeatedly wash the residue and the aliquot in the volumetric flask. After that, the volume was increased to 250 ml. The solution was subsequently diluted 100 times to determine the calcium content. A flame photometer was used to examine sodium and potassium, and an Atomic Absorption Spectrophotometer (Varian AA 575 series) was used to quantify the mineral elements (Ca, Mg, K, Na, Cu, Fe, Zn, and Mn). Results were expressed per 100 g of dried sample, and all measurements were carried out in triplicate (Biwas *et al.,* 2017).

2.4 Preparation of the methanol extract by Soxhlet extraction

As *T. cordifolia* stems were dried and ground into a fine powder, 30 g of the powder was placed in a thimble inside a Soxhlet apparatus Figure 3. After adding 0.3% of methanol to the round-bottom flask, a continuous reflux extraction was performed for 14 h. Methanol vapours rose during the process, condensed in the condenser, and then passed through the plant material in the thimble. The cycle repeated until complete extraction was achieved, as the solvent level automatically drained back into the flask once it reached the siphon point. A rotary vacuum evaporator was used to filter and concentrate the methanolic extract, which was then stored at 4°C until further analysis. Multiple Soxhlet runs were conducted to obtain enough extract.

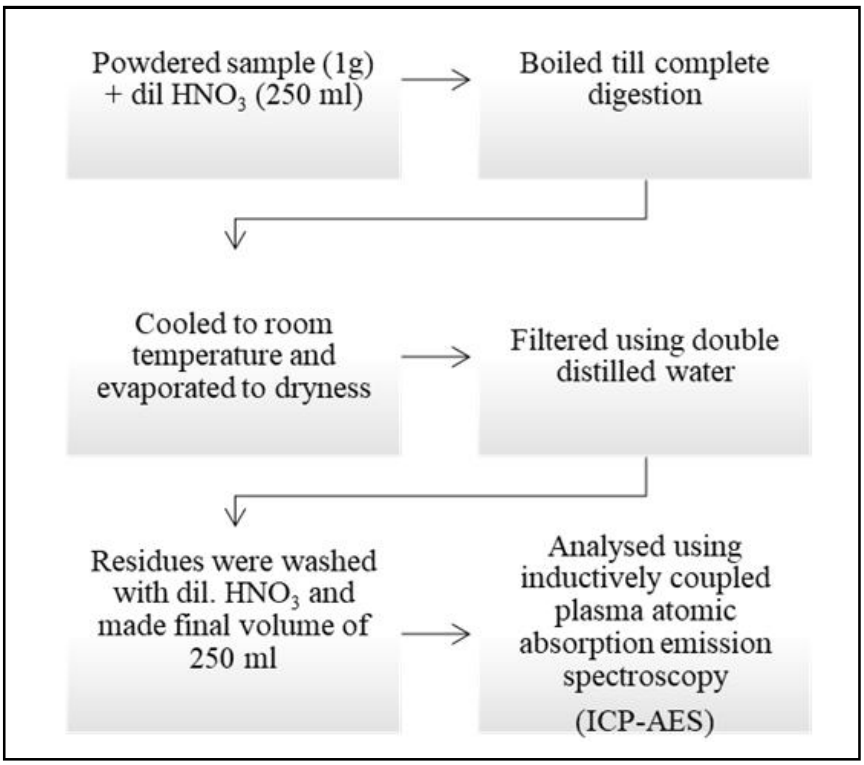

Figure 2: The mineral analysis approach is illustrated schematically.

