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Antimicrobial, Antioxidant, Cytotoxic Activity And Phytochemical Screening Of Secondary Metabolites From Urtica Dioica

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Abstract

Urtica dioica, also called as stinging nettle which has been traditionally used for medicinal properties. The aim of this study to evaluate the antimicrobial, antioxidant, cytotoxic activities, and phytochemical screening of secondary metabolites that extracted from the plant (Urtica Dioica). Plant leaves were collected, identified, and processed to obtain methanolic extracts. The extraction process was followed by comprehensive phytochemical screening and experimental assays to evaluate the biological properties of the extracts. Phytochemical analysis confirmed the presence of key secondary metabolites, including polyphenols, alkaloids, tannins, triterpenes, and saponins. Gas Chromatography-Mass Spectrometry analysis further identified major bioactive compounds such as quercetin (3.43%), kaempferol (0.03%), caffeic acid (0.03%) beta- sitosterol (0.05%), and ferulic acid (0.5%). The antibacterial activity, assessed via the agar well diffusion method, demonstrated notable inhibitory effects against bacterial strains, with the highest activity observed against P. mirabilis (26.5 mm) followed by E. coli (26 mm), P. aeruginosa (24.5 mm), S. mutans (21 mm), and

B. subtilis (18.5 mm) at a concentration of 100 mg/ml. Antifungal activity revealed inhibition zones of 16.2 mm and 14.5 mm for Candida albicans and Aspergillus niger, respectively. Cytotoxic activity was evaluated using the brine shrimp lethality assay, where lethality rates were concentration- dependent, with the highest activity recorded at 1000 µg/ml (20.67%). The DPPH radical scavenging assay was used to measure antioxidant activity, demonstrating the methanolic extract's capacity to efficiently neutralize free radicals. The findings highlight Urtica dioica's potential as a rich source of bioactive compounds with significant therapeutic applications, particularly for antimicrobial, antioxidant, and cytotoxic purposes. These results emphasize the plant's utility in pharmaceutical and nutraceutical industries.

Keywords: Urtica dioica, stinging nettle, DPPH, GC-MS, Antioxidant, Methanolic extracts.

INTRODUCTION

People utilize it for a variety of things, including food, medicine, and spices. Plants have played a significant role in people's daily lives because of their nutritious qualities. They satisfy people's demands by containing vital nutritional components like vitamins and minerals. Many plant species are also highly significant from a medical perspective in addition to these characteristics. (Sevindik et al., 2023). Stinging nettle, or Urtica dioica L., is an herbaceous perennial flowering plant that is native to Eurasia, a member of the genus Urtica and family Urticaceae, and is used interchangeably in medicine. Nettles are an easy-to-digest food that is rich in pro-vitamin A, vitamin C, and minerals, particularly iron. It is thought to have an impact on the metabolism of fats and proteins and enhance their functionality (Dhouibi et al., 2020). Stinging nettle or UD (Urtica dioica) produces skin irritation which causes red bumps and welts and stinging pain when it makes contact with humans. The liquid substance within its hair follicles duplicates similar to a hypodermic needle to generate pricking effects which cause different types of skin irritation. Intelligence indicates that the original usage of "nettle" emerged from Anglo-Saxon derivative of "needle." Two components working together create the stinging nettle dermatitis reaction (Cummings and Olsen, 2011).

The chemical substances responsible for the dermatitis reaction are multiple including formic acid, tartaric acid as well as alkaloids, enzymes, histamine, acetylcholine, 5- hydroxytryptamine, salts and proteins (Bhusa et al., 2022). Despite its irritating

properties Urtica dioica establishes strong successful populations across various habitats primarily growing in nutrient-rich soil before forming clustered vegetative patches. The scientific term Urtica dioica combines Urtica from the Latin word urere meaning "to burn" with dioica from Greek dis (two) and oikia (house) to describe plants having separate sexes. When looking at Urtica dioica growth one can identify its dioecious characteristics because male and female flowers appear on individual plants. (Grauso et al., 2020).

