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Bioactive Phytochemicals of *Xanthium strumarium* L.: Natural Weapons Against Microbial Pathogens

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Abstract

The rise of multidrug-resistant microbial pathogens has renewed interest in medicinal plants as a source of natural antimicrobial agents. *Xanthium strumarium* L., a widely used ethnomedicinal plant, is known for its diverse bioactive phytochemicals. This study investigates the phytochemical composition and antimicrobial potential of *X. strumarium* L. using ultrasonication-assisted extraction (UAE) to enhance bioactive compound recovery. Qualitative and quantitative analyses revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides, with ethanolic extracts showing the highest concentrations. Antimicrobial assays against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* demonstrated significant inhibitory activity, correlating strongly with flavonoid and phenolic content. The results validate the traditional medicinal uses of *X. strumarium* L. and highlight its phytochemicals as effective natural antimicrobial agents.

Keywords: *Xanthium strumarium*, phytochemicals, antimicrobial activity, secondary metabolites, ultrasonication.

1. Introduction:

The rapid emergence of microbial resistance to conventional antibiotics has become a major global health concern, necessitating the exploration of alternative and effective antimicrobial agents. In this context, medicinal plants have gained renewed scientific attention due to their rich reservoir of bioactive phytochemicals capable of inhibiting pathogenic microorganisms. Plant-derived secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides are known to exhibit broad-spectrum antimicrobial activity through diverse mechanisms, including

disruption of microbial cell walls, inhibition of enzyme systems, and interference with nucleic acid synthesis (Matei AT, *et al*., 2025).

Xanthium strumarium L., commonly known as cocklebur, belongs to the family Asteraceae and is widely distributed across tropical and subtropical regions. The plant has been extensively used in traditional medicine systems for the treatment of skin infections, inflammation, wounds, fever, and microbial diseases (Fakudze NT, *et al*., 2023). Various parts of the plant, particularly leaves and stems, are reported to contain biologically active compounds with therapeutic potential. Despite its ethnomedicinal importance, scientific validation of its antimicrobial efficacy in relation to specific phytochemical constituents remains limited (Fan W, *et al*., 2019).

Recent advancements in extraction technologies have significantly improved the recovery of plant bioactive compounds. Ultrasonication-Assisted Extraction (UAE) has emerged as an efficient and environmentally friendly technique that enhances the release of intracellular phytochemicals by disrupting plant cell walls through acoustic cavitation. This method offers advantages such as reduced extraction time, lower solvent consumption, and improved yield of thermolabile compounds compared to conventional extraction methods (Islam M, *et al*., 2023).

Understanding the relationship between phytochemical composition and antimicrobial activity is crucial for identifying plants with potential pharmaceutical applications. Establishing such correlations not only validates traditional medicinal claims but also aids in the development of plant-based antimicrobial formulations. Therefore, the present study focuses on the comprehensive evaluation of bioactive phytochemicals present in *X. strumarium* L. and their role as natural weapons against selected bacterial and fungal pathogens, using Ultrasonication-Assisted Extraction to optimize bioactive recovery (Vaou N, *et al*., 2021).

2. Literature Review

Sofowora A. (1993) reported that medicinal plants are important sources of therapeutic agents due to their rich diversity of bioactive phytochemicals and long history of use in traditional medicine. Cowan (1999) studied plant-derived secondary metabolites and reported that compounds such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides possess strong antimicrobial activity by disrupting microbial cell membranes, inhibiting enzymatic functions, and

interfering with nucleic acid synthesis. He further emphasized the potential of plant-based compounds as alternatives to synthetic antimicrobial agents. Ahmed *et al.* (2020) analysed *Xanthium strumarium* L. (Asteraceae) and reported that the plant contains diverse bioactive phytochemicals including flavonoids, phenolic compounds, sesquiterpene lactones, and alkaloids, which are responsible for its various biological and medicinal activities. The authors also highlighted the traditional use of the plant in treating skin infections, wounds, inflammation, fever, and microbial diseases. Suresh *et al.* (2018) investigated the antimicrobial activity of crude extracts of *X. strumarium* and demonstrated significant antibacterial and antifungal effects against both Gram-positive and Gram-negative microorganisms. Their study reported that ethanol and aqueous extracts were more effective due to their ability to extract polar bioactive compounds. However, the study mainly focused on antimicrobial screening without establishing a clear correlation between phytochemical content and antimicrobial efficacy.

