



Phytochemical determination and evaluation of biological potential of *Tinospora cordifolia*

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Abstract

The current study aimed to perform phytochemical analysis of primary and secondary metabolites in stem extract of *Tinospora cordifolia* using three different solvents-water, methanol and petroleum extract. This was followed by evaluation of the antibacterial, antioxidant, and antidiabetic activities of the plant's extracts. The study showed presence of proteins, tannins, alkaloids, flavonoids, phytosterols, saponins and terpenoids in different plant extracts in variable amount. The findings of the study also showed potent antioxidant activity of all the tested plant extracts. Also, the extracts demonstrated potent antibacterial activity against *E. coli* and *S. aureus* along with potent antifungal efficacy against *A. niger* and *Penicillium chrysogenum*. The findings of the current research will provide a solid foundation for advanced phytochemical, pharmacological, molecular, and clinical studies by integrating the traditional knowledge with modern science that could lead to innovative medications, standardized herbal products, and environmentally friendly medicinal plant use.

Keywords: *Tinospora cordifolia* stem, extraction, phytochemicals, biological potential etc.

Introduction

The study of traditional medicine and therapeutic methods that are frequently intricately linked to ecological and cultural systems is known as ethnomedicine. Plants remain the main source of medicines in many parts of the world, with indigenous populations in South America, Africa, and India depending on their native flora for medicinal purposes. These medical systems provide insightful information on the prospective use of medicinal plants, many of which have not yet received adequate scientific investigation. Local populations of Rajasthan, in northwest India, have a long history of using a wide variety of medicinal plants in their ethnomedical practices. Numerous plants with medicinal qualities that have been utilized in traditional medicine for millennia can be found in Ajmer in particular. With an emphasis on plants that have demonstrated encouraging biological activity, the ethnomedicinal survey carried out in Ajmer aimed to record and validate the local knowledge of medicinal plants (Mittal *et al.*, 2014; Bharathi *et al.*, 2018) [25, 26].

Drugs derived from plants have many advantages over those made from chemicals, which makes them indispensable in contemporary medicine. Because of their chemical diversity and many therapeutic effects, plant-derived medicines continue to be essential even if synthetic medications have revolutionized healthcare. Numerous plant substances, including terpenoids, alkaloids, flavonoids, and glycosides, have anti-inflammatory, anti-cancer, antioxidant, and antibacterial qualities. They frequently target several biological pathways at once, which lowers the possibility of adverse effects. Because they provide a wide range of bioactive chemicals with substantial therapeutic potential, plants have long been a mainstay of traditional medicine. Secondary metabolites like alkaloids, flavonoids, terpenoids, glycosides, and phenolic compounds are the main source of a plant's therapeutic value. These molecules are essential to the plant's defensive systems against infections, herbivores, and environmental stresses. Because of their antibacterial, anti-inflammatory, and anticancer properties, terpenoids such as like artemisinin from

Artemisia annua are prized (Nair *et al.*, 2004; Dhama *et al.*, 2016; Aranha *et al.*, 2012) [27, 28, 29].

The various secondary metabolites that plants generate, including alkaloids, flavonoids, terpenoids, phenolic chemicals, and glycosides, are substantially responsible for the plants' well-known strong antibacterial qualities. These bioactive substances aid in plants' defense against infections, and their antimicrobial properties are currently being used for purposes related to human health. The presence of secondary metabolites like flavonoids, phenolic acids, carotenoids, and terpenoids is largely responsible for the long-known antioxidant action of plants. Reactive oxygen species (ROS), which are toxic byproducts of cellular metabolism that lead to oxidative stress and the development of numerous chronic diseases, including as cancer, diabetes, cardiovascular disease, and neurological disorders, are naturally scavenged by these substances.