Urtica dioica is not only valued for its medicinal properties but also as a nutritious food source. Every component of the Urtica dioica plant serves as edible food though youth foliage and leaves function mostly in soups and teas and smoothies. Urtica dioica leaves contain a rich blend of vitamins A C and K with added magnesium calcium and iron to make a valuable dietary supplement. The nutritional value of its leaves gets further improvement because of its high protein content. Heat treatment removes the burning protective hairs that make Urtica dioica edible for human consumption. The Urtica dioica plant seeds can be enjoyed as seasonings or baked goods additions because of their nutty flavor (Idris et al., 2021).

Bioactive substances from Urtica dioica fight inflammation by blocking pro-inflammatory cytokines and enzymes which reduces pain and swelling experienced by patients with arthritis and gout. The plant's quality as a diuretic helps remove toxic substances from the body while it provides treatment for all conditions related to urinary tract infections and hypertension as well as kidney stones. Urtica dioica has antihistamine properties which turn it into a wonderful natural allergy remedy that combats hay fever symptoms and various allergy reactions. Research indicates that plant extracts exhibit promising potential to control blood sugar by enhancing insulin sensitivity while reducing glucose in patients with diabetes. Urtica dioica presence in herbal skincare products helps promote hair growth while fighting off dandruff symptoms and treating eczema and acne (Taheri et al., 2022).

Almost fifty chemicals in Urtica active fraction have known chemical structures that exist both in hydrophilic and lipophilic states. Research on worldwide phytochemical composition of Urtica species reveals only minor substances including sterols together with triterpenes and coumarins along with phenols and lignans and

ceramides and fatty acids. Nettle diverts these substances unevenly throughout its varied plant organs (Majedi, et al., 2021).

The multiple bioactive constituents in Urtica species demonstrate preventive and therapeutic functions in communicable diseases and non-communicable diseases. Beta-sitosterol, transferulic acid, dotriacontane, erucic acid, ursolic acid, scopoletin, rutin, quercetin, and phy-droxylbenzalcohol are a few noteworthy substances. The stinging effect of nettle hairs is attributed to their liquid composition, which includes formic acid, leukotrienes, 1% acetylcholine, small amounts of histamine (1 in 500 to 1 in 2000), and serotonin (5- hydroxytryptamine) (Marotti et al., 2022). The aerial portions of the plant contain essential ketones at 38.5% followed by essential esters at 14.7% together with free alcohols at 2% and additionally include nitrogenous compounds, phenols, aldehydes, p-sitosterol, formic acid, acetic acid, chlorophyll, phytol, vitamins and carotenoids. The chromatographic examination of aerial parts revealed several organic acids such as caffeic, ferulic, caffeoylmalic, chlorogenic and sinapic acids. Scientific studies have revealed that the flowers contain five distinct flavonoids including isorhamnetol 3-O-glucoside and quercetol 3-O-glucoside as well as kaempferol 3-O-glucoside and isorhamnetol 3-O-rutinoside and quercetol 3-O-rutinoside. Additionally, p- sitosterol p-sitoster (Uğur et al., 2023).

The roots have complex chemical groups which include polysaccharides like glycans, glucogalacturonans, and arabino galactan acid together with fatty acids like hydroxyl octadecadienoic acid and lectins, ceramides and terpene diols and glucosides. The essential oil composition research on Urtica dioica and Urtica pilulifera has recognized significant compounds such as hexahydrofarnesyl acetone, 1,8-cineole, α-ionone, β-ionone, farnesyl acetone, methylbenzene, (−)-limonene, 3-carene, (+)-limonene, gamma-terpinene, vanillin, butyl acetate, 1,2-benzenedicarboxylic acid, and 7-acetyl-6-ethyl-1,1,4,4-tet Researchers must conduct additional studies to determine phytochemical characteristics of these plants because of their substantial diversity potential (El Haouari et al., 2019). The interaction of plant metabolites and biological activity is a function of geography and environment in conjunction with genetic differences within this system. Most plants of wild origin constituting the same species have probably exhibited variations between their metabolic fingerprint and biological

activity when compared to Urtica dioica occurring across various places. The dissimilarities between samples arise due to climate factors in addition to soil structure and environmental factors and genetic differences among these plant organisms. The varying properties of plants as medicine vary due to these factors influencing their medicinal properties which include antibacterial and antioxidant activities as well as anticancer activity (Batool et al., 2017).