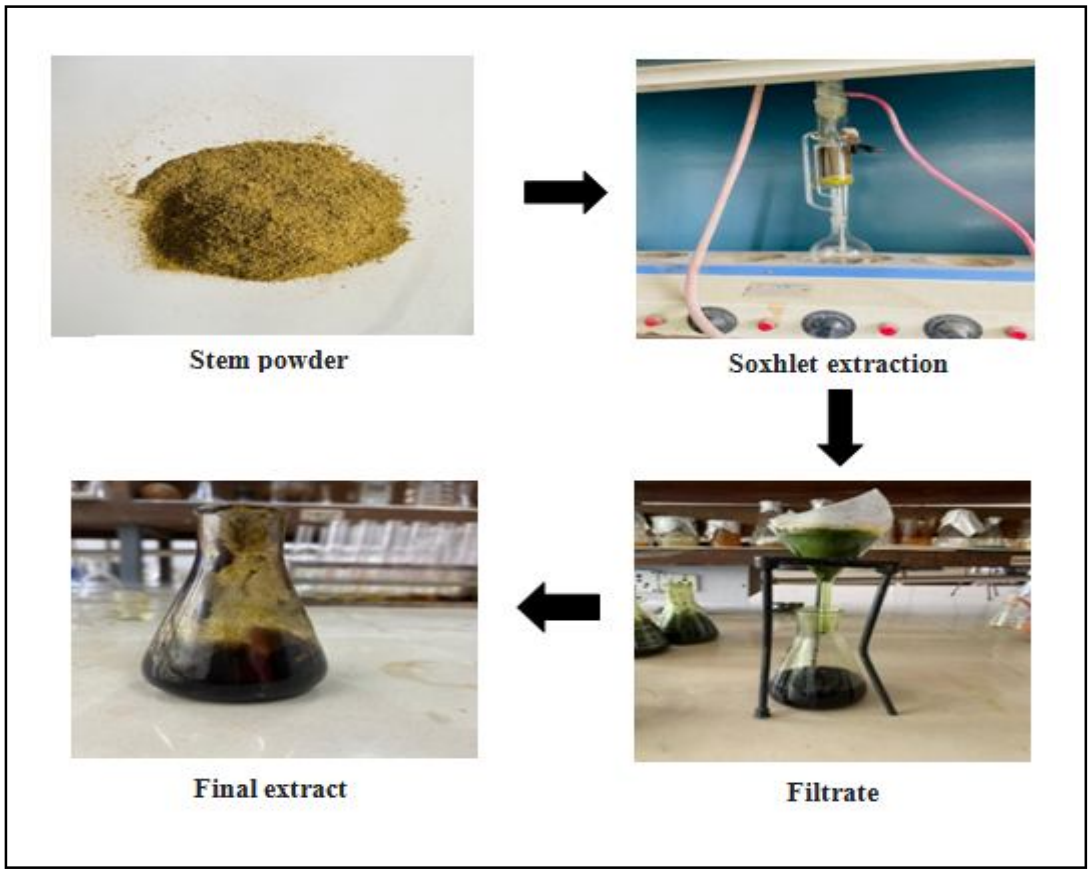

Figure 3: Preparation of methanol extract.

2.5 Yield estimation

The yield of the methanol extract was calculated using the formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of final extract}}{\text{Weight of sample taken}} \times 100$$

2.6 Fractionation of methanolic extract

The methanolic extract obtained through Soxhlet extraction was fractionated using liquid-liquid partition chromatography (Figure 4). A portion of the extract (5.0 g) was dissolved in 10.0 ml of methanol and sequentially partitioned with hexane, dichloromethane, and chloroform using a separatory funnel (Figure 5). Each recovered fraction was concentrated using a rotary vacuum evaporator and stored at 4°C until further analysis.

2.7 Phytochemical screening of *T. cordifolia* extract and its fractions

2.7.1 Qualitative analysis

To find the existence of key classes of bioactive chemicals, standard phytochemical assays (Table 1) were used to analyse the methanolic extract and its fractions qualitatively (Kaur *et al.,* 2016).

Figure 4: Fractionation of methanol extract.

