Bioactive Phytocompounds and Antimicrobial Potency of *Azadirachta indica* and *Tinospora cordifolia*: Insights into Their Ethnopharmacological Relevance

JayLaxmi[1]& Meenakshi Kumari[2]

Research Scholar Department of Botany, Career Point University Kota Rajasthan, India

Associate Professor Department of Botany, Career Point University Kota Rajasthan, India

Abstract: - The present study aimed to investigate the plant derived secondary metabolites and ability to suppress microbial growth of ethanolic and methanolic extracts of *Azadirachta indica* and *Tinospora cordifolia*. Qualitative screening detected naturally occurring plant chemicals (alkaloids, flavonoids, phenols, tannins, glycosides, and saponins), with variation observed based on the solvent used. Among the tested compounds, glycosides and phenolic compounds were notably abundant. Antimicrobial activity, assessed through the disc diffusion method, showed significant zones of inhibition, with *Azadirachta indica* exhibiting a maximum zone of 31 mm and *Tinospora cordifolia* 30 mm, comparable to the standard antibiotic Gentamycin. These findings support the traditional use of these medicinal plants and suggest their potential for development into natural antimicrobial agents. Further studies on compound isolation, mechanism of action, and in vivo efficacy are recommended. The findings suggest that both plants possess unique and complementary phytochemical profiles that contribute to their respective antimicrobial properties, supporting their continued use in herbal medicine and potential applications in antimicrobial drug development.

Keywords:Germicidal action, Enterobacter, *Azadirachta indica*, *Guduchi*, Plant polyphenol.

1. **Introduction: -** Plant-based remedies and their formulations servea pivotal role in both traditional and modern healthcare systems across the globe. Despite their widespread use, concerns regarding the quality consistency of herbal formulations remain a major challenge. This inconsistency arises due to the inherent complexity of herbal medicines, which often contain multiple active compounds that may vary depending on variables like plant type, geographical origin, harvesting methods, and processing approaches. To address these issues, advanced analytical methods such as chemical fingerprinting and multi-component quantitative analysis have been widely adopted. Chemical fingerprinting covers a broad perspective of the entire chemical configuration of a herbal product, enabling the comparison of different batches for overall consistency. On the other hand, multi-component quantitative analysis focuses on accurately measuring the concentration of key bioactive constituents, ensuring that the therapeutic components remain within specified limits(Zhang et al., 2025).Abiotic and biotic factors that change the concentration of bioactive compounds in medicinal plants and highlights the types of contaminants that can compromise product safety. Natural remedies and plant-based therapies are widely regarded as safe, natural, and beneficial to health. However, with their growing popularity and widespread use, it becomes essential to address concerns related to their safety, quality, and user awareness. Medicinal plant materials is sourced from the wild, where varying environmental and biological factors influence the production of phytochemicals. This variability can lead to inconsistent levels of active compounds, potentially affecting both the efficacy and safety of the final products. Moreover, certain secondary metabolites produced by plants for defense can have toxic effects on humans, including mutagenic, genotoxic, or carcinogenic outcomes. The quality of herbal products is further threatened by contamination from natural elements or human activities, sometimes resulting in serious adverse reactions or fatalities (van Wyk & Prinsloo, 2020).Herbal products are gaining prominence in oral healthcare due to their phytochemical content and natural therapeutic potential. Plant-derived secondary metabolites—such as polyphenols, flavonoids, tannins, terpenes, terpenoids, and alkaloids—play vital roles in maintaining oral health. These compounds can neutralize harmful agents, promote the growth of beneficial oral microbes, and inhibit pathogenic bacteria responsible for oral diseases. The phenolic compounds, known for their antioxidant and antimicrobial effects, and terpenes/terpenoids, recognized for their structural

diversity and biological activity. medicinal plants used in various formulations to manage and prevent dental caries, gingivitis, and periodontitis. The evidence supports the use of specific plant-based compounds in oral health care, offering a scientific basis for their integration into modern pharmaceutical and dental formulations (Arzani et al., 2025).*A. indica*, commonly known as neem, is rightfully described as a "miracle tree" due to its extensive medicinal, pesticidal, and industrial significance. Ancients times neem tree holds prominent place holds a prominent place in traditional medicine and agro-practices. Biologically active compounds such as nimbinin and nimbidin are noted for their medicinal value, highlighting neem's wide pharmacological spectrum. *Azadirachtin* identified as the most potent, especially for its insecticidal action. These bioactive compounds validate the traditional uses of neem and provide a solid foundation for its commercial exploitation in biopesticides, pharmaceuticals, and cosmetic products (Arzani et al., 2025). Tinospora cordifolia contains diverse bioactive compounds, including alkaloids, diterpenoids, glycosides, flavonoids, steroids, polysaccharides, and fatty acids, which contribute to its immunomodulatory, anti-inflammatory, antimicrobial, anticancer, and metabolic health benefits. It is also utilized in culinary and commercial health-support products (Beohar et al., 2024a). The leaf extract of *T. cordifolia* acts as a capping in ecofriendly synthesis of silver nanoparticles (AgNPs) and characterized using various analytical techniques. UV-Vis approved the composition of AgNPs by showing a detecting a Plasmon resonance. X-ray diffraction (XRD) confirmed the nanocrystalline nature of the material, infrared (IR) spectroscopy identified the functional groups involved in nanoparticle stabilization, and scanning transmission electron microscopy (STEM) provided detailed insights into their size, shape, and morphology. Thermogravimetric analysis (TGA) was used for evaluating the thermal behavior of nanoparticles. Thermogravimetric analysis, along with other techniques, confirmed the thermal stability, composition, and successful green synthesis of *T. cordifolia*-mediated silver nanoparticles (Lekkala et al., 2025). Neem-derived silver nanoparticles (35.2 μg/mL) showed strong antioxidant activity and significant antidiabetic effects in alloxan-induced mice, restoring organ tissues and offering a cost-effective natural therapeutic option (Tahir et al., 2025).

