

Screening of Bioactive Phytochemicals in Methanolic Leaf and Stem Extracts of *Thuja occidentalis* and *Araucaria heterophylla*: A Qualitative Approach

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Abstract

The present investigation provides a preliminary qualitative phytochemical evaluation of methanolic leaf and stem extracts obtained from *Thuja occidentalis* and *Araucaria heterophylla* to identify the major classes of bioactive secondary metabolites present in these medicinal plant species. Comparative phytochemical screening was performed to assess similarities and differences in the chemical composition of the two plants. The analysis of methanolic leaf and stem extracts of *T. occidentalis* (TOLMe and TOSMe) revealed the occurrence of several pharmacologically important phytoconstituents, including phenolic compounds, flavonoids, tannins, alkaloids, phytosterols, terpenoids, and triterpenoids. These metabolites are widely recognized for their antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties, suggesting the medicinal significance of this species. In contrast, the methanolic leaf and stem extracts of *A. heterophylla* (AHLMe and AHSMe) exhibited a comparatively broader spectrum of phytochemicals. The extracts tested positive for carbohydrates, phenolic compounds, flavonoids, glycosides, saponins, alkaloids, tannins, terpenoids, diterpenoids, and lipids or fixed oils. The presence of these diverse classes of bioactive constituents indicates the rich phytochemical composition of *A. heterophylla* and highlights its potential as a valuable source of naturally occurring therapeutic compounds. Overall, the comparative findings demonstrate that both plant species possess an abundance of biologically active metabolites, although *A. heterophylla* displayed greater phytochemical diversity than *T. occidentalis*. These preliminary observations provide a scientific basis for future quantitative

phytochemical analyses, compound isolation, and pharmacological investigations aimed at validating the therapeutic potential and possible pharmaceutical applications of these medicinal plants.

Keywords-: *Thuja occidentalis*, Phytochemical analysis · *Araucaria heterophylla*

I. Introduction

Medicinal plants continue to represent one of the most valuable reservoirs of structurally diverse bioactive compounds for pharmaceutical, nutraceutical, and industrial applications. Plant-derived secondary metabolites, including alkaloids, flavonoids, terpenoids, phenolics, tannins, and glycosides, possess a broad spectrum of biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, antiviral, and cardioprotective properties. Despite significant advances in synthetic chemistry, natural products remain indispensable in modern drug discovery, with nearly half of newly approved therapeutic agents originating directly or indirectly from natural sources (Newman & Cragg, 2020; Atanasov et al., 2021).

Although many medicinal plants have been extensively investigated, ornamental species are increasingly recognized as valuable reservoirs of pharmacologically important phytochemicals. A classical example is *Catharanthus roseus*, whose alkaloids, vincristine and vinblastine, revolutionized cancer chemotherapy. Similarly, several ornamental gymnosperms have recently attracted scientific interest because of their unique phytochemical composition and diverse biological activities, highlighting their potential for pharmaceutical and industrial applications (Atanasov et al., 2021).

Araucaria heterophylla (Salisb.) Franco, belonging to the family Araucariaceae, is an evergreen conifer widely cultivated as an ornamental and decorative tree throughout tropical and subtropical regions. The Araucariaceae family comprises only three extant genera (*Araucaria*, *Agathis*, and *Wollemia*) and approximately 40 species distributed mainly in the Southern Hemisphere. Members of this family are characterized by abundant resin production, which serves as a natural defense mechanism against microbial infection and environmental stress. Recent phytochemical investigations have revealed that *A. heterophylla* contains diterpenoids, biflavonoids, phenolic compounds, and volatile constituents exhibiting antioxidant, antimicrobial, gastroprotective, and anti-inflammatory activities, suggesting its considerable therapeutic potential (Abdallah et al., 2022; Elshamy et al., 2023).

Similarly, *Thuja occidentalis* L. (family Cupressaceae), commonly known as northern white cedar or arborvitae, has been used extensively in traditional medicine across Europe and North America. Different plant parts have historically been employed for the management of respiratory disorders, rheumatism, skin diseases, urinary tract infections, migraine, and benign tumors. Contemporary pharmacological

studies have demonstrated that extracts of *T. occidentalis* possess antiviral, antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, antidiabetic, and anticancer activities, largely attributed to the presence of essential oils, flavonoids, lignans, tannins, and monoterpenes such as thujone and borneol (Salehi et al., 2021; Sharifi-Rad et al., 2022).