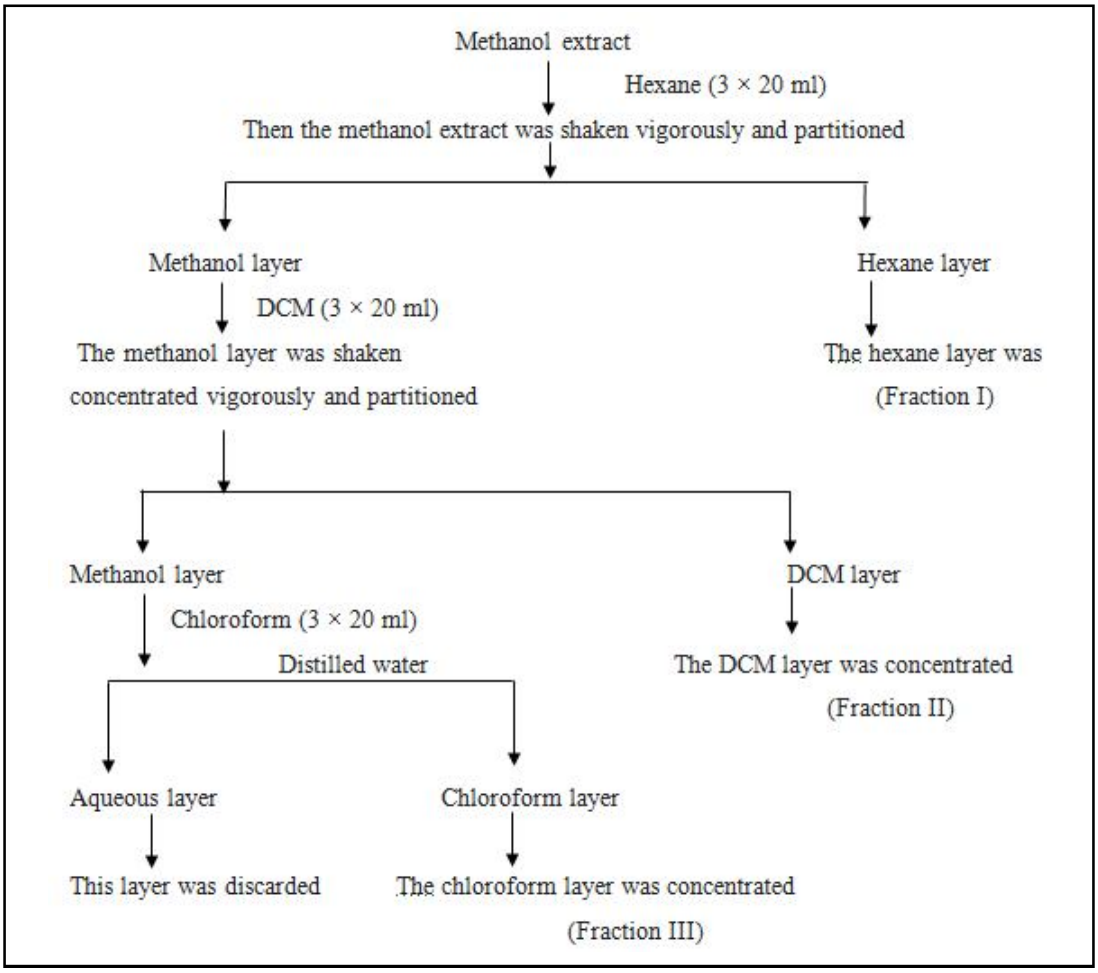

Figure 5: Fractionation of the methanol extract of *T. cordifolia* stem.

2.7.1.1 Ferric chloride test phenols

Three to four drops of ferric chloride solution were added to about two millilitres of plant extract. Phenolic chemicals were present because a bluish-green tint formed.

2.7.1.2 The froth test for saponins

Three to four drops of distilled water were added to two millilitres of extract, and the mixture was shaken vigorously. The presence of saponins was confirmed by the production of a persistent lather.

2.7.1.3 The test for tannins (Wohler's)

Three to four drops of lead acetate solution were added to two millilitres of extract. The presence of tannins was shown by the formation of a white precipitate.

2.7.1.4 Flavonoids (*via* Shindo's test)

Five minutes were spent boiling a mixture comprising 0.5 ml of magnesium turnings and 1.3 ml of extract. Flavonoids were proven to be present when an orange to red colouring developed.

2.7.1.5 Steroids and terpenoids (Liebermann-Burchard test)

One millilitre of extract was combined with one millilitre of chloroform and four millilitres of acetic anhydride. After that, three to four drops of concentrated sulphuric acid were added to the test tube's sidewalls. Steroids were identified by a red upper layer, and terpenoids by a green lower layer.

2.7.1.6 Alkaloids (Dragendorff's test)

One millilitre of extract was treated with 3-4 drops of Dragendorff's reagent. The formation of an orange precipitate confirmed the presence of alkaloids.

2.7.1.7 Cardiac glycosides (Baljet test)

One millilitre of extract was mixed with 3 ml of sodium picrate methanolic solution, followed by 1 ml NaOH hydroxide solution. The formation of an orange precipitate indicated the presence of cardiac glycosides.

Table 1: Phytochemical analysis of various compounds in extracts

Chemical	Test used	Phytochemical analysis	Observations
Phenols	Ferric chloride test	Plant extract (2 ml) + Ferric chloride (3-4 drops)	Appearance of a bluish-green colour
Saponins	Froth test	Plant extract (2 ml) + distilled water (3-4 drops) + vigorous shaking	Lather formation
Tannins	Wohler's test	Plant extract (2 ml) + lead acetate (3-4 drops)	White precipitates formed
Flavonoid	Shindo's test	Plant extract (1.3 ml) + Mg turnings (0.5 ml) + boil for 5 min	Orange to red colouration
Terpenoid/ steroids	Lebermann-Burchard test	Plant extract (1 ml) + chloroform (1 ml) + acetic anhydride (4 ml) + conc. H_2SO_4 (3-4 drops) along the sides of the test tube	Steroids-upper red layer terpenoids-lower green layer
Alkaloids	Dragondroff's test	Plant extract (1 ml) + Dragondroff's reagent (3-4 drops)	Orange precipitates formed
Cardiac glycoside	Baljet test	Plant extract (1 ml) + sodium picrate methanolic solution (3 ml) + N-sodium hydroxide solution (1 ml)	Orange precipitates formed