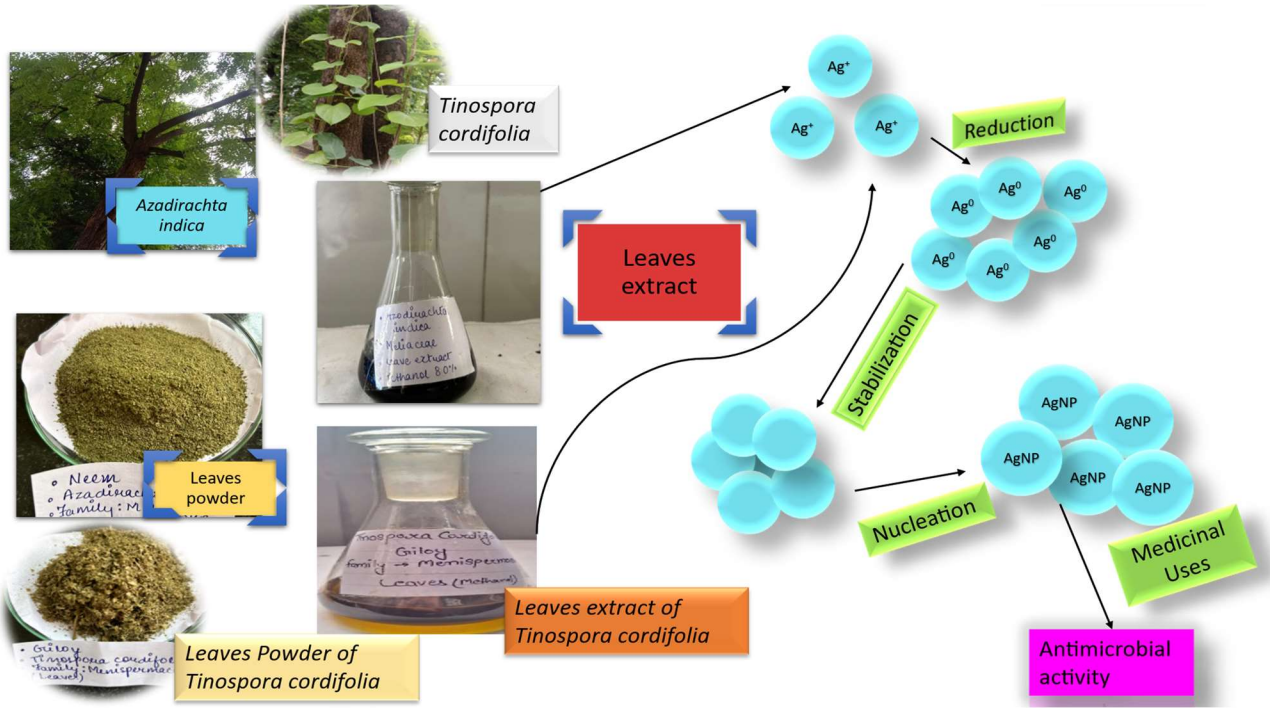

Figure 1:-The diagram represents the bio synthesis (AgNPs) using Azadirachta indica leaf extracts (AgNPs) usin and *Tinospora cordifolia*. It includes images of both plants, their dried leaf powders, and aqueous extracts. The leaf extracts act as (Ag^{+}) are reduced to elemental silver (Ag^{0}) through phytochemical-mediated reduction. Stabilization prevents particle aggregation. Nucleation leads to the formation of AgNPs. The process involves three key steps: reduction, stabilization, and nucleation, resulting in the biosynthesis of silver nanoparticles. The synthesized AgNPs exhibit significant antimicrobial activity, attributed to capping, making them effective against a broad range of pathogenic microorganisms.

This study investigates and compares the bioactive compounds in *Azadirachta indica* and *Tinospora cordifolia* leaf extracts, known for their therapeutic potential. It emphasizes a phytochemical-centric approach, with sustainable silver nanoparticle synthesis providing additional functional insights. Their antimicrobial efficacy against selected bacterial strains is also evaluated to explore pharmaceutical and biomedical applications.

2. Material & Method: -

2.1 Plant Collection: -The study was conducted in the region Bijasan Mataji Temple, located in Baran District, Rajasthan, near the Madhya Pradesh border. It is characterized by rich native vegetation and is traversed by the 3 km-long Parvati River. Baran, covering an area of approximately 6992

km², is renowned for its ecological diversity and cultural significance. During the study, leaves of *A. indica* and *T. cordifolia* were collected from the high-altitude forest regions of Gugor Chhabra in Baran District, based on their recognized traditional use in Indian systems of alternative medicine by local healers. The collected plant specimens were subsequently authenticated at the Botany department CPU Kota

2.2 Preparation of Plant Extracts: -

Leaves of both plants were cleaned with filtered water, shade-dried for three weeks, and ground into powder using an electric blender (Figure-2).

Figure 2: Complete plant leaves and powder of *A. indica and T. cordifolia*

The powdered samples were stored in clean, airtight plastic containers, properly labeled with plant name and collection details. Crude plant extracts were prepared using ethanol and methanol as solvents. Soxhlet extraction was employed for approximately 20 hours to extract the bioactive compounds from the powdered leaf material. Filtered extracts were refrigerated in airtight containers for subsequent phytochemical and antimicrobial analyses (Figure-3).

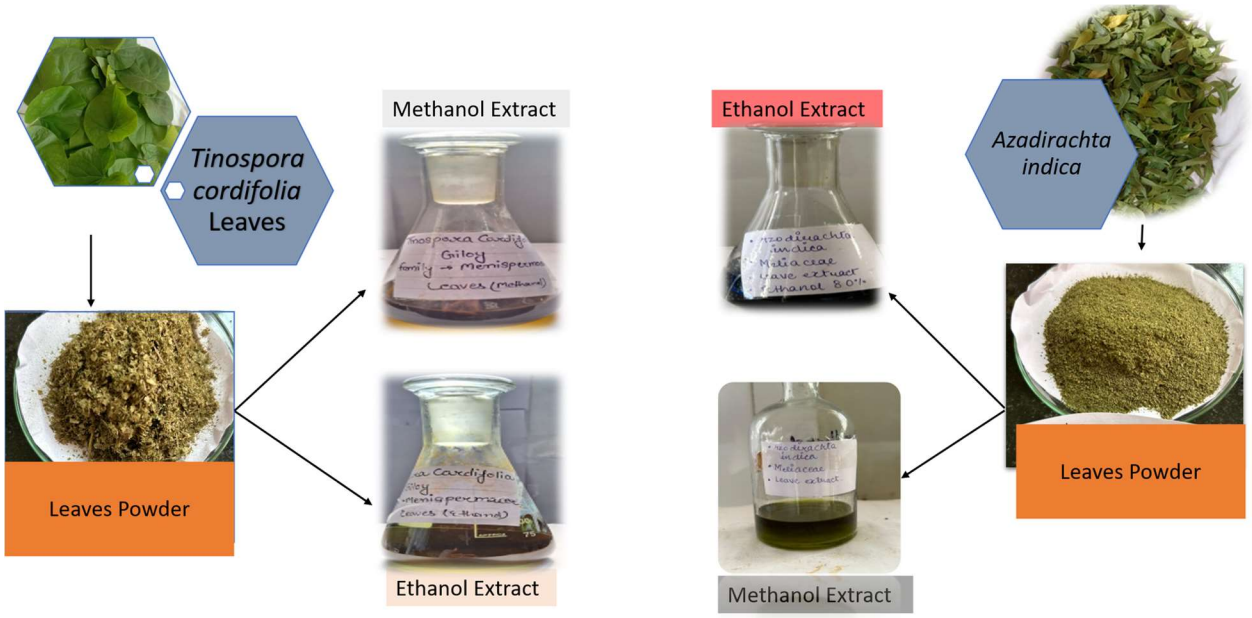

Figure 3:-This diagram illustrates the preparation and extraction process of *Tinospora cordifolia* and *Azadirachta indica* leaf powders for phytochemical analysis.

2.3 Solvent Extraction: - The air-dried and powdered leaves of *Azadirachta indica* and *Tinospora cordifolia* were subjected to solvent extraction using a Soxhlet apparatus. For each plant, 50 grams of powdered leaf material were extracted with ethanol and methanol in a 1:10 (w/v) ratio. The extraction process was carried out for 6 to 8 hours for each solvent. The resulting extracts were filtered using Whatman No. 1 filter paper and subsequently concentrated under reduced pressure using a rotary evaporator. The dried crude extracts were then transferred into sterile, airtight amber-colored bottles and stored at 4°C until further analysis for phytochemical constituents and antimicrobial properties.

2.4 Plant metabolites assesment- Ethanolic and methanolic extracts of *A. indica* and *T. cordifolia* were screened to identify primary and secondary metabolites. Specific tests were conducted to detect the presence of various bioactive compounds (Figure-4).

