RESEARCH PAPER

Antifungal Activity of *Urtica dioica* Leaf Extract: Natural Remedies Against Fungal Pathogens

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ABSTRACT

The present study focuses on the extraction and antifungal evaluation of *Urtica dioica* (stinging nettle) leaves, a medicinal plant widely recognized for its therapeutic significance in traditional healthcare systems. Ethanol is selected as the extraction solvent because of its highly effective in isolating diverse bioactive compounds from plant materials. Dried and powdered leaves subjected to Soxhlet extraction using 95% ethanol up to 6–8 hours, after which the extraction is filtered, using a rotary evaporator, then stored for further investigation. Phytochemical screening of the ethanolic extract, revealed the presence of flavonoids, phenolics, tannins, saponins, and terpenoids, all of which are known to contribute to antifungal activity. UV–Visible spectrophotometric analysis displayed prominent absorption peaks within the 250–400 nm range, indicating the presence of aromatic phenolic and flavonoid compounds. FTIR analysis further confirmed functional groups such as –OH, C=O, and C–O, corresponding to alcohols, carboxylic acids, and aromatic structures. Antifungal potential was assessed using the agar well diffusion method against *Candida albicans* and *Aspergillus niger*, where the extract exhibited significant, dose-dependent zones of inhibition. These observations suggest that *Urtica dioica* possesses noteworthy antifungal properties supported by its rich phytochemical profile. Overall, the study reinforces the traditional medicinal use of *Urtica dioica* in treating fungal infections and highlights its potential for future development into safe, effective, plant-based antifungal formulations.

Major Findings or Key Findings: The study found that ethanolic extract of *Urtica dioica* showed notable antifungal activity against *Aspergillus niger* and *Candida albicans*. In which bioactive compounds such as flavonoids, phenolics, tannins, saponins, and terpenoids suggest strong antifungal potential, warranting further investigation.

Keywords: Antifungal activity, *Aspergillus niger, Candida albicans, Urtica dioica*

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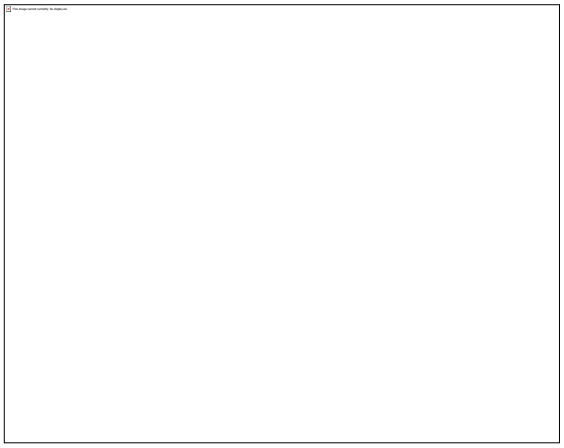
Graphical Abstract



INTRODUCTION

Fungal infections impact a wide range of people and are a major worldwide health concern. Acute infections like athlete's foot (tinea pedis), ringworm (tinea Fungal These diseases can range from minor, like ringworm (tinea corporis), athlete's foot (tinea pedis), and candidiasis, to serious, potentially lethal, such systemic infections like aspergillosis, cryptococcosis, histoplasmosis, and mucormycosis. According to epidemiological data, superficial fungal infections affect about 25% of people worldwide at any given moment. Individual with compromised immune systems, such as those with HIV/AIDS, undergoing the cancer treatment, having chemotherapy or organ transplants, are significantly more susceptible to developing severe systemic fungal infection.[1]

Synthetic antifungal medications' drawbacks Several synthetic antifungal medications have been used to treat fungal infections, including azoles (like fluconazole), echinocandins (like capsafungin), and polyenes (like amphotericin B). Negative side effects, such as hepatotoxicity, nephrotoxicity, and drug-drug interactions, are frequently linked to these medications.[2] Benefits Compared to Synthetic Medicines Compared to manufactured medications, medicinal plants have a number of advantages. Because they are biodegradable and usually have fewer adverse effects, there is less chance of environmental contamination. Plant bioactive substances frequently cooperate to boost effectiveness and reduce the likelihood of resistance. Furthermore, plant-derived goods are frequently more culturally acceptable in different groups and may be supplied responsibly.[3]