Among many plants investigated for antibacterial activity, Urtica dioica has been a promising candidate. Phytochemicals that can be used to fight bacterial infections. Methanol- and ethanol-based Urtica dioica extracts have been shown to inhibit the growth of many pathogenic bacteria in studies. These include Staphylococcus aureus, Escherichia coli, Pasteurella multocida and other Gram positive and Gram negative bacteria. However, these suggest that Urtica dioica has antibacterial agents that can be used to explore novel antibacterial medications against bacterial diseases. This point advises further studies to identify the exact components triggering the antibacterial activity of Urtica dioica and its potential therapeutic usages (Khan et al., 2023).

One of the main antibacterial activities of Urtica dioica is the blocking of the external bacterial cell membrane. A bacterial cell membrane is a protective boundary which has control mechanisms for permitting essential organic nutrition substances valued by the cell as well as shielding the bacterium from other harms. Most of the bioactive compounds of Urtica dioica contain flavonoids and phenolics which decrease the bilayer's lipid permeability barrier. Thus this leads to the destruction of bacterial cells, in which critical constituents of the cell such as nucleotides and proteins, are expelled from the cell and cell death occurs. Although Gram- negative bacteria may possess an extra layer of defense due to their outer membrane, this method is very effective on both Gram-positive and Gram-negative bacteria. (Elkelish et al., 2024). Inhibiting the formation of bacterial cell walls is another significant antibacterial strategy. A few of Urtica dioica's phytochemicals prevent peptidoglycan, a crucial structural element of bacterial cell walls, from being biosynthesised. Through the suppression of peptidoglycan cross-linking enzymes, these chemicals weaken the bacterial cell wall, increasing its vulnerability to osmotic pressure. Bacterial cells eventually become brittle

and ultimately burst, which inhibits them from surviving and developing (Mohammed et al., 2022).

Moreover, Urtica dioica performs a critical role in significant metabolic pathways by inhibiting bacterial enzymes. The specific bioactive compounds in the plant influence the bacterial enzymes that are responsible for nucleic acid synthesis, protein synthesis, and cellular respiration. By inhibiting these enzymes, Urtica dioica increases so-called pathogen infection potential decreases; therefore, pathogens cannot develop and multiply. One of the extensively researched plants for possessing properties to fight against bacterial infections is stinging nettle- Urtica dioica. It acts through various pathways, thus forming a copartner in the battle against resistant strains of bacteria growth. Its major constituents include bioactive components such as phenolics, flavonoids, and tannins which are related to destroy bacteria membranes and inhibit vital processes for their survival and growth. The effects of compounds from Urtica dioica on multiple bacterial activities at once pose a resistance risk avoided at present as a grave concern within modern medicine (Gulhan et al., 2022).

Methodology

Study Overview and Design

This experimental research was conducted at the Sarhad University of Science and Information Technology (SUIT), Peshawar, specifically within the Microbiology Laboratory of the Sarhad Institute of Allied Health Sciences. The study, which spanned a 12-month period from February to December 2024, employed an experimental design to manipulate Urtica dioica (Stinging Nettle) plant material for the extraction of secondary metabolites, phytochemical profiling, and the assessment of antioxidant and biological activities.

Sample Collection and Preparation

The leaves of Urtica dioica were harvested from various agricultural regions in Peshawar. The botanical identity of the samples was officially verified by the Department of Botany. Following collection, the plant parts (leaves, stems, and roots) were washed with distilled water to eliminate impurities and subsequently shade-dried at room temperature to prevent the thermal degradation of bioactive compounds. The dried material was then

pulverized into a fine powder using a mortar and pestle and stored in airtight containers at 4°C.