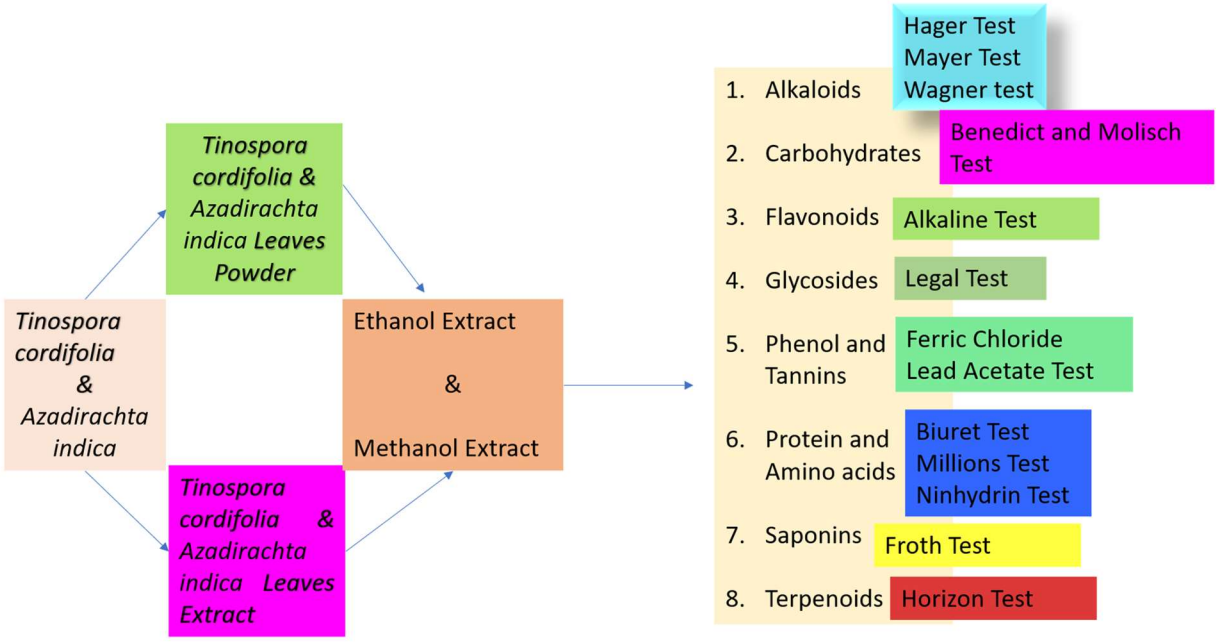

Figure 4:- The diagram illustrates the workflow for phytochemical analysis of *Tinospora cordifolia* and *Azadirachta indica*, from leaf powder preparation to ethanol and methanol extraction. Extracts are qualitatively screened for secondary metabolites using specific colorimetric and reagent-based tests, with test names shown in a color-coded format.

2.4.1 Analysis of Primary Metabolites

2.4.1.1 Carbohydrates Test: Molisch's test, is a qualitative biochemical assay used to detect the presence of carbohydrates in a sample. The reddish-violet ring forms because carbohydrates undergo dehydration by concentrated sulfuric acid to form furfural or hydroxymethylfurfural, which then reacts with Molisch's reagent (α-naphthol) to produce the characteristic color. 2 ml of extract was treated with 2 drops of Molisch's reagent and carefully layered with 2 ml concentrated H_2SO_4; formation of a reddish-violet ring confirmed carbohydrates. For Benedict's test, 1 ml of extract was mixed with 3 ml Benedict's reagent, heated in a boiling water bath for 10 minutes, and allowed to cool; the appearance of green, yellow, or red precipitates indicated reducing sugars. Benedict's reagent was prepared by dissolving sodium citrate and sodium carbonate in distilled water, followed by addition of copper sulfate solution after cooling.

The test was carried out following the method by Ramakrishnan *et al*., (1994). 2 ml of aliquot of the ethanolic and methanolic extract was treated with 2 drops of Molisch's reagent. After shaking and holding the test tube in slanting position, 2 ml of concentrated sulphuric acid was added along the side of the test tube. The reddish violet ring at the junction of two solutions indicates the presence of carbohydrates. For benedict's test, 1 ml of extract was mixed with 3 ml Benedict's solution and heated in a boiling water bath for 10 minutes; green, yellow, or red precipitates upon cooling indicated reducing sugars. Benedict's reagent was prepared by dissolving sodium citrate and sodium carbonate in boiling distilled water, then adding copper sulfate solution after cooling.

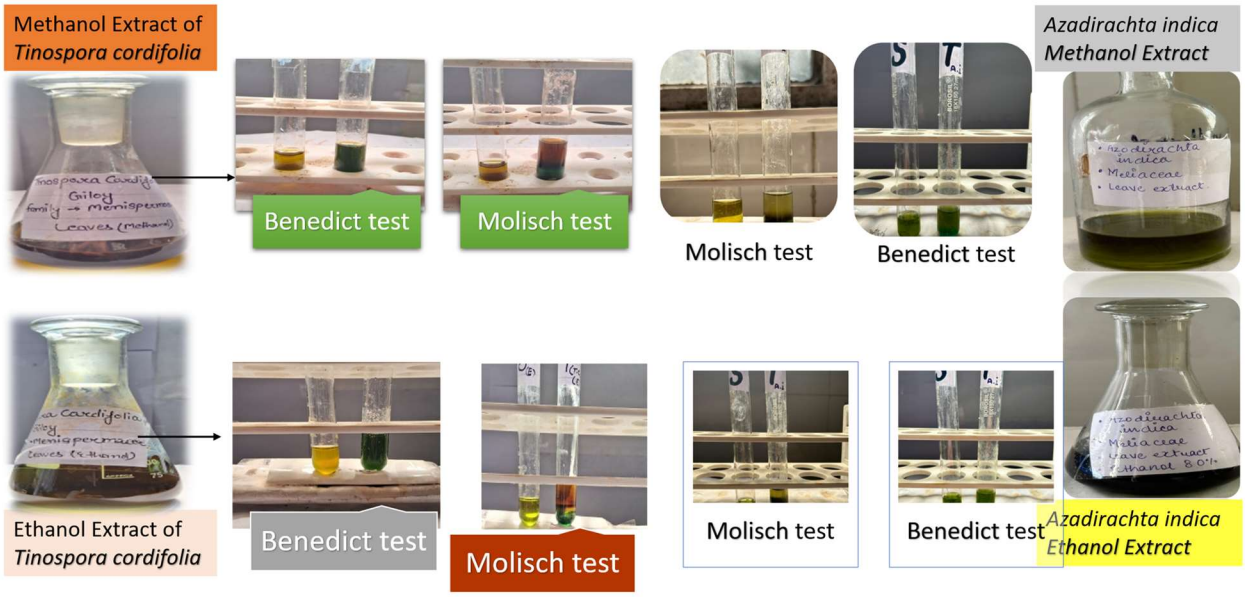

Figure 5This diagram illustrates the phytochemical analysis process for *Tinospora cordifolia* and *Azadirachta indica* leaves, highlighting the detection of carbohydrates using Benedict's and Molisch's tests. The workflow begins with leaf powder preparation, followed by ethanol and methanol extraction.

2.4.1.2 Protein Test: Protein detection was carried out using Millon's and Biuret tests. In Millon's test, 2 ml of extract was treated with 2 drops of Millon's reagent, producing a white creamy precipitate that turned brick red upon heating, indicating proteins. In

Biuret's test, 2 ml of filtrate was treated with a few drops of copper sulfate solution, followed by 1 ml of 95% ethanol and excess potassium hydroxide pellets; a violet or pink color in the ethanolic layer confirmed the presence of proteins (Figure-6).