Natural products isolated from terrestrial plants continue to play a fundamental role in healthcare systems worldwide. Traditional medical systems, including Ayurveda, Unani, Siddha, Traditional Chinese Medicine, and numerous indigenous healthcare practices, rely extensively on medicinal plants for disease prevention and treatment. According to the World Health Organization, approximately 80% of the global population still depends on traditional herbal medicine for primary healthcare, emphasizing the continued importance of medicinal plants in public health and pharmaceutical research (WHO, 2023).

India is recognized as one of the world's richest centers of medicinal plant diversity, harboring thousands of plant species with ethnopharmacological significance. The country's rich botanical resources and long history of traditional medicine have encouraged extensive exploration of indigenous plants for the discovery of novel bioactive compounds. Secondary metabolites synthesized by medicinal plants function primarily as ecological defense molecules but simultaneously exhibit remarkable pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, and neuroprotective effects. Consequently, phytochemical investigation has become an essential step in identifying biologically active constituents and validating the therapeutic potential of medicinal plants (Atanasov et al., 2021; Nirmala et al., 2023).

Qualitative phytochemical screening serves as a preliminary yet indispensable approach for detecting major classes of secondary metabolites prior to detailed chemical characterization. In recent years, advanced analytical techniques such as gas chromatography-mass spectrometry (GC–MS), liquid chromatography-mass spectrometry (LC–MS/MS), high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance (NMR) spectroscopy have significantly improved the identification and characterization of plant metabolites. Among these techniques, GC–MS remains one of the most reliable analytical platforms for identifying volatile and semi-volatile phytoconstituents in medicinal plants due to its high sensitivity, reproducibility, and extensive spectral libraries (Anand et al., 2022; Elshamy et al., 2023).

Beyond pharmaceutical applications, medicinal plants have also gained considerable attention as environmentally sustainable resources for industrial purposes. In particular, plant extracts are increasingly being investigated as eco-friendly corrosion inhibitors for metallic materials. Metals employed in

transportation, construction, marine engineering, and manufacturing industries are susceptible to corrosion because refined metals naturally tend to return to their thermodynamically stable oxide or mineral forms. Conventional synthetic corrosion inhibitors, although effective, often exhibit high toxicity, poor biodegradability, and environmental persistence. Consequently, green corrosion inhibitors derived from renewable plant sources have emerged as attractive alternatives due to their biodegradability, low toxicity, cost-effectiveness, and abundance of naturally occurring phytochemicals capable of adsorbing onto metal surfaces and reducing corrosion rates (Verma et al., 2022; Quraishi et al., 2023).

Considering the increasing demand for natural bioactive compounds and environmentally sustainable technologies, comprehensive phytochemical evaluation of medicinal and ornamental plant species has become increasingly important. Therefore, the present study focuses on the qualitative phytochemical screening of methanolic leaf and stem extracts of *Thuja occidentalis* and *Araucaria heterophylla* to identify the major classes of secondary metabolites that may contribute to their pharmacological and industrial applications.

II. Materials and Methods

Plant Material Collection

Fresh and healthy specimens of *Thuja occidentalis* and *Araucaria heterophylla* were collected from the Shivpuri district of Madhya Pradesh, India, during the appropriate growing season. The selected plants were free from visible signs of disease, insect infestation, and mechanical damage to ensure the authenticity and quality of the plant material used in the study. The collected specimens were transported to the laboratory in sterile polyethylene bags for further processing. Botanical identification and taxonomic authentication were carried out using standard floristic keys and authenticated by a qualified plant taxonomist. Voucher specimens were prepared and deposited in the departmental herbarium for future reference following established botanical documentation procedures (Jain & Rao, 1977; APG IV, 2016).

Thuja occidentalis

This species belongs to the Cupressaceae family. It has traditionally been used to address rheumatism, migraine, and uterine tumors, and research has further documented anticancer, antiviral, anti-inflammatory, insecticidal, and antidiabetic activity (Figure 1).