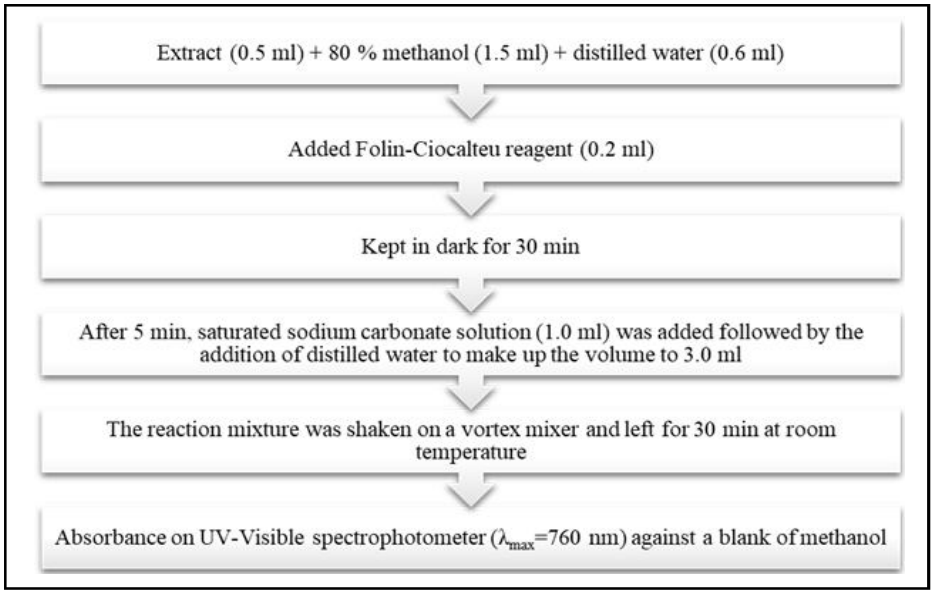

Figure 6: Determination of total phenolic content.

2.7.2 Quantitative analysis

2.7.2.1 Assessment of the methanol extract's and its fractions' total phenolic content (TPC)

The total phenolic content (TPC) of the methanolic extracts and fractions of the stem was estimated following the method illustrated in Figure 6. Quantification was performed using the linear equation obtained from the gallic acid standard curve (Figure 7), and results were expressed as mg gallic acid equivalents per gram of sample (mg GAE g^{-1}). All analyses were conducted in triplicate.

2.7.2.2 Assessment of the methanol extract's and its fractions' total flavonoid content (TFC)

The total flavonoid content (TFC) of the methanolic extract and its fractions was determined following the method described in Figure 8. Quantification was based on the linear equation obtained from the quercetin standard curve (Figure 9), and results were expressed as milligrams of quercetin equivalents per gram of sample (mg QE g^{-1}). All measurements were carried out in triplicate.

2.7.2.3 Measurement of total alkaloid concentration (TAC) in the methanol extract and its fractions

The total alkaloid content of the methanolic extract and its fractions was estimated following the method outlined in Figure 10. Quantification was carried out using the linear equation derived from the berberine standard curve (Figure 11), and results were expressed as milligrams of berberine equivalents per gram of sample (mg B g^{-1}). All analyses were performed in triplicate.

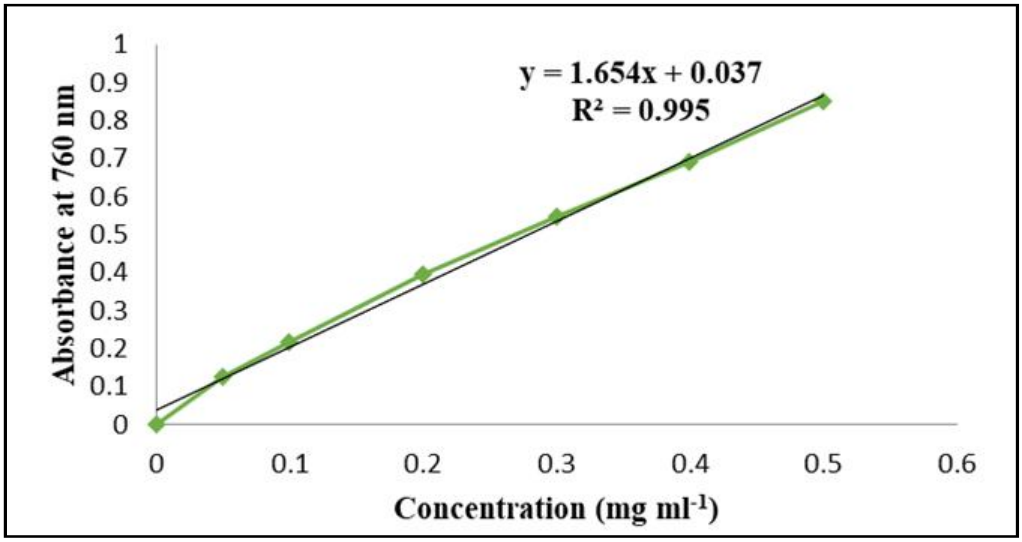

Figure 7: Standard curve of gallic acid.