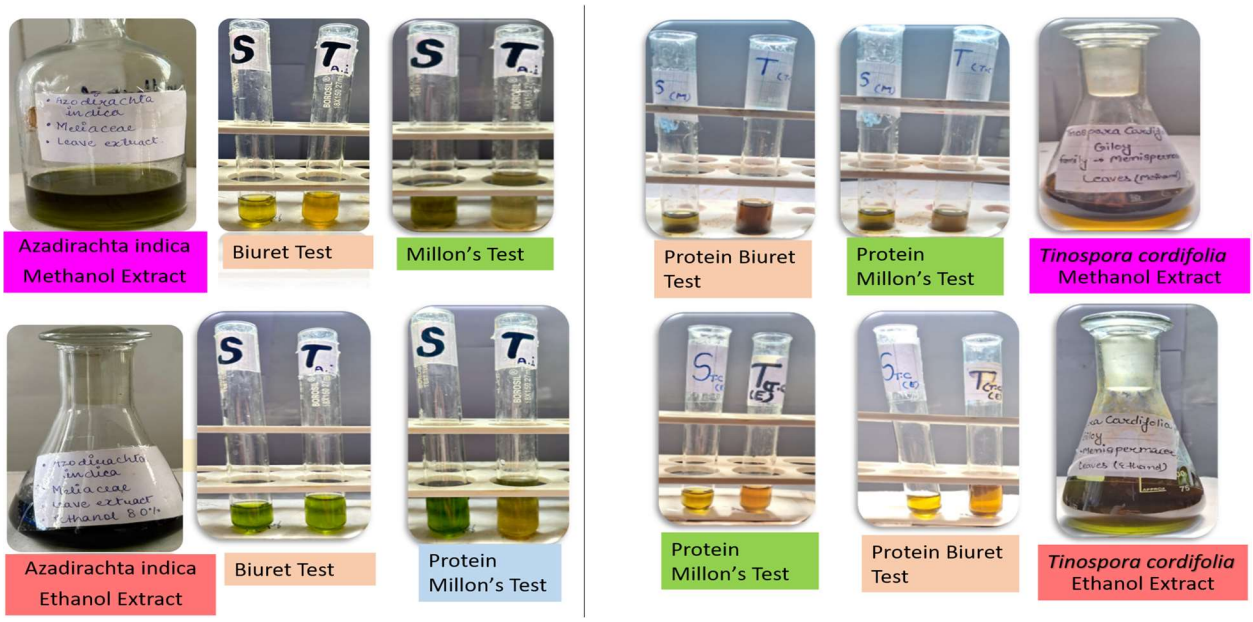

Figure 6: -Qualitative Analysis of Proteins Using Millon's and Biuret Tests

2.4.1.3 Amino acid Test: - Two drops of ninhydrin solution (prepared by dissolving 10 mg ninhydrin in 200 ml acetone) were added to 2 ml of the aqueous filtrate. Development of a purple coloration signified the presence of amino acids (Figure-7).

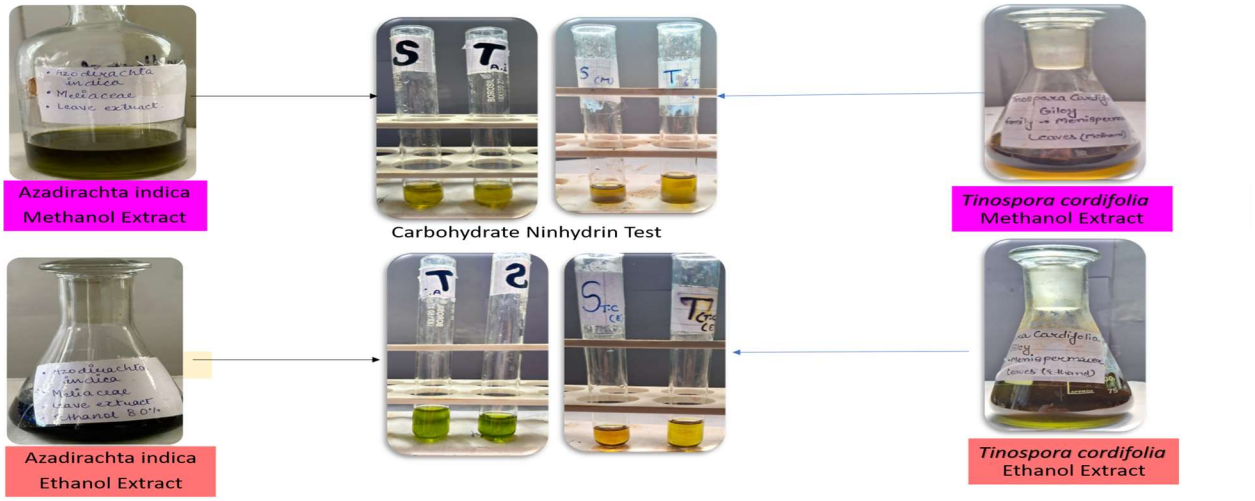

Figure 7:-The diagram illustrates a comparative analysis of *Azadirachta indica* and *Tinospora cordifolia* leaf extracts prepared using methanol and ethanol. It shows the color responses of these extracts to carbohydrate and Ninhydrin tests, highlighting variations in phytochemical presence. Methanolic extracts show darker tones, while ethanolic extracts appear lighter, indicating solvent-based differences in compound extraction.

2.4.2 Analysis of Secondary Metabolites

2.4.2.1Test for Alkaloids: - Alkaloids were identified using Mayer's and Wagner's tests. For Mayer's test, 4 ml of the plant extract was carefully mixed with a few drops of Mayer's reagent along the side of the test tube, where the formation of a white or creamy precipitate signified a positive reaction. Mayer's reagent was prepared by dissolving mercuric chloride in water, preparing a separate potassium iodide solution, then combining and diluting to 100 ml. In Wagner's test, 4 ml of extract was combined with 1 ml of Wagner's reagent, and the appearance of a reddish-brown precipitate indicated the presence of alkaloids. Wagner's reagent was made by dissolving iodine and potassium iodide in water and adjusting the volume to 100 ml (Figure-8 & 9).

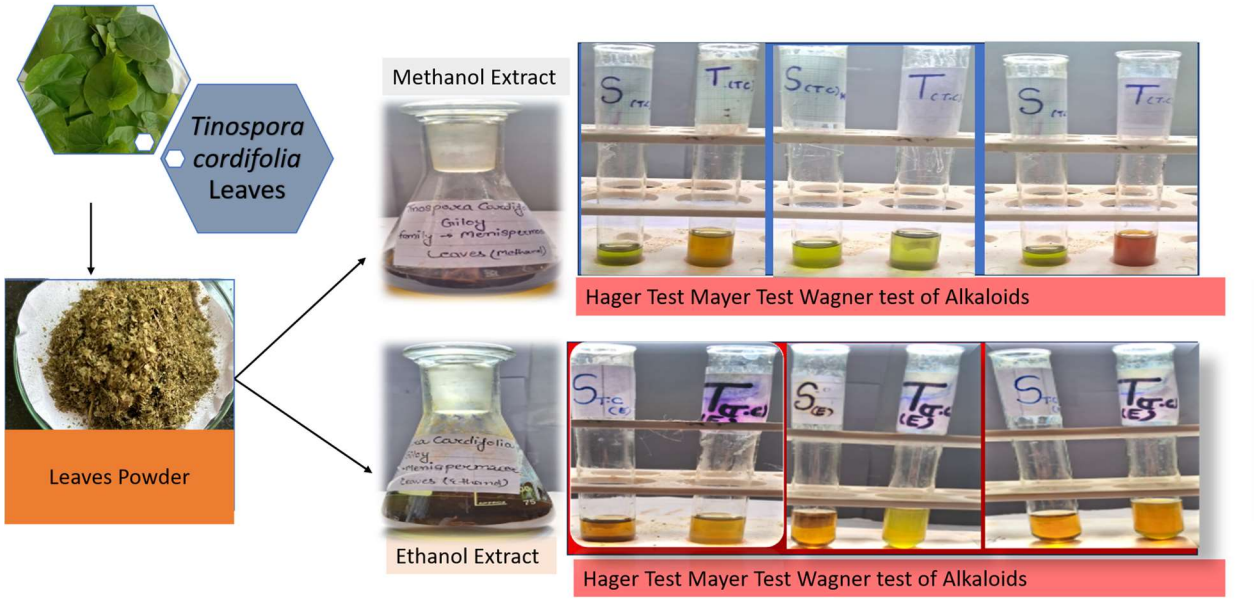

Figure 8:- This diagram illustrates the alkaloid detection in methanolic leaf extract of *Tinospora cordifolia* using Hager's, Mayer's, and Wagner's tests. The methanol extract appears in a labeled flask, and test tubes corresponding to each test are shown. Hager's test resulted in a yellow precipitate, Mayer's test displayed a creamy white color, and Wagner's test showed a reddish-brown precipitate. These distinct color changes confirm the presence of alkaloids in the methanolic extract.