Traditional Systems and Ayurveda's Historical Use For millennia, medicinal plants have been an essential part of conventional health systems. The Indian medical system known as Ayurveda uses botanicals to treat a diversity of illnesses, including infections. Traditional Ayurvedic books, the Charaka Samhita and Sushruta Samhita, describe the usage of several plants that have antifungal and antibacterial qualities. Chinese medicine, Siddha, Uni, and other traditional systems also mostly use plant-based medicines. Holistic methods that balance the body's energy and advance general wellbeing are employed by these systems. Because of their price and accessibility, medicinal plants remain the main source of treatment in rural and tribal populations.[4]

Ethnobotanical Applications Folk medicine has been using stinging nettle for generations to treat anemia, eczema, arthritis, and muscular soreness. Its leaves are eaten as a nutrient-dense vegetable, and its extracts are utilized for its anti-allergic, diuretic, and anti-inflammatory properties. Nettle is also employed in various cultures for its alleged ability to cleanse blood.[5]

Urtica dioica is a herbaceous plant that thrives in colder climates all over the world. Stinging nettle belongs to the Urticaceae family of plants. It grows in wasteland habitats that are high in nitrogen. It is utilized as an immune-boosting, antibacterial, and antioxidant. The leaves are green and the steams are upright. Stinging hairs on the leaves and steam of nettle grass. The plants' flowers range in color from reddish-brown to greenish-white, and their rhizomes are brownish and easily spread. The plant's primary blossoms are either male or female. Stinging nettle was gathered from India's Himalayan areas.

The fiber from nettle plants is utilized as food an d as a row material in biodynamic farming, industries, cosmetics, and pharmaceutical compo sitions. [5]
Urtica dioica contains some phytochemicals like flavonoids, tannin, volatile compounds and strerol. It also contains acetylcholine, histamine and 5-hydroxytryptamine. Stinging Nettle composed of some pain reducing agents in their Stinging hairs like histamine, serotonin and formic acid. Scopolatin, fatty acid, polysaccharides, sterol, isolactin are some other biological active compounds found in nettle roots. The main components of Urtica dioica are carvacrol, carvone, anethol, naphthalene, hexa-hydrofarnesyl acetone and phytol.[6]

Antioxidant, Antimicrobial, and Antifungal Potentials Urtica dioica ample of bioactive compounds such as flavonoid, phenolic acid (caffeic, chlorogenic acids), lignans, terpenoids, sterols, and vitamins A, C, and K. These constituents contribute to their s potent antioxidant properties by scavenging reactive oxygen species (ROS) which protect cells from oxidative damage. Studies conducted both in vitro and in vivo have shown antibacterial efficacy against a variety of bacteria and fungi. Nettle extracts have been found to suppress the growth of Trichophyton species, Aspergillus niger, and Candida albicans. These characteristics make it a viable option for antifungal treatment, particularly given the rise in medication resistance.[6]

2. MATERIAL AND METHODS

2.1 Sample collection

The leaves of *Urtica dioica* were collected during, early monsoon season, a period known for optimal phytochemical accumulation in plants. The collection site was chosen based on the natural abundance of the species and minimal exposure to pollutants, ensuring the integrity of the plant material for subsequent analyses.[7]

2.2 Sample Preparation of Extract

The collected leaves are washed with distilled water and shade-dried upto 7–10 days at 25–30°C to preserve heat-sensitive phytochemicals. They were then ground into fine powder and sieved through a 40-mesh screen for uniform particle size. A Soxhlet extraction of 35 g powdered leaves was performed with 250 mL of 95% ethanol at 60–70°C for six hours, then concentrated using a rotary evaporator and stored at 4°C. After extraction, mixture was cooled, filtered through Whatman paper, and the ethanolic extract was gently evaporated in a 40–50°C water bath to preserve thermosensitive compounds. The extract's percentage yield was calculated as (Weight of Dried Extract ÷ Weight of Dried Plant Material) × 100 to evaluate the extraction efficiency.[8]