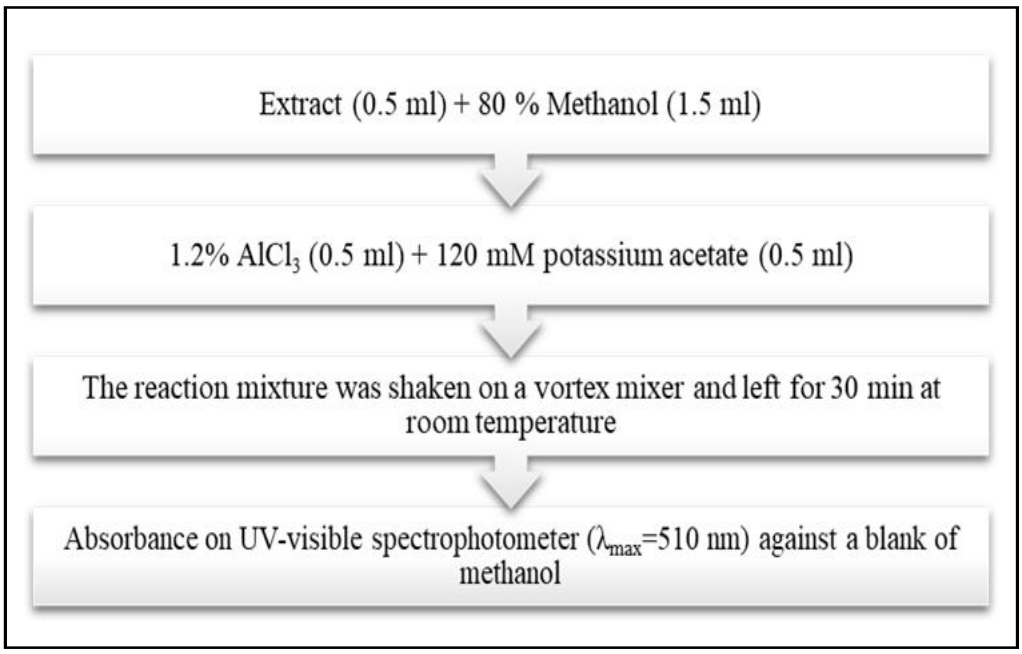

Figure 8: Determination of total flavonoid content.

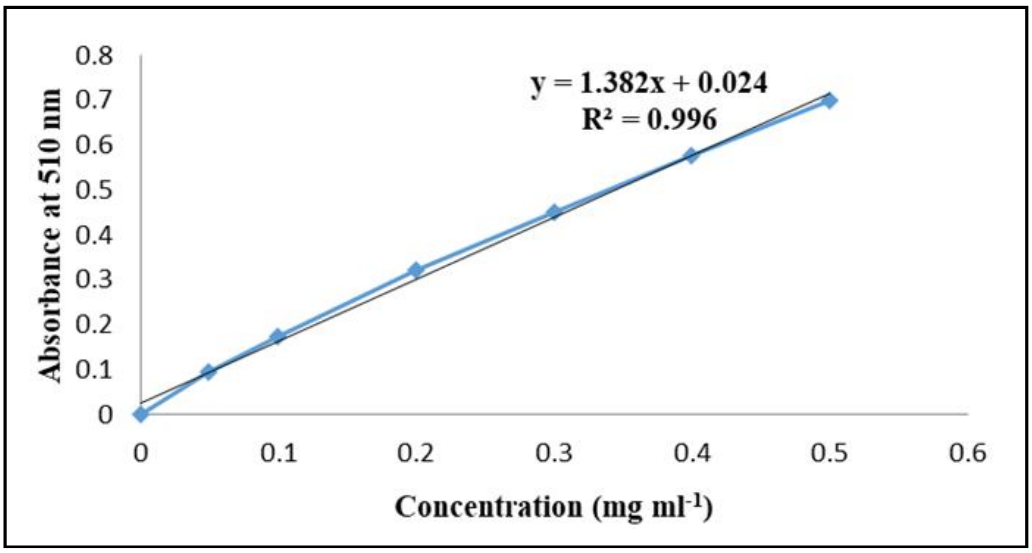

Figure 9: Standard curve of quercetin.