Patil *et al.* (2021) reviewed advanced extraction techniques and reported that Ultrasonication-Assisted Extraction (UAE) is an efficient and eco-friendly method for enhancing phytochemical recovery. The authors explained that UAE improves extraction efficiency by disrupting plant cell walls through acoustic cavitation, while reducing extraction time and degradation of heat-sensitive compounds. Despite these advantages, limited studies have applied UAE specifically to *X. strumarium* for antimicrobial evaluation.

The present research addresses these gaps by employing UAE to obtain phytochemically rich extracts from both leaves and stems of *X. strumarium* L. and by establishing a direct correlation between phytochemical content and antimicrobial activity. This study provides scientific validation for the traditional use of the plant and supports its potential as a natural source of antimicrobial agents.

3. Materials and Methods

Plant Material Collection: Fresh leaves and stems of *X. strumarium* L. were collected from Nandurbar district and authenticated by a plant taxonomist Prof. Dr. M. B. Patil. Voucher specimens were deposited in the departmental herbarium.

Ultrasonication Assisted Extraction (UAE): Dried leaves and stems of *X. strumarium* L. were separately powdered using a mechanical grinder and sieved to obtain uniform particle size. Five grams of each powdered sample were mixed with 50 mL of petroleum ether, chloroform, ethanol, and distilled water. The mixtures were subjected to ultrasonication-assisted extraction using an ultrasonic bath at 35 kHz and 30 ± 2°C for 30 minutes to enhance phytochemical release. Ultrasonication facilitated the disruption of plant cell walls through acoustic cavitation, enhancing the release of intracellular bioactive phytochemicals into the solvent. (Patil, P. S., *et al*., 2021) After sonication, the extracts were cooled, filtered through Whatman No. 1 filter paper, and concentrated under reduced pressure using a rotary evaporator. The dried extracts were weighed and stored at 4°C for further analysis. (Kumar A, *et al*., 2023).

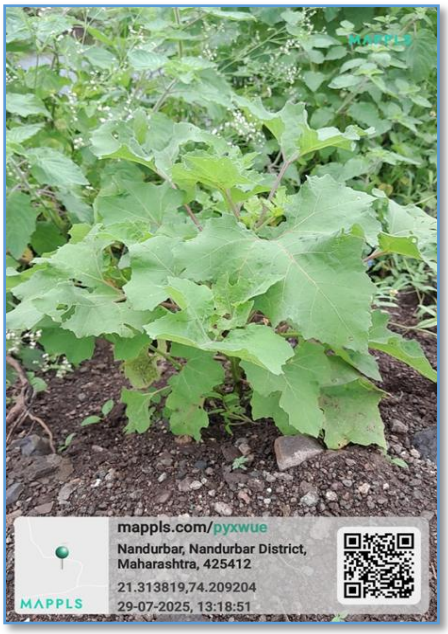


Fig. 1. *X. Strumarium* L. Whole Plant



Fig. 2. *X. strumarium* L. Plant parts

Phytochemical Analysis

Qualitative Phytochemical Screening: Preliminary phytochemical analysis revealed the presence of various secondary metabolites in both leaves and stem extracts. The intensity of phytochemical presence varied according to solvent polarity.

Test for Alkaloids (Mayer's Test): About 2 mL of plant extract was taken in a test tube and a few drops of Mayer's reagent were added. The formation of a cream or pale-yellow precipitate indicated the presence of alkaloids.

Test for Flavonoids (Shinoda Test): To 2 mL of plant extract, a small amount of magnesium turnings was added, followed by the careful addition of a few drops of concentrated hydrochloric acid. The appearance of a pink, crimson, or red coloration confirmed the presence of flavonoids.

Test for Phenolics and Tannins (Ferric Chloride Test): To 2 mL of plant extract, 2-3 drops of 5% ferric chloride solution were added. The formation of a blue, green, or black coloration indicated the presence of phenolic compounds and tannins.

Test for Saponins (Froth Test): About 2 mL of plant extract was diluted with 5 mL of distilled water and shaken vigorously for 2 minutes. The formation of a stable froth persisting for more than 10 minutes indicated the presence of saponins.

Test for Glycosides (Keller-Kiliani Test): To 2 mL of plant extract, 1 mL of glacial acetic acid containing a trace amount of ferric chloride was added. This mixture was carefully underlaid with 1 mL of concentrated sulfuric acid along the side of the test tube. The appearance of a brown ring at the interface confirmed the presence of cardiac glycosides.