Extraction and Phytochemical Screening

For the extraction process, the powdered plant material was macerated in 80% methanol at a 1:10 (w/v) ratio for 24 to 72 hours with periodic agitation. The mixture was filtered through Whatman filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure at temperatures below 50°C. The resulting crude extract was stored at 4°C. Qualitative phytochemical analysis was performed to detect various metabolite groups. Polyphenols were identified via a green color reaction with ferric chloride ($FeCl_3$), while alkaloids were determined through the cyanidin reaction, where a color shift from orange to purple-red indicated the presence of flavonoids/alkaloids. Tannins were differentiated by $FeCl_3$ into gallic (blue-black) or condensed (greenish-blue) types. Triterpenes were identified by a golden-yellow layer in a chloroform-sulfuric acid test, and saponins were confirmed by the formation of a persistent 1 cm foam layer after vigorous agitation in distilled water.

Biological and Antimicrobial Assays

The antibacterial activity was evaluated using the agar well diffusion method against Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans, and Bacillus subtilis. Wells (6 mm) were inoculated with 40 µL of the extract (100 mg/mL), with ampicillin and sterile water serving as controls. Zones of inhibition were measured after a 24-hour incubation at 37°C. Antifungal activity was tested against Aspergillus niger and Candida albicans using the agar incorporation method on Potato Dextrose Agar (PDA), with results observed after 72 hours of incubation at 28°C.

GC-MS Analysis and Cytotoxicity

The chemical constituents of the methanolic extract were characterized using Gas Chromatography-Mass Spectrometry (GC-MS) on an Agilent 7890 instrument. A 1 µl sample was scanned for 45 minutes, and compounds were identified by comparing their mass-to-charge ratios and spectra against standard library peaks. Cytotoxicity was assessed via the Brine Shrimp Lethality Assay (BSLA). Brine shrimp (Artemia salina) nauplii were hatched in an aerated seawater solution (27 g/L sea salt). Ten nauplii were exposed to varying concentrations of the extract (10, 100, and 1000 µg/mL) in triplicate.

After 24 hours, the mortality rate was recorded, and the LC_{50} value was calculated using the probit method.

Antioxidant Activity

The antioxidant potential was quantified using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A 1 mL sample was mixed with 2 mL of DPPH solution and kept in the dark for 30 minutes. The absorbance was measured at 517 nm using a spectrophotometer, with ascorbic acid serving as the re

DPPH Scavenging Activity (%) = Absorbance of Control −Absorbance of Sample×100
Absorbance of Control

CHAPTER 4

RESULTS

4.1.Phytochemical Screening

Phytochemical analysis of the Urtica dioica methanolic extract confirmed the presence of secondary metabolites, including polyphenols, alkaloids, tannins, triterpenes, and saponins.

Phytochemical Compound	Test Performed	Observation	Result
Polyphenols	Reaction with Ferric Chloride ($FeCl_3$)	Greenish coloration	+
Alkaloids	Cyanidin Reaction	Purple-red coloration	+
Tannins	Reaction with 1% Ferri c Chloride ($FeCl_3$)	Blue-black (Gallic) / Greenish-blue (Condensed)	+
Triterpenes	Sulfuric Acid Treatment	Golden-yellow layer	+
Saponins	Foam Formation Test	Persistent foam (1 cm layer)	+

Table 4.1: Phytochemical analysis of the Urtica dioica.

4.2. Antibacterial Activity

To determine the antibacterial properties of the crude methanolic extracts of Urtica dioica antibacterial activity was tested against five selected bacterial strains using the agar well diffusion method. The methanolic extracts exhibited potent activities against all of the selected bacterial strains, as detailed in Table 1. The highest mean antibacterial activity of the methanolic extract was observed against P. mirabilis (26.5 mm), followed by E. coli (26 mm), P. aeruginosa (24.5 mm), S. mutans (21 mm), and B. subtilis (18.5 mm) at a concentration of 100 mg/ml.

Bacterial Strain	**Mean Antibacterial Activity (mm)**
P. mirabilis	26.5
E. coli	26
P. aeruginosa	24.5
S. mutans	21
B. subtilis	18.5

Table 4.2: Antibacterial activityof Urtica dioica extracts against bacterial strains.

4.3. Antifungal Activity

The methanolic extract of Urtica dioica showed notable antifungal activity, with inhibition zones of 14.5 mm against Aspergillus niger and 16.2 mm against Candida albicans.