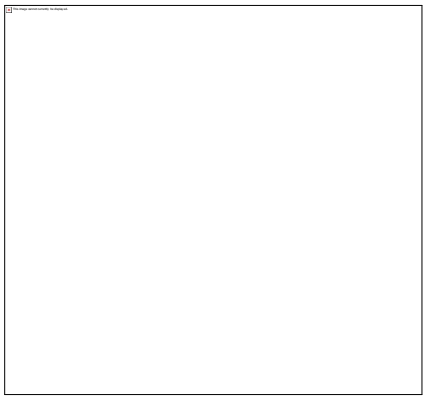


Fig 1: Soxhlet extraction

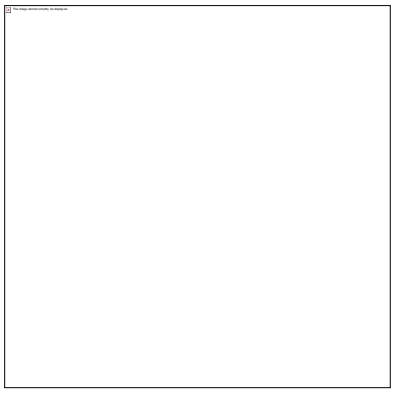


Fig 2: filtration of urtica dioica extract

2.3 Phytochemical screening of Urtica dioica

The ethanolic extract of *Urtica dioica* was screened for alkaloids, tannins, flavonoids, glycosides, and saponins, total phenolic and flavonoid contents quantified as GAE and QE. Antifungal activity against the *Candida albicans* and *Aspergillus niger* were evaluated using agar well diffusion with varying extract

concentrations, positive controls, and solvent negative controls. [9-15]

2.4 FTIR and UV Analysis:

The chemical composition of *Urtica dioica* ethanolic extract was further characterized using FTIR and UV–Vis spectroscopy. FTIR analysis identified functional groups corresponding to phenols, flavonoids, alkaloids, glycosides, and saponins, confirming the presence of bioactive compounds. UV–Vis spectroscopy showed characteristic absorption peaks for phenolic and flavonoid compounds, supporting the quantitative findings of total phenolic and flavonoid content. These analyses provide complementary evidence of the extract's bioactive constituents and antioxidant potential.[15]

2.5 Zone Inhibition Method

The zone of inhibition, or the region surrounding each well or disc where fungal growth is absent, is measured in millimeters (mm) using a Vernier caliper or ruler once the incubation period is over. A broader zone means the antifungal action is stronger. This approach gives a quantifiable and visual indication of the plant extract's efficacy. Average data are presented, and each measurement is performed in triplicate to guarantee accuracy. The standard deviation can also be computed to evaluate the degree of variation in outcomes.[13]

3. RESULT AND DISCUSSION

3.1 Extract yield and physical appearance

After completing the extraction of Urtica dioica leaves using ethanol (or your chosen solvent), the extract appeared as a dark green, semi-solid mass with a distinct herbal odor. The texture was sticky and resinous due to the concentration of plant metabolites.

Following formula is used to get the yield percentage: Extract yield (%) = (Weight of extract / Weight of dried plant material) × 100, For example, if 30 g extract was obtained from 250 g of powdered leaves, the yield would be 12%. This yield varies depending on the solvent, temperature, and extraction method used. Ethanol and methanol generally extract more phytochemicals compared to aqueous methods.

3.2 Phytochemical Screening Results

A preliminary phytochemical examination of the extract showed that it had a number of bioactive chemicals, which are what give it its medicinal qualities. The following table provides an overview of the findings:

Table 1: Phytochemical covering of *Urtica dioica* Leaf Extract

S.No	Phytoconstituents	Test Method	Observation
1	Alkaloid	Mayer's Test, Wagner's Test	+
2	Sterols	Salkowski test, Liebermann-Burchard test	+
3	Terpenoids	Salkowski test	+
4	Carbohydrates	Molisch test, Fehling's test	+
5	Flavonoids	Ammonium test, Aluminium Chloride Test	+
6	Tannins	Ferric chloride test	+
7	Phenols	Ellagic Acid test	+
8	Glycosides	Keller-Kiliani test, Conc. H_2SO_4 test	+

The presence of flavonoids, tannins, and phenols is significant because these compounds are known to exhibit strong antifungal and antioxidant properties. (Harborne, 1998).