Considering all these things, the present study entitled as "Investigation of some selected medicinal plants of Ajmer district with special reference to *Tinospora cordifolia* (Wild.) Mires ex Hook. & Thoms." was conducted in order to perform phytochemical analysis of primary and secondary metabolites in *Tinospora cordifolia* using three different solvents-water, methanol and petroleum extract. Thereafter, the researchers evaluated the antibacterial, antioxidant, and antidiabetic activities of the plant's extracts. The findings of the current research will provide a solid foundation for advanced phytochemical, pharmacological, molecular, and clinical studies by integrating the traditional knowledge with modern science that could lead to innovative medications, standardized herbal products, and environmentally friendly medicinal plant use.

Materials and Methods

For the study, *Tinospora cordifolia* was selected. Detailed phytochemical and biological properties are given in this section.

Collection and processing of plant material

Stem of *Tinospora cordifolia* was collected from Local area of Jaipur, Rajasthan. It was washed with running tap water to remove impurities of dust and dirt and then with distilled water to remove ionic impurities. It was cut into pieces using sterile knife. Then dried in shade. Dried material was grinded using mixer grinder to make fine powder. It was stored in refrigerator for further use.

Extraction of the collected plant part

The processed plant material was extracted in different solvents based on polarity like polar (water), mid polar (methanol) and non-polar (pet ether). Three different solvents water, methanol, and petroleum ether were used to extract the stem of *Tinospora cordifolia* (Giloy). The extractive values were recorded in mg per gram of dry weight.

Evaluation of biological potential

All samples were evaluated for their antioxidant (DPPH free radical scavenging activity), antibacterial and antifungal (agar well diffusion assay).

Results

Extractives in stem of *Tinospora cordifolia* (Giloy) using different solvents

Different solvents such as water, methanol and petroleum ether were used for extraction of stem of *Tinospora cordifolia* (Giloy). The results showed that water showcased the maximum extractive value of 552 mg/g dry weight, and the extract was in powder form. This maybe attributed to the fact that water being a polar solvent proves to be a good solvent for extraction of most of the active chemicals in this plant.

Thereafter, methanol extract showcased the second highest extractive value, at 231 mg/g dry weight, and it also made a powder form extract. The methanol extractive value was lower than the water extractive value, but it was still good. This means that methanol is also a good solvent for getting

valuable compounds out of this plant. Petroleum ether had the lowest extractive value, at only 21 mg/g dry weight. The texture of this extract was sticky, not powdery. The poor yield indicates that fewer chemicals in the stem of *Tinospora cordifolia* are soluble in petroleum ether, a non-polar solvent. This means that the stem doesn't include a lot of non-polar substances like oils and waxes.

Table 1: Extractive value of different extracts of stem of *Tinospora cordifolia*

Solvent name	Extractive value (mg/g.dw)	Extract texture
Water	552	Powder
Methanol	231	Powder
Pet ether	21	Sticky

Qualitative test for phytochemical determination of the plant extracts

We used established procedures to do a qualitative phytochemical screening on three distinct extracts of the stem of *Tinospora cordifolia*: water, methanol, and petroleum ether. The Fehling test was found to be negative in all the cases indicating absence of any free reducing sugars in the extracts. The Ninhydrin technique showed that water and methanol were dark purple, which is a sign that proteins are present. The petroleum ether extract looked brown, indicating absence of proteins. The Gelatin and NaCl technique was used to test the tannins. Only the methanol extract was the only one that showed a positive outcome, with a green precipitate. Mayer's test to detect alkaloids was found to be positive in case of water and methanol extracts that became red while petroleum extract was found to be negative. The FeCl₃ test for flavonoids was found to be positive in all the three cases showing that water, methanol, and petroleum ether were equally potent for extraction of flavonoids. Libermann Burchard's assay for detection of phytosterols also tested positive in all the cases. Saponins were found to be present only in methanol and petroleum ether extract. Terpenoids were found only in water and methanol extracts.