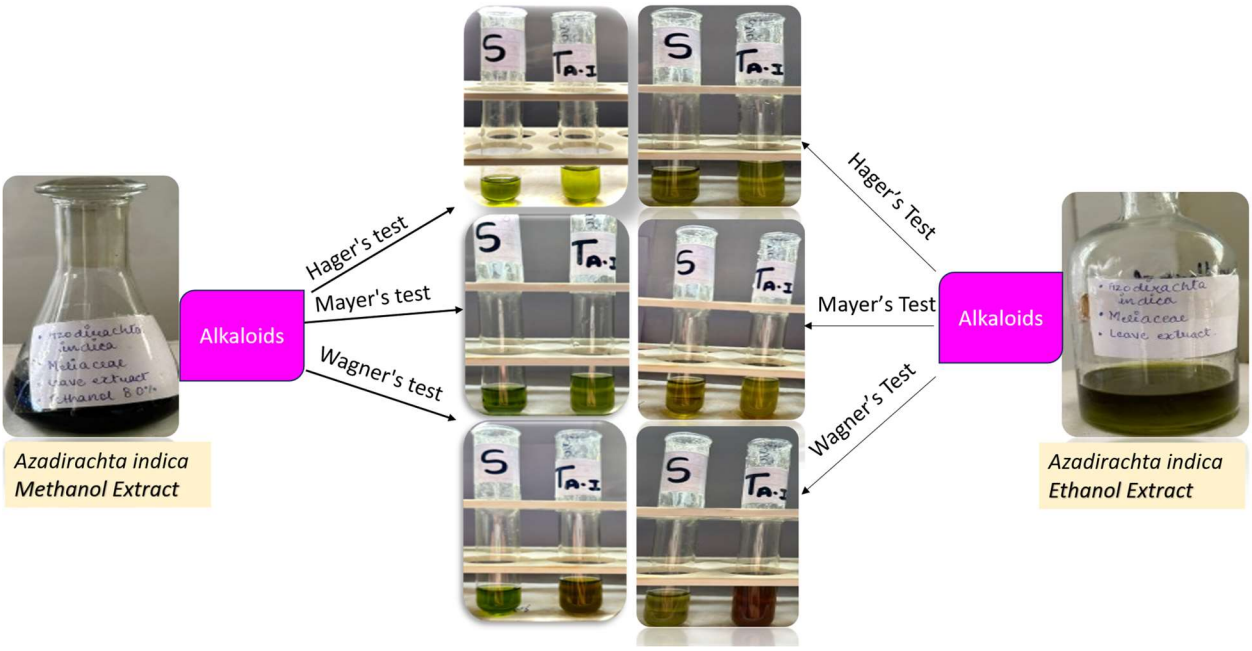

Figure 9: - This diagram represents the alkaloid analysis in ethanolic leaf extract of *Tinospora cordifolia*, tested with Hager's, Mayer's, and Wagner's reagents. The ethanolic extract is shown in a flask at the bottom. Corresponding test tubes reveal color changes: Hager's test gives a yellow color, Mayer's test shows a faint white to yellowish precipitate, and Wagner's test results in a reddish to orange hue. These observations confirm alkaloid presence in the ethanolic extract, with differences in intensity compared to the methanol extract.

2.4.2.1 Test for Tannins and Phenols: - The presence of tannins and phenolic compounds was assessed using Ferric Chloride and Lead Acetate tests on ethanolic and methanolic extracts. In the Ferric Chloride test, 3 ml of extract was mixed with 1 ml of 5% ferric chloride solution; a bluish-black coloration indicated tannins and phenols. For the Lead Acetate test, 3 ml of extract was combined with 1 ml of 10% lead acetate solution, and the appearance of a dense white precipitate confirmed phenolic compounds (Figure-10).

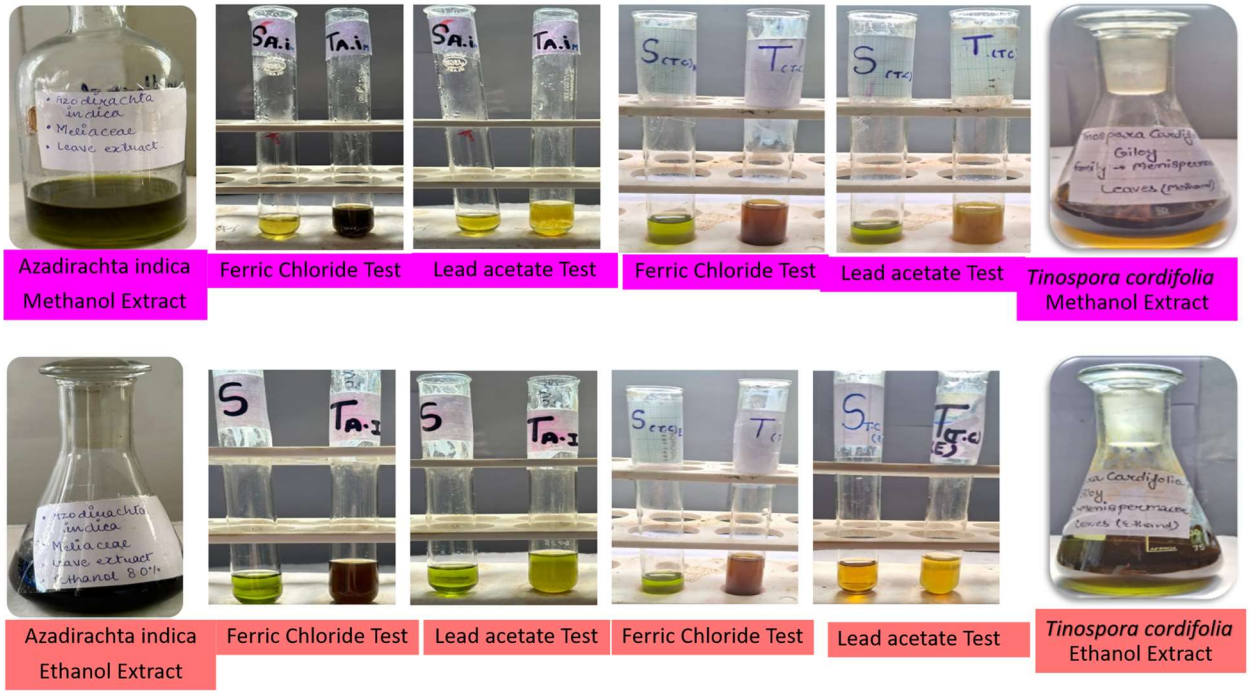

Figure 10:-The diagram illustrates the qualitative analysis of tannins and phenolic compounds in methanolic and ethanolic extracts using Ferric Chloride and Lead Acetate tests. Test tubes containing the extracts are shown reacting with specific reagents. In the Ferric Chloride test, the appearance of a bluish-black coloration confirms the presence of tannins and phenols, while in the Lead Acetate test, the formation of a bulky white precipitate indicates phenolic content. The diagram visually compares the results for both methanol and ethanol extracts, demonstrating the effectiveness of each solvent in extracting phenolic compounds.

2.4.2.2 Qualitative Phytochemical Screening for Flavonoids, Saponins, Terpenoids, and Glycosides: - Ethanolic and methanolic leaf extracts were subjected to qualitative characters for bioactive substance. For flavonoids, 3 ml of extract was mixed with a few drops of 10% sodium hydroxide solution; a yellow color that disappeared upon addition of dilute acid indicated a positive result. Saponins were tested using the froth method by combining 1 ml of extract with 50 mg sodium carbonate and 1.5 ml distilled water, shaking vigorously for 5 minutes; a stable froth persisting for at least 15 minutes confirmed saponins. Terpenoids were detected by Horizon's test, wherein 1 ml of extract was treated with 2 ml trichloroacetic acid, and the formation of a yellow to red precipitate signified a

positive result. Glycosides were identified using Legal's test by adding 1 ml pyridine and 1 ml sodium nitroprusside to 2 ml of extract, with a pink or red color indicating their presence (Figure-11).