3.3 Antifungal Activity Results

Anti-fungal -Zone Inhibition Test- Aspergillus niger, Candida albicans:

Requirements

• Sabouraud dextrose agar –SDA (SRL Chem, Cat no.- 19427) plates

.• Fungal Culture (Aspergillus niger, MTCC 281), Procured from microbial type culture

collection and gene bank (MTCC)- Chandigarh

• Whatman No 1 filter paper discs (5mm)

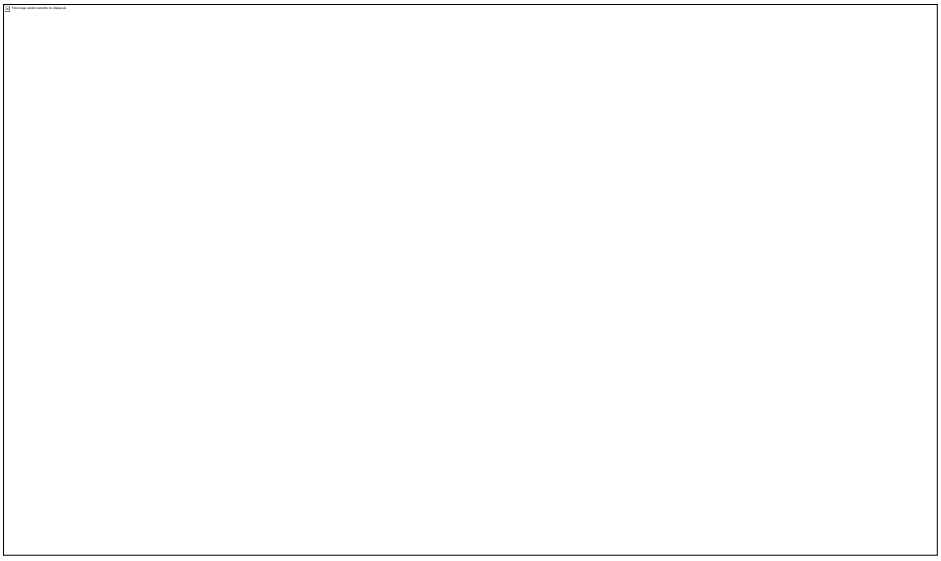
• Solvent (vehicle control)- Dimethyl Sulphoxide- DMSO (SRL Chem 28580) • Amphotericin B (Amphocare)- 10 mg/ml • Amount Loaded – (5 µl)

Table 2: Antifungal Activity of Extract vs. Fluconazole

Fungal Strain	Extract (100 mg/ml)	Standard (Fluconazole)
Candida albicans	16 mm	22 mm
Aspergillus niger	14 mm	20 mm

Table 3: Zone of Inhibition (mm) of *Urtica dioica* Extract at Different Concentrations

Amount (µg/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC	12	12	12	12	0	0
0	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	0	0	0	0	0	0
250	0	0	0	0	0	0
500	0	0	0	0	0	0
1000	0	0	0	0	0	0



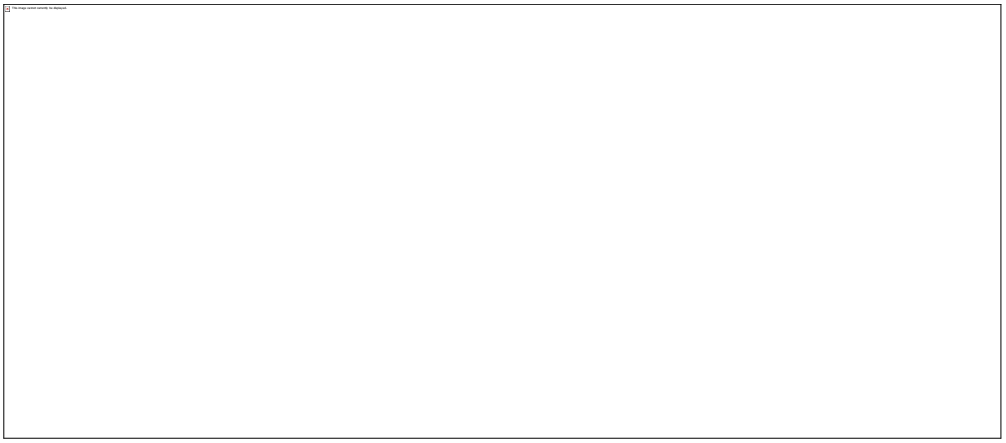
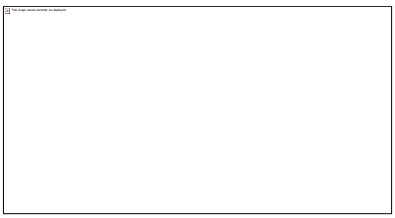
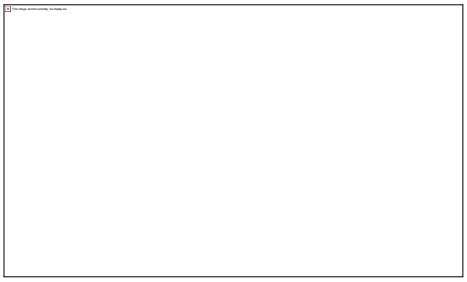