Table 2: Qualitative Phytochemical determination in different extracts of stem of *Tinospora cordifolia*

Phytochemical test and method	plant extract	observation	Result
Total carbohydrates (Fehling method)	Water	blue	-ve
	Methanol	blue	-ve
	Pet ether	blue	-ve
Protein (Ninhydrin)	Water	Dark purple	+ve
	Methanol	Dark purple	+ve
	Pet ether	brown	-ve
Tannins (Gelatin + NaCl)	Water	Transparent (no ppt)	-ve
	Methanol	Green ppt	+ve
	Pet ether	Transparent (No ppt)	-ve
Alkaloids (Mayer's method)	Water	Red	+ve
	Methanol	Red	+ve
	Pet ether	yellow	-ve
Flavonoids (FeCl ₃ test)	Water	Yellow to disappaearr	+ve
	Methanol	Yellow to disapperar	+ve
	Pet ether	Yellow to disappear	+ve
Phytosterols (Libermann Burchard's test)	Water	Brown	+ve
	Methanol	Dark purple	+ve
	Pet ether	brown	+ve
Saponins (Sofowora)	Water	No frothing	-ve
	Methanol	frothing	+ve
	Pet ether	frothing	+ve
Terpenoids (Salkowski)	Water	Ring +	+ve
	Methanol	Ring +	+ve
	Pet ether	No ring -	-ve

Quantitative Phytochemical Analysis of Stem of *Tinospora cordifolia*

The quantitative estimation of major phytochemicals was carried out in the stem of *Tinospora cordifolia* and the results were expressed in mg per gram of dry weight. Among all the phytochemicals tested, proteins were found in the highest amount, with a value of 17.55±0.12 mg/g dry weight. This was closely followed by carbohydrates, which were recorded at 15.50±0.14 mg/g dry weight. Lipids were the third most abundant compound, found at 8.68±0.41 mg/g dry weight. Although lower than proteins and

carbohydrates, lipids were still present in a noticeable quantity compared to the other secondary metabolites. Phenols were recorded at 4.61±0.44 mg/g dry weight, making them the most abundant secondary metabolite among all the compounds tested. Flavonoids were found at 1.48±0.50 mg/g dry weight while alkaloids were present in a relatively small amount of 0.51±0.012 mg/g dry weight. Phytosterols were found to be in the least amount among all tested compounds, with a value of 0.24±0.11 mg/g dry weight.

Table 3: Quantitative Phytochemical determination in stem of *Tinospora cordifolia*

Phytochemical name	Quantity (mg/g.dw)
Carbohydrates	15.50±0.14
Proteins	17.55±0.12
Lipids	8.68±0.41
Phenols	4.61±0.44
Flavonoids	1.48±0.50
Alkaloids	0.51±0.012
Phytosterols	0.24±0.11

DPPH free radical scavenging activity of different extracts of stem of *Tinospora cordifolia*

The antioxidant activity of water, methanol, and petroleum ether extracts of *Tinospora cordifolia* stem was evaluated using the DPPH free radical scavenging method at five different concentrations ranging from 20 µg/ml to 100 µg/ml. The water extract showed the highest free radical scavenging activity among all three extracts at every concentration tested, with activity started at 35.78±0.13% at

20 µg/ml and gradually increasing to 48.21±0.49% at 100 µg/ml (IC₅₀ value: 110.43±3.38 µg/ml). This was followed by petroleum ether extract showed moderate antioxidant activity, with values ranging from 29.14±0.20% at 20 µg/ml to 39.98±0.38% at 100 µg/ml (IC₅₀ value was 165.72±4.04 µg/ml). The methanol extract showed the lowest free radical scavenging activity ranging from 19.05±0.26% at 20 µg/ml to 27.42±0.33% at 100 µg/ml (IC₅₀ value 305.9±1.05 µg/ml).