Figure 1: Image of *Thuja occidentalis*

Araucaria heterophylla

Araucaria is part of the Araucariaceae family, a compact family containing only 38 species across the genera Araucaria and Agathis. *A. heterophylla* is a familiar columnar tree, commonly shaped and marketed as an ornamental Christmas tree, and studies of its resin have reported gastroprotective chemical and biological properties.



Figure 2: Image of *Araucaria heterophylla*

Preparation of Plant Extract

Fresh plant material was air-dried and coarsely powdered. After defatting, the powder underwent successive methanolic extraction using a Soxhlet apparatus, with the process kept below 60°C throughout. A rotary vacuum evaporator was then used to remove residual solvent from the extract under reduced

pressure. Soxhlet extraction was chosen for this work because it is straightforward to run, relatively quick, cost-effective (requiring only a single sample for the full extraction), easy to monitor for completion, and being a closed system carries a lower risk of contamination.



Figure 3: Soxhlet assembly

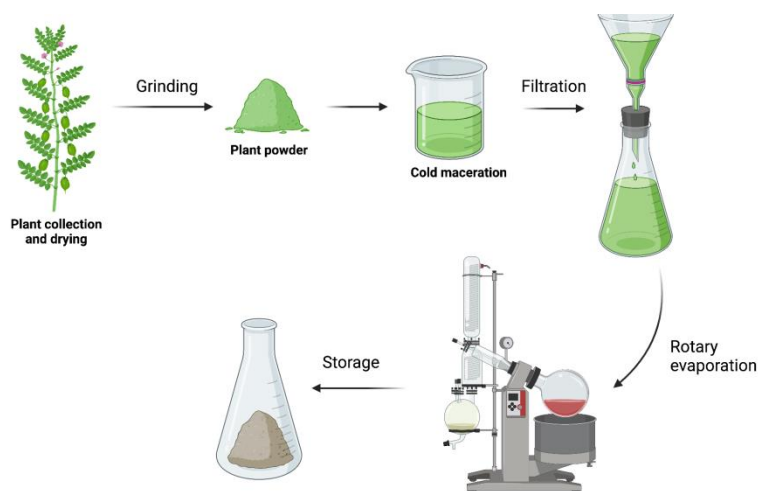


Figure 4: Process of making the plant extract

Materials

A separate portion of this study involved metal-sheet analysis including weight-loss measurement, electrochemical impedance spectroscopy, potentiodynamic polarization, and surface examination. Metal sheets were cut into 2.5 cm × 2.5 cm squares and polished using emery papers of grade 320, 600, 1000,

1500, and 2000 in sequence to achieve a uniform surface. The samples were then degreased with acetone, rinsed with distilled water, air-dried, and subsequently immersed in the corrosive test media.

Phytochemical Screening

Extracts were screened for phytochemical content following standard qualitative methods. This initial screening aimed to detect the range of phytochemicals present in each plant, including terpenoids, glycosides, alkaloids, flavonoids, tannins, saponins, and carbohydrates.

Test for Carbohydrates

Molisch's test: roughly 1 mL of test sample was treated with 2-3 drops of α -naphthol, followed by concentrated sulfuric acid added carefully down the side of the tube. A purple ring forming at the interface of the two liquids indicated the presence of carbohydrates.

Test for Alkaloids

A whitish-yellow precipitate forms when Mayer's reagent is added to an alkaloid-containing solution. After evaporating the alcoholic extract, the residue was heated with 2% hydrochloric acid in a water bath, cooled, and filtered; Mayer's reagent was then added in small amounts, and the mixture was checked for turbidity or yellow precipitation.

Test for Glycosides

Keller-Kiliani test: about 2 mL of extract was dissolved in 3 mL glacial acetic acid together with 1 mL of 5% ferric chloride solution. This mixture was layered carefully onto 2 mL of concentrated sulfuric acid, and any resulting color change was recorded.

Test for Flavonoids

Lead acetate test: extract was mixed with lead acetate solution; a yellow precipitate signals the presence of flavonoids. Alkaline reagent test: 1 mL of extract was combined with dilute NaOH, with a yellow color again indicating flavonoids.