Phytochemical screening by GC-MS Gas chromatography-mass spectrum. The GC-MS screening of Urtica dioica revealed the presence of several phytochemicals. Quercetin was identified as the most abundant compound with a retention time of 1.98 minutes and a % area of 3.43. Kaempferol and caffeic acid were detected at 8.6 minutes with % areas of 0.03 each. Beta-sitosterol was observed at 12.53 minutes with a % area of 0.05, and ferulic acid was detected at 27.71 minutes with a % area of 0.5. The retention times, molecular formulas, molecular weights, and percentage areas were derived from the GC-MS data, providing detailed insights into the chemical composition and presence of these compounds given in table 4.3.

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S. No.	Retention time (min)	Compound	Molecular Formula	Chemical Formula	Molecular Weight (g/mol)	% Area
1	1.98	Quercetin	3,3',4',5,7-Pentahydroxyflavone	$C_{15}H_{10}O$	302.24	3.43
2	8.6	Kaempferol	3,4',5,7-Tetrahydroxyflavone	$C_{15}H_{10}O_6$	286.24	0.03
3	8.6	Caffeic acid	2-Propenoic acid, 3-(3,4-dihydroxyphenyl	$C_9H_8O_4$	180.16	0.03
4	12.53	Beta-sitosterol	24-Ethylcholest-5-en-3β-ol	$C_{29}H_{50}O$	414.71	0.05
5	27.71	Ferulic acid	4-Hydroxy-3-methoxycinnamic acid	$C_{10}H_{10}O_4$	194.19	0.5

Table 4.3: Phytochemical screening by GC-MS Gas chromatography-mass spectrum.

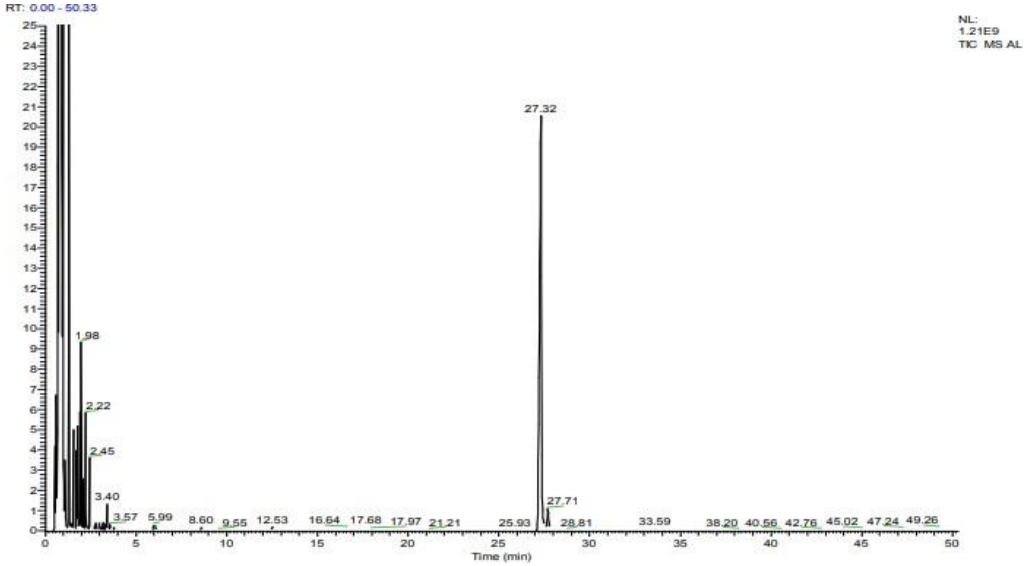

Fig 4.2: Phytochemicals screening by GC-MS of Urtica dioica.

4.5. Cytotoxic Activity

Cytotoxic activity reveals lethality rates increased slightly with higher concentrations: at 30 µg/ml, the lethality was 16.67%, at 100 µg/ml it was 18.67%, and at 1000 µg/ml, it was 20.67%. This indicates that the test substance has a dose-dependent effect on the shrimps, with higher concentrations leading to slightly more mortality.