Table 4: DPPH free radical scavenging assay of different extracts of stem of *Tinospora cordifolia*

Extract name	% Free Radical scavenging activity at various concentrations					IC 50 (µg/ml)
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)	
Water	35.78±0.13	39.04±0.46	43.21±0.22	44.98±0.41	48.21±0.49	110.43±3.38
Methanol	19.05±0.26	20.82±0.56	23.31±0.20	25.79±0.24	27.42±0.33	305.9±1.05
Pet ether	29.14±0.20	32.59±0.36	34.78±0.40	39.09±0.46	39.98±0.38	165.72±4.04

Antibacterial activity

Against *E. coli*: The antibacterial efficacy of water, methanol, and petroleum ether extracts from the stem of *Tinospora cordifolia* was evaluated against *Escherichia coli* (*E. coli*) at four distinct concentrations: 25, 50, 75, and 100 µg/ml. The results showed that only the water extract was able to kill germs at all four concentrations that were tested starting from an inhibition zone of 7 mm at the lowest dose of 25 µg/ml and progressing to 8 mm at 50 µg/ml, 9 mm at 75 µg/ml, and 10 mm at 100 µg/ml. This shows that the water extract worked against *E. coli* even at the lowest concentration, and it worked better as the concentration went up. At 25 µg/ml, the methanol extract didn't work, but it started to work at 50 µg/ml. The inhibition zone was 7 mm and the activity index was 0.23 at 50 µg/ml. It went up to 8 mm at 75 µg/ml and 10 mm at 100 µg/ml, with activity index values of 0.25 and 0.31, respectively. The extract from petroleum ether showed the least antibacterial activity of the three with the first sign of activity occurring at 75 µg/ml, with a 7 mm inhibition zone and an activity index of 0.22. The inhibition zone stayed at 7 mm at 100 µg/ml, although the activity index dropped to 0.20. This means that

petroleum ether extract doesn't work very well against *E. coli* and needs to be more concentrated to have any effect at all.

Against *S. aureus*: We evaluated the antibacterial properties of water, methanol, and petroleum ether extracts of *Tinospora cordifolia* stem against *Staphylococcus aureus* (*S. aureus*) at four different concentrations: 25, 50, 75, and 100 µg/ml.

The results showed that the water extract had an inhibitory zone of 7 mm at both 25 µg/ml and 50 µg/ml with clear increase in activity which progressed to 11 mm at 75 µg/ml and 12 mm at 100 µg/ml. This means that the water extract acts better against *S. aureus* when it is more concentrated. The methanol extract was the most effective at killing *S. aureus* of the three extracts, starting with an inhibitory zone of 7 mm at 25 µg/ml and growing quickly to 12 mm at 50 µg/ml, 14 mm at 75 µg/ml, and 15 mm at 100 µg/ml. The petroleum ether extract also worked rather well against *S. aureus* with activity starting with inhibition zone of 8 mm at 25 µg/ml and progressing to 10 mm, 12 mm and 13 mm at 50 µg/ml, 75 µg/ml and 100 µg/ml respectively.

Table 5: Antibacterial activity of different extracts of stem of *Tinospora cordifolia* against *E. coli*

Bacteria name	Extract name	25 µg/ml		50 µg/ml		75 µg/ml		100 µg/ml	
		IZ	AI	IZ	AI	IZ	AI	IZ	AI
<i>E. coli</i>	Water	7	0.29	8	0.26	9	0.29	10	0.31
	Methanol	NA	-	7	0.23	8	0.25	10	0.31
	Pet ether	NA	-	NA	-	7	0.22	7	0.2
	Standard	24		30		31		32	
<i>S. aureus</i>	Water	7	0.22	7	0.2	11	0.30	12	0.31
	Methanol	7	0.22	12	0.34	14	0.38	15	0.39
	Pet ether	8	0.25	10	0.28	12	0.33	13	0.34
	Standard	31		35		36		38	

IZ- Inhibition zone (mm); AI- Activity index

Antifungal potential of the extracts

Against *A. niger*: The antifungal activity of water, methanol, and petroleum ether extracts of *Tinospora cordifolia* stem against *Aspergillus niger* was tested at four different concentrations: 25, 50, 75, and 100 µg/ml. The results showed that both water and petroleum ether extracts were ineffective against the fungus at 25 µg/ml or 50 µg/ml with initiation in activity initially seen at 75 µg/ml (7 mm inhibition zone) and growing to 9 mm at 100 µg/ml. This means that both water and petroleum ether extract needs a higher concentration to work against *A. niger*. The methanol extract worked a little better than the other two. It started to work at 50 µg/ml, with an inhibition zone of 7 mm, growing to 8 mm at 75 µg/ml and reaching 9 mm inhibition zone at the greatest dose of 100 µg/ml.