Test for Saponins

Froth test: approximately 0.2 g of extract was mixed with 5 mL distilled water and shaken thoroughly before boiling; persistent foaming indicates the presence of saponins.

Test for Steroids

A 4 mg portion of extract was treated with 0.5 mL acetic anhydride and 0.5 mL chloroform, followed by concentrated sulfuric acid; a greenish-blue color confirms the presence of steroids.

Test for Proteins and Amino Acids

Ninhydrin test: 3 mL of test solution was heated with roughly three drops of 5% ninhydrin and boiled for 10 minutes in a water bath, with any color change noted. Biuret test: treatment of the sample with 1% CuSO₄ and 4% NaOH produces a pink or violet color when proteins are present.

Test for Tannins

Ferric chloride test: a small quantity of extract was heated with water and filtered; adding two drops of ferric chloride to the filtrate produced a blue-black or greenish-black precipitate when tannins were present.

Test for Phytosterols

Concentrated sulfuric acid was added to the extract, shaken, and left to stand; a red color developing in the lower layer indicates phytosterols.

Test for Cholesterol

The extract was treated with 2 mL chloroform and 10 drops of concentrated acetic anhydride, followed by a drop of concentrated sulfuric acid; a red-rose color confirms cholesterol.

Test for Terpenoids

Extract was combined with 2 mL chloroform and 3 mL concentrated sulfuric acid and heated in a water bath; a grey color developing in the solution indicates terpenoids.

Test for Diterpenes

Extract dissolved in distilled water was treated with 3-4 drops of copper acetate solution; a green color indicates diterpenes.

Test for Lignin

Extract treated with gallic acid produces an olive-green color when lignin is present.

Test for Carotenoids

Extract was evaporated to dryness and treated with a saturated solution of antimony trichloride in chloroform; a shift from bluish-green to red indicates carotenoids.

Test for Anthocyanins

Sample was treated with 2 mL HCl followed by 1-2 mL ammonia solution; a pinkish-red solution turning bluish-violet upon ammonia addition indicates anthocyanins.

Test for Phenols

A small amount of leaf extract was warmed with water, and about 2 mL of 5% ferric chloride solution was added; the appearance of a green or blue color indicates phenolic compounds.

Test for Fats and Oils

Spot test: a drop of extract was placed on filter paper and left to air-dry; an oily stain remaining after the solvent evaporated confirmed the presence of fixed oils.

III. Results and Discussion

Phytochemical Estimation

Table 1. Qualitative phytochemical screening of *Thuja occidentalis* and *Araucaria heterophylla* leaf and stem extracts prepared in methanol (TOLMe: *T. occidentalis* leaf, methanol; TOSMe: *T. occidentalis* stem, methanol; AHLMe: *A. heterophylla* leaf, methanol; AHSMme: *A. heterophylla* stem, methanol)

Phytoconstituent	TOLMe	AHLMe	TOSMe	AHSMme
Carbohydrates	-ve	+ve	-ve	+ve
Alkaloids	+ve	+ve	+ve	+ve
Glycosides	-ve	+ve	-ve	+ve
Flavonoids	+ve	+ve	+ve	+ve
Saponins	-ve	+ve	-ve	+ve
Proteins and amino acids	-ve	-ve	-ve	-ve
Tannins	+ve	+ve	+ve	+ve
Phytosterols	+ve	-ve	+ve	-ve
Cholesterol	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve
Triterpenoids	+ve	+ve	+ve	+ve
Lignin	-ve	+ve	-ve	+ve
Carotenoids	-ve	-ve	-ve	-ve
Anthocyanins	-ve	-ve	-ve	-ve
Oils and fats	+ve	+ve	+ve	-ve
Phenolic compounds	+ve	+ve	+ve	+ve

Screening of the *T. occidentalis* extracts (TOLMe and TOSMe) confirmed the presence of phenolic compounds, flavonoids, tannins, alkaloids, phytosterols, terpenoids, and triterpenoids, alongside tannins and alkaloids more broadly. The *A. heterophylla* extracts (AHLMe and AHSMme), by contrast, showed a wider phenolic profile that included carbohydrates, flavonoids, glycosides, saponins, alkaloids, tannins, terpenoids, diterpenoids, and oils/lipids.