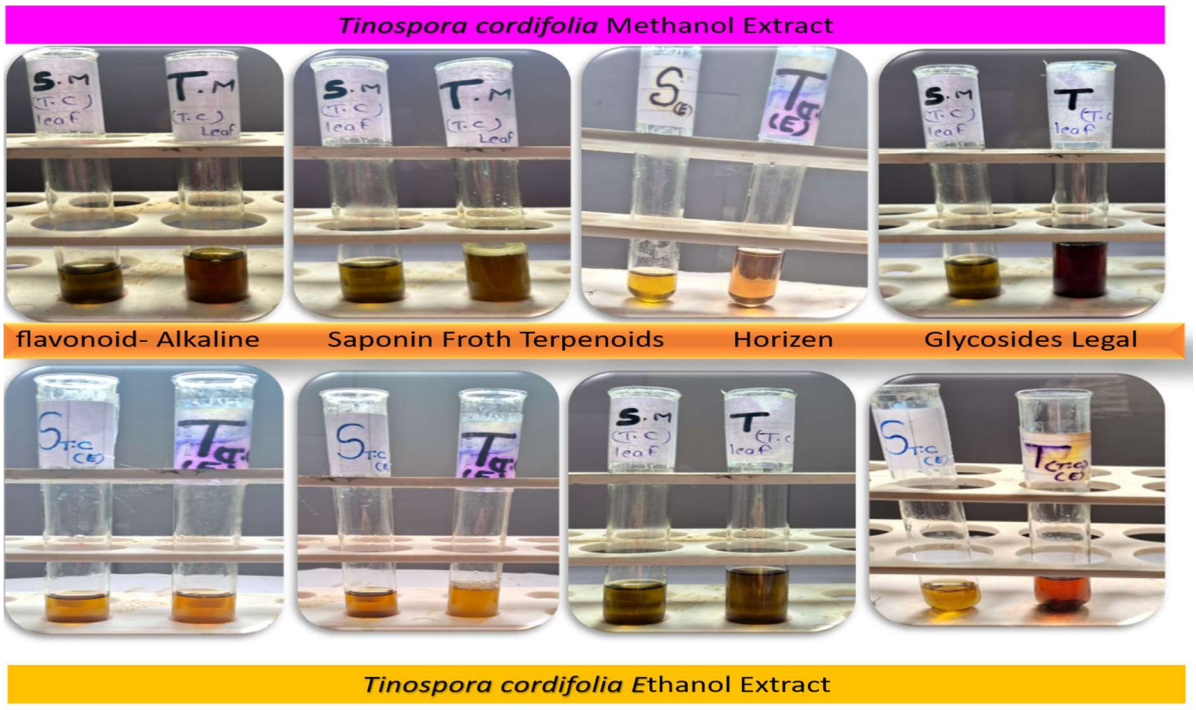

Figure 11 *Qualitative Phytochemical Screening of Azadirachta indica Leaf Extracts in Methanol and Ethanol*

2.4.3 Antibacterial Activity Against *Enterobacter aerogenes*: - *Enterobacter aerogenes* a clinically relevant, antibiotic-resistant Gram-negative bacterium, was sourced from a certified collection and cultured under sterile conditions at 37 °C for viability. The prepared plant extracts were used for antimicrobial screening. Agar well diffusion methods was used for antibacterial activity.

Figure 13: The figure illustrates the antibacterial effects of methanolic leaf extracts of *A. indica* and *T. cordifolia* against *Enterobacter* spp. using the agar well diffusion method.

Mueller-Hinton Agar (MHA) plates, 4 mm in depth and 100 mm in diameter, were formulated with the pH adjusted to 7.2. The bacterial inoculum was standardized to a 0.5 McFarland turbidity standard (≈1 × 10^8 CFU/mL) and evenly spread over the agar surface with a sterile cotton swab to form a uniform lawn. Wells of 6 mm diameter were created using a sterile borer, and each was loaded with 50–100 µL of plant extract solution in methanol or ethanol. Control wells contained standard antibiotics for reference, while solvent blanks were included to eliminate solvent effects. Plates were incubated at 37 °C for 24–48 hours, after which antibacterial activity was determined by measuring the inhibition zones in millimeters, allowing comparison with standard antibiotics and selection of potent extracts for further MIC analysis.

3. **Results and Discussion**

3.1 Primary and Secondary Biochemical Tests of *Azadirachta indica* and *Tinospora cordifolia*: The phytochemical analysis of *Azadirachta indica* leaf extracts revealed variations in the detection of compounds depending on the solvent used.

Table-1: Qualitative Phytochemical Screening of *Azadirachta indica*and *Tinospora cordifolia*Leaf Extracts Using Ethanolic and Methanolic Solvents

S. No.	Name of Phytochemicals	Tests Methods	Ethanolic extract of *A. indica (A.I.)* & *T. cordifolia (T.C.)*		Methanolic extract of *A. indica (A.I.)* & *T. cordifolia (T.C.)*	
			A.I.	*T.C.*	*A.I.*	*T.C.*
1.	Carbohydrates and Reducing sugar	Molisch's test	++	++	++	+++
		Benedict's test	+++	+++	+++	+++
2.	Proteins	Millon's test	+	+	+	++
		Biuret test	++	-	++	++
3.	Amino Acids	Ninhydrin test	-	+	-	+
4.	Alkaloids	Mayer's test	+	++	+	+
		Wagner's test	+++	-	+++	+++
		Hager's test	++	+	++	++
5.	Phenols and Tannins	Ferric Chloride test	+++	++	+++	+++

		Lead Acetate test	+	+	+++	+
6.	Flavonoids	Alkaline reagent test	+	+	+	+
7	Saponins	Froth Test	-	++	++	++
8	Terpenoids	Horizon's Test	+	++	+	+
9	Glycosides	Legal Test	+++	+++	+++	+++