Against *Penicillium chrysogenum*: We tested the antifungal properties of extracts of *Tinospora cordifolia* stem in water, methanol, and petroleum ether against *Penicillium chrysogenum* at four different concentrations: 25, 50, 75, and 100 µg/ml. The results showed that water extract did not work at 25 µg/ml or 50 µg/ml. At 75 µg/ml, it began to demonstrate antifungal action, with an inhibition zone of 7 mm that grew to 9 mm at 100 µg/ml. The methanol extract didn't work at 25 µg/ml, but it did work at 50 µg/ml and 75 µg/ml with an inhibition zone of 7 mm in both the cases which grew to 8 mm at 100 µg/ml. The petroleum ether extract didn't work at 25 µg/ml either, but like the methanol extract, it did work at 50 µg/ml with an inhibition zone of 7 mm that progressed to 8 mm and 9 mm at 75 µg/ml and 100 µg/ml respectively.

Table 6: Antifungal activity of different extracts of stem of *Tinospora cordifolia* against *A. niger*

Name of Fungi	Extract name	25 µg/ml		50 µg/ml		75 µg/ml		100 µg/ml	
		IZ	AI	IZ	AI	IZ	AI	IZ	AI
<i>Aspergillus niger</i>	Water	NA	-	NA	-	7	0.33	9	0.40
	Methanol	NA	-	7	0.38	8	0.38	9	0.40
	Pet ether	NA	-	NA	-	7	0.33	9	0.40
	Standard	8		18		21		22	
<i>Penicillium chrysogenum</i>	Water	NA	-	NA	-	7	0.30	9	0.37
	Methanol	NA	-	7	0.33	7	0.30	8	0.33
	Pet ether	NA	-	7	0.33	8	0.34	9	0.37
	Standard	20		21		23		24	

IZ- Inhibition zone (mm); AI- Activity index

Discussion

The current study was conducted with an aim to investigate phytochemical composition and biological potential of stem extracts of *Tinospora cordifolia*. For this, three different solvents, namely, water, methanol and petroleum ether were used to extract stem isolate of *Tinospora cordifolia*. The study showed that extractive values acquired using various solvents exhibited a distinct polarity-dependent pattern. Water had the highest extractive value (552 mg/g), followed by methanol (231 mg/g), and petroleum ether had the lowest (21 mg/g). This suggests that most of the phytoconstituents in the stem are polar. This trend is in line with other studies that found that *T. cordifolia* has a lot of polar chemicals, like glycosides, polysaccharides, phenolics, and alkaloids, that may be easily extracted in water or alcohol (Sharma *et al.*, 2012) [2]. The very low yield in petroleum ether indicates that there are not many non-polar compounds in the stem, including fixed oils and waxes. The higher extractive value of water also backs up the traditional usage of Giloy aqueous decoctions (Kadha) in Ayurveda (Krishna *et al.*, 2024; Modi *et al.*, 2021; Modi *et al.*, 2020; Ilaiyaraja *et al.*, 2011) [4, 5, 6, 7].

Thereafter, Qualitative analysis was conducted to determine phytochemical analysis of plant extracts. The results showed lack of reducing sugars in all the extracts while proteins were detected in both water and methanol extracts, indicating that polar solvents efficiently isolated proteinaceous substances or peptides, potentially enhancing immunomodulatory action. Alkaloids were found in both water and methanol extracts, which backs with earlier research that showed that isoquinoline alkaloids like magnoflorine and berberine can dissolve in polar solvents (Upadhyay *et al.*, 2010) [1]. All the three extracts contained flavonoids and phytosterols, which shows that they are found in many places in the stem matrix. Flavonoids are renowned for their powerful anti-inflammatory and antioxidant properties, whereas phytosterols help decrease cholesterol and fight inflammation (Saha & Ghosh, 2012) [3]. Thereafter, results showed that tannins were present in the methanol extract, which means that methanol was better at getting polyphenolic chemicals with intermediate polarity. Saponins were found in methanol and petroleum ether extracts, but not in water extracts (Reddy *et al.*, 2015; Sharma *et al.*, 2019; Priya *et al.*, 2022) [8, 9, 10].