IV. Conclusion

Across both species examined, tannins and alkaloids were consistently detected. The *A. heterophylla* extracts additionally showed a broader phenolic profile encompassing carbohydrates, flavonoids, glycosides, saponins, alkaloids, tannins, terpenoids, diterpenoids, and lipids/oils. Taken together, the phytochemical screening of the *T. occidentalis* extracts (TOLMe and TOSMe) confirmed the presence of phenolic compounds, flavonoids, tannins, alkaloids, phytosterols, terpenoids, and triterpenoids, underscoring the phytochemical richness of both plants examined in this study.

References

- Abdallah, E. M., Elshamy, A. I., Mohamed, T. A., & Hegazy, M. E. F. (2022). Phytochemistry and biological activities of *Araucaria* species: A comprehensive review. *Plants*, 11(18), 2421.
- Anand, U., et al. (2022). Recent advances in GC–MS-based metabolomics for medicinal plant research. *Journal of Pharmaceutical and Biomedical Analysis*, 214, 114727.
- APG IV. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, 181(1), 1–20. <https://doi.org/10.1111/boj.12385>
- Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & International Natural Product Sciences Taskforce. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, 20(3), 200–216.
- Bridson, D., & Forman, L. (Eds.). (1999). *The Herbarium Handbook* (3rd ed.). Royal Botanic Gardens, Kew.
- Elshamy, A. I., Mohamed, T. A., & Hegazy, M. E. F. (2023). Phytochemical diversity and biological potential of coniferous medicinal plants. *Molecules*, 28(6), 2695.
- Funk, V. A., Hoch, P. C., Prather, L. A., & Wagner, W. L. (2005). The importance of vouchers. *Taxon*, 54(1), 127–129. <https://doi.org/10.2307/25065309>
- Hicks, J. A., & Hicks, P. M. (1978). A selected bibliography of plant collection and herbarium curation. *Taxon*, 27(1), 63–99. <https://doi.org/10.2307/1220483>
- Jain, S. K., & Rao, R. R. (1977). *A Handbook of Field and Herbarium Methods*. Today & Tomorrow's Printers and Publishers, New Delhi.
- Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over nearly four decades (1981–2019). *Journal of Natural Products*, 83(3), 770–803.

- Nirmala, M., Kumar, R., & Singh, P. (2023). Medicinal plants of India: Phytochemical diversity and therapeutic applications. *South African Journal of Botany*, 157, 468–483.
- Quraishi, M. A., Verma, C., & Ebenso, E. E. (2023). Green corrosion inhibitors: Progress, challenges, and future prospects. *Journal of Molecular Liquids*, 372, 121143.
- Salehi, B., Sharifi-Rad, J., et al. (2021). Therapeutic potential of *Thuja occidentalis*: A comprehensive review of phytochemistry and pharmacology. *Frontiers in Pharmacology*, 12, 717574.
- Sharifi-Rad, J., et al. (2022). Plant-derived bioactive compounds in disease prevention and therapy. *Biomolecules*, 12(4), 557.
- Simpson, M. G. (2019). *Plant Systematics* (3rd ed.). Academic Press. (Chapter: Plant Collecting and Documentation).
- Thiers, B. (2025). *Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff*. New York Botanical Garden. Available at: [Index Herbariorum](#)
- United States Department of Agriculture (USDA-ARS). (2024). *Herbarium Vouchers: Guidelines for Preparing Voucher Specimens*. Available at: [USDA Herbarium Vouchers](#)
- University of Florida Herbarium. (2025). *Herbarium Methodologies*. Available at: [Herbarium Methodologies](#)
- University of Florida Herbarium. (2025). *Preparation of Plant Specimens for Deposit as Herbarium Vouchers*. Available at: [University of Florida Herbarium guide](#)
- World Health Organization. (2023). *WHO traditional medicine strategy and global report on traditional, complementary and integrative medicine*. Geneva: World Health Organization.