Quantitative Phytochemical Estimation:

Determination of Total Phenolic Content (TPC): Total phenolic content was estimated using the Folin Ciocalteu method. Absorbance was measured at 765 nm using a UV-Visible spectrophotometer, and results were expressed as mg gallic acid equivalents (GAE) per gram of extract.

Determination of Total Flavonoid Content (TFC): Total flavonoid content was determined by the aluminium chloride colorimetric method. Absorbance was recorded at 415 nm, and results were expressed as mg quercetin equivalents (QE) per gram of extract.

Estimation of Alkaloid Content: Total alkaloid content was determined following standard gravimetric procedures and expressed as mg/g of dry extract (Harborne JB, 1998).

Antimicrobial Activity: The antimicrobial activity of *Xanthium strumarium* L. leaf and stem extracts was evaluated against selected pathogenic microorganisms:

Bacterial strain: Gram$^-$ bacterium: *Escherichia coli*, Gram$^+$ bacteria: *Staphylococcus aureus, Bacillus subtilis.*

Fungal strain: *Candida albicans*

All microbial cultures were obtained from a recognized microbiology laboratory and maintained on appropriate culture media under standard conditions (Beyatli A, 2024).

Preparation of Extracts for Antimicrobial Assay: Ultrasonication-assisted extracts of leaves and stems were prepared using ethanol, distilled water, chloroform, and petroleum ether. The dried extracts were re-dissolved in their respective solvents to obtain known concentrations and filtered through sterile filters before use. Medicinal plants have been the main source of therapeutic remedies since ancient times, despite their crucial significance in modern medicine. Medicinal plants may be a viable substitute for inefficient antibiotics in the fight against infectious diseases, according to a few scientific studies published over the previous thirty years (Abdallah EM, *et al*., 2023).

Agar Well Diffusion Method: The antimicrobial activity was assessed using the agar well diffusion method. Nutrient agar plates (for bacteria) and Sabouraud dextrose agar plates (for fungi) were prepared and sterilized. Fresh microbial suspensions were evenly spread over the agar surface using sterile cotton swabs. Wells of 6 mm diameter were aseptically punched into the agar using a sterile corkborer. A fixed volume of each plant extract was carefully introduced into the wells. Plates were incubated at: 37°C for 24 hours for bacterial strains, 28°C for 48 hours for Candida albicans After incubation, the zones of inhibition around the wells were measured in millimetres (mm).

Controls:

Positive control: Standard antibiotic Ampicillin (for bacterial strains).

Negative control: Corresponding extraction solvent (ethanol, distilled water, chloroform, or petroleum ether). The use of controls ensured the reliability and validity of the antimicrobial assay (Balouiri M, *et al.*,2016).

Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of the leaf and stem extracts was determined using the broth dilution method. Serial dilutions of each extract were prepared in sterile nutrient broth. A standardized inoculum of each test microorganism was added to the tubes. The tubes were incubated under appropriate conditions. The MIC was defined as the lowest concentration of extract that showed no visible microbial growth after incubation. (Elshobary ME, *et al.*, 2025)

4. Result and Discussions:

Phytochemical Analysis of *Xanthium strumarium* L.: Ultrasonication-assisted extraction of leaves and stems of *Xanthium strumarium* L. using solvents of varying polarity resulted in the recovery of diverse bioactive phytochemicals. The phytochemical profile varied significantly depending on the plant part and extraction solvent.

Qualitative Phytochemical Analysis: Qualitative screening confirmed the presence of major secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides in the extracts. Ethanolic extracts exhibited the richest phytochemical profile, particularly in leaf samples.

Table 1: Qualitative phytochemical constituents of *X. strumarium* L.

Phytochemical	Water	Ethanol	Chloroform	Petroleum ether
Leaves				
Alkaloids	+	++	+	+
Flavonoids	++	+++	+	-
Phenolics & Tannins	++	+++	+	-
Saponins	+	++	-	-
Glycosides	+	++	+	-
Stems				
Alkaloids	+	++	+	+
Flavonoids	+	++	+	-
Phenolics & Tannins	+	++	+	-
Saponins	+	+	-	-
Glycosides	+	+	+	-

(+ present, ++ moderately present, +++ strongly present, - absent)

Leaves showed a higher intensity of phytochemicals than stems. Ethanolic extracts displayed strong presence (+++) of flavonoids and phenolics, indicating efficient extraction by

ultrasonication. Petroleum ether extracts contained minimal phytochemicals, mainly non-polar compounds.