This may be attributed to the fact that solvents were selective and the extraction process was different. Terpenoids were found in polar extracts, which is in line with previous research that found diterpenoid lactones to be the main parts of Giloy stem. The qualitative research substantiates that *T. cordifolia* stem encompasses various groups of pharmacologically active secondary metabolites.

Quantitative phytochemical analysis indicated that proteins (17.55±0.12 mg/g) and carbs (15.50±0.14 mg/g) are the predominant main metabolites. These substances add nutritional value, but they may also help the immune system work better. Phenols (4.61±0.44 mg/g) were the most abundant secondary metabolites, followed by flavonoids (1.48±0.50 mg/g), alkaloids (0.51±0.012 mg/g), and phytosterols (0.24±0.11 mg/g). The high amount of phenolic compounds is strongly linked to the antioxidant activity seen in the DPPH experiment. Phenolic chemicals transfer hydrogen atoms or electrons to free radicals to neutralize them. This stops oxidative stress from damaging cells. Previous research has indicated substantial total phenolic and flavonoid concentrations in *T. cordifolia* stem extracts (Saha & Ghosh, 2012) [3], corroborating the current results (Asha *et al.*, 2025; Royani *et al.*, 2023; Singh *et al.*, 2014) [12, 13, 14].

The DPPH free radical scavenging experiment showed that the antioxidant activity of all the extracts increased in a clear dose-dependent way. The aqueous extract showcased the lowest IC₅₀ value, indicative of its highest antioxidant power, which maybe attributed to its ability to extract polar phenolic chemicals. On the contrary, the petroleum ether extract had moderate activity and methanol extract had lesser activity. These results are in line with other previously published studies that show that water extracts of *T. cordifolia* have considerable effects on scavenging radicals and protecting against oxidative stress (Upadhyay *et al.*, 2010; Praiwala *et al.*, 2018; Nath *et al.*, 2023; Mishra *et al.*, 2014) [1, 15, 16, 17, 24].

Thereafter, researchers evaluated the antibacterial activity results that showed that all extracts inhibited both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. The aqueous extract was found to be effective against *E. coli* even at the lowest concentration, however, the methanol needed greater doses and the petroleum ether showed little activity. This maybe attributed to the presence of lipopolysaccharide barrier in Gram-negative bacteria, which prevents the infiltration of phytochemicals. On the contrary, all of the extracts worked better and more consistently against *S. aureus*, with the methanol extract having the biggest inhibition zone. Gram-positive bacteria lack the protective outer layer that renders them prone to being affected by plant-based substances. One probable reason for the higher efficacy of methanol extract maybe presence of higher amount of alkaloids and tannins, which are known to kill bacteria (Saini *et al.*, 2024; Kumar *et al.*, 2020; Shivakumar *et al.*, 2022) [18, 19, 20].

At higher concentrations, the antifungal activity against *Aspergillus niger* and *Penicillium chrysogenum* was found to be only moderately strong with none of the extracts showing efficacy at 25 µg/ml. However, the methanol extract started working at lower doses however antifungal activity was not as strong as the antibacterial activity. This may be attributed to the fact that cell walls of fungi are made up of chitin and ergosterol, which can make it harder for phytochemicals to get through. The dose-dependent

increase in the inhibitory zone shows presence of antifungal chemicals in the stem. Antibacterial activity was stronger than antifungal activity, which suggests that the phytoconstituents of *T. cordifolia* may work better against bacteria than fungi (Desai *et al.*, 2012; Kumar *et al.*, 2018; Gupta *et al.*, 2024; Nath *et al.*, 2023) [16, 21, 22, 24].