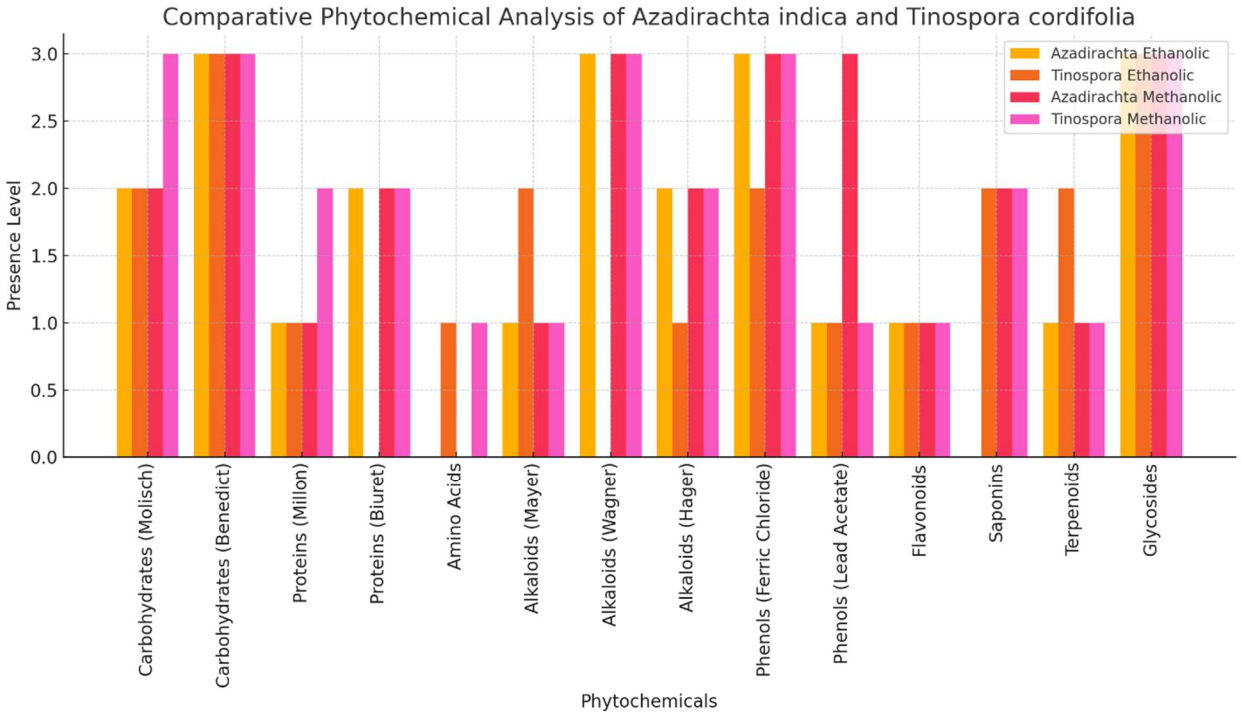

Figure 12: -Here is the comparative bar graph showing the phytochemical presence in ethanolic and methanolic extracts of *Azadirachta indica* and *Tinospora cordifolia*. The intensity of presence is quantified based on the test results (+, ++, +++, or -).

Bioactive compounds analysis of the ethanolic and methanolic extracts of both plants showed the appearance of several bioactive substances. Carbohydrates and reducing sugars were strongly

present in all extracts, particularly in the methanolic extract of *T. cordifolia* as indicated by intense reactions in both Molisch's and Benedict's tests. However, (Sharma *et al*., 2019), did not include carbohydrate screening in its phytochemical analysis. This highlights an additional dimension in the current investigation, suggesting that *A. indica* leaves not only possess antimicrobial and antioxidant compounds but also contain primary metabolites like carbohydrates that may support overall plant bioactivity and stability. Other medicinal plants such as *Vernonia amygdalina* also show comparable carbohydrate levels, though specific tests may reveal weaker simple sugar detection in that plant (Low *et al*., 2017 and Erhabor&Erhabor, 2024). Proteins were moderately detected, with the Biuret test showing stronger positivity in the methanolic extract of plants, showed mild reactions in both tests. It exhibited moderate presence, evidenced by Millon's (+) and Biuret (++) tests in methanol; this aligns with literature reporting approximately 7–22% crude protein in *A. indica* leaf flour(Mishra *et al*., 2014). Similar result was found with (Andersa *et al.,* 2024) who reported crude protein content in neem leaf flour ranging from 7–22 % of dry weight.Amino acids were detected only in *T. cordifolia* extracts through the Ninhydrin test, indicating the presence of trace levels of free amino acids. This contrasts with aqueous-based studies, such as (Darshane& Pandit, 2023), who reported detectable levels of amino acids in *A. indica* using water as a solvent, indicating that alcoholic solvents may not be effective for extracting free amino acids.Alkaloids were strongly present in *A. indica*, especially in the methanolic extract, as shown by Wagner's (+++), Hager's (++), and Mayer's (+) tests. In contrast, *T. cordifolia* showed moderate alkaloid content, with a relatively weaker response. Similar result was found with (Mishra *et al*., 2014), who reported the confirmation of their abundance and bioactivity in *A. indica* and *T. cardifolia.* Alkaloids are known for antimicrobial and analgesic effects and have been reported as abundant in both plants *(Liang et al*., 2024). Phenols and tannins were abundant in all samples, with *A. indica* methanolic extract displaying the highest intensity, particularly in the Ferric chloride and Lead acetate tests, suggesting strong antioxidant potential. Phenols and tannins were also strongly detected (Ferric Chloride ++ in ethanol, +++ in methanol; Lead Acetate + in both), highlighting their antioxidant and antimicrobial potential, as supported by recent analyses reporting high phenolic and tannin contents in these plants (Mishra *et al*., 2014).Flavonoids were mildly present in all extracts, as indicated by weak positive results in the alkaline reagent test. This contrasts with the moderate-to-high flavonoid content observed in aqueous or acetone extracts as reported by (Saleem et al., 2018). Flavonoids were weakly detected

(+ in both extracts), indicating lower concentration than typically reported in alcoholic extracts (Nadeeshani *et al.,* 2022). Saponins were only observed in the methanolic extract, reflecting the effect of extraction effectiveness on extraction efficiency. This aligns with the study by (Bolaji et al., 2024). Terpenoids were weakly detected in both solvents, indicating low concentrations yet suggesting potential anti-inflammatory and antimicrobial properties. Câmara *et al.,* 2024; Rai *et al*., 2023 investigated similar results and suggested that non-polar solvents or supercritical CO_2 methods may yield higher terpenoid content. Glycosides were strongly confirmed through Legal's test in both extracts, indicating cardiotonic or anti-inflammatory potential. The differential extraction results underscore the role of solvent selection in phytochemical profiling. Glycosides were strongly detected in both ethanol and methanol extracts using Legal's test, which aligns with (Andersa et al., 2024), who documented abundant cardiac and flavonoid glycosides in both plants extracts. These constitutes are recognized for their cardiotonic, anti-inflammatory, and immunomodulatory properties. Overall, methanol extracted more proteins, phenols, and alkaloids, while ethanol was more effective for terpenoids and specific alkaloid forms. The variation in phytochemical detection between ethanolic and methanolic extracts arises due to differences in solvent polarity and solubility profiles of the bioactive compounds. Methanol, being more polar, tends to extract phenolic compounds, proteins, and certain alkaloids more efficiently, whereas ethanol can better solubilize moderately polar compounds like terpenoids and specific glycosidic forms. The phytochemical screening of ethanolic and methanolic leaf extracts revealed a diverse array of bioactive compounds with varying solubility profiles.

3.2 Natural Defense Against Drug-Resistant Bacteria: - The agar well method identified that both *A. indica* and *T. cordifolia* leaf extracts possess significant antimicrobial activity against *Enterobacter aerogenes*, as indicated by clear zones of inhibition around the wells. *Table-3* Comparative Antibacterial Activity of *A. indica* and *T. cordifolia* Against *Enterobacter aerogenes*

Plant	Plant Leaves Weight		Zone of inhibition (mm)			Control use Gentamycin (µg/disc)
	Fresh weight	Dry Weight	(A)	B	C	
A. indica	10.754/gm	2.356/gm	31mm	28mm	27mm	30 µg/disc

T. cordifolia	10.534/gm	2.45/gm	28MM	30MM	28MM	30 µg/disc

The antibacterial activity of *A. indica* and *T. cordifolia* leaf extracts against *Enterobacter aerogenes* was assessed using the agar well diffusion method with fresh and dry leaf weights. *A. indica* showed inhibition zones of 31, 28, and 27 mm, while *T. cordifolia* exhibited 28,30-, and 28-mm. Gentamycin (30 µg/disc) served as the positive control. The slightly higher activity of *A. indica* may be linked to its abundant alkaloids and phenolics. Results confirm both plants' potential as natural antibacterial agents, with consistent replicates supporting their reproducibility for phytomedicinal applications.

4. Conclusion

A comparative phytochemical analysis of *Azadirachta indica* and *Tinospora cordifolia* using ethanolic and methanolic extracts revealed multiple bioactive compounds. Both species contained key therapeutic phytoconstituents, though differences in compound presence and intensity were noted based on the extraction solvent. *A. indica* methanolic extract showed a strong presence of reducing sugars, alkaloids, phenols, and glycosides, while *T. cordifolia* methanolic extract exhibited a richer presence of proteins, saponins, and amino acids. Notably, both plants tested positive (++ or +++) for glycosides across all extracts, suggesting their potential role in bioactivity. Antimicrobial studies demonstrated that both plant extracts produced significant zones of inhibition against the test organism, with *A. indica* showing a maximum inhibition zone of 31 mm, and *T. cordifolia* showing 30 mm, compared to the standard antibiotic Gentamycin (30 µg/disc), which showed 27–28 mm. These findings indicate that the crude extracts possess potent antimicrobial activity, likely attributable to the synergistic effect of the phytochemicals present. Thus, the results support the traditional use of these plants for therapeutic applications and highlight their potential as natural sources for antimicrobial agents. Advanced studies on their mechanisms, synergistic effects with antibiotics, and formulation into herbal drugs are warranted. Incorporation into nanocarriers and subsequent *in vivo* evaluations could further enhance their therapeutic potential.