Quantitative Phytochemical Analysis: Quantitative estimation revealed significant variations in total phenolic, flavonoid, and alkaloid content among different extracts.

Table 2: Quantitative phytochemical constituents of *X. strumarium* L.

Extract	Plant Part	Total Phenolics (mg GAE/g)	Total Flavonoids (mg QE/g)	Alkaloids (mg/g)
Distilled Water	Leaves	74 ± 1.5	68 ± 1.3	20 ± 0.9
	Stems	61 ± 1.2	55 ± 1.1	17 ± 0.8
Ethanol	Leaves	**92 ± 2.0**	**82 ± 1.7**	**28 ± 1.1**
	Stems	78 ± 1.6	69 ± 1.4	22 ± 1.0
Chloroform	Leaves	48 ± 1.1	42 ± 1.0	15 ± 0.7
	Stems	39 ± 0.9	35 ± 0.8	12 ± 0.6
Petroleum Ether	Leaves	22 ± 0.6	18 ± 0.5	8 ± 0.4
	Stems	17 ± 0.5	14 ± 0.4	6 ± 0.3

Ethanolic leaf extracts showed maximum phenolic and flavonoid content. Leaves consistently contained higher levels of phytochemicals than stems. Ultrasonication significantly enhanced the extraction of bioactive compounds, especially polar metabolites.

Antimicrobial Activity: The antimicrobial activity of leaf and stem extracts was evaluated against selected bacterial and fungal pathogens. The results are expressed as zones of inhibition (mm).

Table 3: Antibacterial activity of *X. strumarium* L. extracts

Extract	Plant part	*E. coli*	*S. aureus*	*B. subtilis*
Distilled water	Leaves	16 ± 0.8	18 ± 0.9	17 ± 0.8
	Stems	13 ± 0.7	15 ± 0.8	14 ± 0.7
Ethanol	Leaves	21 ± 1.1	24 ± 1.2	22 ± 1.0
	Stems	18 ± 0.9	20 ± 1.0	19 ± 0.9
Chloroform	Leaves	14 ± 0.7	16 ± 0.8	15 ± 0.7
	Stems	11 ± 0.6	13 ± 0.7	12 ± 0.6
Petroleum ether	Leaves	10 ± 0.5	12 ± 0.6	11 ± 0.5
	Stems	8 ± 0.4	10 ± 0.5	9 ± 0.4

Leaf extracts showed significantly higher antimicrobial activity than stem extracts. Ethanolic extracts exhibited the strongest antibacterial and antifungal effects, correlating with

higher phytochemical content. Petroleum ether extracts showed minimal activity, suggesting that non-polar compounds play a limited role in antimicrobial efficacy. The results clearly demonstrate that ultrasonication-assisted ethanolic extracts of *Xanthium strumarium* L. leaves are rich in bioactive phytochemicals and exhibit significant antimicrobial activity, confirming the role of secondary metabolites as natural weapons against microbial pathogens.

Table 4: Antifungal activity against *Candida albicans*

Extract	Plant part	Zone of inhibition (mm)
Distilled water	Leaves	15 ± 0.7
	Stems	12 ± 0.6
Ethanol	Leaves	19 ± 0.9
	Stems	16 ± 0.8
Chloroform	Leaves	13 ± 0.6
	Stems	10 ± 0.5
Petroleum ether	Leaves	9 ± 0.4
	Stems	7 ± 0.3

Ultrasonication enhanced extraction efficiency by disrupting plant cell walls and releasing intracellular metabolites. Ethanolic extract exhibited the highest phytochemical content, explaining its superior antimicrobial activity. Flavonoids and phenolics are likely the primary contributors to antibacterial and antifungal effects. The study provides a scientific basis for traditional medicinal uses of *X. strumarium* L. UAE offers a green and efficient method for extracting bioactive compounds for pharmaceutical applications.

5. Conclusion:

The present study demonstrates that *Xanthium strumarium* L., a widely used medicinal plant, is a rich source of bioactive phytochemicals such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides. Using Ultrasonication-Assisted Extraction (UAE), we were able to efficiently extract these compounds from both leaves and stems, with ethanolic extracts showing the highest concentrations. *Xanthium strumarium* L., particularly its ethanolic leaf extract, is a promising natural antimicrobial agent. The study highlights the importance of selecting appropriate plant parts and extraction methods to maximize bioactive compound recovery. This plant has the

potential for therapeutic and pharmaceutical applications, offering a natural alternative in the fight against multidrug-resistant pathogens.

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