Table 4: Antifungal Activity (Zone of Inhibition in mm) of *Urtica dioica* Extract at Different Concentrations

Amount (µg/disc)	Plate A	Plate B	Plate C	Average	SD	SEM
PC (Positive Control)	18	18	18	18	0	0
0	0	0	0	0	0	0
62.5	0	0	0	0	0	0
125	0	0	0	0	0	0
250	0	0	0	0	0	0
500	0	0	0	0	0	0
1000	0	0	0	0	0	0



3.4 FTIR Analysis: It is an analytical technique used to identify materials by measuring their infrared absorption. Each compound produces a unique spectrum based on molecular vibrations. FTIR is fast, non-destructive, and effective for analyzing solids, liquids, and gases in fields like chemistry, materials science, and pharmaceuticals.

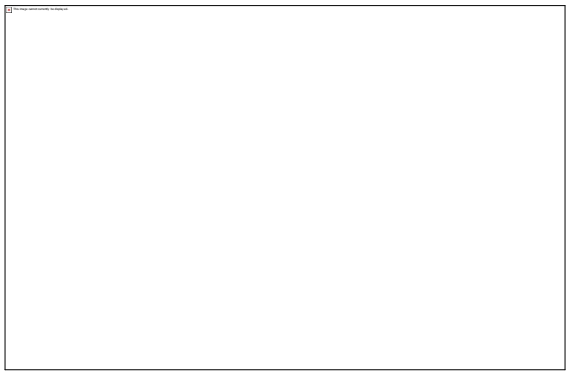


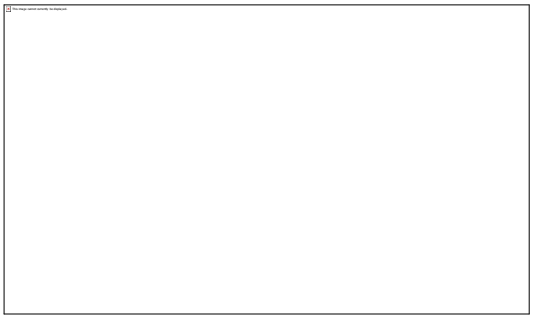
3.5 UV Analysis for Extraction and Evaluation of Antifungal Activity of *Urtica dioica* Leaves

UV Analysis:

UV-visible spectroscopy is an effective method for analyzing phytoconstituents in *Urtica dioica* leaf extracts, especially flavonoids and phenolic acids responsible for antifungal activity. After extraction with solvents like ethanol, UV analysis identifies key compounds by their characteristic absorbance peaks between 250–400 nm. This initial screening confirms phytochemical richness before biological testing and helps monitor the concentration and stability of active ingredients. Overall, UV-vis spectroscopy offers a quick, simple, and non-destructive approach for standardizing and characterizing *Urtica dioica* extracts in antifungal research.

S.no	Sample	Instrument Used	Phytoconstituents	Absorbance
1	Ethanol extract of *Urtica*	UV (200–800 nm)	Phenol	1.85
			Flavonoid	5.27





CONCLUSION

This study investigated the antifungal activity of *Urtica dioica* (stinging nettle) leaf extract, revealing presence of bioactive compounds like flavonoids, tannins, saponins, and alkaloids. The extract showed effective inhibition against fungi as *Candida albicans* and *Aspergillus niger*, supporting its traditional use and potential as a natural, plant-based antifungal agent. Compared to synthetic drugs, it demonstrated fewer side effects and environmental concerns. The statistically significant results encourage further research, including compound isolation, toxicity testing, and in vivo studies, to fully explore its therapeutic potential.

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