5. References

Andersa, K. N., Tamiru, M., Teka, T. A., Ali, I. M., Chane, K. T., Regasa, T. K., & Ahmed, E. H. (2024a). Proximate composition, some phytochemical constituents, potential uses, and safety of neem leaf flour: A review. *Food Science and Nutrition*, *12*(10), 6929–6937. https://doi.org/10.1002/FSN3.4336

Andersa, K. N., Tamiru, M., Teka, T. A., Ali, I. M., Chane, K. T., Regasa, T. K., & Ahmed, E. H. (2024b). Proximate composition, some phytochemical constituents, potential uses, and safety of neem leaf flour: A review. *Food Science & Nutrition*, *12*(10), 6929. https://doi.org/10.1002/FSN3.4336

Arzani, V., Soleimani, M., Fritsch, T., Jacob, U. M., Calabrese, V., & Arzani, A. (2025). Plant polyphenols, terpenes, and terpenoids in oral health. *Open Medicine*, *20*(1), 20251183. https://doi.org/10.1515/MED-2025-1183

Beohar, M., Verma, M., Verma, A., & Gupta, V. (2024a). Updates on Tropical Medicinal Plant Tinospora cordifolia as an Antimicrobial Shrub to Combat Respiratory Infections. *The Natural Products Journal*, *15*(5), E020724231453. https://doi.org/10.2174/0122103155321163240620101820/CITE/REFWORKS

Beohar, M., Verma, M., Verma, A., & Gupta, V. (2024b). Updates on Tropical Medicinal Plant Tinospora cordifolia as an Antimicrobial Shrub to Combat Respiratory Infections. *The Natural Products Journal*, *15*(5), E020724231453. https://doi.org/10.2174/0122103155321163240620101820/CITE/REFWORKS

Bolaji, O., Adeniyi Abolade, Y., Aduwa, S., Bernaldine Isiaka, A., Durodola, O., Adeoye, A., Ojemai Adeoye, T., Adeesan Bamidele, A., OnyenachiAkagbue, B., Baba Aminu, awiya, & Siame, T. (2024). Corresponding author: Mu'awiya Baba Aminu; Email: Potential health and environmental benefits of the identified phytochemicals screening of (Azadirachta indica) neem leaves in Bauchi Metropolis, Bauchi State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, *2024*(03), 68–083. https://doi.org/10.30574/gscbps.2024.26.3.0037

Câmara, J. S., Perestrelo, R., Ferreira, R., Berenguer, C. V., Pereira, J. A. M., & Castilho, P. C. (2024). Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. *Molecules 2024, Vol. 29, Page 3861*, *29*(16), 3861. https://doi.org/10.3390/MOLECULES29163861

Darshane, P., & Pandit, A. (2023). International journal of current medical and pharmaceutical research ancient indian and contemporary dietary factors to plausibly reduce chemotherapy induced leukopenia (cil): a comprehensive & narrative review. *9*(7), 378–385. https://doi.org/10.24327/23956429.ijcmpr202301106

Erhabor, A., &Erhabor, O. (2024). Comparative Analysis of Phytochemical Composition of Aqueous Extracts from Azadirachta indica and Vernonia amygdalina. *Sciences of Phytochemistry*, *3*(2), 91–97. https://doi.org/10.58920/SCIPHY0302270

Evans, W. C. (1997). *Trease and Evans Pharmacognosy* (14th ed.). W. B. Saunders

Lekkala, V. D. V. V., Muktinutalapati, A. V., Lebaka, V. R., Lomada, D., Korivi, M., Li, W., & Reddy, M. C. (2025). Green Synthesis and Characterization of Silver Nanoparticles from Tinospora cordifolia Leaf Extract: Evaluation of Their Antioxidant, Anti-Inflammatory, Antibacterial, and Antibiofilm Efficacies. *Nanomaterials*, *15*(5), 381. https://doi.org/10.3390/NANO15050381/S1

Liang, X., Yang, L., Xu, X., & Chi, X. (2024). The complete plastome of Saxifraga giraldiana Engler and its phylogenetic analysis. *Mitochondrial DNA Part B: Resources*, *9*(9), 1157–1161. https://doi.org/10.1080/23802359.2024.2397983,

Low, J. Y. Q., Lacy, K. E., McBride, R. L., & Keast, R. S. J. (2017). Evidence supporting oral sensitivity to complex carbohydrates independent of sweet taste sensitivity in humans. *PLoS ONE*, *12*(12), e0188784. https://doi.org/10.1371/JOURNAL.PONE.0188784

Mace, M. E. (1963). Histochemical localization of phenols in healthy and diseased tomato roots. *Phytopathology, 16*, 915–925.

Mishra, P., Jamdar, P., Desai, S., Patel, D., & Meshram, D. (2014). Phytochemical analysis and assessment of in vitro antibacterial activity of Tinospora cordifolia. *Int.J.Curr.Microbiol.App.Sci*, *3*(3), 224–234. http://www.ijcmas.com

Nadeeshani, H., Li, J., Ying, T., Zhang, B., & Lu, J. (2022). Nicotinamide mononucleotide (NMN) as an anti-aging health product – Promises and safety concerns. *Journal of Advanced Research*, *37*, 267–278. https://doi.org/10.1016/j.jare.2021.08.003

Nieto Marín, V., Buccini, D. F., Gomes da Silva, V., Fernandez Soliz, I. A., & Franco, O. L. (2025). Nanoformulations of bioactive compounds derived from essential oils with antimicrobial activity. *Nano Trends*, *9*, 100070. https://doi.org/10.1016/J.NWNANO.2024.100070

Ramakrishnan, S., Prasannan, K. D., & Rajan, R. (1994). *Textbook of Medical Biochemistry* (2nd ed.). Orient Longman.

Saleem, S., Muhammad, G., Hussain, M. A., & Bukhari, S. N. A. (2018). A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica. *Phytotherapy Research*, *32*(7), 1241–1272. https://doi.org/10.1002/PTR.6076

SHARMA, G., THAKUR, A., LAL, S., & KUMAR NADDA, R. (2019). Comparative analysis of phytochemical, antibacterial, and antioxidant activity of different extracts of azadirachta indica leaves. *Asian Journal of Pharmaceutical and Clinical Research*, 151–154. https://doi.org/10.22159/AJPCR.2019.V12I3.29286

Tahir, H., Rashid, F., Ali, S., Summer, M., & Afzal, M. (2025). Synthesis, Characterization, Phytochemistry, and Therapeutic Potential of Azadirachta indica Conjugated Silver Nanoparticles: A Comprehensive Study on Antidiabetic and Antioxidant Properties. *Biological Trace Element Research*, *203*(4), 2170–2185. https://doi.org/10.1007/S12011-024-04293-3/METRICS

van Wyk, A. S., & Prinsloo, G. (2020). Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. *South African Journal of Botany*, *133*, 54–62. https://doi.org/10.1016/J.SAJB.2020.06.031

Wagner, H. (1993). *Pharmazeutische Biologie: Drogen und ihre Inhaltsstoffe* (5th ed.). Gustav Fischer Verlag.

Zhang, L., Hu, Y., Zhang, J., Cai, M., Lan, L., & Sun, G. (2025). Application of chemical pattern recognition and similarity evaluation in electrochemical and HPLC-DAD fingerprints for quality consistency study of herbal medicines. *Analytica Chimica Acta*, *1349*, 343830. https://doi.org/10.1016/J.ACA.2025.343830