Dosage (µg/ml)	No. of Shrimps	No. of Survivors	Percent Lethality	Negative Control (Sea Water)	Percent Lethality of Control	Positive Control (7.5 µg/ml Etoposide)	Percent Lethality of Positive Control
10	30	25	16.67%	30	0.00%	Etoposide	70%
100	30	25	18.67%	30	0.00%	Etoposide	70%
1000	30	25	20.67%	30	0.00%	Etoposide	70%

Table 4.4: Brine shrimp lethality assay of extracts.

Antioxidant Activity by DPPH Assay

The antioxidant activity of Urtica dioica extracts was evaluated using the DPPH free radical scavenging assay. Both water and ethanol extracts exhibited significant antioxidant potential. The total antioxidant values were determined to be 4.77 mg/g for the water extract and 5.26 mg/g for the ethanol extract, indicating a higher antioxidant activity in the ethanol extract. The IC50 values, which represent the concentration required to inhibit 50% of DPPH radicals, were calculated as 0.64 mg/mL for the water extract and 0.86 mg/mL for the ethanol extract.

DISCUSSION

Urtica dioica, which are attributed to the presence of bioactive secondary metabolites such as polyphenols, flavonoids, tannins, and alkaloids. The plant's antioxidant properties help combat oxidative stress, which is a major contributor to chronic diseases, while its antimicrobial effects make it a potential candidate for addressing infections caused by pathogenic microorganisms. Additionally, its mild cytotoxic activity highlights its potential for therapeutic applications in cancer treatment with minimal toxicity risks.

Urtica dioica methanolic extract's antibacterial activity, which shown strong inhibition against P. mirabilis (26.5 mm) and other bacterial strains, is consistent with some earlier

research but also shows variations. Binsalah, et al., (2022) reported similar strong antibacterial effects against E. coli and P. aeruginosa but noted slightly lower activity against P. mirabilis and S. mutans. In contrast, Hashem et al., (2022) observed stronger antibacterial activity against Gram-positive bacteria, particularly B. subtilis and S. mutans, but lower efficacy against Gram- negative bacteria, which differs from the present study's findings where Gram-negative bacteria showed higher susceptibility. Ghasemi et al., (2024) also found antibacterial activity against E. coli and P. aeruginosa, but the inhibition zones were less significant than those observed in this study. Therefore, depending on the extraction techniques, the bacterial strains used, and the experimental setup, the antibacterial effects may vary. These variations show how many factors affect the strength of exhibited antibacterial capabilities, such as the particular bacterial strains employed for testing and the extraction method for the plant extract.

This study resulted that the antifungal activity of methanolic extract from Urtica dioica the inhibition zones of 16.2 mm against Candida albicans and 14.5 mm against Aspergillus niger. In another study found that Urtica dioica has an effective antifungal properties against Aspergillus flavus and Fusarium oxysporum and as described by Alfurjany et al., 2024 there was substantial inhibition against both of these two species but relatively smaller inhibition zones than those resulted in this study, against A. niger and C. albicans. the anibacfungal activity of Urtica dioica against Candida albicans and Penicillium sp. The results showed that Penicillium sp. has lower antifungal activity as compared to Candida albicans and this susceptibility of different fungal species to the plant extracts depend on cell wall composition in fungus cell. Therefore it is suggesting that differences in efficacy among various species. Moreover, Rolta et al., (2020) explored that Urtica dioica against a variety of fungal species, including Rhizopus stolonifer, Aspergillus niger, and Candida albicans and Urtica dioica has the ability to inhibit their growth. In contrast to the findings of the present work for A. Niger, the work conducted on Rhizopus stolonifer was found to have no significant activity. It may be due of the intrinsic resistance of the fungal strain or differentiation in extract concentration.