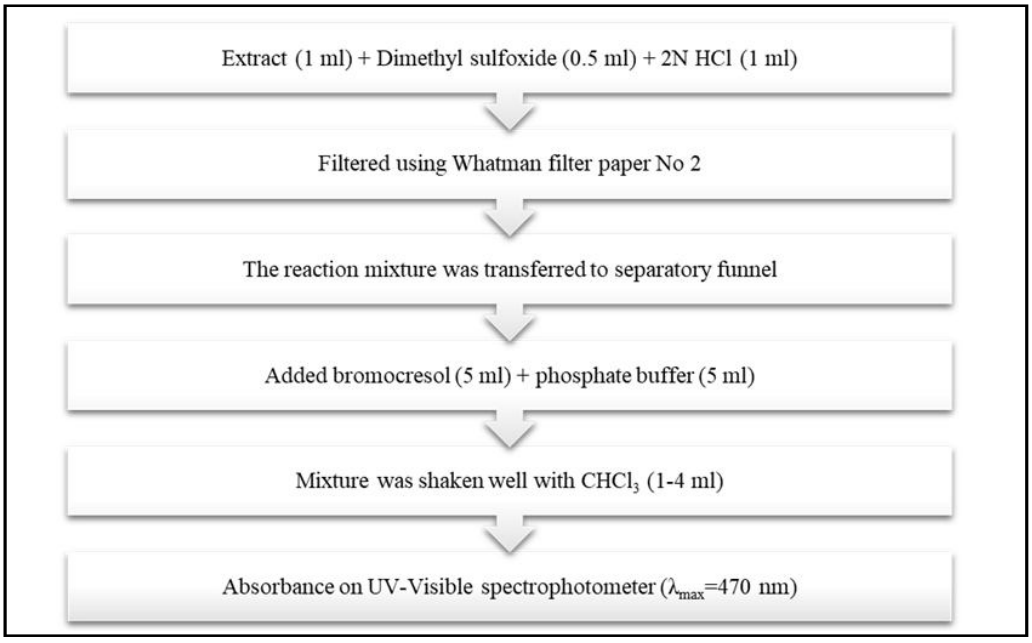

Figure 10: Determination of total alkaloid content.

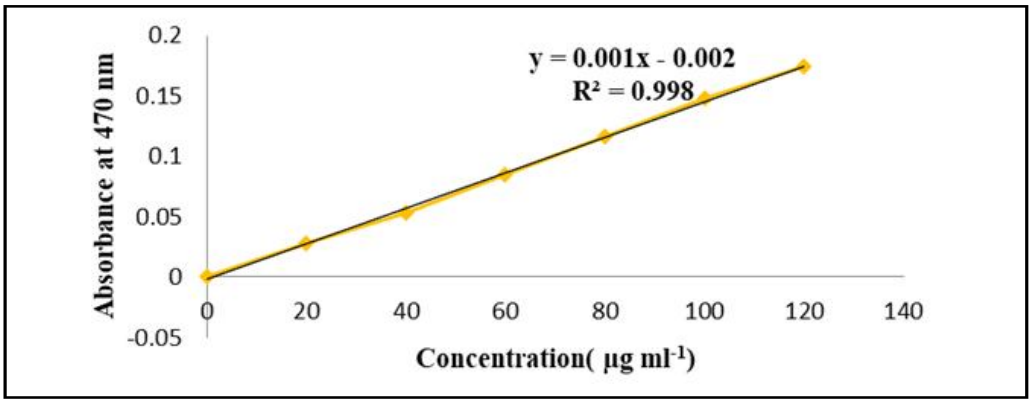

Figure 11: Standard curve of berberine.

2.8 Statistical analysis

The replication data were used to compute the mean and standard deviation. CPCS1 software was used to run a two-way ANOVA on the data. Using SPSS software, the Tukey multiple range test was also used to compare the test components, with a five per cent significance threshold.

3. Results

3.1 Mineral analysis

The mineral content of the methanolic extract and its solvent fractions of the stem of *T. cordifolia* was ascertained using flame photometry for sodium and potassium and atomic absorption spectrophotometry for calcium, magnesium, copper, iron, zinc, and manganese. The results of the investigation showed that different fractions contained critical macro- and micro-elements in variable quantities. These minerals are essential for enzyme activity, electrolyte balance, and the structural integrity of biological tissues, among other physiological processes. The following Table 2 shows the numerical outcomes for every component.

3.2 Yield of *T. cordifolia* stem methanolic extract and fractions

The methanolic extract's and their succeeding solvent fractions' extraction yields were determined by comparing the weight of the dried extract to the original weight of the plant material. The different yield percentages across the fractions were a result of variations in the solvent polarity and phytoconstituent solubility. The findings provide a general picture of the relative abundance of extractable chemicals in each solvent system, as well as the extraction efficiency. The yield values for each fraction are shown in detail in Table 3.