Concluding the findings of the study in a nutshell, the study shows that the stem of *Tinospora cordifolia* is rich in several bioactive phytochemicals and possesses potent antioxidant, antibacterial, and antifungal activity. The antioxidant, antibacterial as well as antifungal capacity maybe attributed to the presence of phenolics, alkaloids, flavonoids, and terpenoids. All these findings validate the age old ethnomedicinal application of the plants and warrant future investigations, aimed at isolation of bioactive components, and investigation of their pharmacological impact.

References

1. Upadhyay AK, Kumar K, Kumar A, Mishra HS. *Tinospora cordifolia* (Guduchi): Validation of traditional uses. International Journal of Ayurveda Research,2010;1(2):112–121. <https://doi.org/10.4103/0974-7788.64405>
2. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Journal of Pharmacognosy and Phytochemistry,2012;1(3):1–7.
3. Saha S, Ghosh S. *Tinospora cordifolia*: Pharmacological aspects. Indian Journal of Pharmacology,2012;44(2):155–160. <https://doi.org/10.4103/0253-7613.93844>
4. Krishna KN, Krishnamoorthy K, Veeraraghavan VP, Jayaraman S. *In vitro* biological properties and in silico studies on *Tinospora cordifolia* stem aqueous extract. Journal of Pharmacy and Bioallied Sciences,2024;16(Suppl 2):S1317-20.
5. Modi B, Koirala N, Aryal SP, Shrestha J, Koirala S, Upadhyaya J, *et al.* *Tinospora cordifolia* (Willd.) Miers: phytochemical composition, cytotoxicity, proximate analysis and their biological activities. Cellular and Molecular Biology,2021;67(1):50-7.
6. Modi B, Kumari Shah K, Shrestha J, Shrestha P, Basnet A, Tiwari I, *et al.* Morphology, biological activity, chemical composition, and medicinal value of *Tinospora cordifolia* (willd.) Miers. Advanced Journal of Chemistry-Section B.,2020;2020:36-54.
7. Ilaiyaraja N, Khanum F. Antioxidant potential of *Tinospora cordifolia* extracts and their protective effect on oxidation of biomolecules. Pharmacognosy Journal,2011;3(20):56-62.
8. Reddy NM, Reddy RN. *Tinospora cordifolia* chemical constituents and medicinal properties: a review. Sch Acad J Pharm,2015;4(8):364-9.
9. Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D. The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon,2019;5(9).
10. Priya LB, Balasubramanian B, Shanmugaraj B, Subbiah S, Baskaran R. Therapeutic Potential of the Medicinal Plant *Tinospora cordifolia*-Minireview. Phytion (0031-9457), 2022, 91(6).
11. Upadhyay N, Ganie SA, Agnihotri RK, Sharma R. Studies on antioxidant activity and total phenolic content of *Tinospora cordifolia* (Miers.) stem using in