The most prevalent photochemical reveled in this study was Quercetin that determined by GC-MS analysis of Urtica dioica extract with a percentage area of 3.43%

and retention time of 1.98 minutes, resulted a potent anti-inflammatory and antioxidant properties, quercetin has been established as a potent bioactive compound in various medicinal plants in many studies, which is similar with our determination. Both kaempferol and caffeic acid were found at 8.6 minutes with 0.03% area each, are known phytochemicals in Urtica dioica, well reported for their antibacterial and antioxidant activities. Being a well-known plant sterol beta-sitosterol that is associated with various health benefits including cholesterol reduction capability, the appearance at 12.53 minutes with % area 0.05 holds significance. Ferulic acid, another phenolic molecule having antioxidant and anti-inflammatory properties, was detected in 27.71 minutes at a percentage area of 0.5. These findings are consistent with previous research demonstrating that Urtica dioica contains these compounds. For instance, in their GC-MS investigation of Urtica dioica, Grauso et al., (2020) detected quercetin, kaempferol, and ferulic acid. These compounds exhibited the same retention lengths, but their relative abundances varied. Contrarily, Naskar et al., (2024) discovered quercetin to be a significant component; however, their research concentrated on higher concentrations of beta-sitosterol and caffeic acid than what is shown here, which may be due to variations in the source plant, extraction methods, or analytical conditions.

A dose-dependent effect on shrimp mortality was indicated by the cytotoxic activity of Urtica dioica methanolic extract, which showed a small rise in lethality rates at higher doses. There was a 16.67% lethality at 30 µg/ml, 18.67% at 100 µg/ml, and 20.67% at 1000 µg/ml.

Urtica dioica may have minor cytotoxic effect, as seen by the comparably low fatality rates despite the extract's some cytotoxic potential. A study by Tona et al., (2020) indicated that when doses in both regimens were lowered, the percentage of mortality dramatically fell ($P < 0.05$). Vincristine sulfate (VCS) and Urtica dioica methanolic extract (MECM) obtained respective LC50 values of 1.63 µg/ml and 550.57 µg/ml. There is another study by Maharjan et al., (2023) using the brine shrimp fatality assay, resulted that the cytotoxic activity of Urtica dioica extracts and showed a more marked dose-dependent cytotoxic impact, with death rates above 50% at higher concentrations (1000 µg/ml). This variance could be driven by various assay methodologies, geographical changes in plant composition, or variations in extraction techniques.

The antioxidant activity of Urtica dioica extracts was investigated using the DPPH free radical scavenging assay, indicating considerable antioxidant potential for both water and ethanol extracts. The total antioxidant values were 4.77 mg/g for the water extract and 5.26 mg/g for the ethanol extract, showing that the ethanol extract displayed stronger antioxidant activity. The IC50 values, representing the concentration necessary to block 50% of DPPH radicals, were estimated as 0.64 mg/mL for the water extract and 0.86 mg/mL for the ethanol extract, with the water extract demonstrating a somewhat better radical scavenging capacity. In contrast Jaiswal et al., (2022) Urtica dioica methanolic extract (MEUD) revealed considerable free radical scavenging activity. The percent DPPH scavenging effect at a concentration of 100 µg/mL was 57.34% for the extract and 69.05% for the reference component, BHA. In a study by Sharma et al., (2021), the antioxidant activity of Urtica dioica was tested using the DPPH radical scavenging assay, revealing results but different from the current study. The IC50 value of the extract was determined to be 72.5 µg/mL, much lower than the IC50 of 105.16 µg/mL observed in the present investigation. Furthermore, at a dose of 100 µg/mL, the percent scavenging effect was measured as 75.12%, greater than the 57.34% obtained in this investigation. These variances may be attributable to variations in extraction procedures, solvent efficiency, plant harvesting conditions, or geographical location, which can alter the content and activity of antioxidant chemicals in Urtica dioica.

CONCLUSION

The study concluded that Urtica dioica is the most valuable medical properties through antimicrobial activity along with the antioxidant and cytotoxic properties screening alongside detailed phytochemical investigations. Testing showed that the methanolic extract possessed strong capabilities against different pathogenic microorganisms in addition to showing both antibacterial and antifungal properties. The DPPH assay confirmed that the plant substances successfully scavenge free radicals which reduces oxidative stress. The extract exhibits low- level cytotoxic behavior which opens doors for developing therapeutic methods while reducing the risk of adverse reactions. The medicinal value of Urtica dioica is strengthened by its secondary metabolites quercetin and kaempferol and beta-sitosterol which enable a scientific understanding of its traditional herbal uses.

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