Table 2: Macro- and micro-minerals composition of the stem of *T. cordifolia*

Element (s)	Composition in the stem of *T. cordifolia* (mg kg^{-1})
Potassium	5972
Magnesium	1700
Phosphorous	1149
Calcium	842
Iron	700
Sodium	671
Boron	23.7
Zinc	23.4
Manganese	13.74
Copper	7.78
Lead	3.80
Arsenic	1.98
Chromium	1.91
Nickel	1.09
Cadmium	0.08

Table 3: Comparison of yield, colour, and nature of methanolic extract and its various fractions

Extract/fraction	Color	Nature	% yield
Methanol extract	Dark brown	Semisolid	8.30
Dichloromethane fraction	Dark green	Solid	5.62
Hexane fraction	Dark brown	Semisolid	4.80
Chloroform fraction	Dark green	Solid	2.58

3.3 Phytochemical analysis

T. cordifolia stem methanolic extract and its solvent fractions were subjected to a preliminary qualitative screening, which identified several bioactive components. Common phytochemical analyses verified that the various fractions included varying amounts of phenols, flavonoids, tannins, saponins, terpenoids, steroids, alkaloids, and cardiac glycosides. The pharmacological characteristics of these substances are well-known and include immunomodulatory, antibacterial, anti-inflammatory, and antioxidant effects. A qualitative indicator of these metabolites' relative abundance was given by the degree of colour changes or precipitate formation that was seen in each test. Table 4 displays the specific outcomes of each test conducted on each extract and fraction.

3.4 Total phenolic content (TPC)

The Folin-ciocalteu technique was used to measure the total phenolic content of the methanolic extract and its solvent fractions of *T. cordifolia* stem. The gallic acid standard curve's linear equation was

used to determine the results, which were then represented as milligrams of gallic acid equivalents per gram of sample (mg GAE g^{-1}). The solvent-specific solubility of phenolic compounds was reflected in the variations in phenolic content between the fractions. The detailed values are presented in Table 5.

3.5 Total flavonoid content (TFC)

Using the aluminium chloride colorimetric method, the total flavonoid concentration of the methanolic extract and its solvent fractions was ascertained. Milligrams of quercetin equivalents per gram of sample (mg QE g^{-1}) were used to express the results of the quantification, which was carried out using the quercetin standard curve. Flavonoid levels in the fractions varied, suggesting that solvents with varying polarity selectively extracted flavonoids. The results are summarized in Table 6.

3.6 Total alkaloid content (TAC)

According to the bromocresol green complex production method, the total alkaloid content of the methanolic extract and its solvent fractions was evaluated. The concentrations were represented as milligrams of berberine equivalents per gram of sample (mg B g^{-1}) and were computed using the berberine standard curve. The observed differences between fractions demonstrated how the type of solvent affects the effectiveness of alkaloid extraction. The detailed results are provided in Table 7.

Table 4: Phytochemical analysis of methanol extract and various fractions of the stem of *T. cordifolia*

Chemical constituents	Test	MEOH extract of the stem	Hexane fraction	DCM fraction	Chloroform fraction
Phenol	Ferric chloride	+	+	+	+
Saponins	Froth test	+	-	-	+
Alkaloids	Dragondroff's test	+	-	+	+
Tannin	Wohler's test	+	+	+	-
Flavonoid	Shindo's test	+	+	+	+
Terpenoid	Lebermann-Burchard reaction	+	+	-	-
Steroid	Lebermannburchard reaction	+	+	+	-
Cardiac glycoside	Baljet test	+	+	-	+

'+' represents presence, '-' represents absence

Table 5: Total phenolic content (TPC) of methanol extract, fractions of dichloromethane, chloroform, and hexane

Components	Total phenols mg GAE g^{-1}
Methanol extract	2.90 ± 0.36
Dichloromethane fraction	2.65 ± 0.68
Hexane fraction	1.77 ± 0.56
Chloroform fraction	2.20 ± 0.47

Table 6: Total flavonoid content (TFC) of methanol extract, fractions of dichloromethane, chloroform, and hexane

Components	Total alkaloids mg B g^{-1}
Methanol extract	0.093 ± 0.45
Dichloromethane fraction	0.032 ± 0.56
Chloroform fraction	0.082 ± 0.24

Table 7: Total alkaloid content of methanol extract, fractions of dichloromethane, and chloroform

Components	Total flavonoids mg QE g^{-1}
Methanol extract	3.79 ± 0.48
Dichloromethane fraction	3.46 ± 0.52
Hexane fraction	2.55 ± 0.59
Chloroform fraction	2.75 ± 0.76

4. Discussion

The macro-and micro-mineral profile of powdered *T. cordifolia* stem is presented in Table 2. The macro minerals sodium, magnesium, phosphorus, potassium, calcium, chromium, and nickel were discovered to be abundant in the stem. Along with trace levels of heavy metals like arsenic, cadmium, and lead, it also included micronutrients, including boron, zinc, manganese, copper, and iron. The element with the highest concentration among those found was potassium, whilst the one with the lowest concentration was cadmium.