- vitro* models. American Journal of Phytomedicine and Clinical Therapeutics,2013;1(8):617-27.
12. Asha G, Lekshmi S, Najmathunnisa KP, Rajva T, Krishnakumar V, Hussan KS. Exploring the anticancer potential of *Tinospora cordifolia* with computational insights into EGFR inhibition. In Silico Pharmacology,2025;13(3):208.
 13. Royani A, Hanafi M, Julistiono H, Dinoto A, Lotulung PD, Manaf A. The potential of *Tinospora cordifolia* extracts as antibacterial material against *Pseudomonas aeruginosa*. Trends in Sciences,2023;20(1):3884.
 14. Singh K, Panghal M, Kadyan S, Chaudhary U, Yadav JP. Antibacterial activity of synthesized silver nanoparticles from *Tinospora cordifolia* against multi drug resistant strains of *Pseudomonas aeruginosa* isolated from burn patients. Journal of Nanomedicine & Nanotechnology,2014;5(2):1.
 15. Praiwala B, Priyanka S, Raghu N, Gopenath N, Gnanasekaran A, Karthikeyan M, *et al.* *In vitro* antibacterial activity of *Tinospora cordifolia* leaf extract and its phytochemical screening. Journal of Biomedical Sciences,2018;5(2):10-7.
 16. Nath H, Khataniar A, Bania KK, Mukerjee N, Al-Hussain SA, Zaki ME, *et al.* Nano-functionalization and evaluation of antimicrobial activity of *Tinospora cordifolia* against the TolB protein of *Pseudomonas aeruginosa*—An antibacterial and computational study. Frontiers in Microbiology,2023;14:1138106.
 17. Mishra P, Jamdar P, Desai S, Patel D, Meshram D. Phytochemical analysis and assessment of *in vitro* antibacterial activity of *Tinospora cordifolia*. International Journal of Current Microbiology and Applied Sciences,2014;3(3):224-34.
 18. Saini R, Mishra RK, Kumar P. Green synthesis of reduced graphene oxide using the *Tinospora cordifolia* plant extract: exploring its potential for methylene blue dye degradation and antibacterial activity. ACS omega,2024;9(18):20304-21.
 19. Kumar N, Khurana SP, Pandey VN. Antifungal activity of essential oil of *Tinospora cordifolia* against storage fungi of wheat. Medicinal Plants-International Journal of Phytomedicines and Related Industries,2020;12(1):150-7.
 20. Shivakumar VH, Tegginamani AS, Zain NM. Antimicrobial efficiency of *Tinospora cordifolia* and *Ocimum tenuiflorum* against *Streptococcus mutans* and *Candida albicans*. Journal of Oral and Maxillofacial Pathology,2022;26(4):470-5.
 21. Desai S, Metrani R, Vantamuri S, Ginigeri V, Phadke K, Hungund B. Phytochemical analysis, antimicrobial and antitumour screening of endophytes of *Tinospora cordifolia*. methods,2012;1(4):5-6.
 22. Kumar V, Singh S, Singh A, Dixit AK, Srivastava B, Sidhu GK, *et al.* Phytochemical, antioxidant, antimicrobial, and protein binding qualities of hydro-ethanolic extract of *Tinospora cordifolia*. Journal of Biologically Active Products from Nature,2018;8(3):192-200.
 23. Gupta PK, Kaur M, Chitara MK, Mishra D, Kushwaha KP. GC-MS Profiling of *Tinospora cordifolia* (Giloy) stem extract for identification of antifungal compounds against *Macrophomina phaseolina* causing dry root rot of Mungbean [*Vigna radiata* (L.) Wilczek].
 24. Nath H, Khataniar A, Bania KK, Mukerjee N, Al-Hussain SA, Zaki ME, *et al.* Nano-functionalization and evaluation of antimicrobial activity of *Tinospora cordifolia* against the TolB protein of *Pseudomonas aeruginosa*—An antibacterial and computational study. Frontiers in Microbiology,2023;14:1138106.
 25. Mittal J, Sharma MM, Batra A. *Tinospora cordifolia*: a multipurpose medicinal plant-A. Journal of Medicinal Plants,2014;2(2):33.
 26. Bharathi C, Reddy AH, Nageswari G, Lakshmi BS, Soumya M, Vanisri DS, *et al.* A review on medicinal properties of *Tinospora cordifolia*. International Journal of Scientific Research and Review,2018;7(12):585-98.
 27. Nair PR, Rodriguez S, Ramachandran R, Alamo A, Melnick SJ, Escalon E, *et al.* Immune stimulating properties of a novel polysaccharide from the medicinal plant *Tinospora cordifolia*. International immunopharmacology,2004;4(13):1645-59.
 28. Dhama K, Sachan S, Khandia R, Munjal A, MN Iqbal H, K. Latheef S, *et al.* Medicinal and beneficial health applications of *Tinospora cordifolia* (Guduchi): a miraculous herb countering various diseases/disorders and its immunomodulatory effects. Recent patents on endocrine, metabolic & immune drug discovery,2016;10(2):96-111.
 29. Aranha I, Clement F, Venkatesh YP. Immunostimulatory properties of the major protein from the stem of the Ayurvedic medicinal herb, guduchi (*Tinospora cordifolia*). Journal of ethnopharmacology,2012;139(2):366-72.