Methanol was used as the solvent in the Soxhlet extraction process to create the methanolic extract of the stem. The resulting extract was further separated using chloroform, hexane, and dichloromethane. The maximum extraction yield of 8.30 percent was found in the methanolic extract. The maximum yield (5.62%) was obtained from the dichloromethane fraction, followed by hexane (4.80%) and chloroform (2.58%). Praveen and Rajesh (2018) observed similar results, giving methanol, ethanol, and chloroform extracts of the stem yields of 4.5%, 4.8%, and 2.3%, respectively. The comparative percentage yields of the methanolic extract and its fractions are presented in Table 3.

Using chemical reagents, a crucial first step in identifying the types of bioactive chemicals in the extracts is phytochemical screening. Using distinctive color changes or precipitate formation, the presence of chemicals was verified. The existence of phenols, tannins, alkaloids, flavonoids, saponins, terpenoids, steroids, and cardiac glycosides was discovered through screening, and each of these substances produced unique qualitative results. Flavonoids and phenols were

found in all fractions, including the methanolic extract. Alkaloids were not found in the hexane fraction, although they were in the methanolic extract, dichloromethane, and chloroform fractions. Terpenoids and saponins were not found in the dichloromethane fraction, whereas steroids and tannins were not present in the chloroform fraction.

The total phenolic content (TPC) of the methanolic extract and its dichloromethane, chloroform, and hexane fractions is presented in Table 5. Among the examined samples, the phenolic content varied between 1.77 and 2.90 mg GAE g^{-1}. The TPC of the methanolic extract was the greatest at 2.90 mg GAE g^{-1}, followed by the dichloromethane and chloroform fractions at 2.65 and 2.20 mg GAE g^{-1}, respectively. This is due to its intermediate polarity, which allows it to dissolve a diverse range of phenolics, including both polar and moderately non-polar molecules. Furthermore, methanol has a low molecular weight and high diffusibility, allowing for improved penetration into plant tissues and more efficient solubilisation of bioactive chemicals. Its capacity to create hydrogen bonds facilitates the extraction of hydroxyl-rich phenolic structures. These features, taken together, explain the greater phenolic recovery in methanolic extracts. The hexane fraction had the lowest TPC (1.77 mg GAE g^{-1}). The methanol, chloroform, and hexane extracts of *T. cordifolia* stem contained 7.6, 6.0, and 4.8 µg g^{-1} of phenolics, respectively, according to similar patterns observed by Kaur *et al.* (2016).

The total flavonoid content (TFC) of the methanolic extract and its dichloromethane, chloroform, and hexane fractions is summarized in Table 6. The examined samples had TFC values ranging from 2.55 to 3.79 mg QE g^{-1}. The maximum concentration of flavonoids (3.79 mg QE g^{-1}) was found in the methanolic extract, which was followed by the dichloromethane (3.46 mg QE g^{-1}) and chloroform (2.75 mg QE g^{-1}) fractions. The hexane fraction has the lowest flavonoid concentration (2.55 mg QE g^{-1}). Similar results were reported by Kaur *et al.* (2016), who found that the TFC of *T. cordifolia* stem extracts in methanol, chloroform, and hexane was 10.8, 50.6, and 3.6 µg g^{-1}, respectively.

The total alkaloid content of the methanol extract and its dichloromethane and chloroform fractions is shown in Table 7. Among the examined samples, the alkaloid concentration varied between 0.032 and 0.093 mg B g^{-1}. The largest amount of alkaloids (0.093 mg g^{-1}) was found in the methanol extract, which was followed by the dichloromethane fraction (0.032 mg g^{-1}) and the chloroform fraction (0.082 mg g^{-1}).

5. Conclusion

Bioactive chemicals found in medicinal plants have long been known to improve human health and prevent disease. The results of this study shows that the stem of *T. cordifolia* is a good source of chemicals with pharmacological and nutritional value. Essential minerals like potassium, calcium, magnesium, and sodium, followed by trace elements like zinc, manganese, and iron were found in considerable amount. These minerals are vital for preserving physiological balance. According to a phytochemical analysis, the plant is rich in bioactive components, including phenols, flavonoids, and alkaloids, with the highest amounts found in methanol extract. The plant's established use in traditional medicine to strengthen immunity, treat metabolic diseases, and fight oxidative stress is well-supported by these chemicals' well-known antioxidant, anti-inflammatory, antibacterial, and immunomodulatory qualities. The most effective solvent extract for bioactive chemical extraction was found to be methanol, indicating that it may be a good choice for further extraction and isolation research. The findings provide compelling scientific evidence in support of *T. cordifolia's* traditional use and demonstrate its potential for the development of herbal remedies, nutritional supplements, and innovative medicinal substances. To thoroughly examine and evaluate its therapeutic uses, further study is advised, including bioavailability studies and in vivo pharmacological assessments.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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