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Research Article

Phytochemical Profiling and Antifungal Activity of Ethanolic Root Extract of Water Hyacinth (*Eichhornia crassipes*) against Selected Fungal Pathogens

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ABSTRACT

The present study aimed to investigate the phytochemical composition and antifungal potential of ethanolic root extracts from water hyacinth (*Eichhornia crassipes*), along with spectroscopic and chromatographic profiling to identify bioactive constituents. The extract exhibited a moderately acidic pH of 4.5. Qualitative phytochemical screening confirmed the presence of secondary metabolites including tannins, lysine, flavonoids, saponins, alkaloids, and terpenes, highlighting the efficiency of ethanol as a solvent for extracting semi-polar compounds. Antifungal activity was assessed via the well diffusion method against several phytopathogenic fungi. Notably, 30% and 40% ethanol extracts significantly inhibited the growth of *Curvularia* sp., *Fusarium errundifyme*, and *F. moniliforme*, showing superior performance over standard antibiotics such as amoxicillin and gentamicin. However, minimal inhibitory effects were observed against *F. proliferatum*, *F. oxysporum*, and *Sclerotium rolfsii*. GC-MS analysis identified approximately 21 organic compounds, primarily long-chain aldehydes and complex ethers, which are commonly associated with plant-based bioactivity. UV-visible spectrophotometry revealed three major absorption peaks (232.5 nm, 287.5 nm, and 322.0 nm), indicative of π→π* transitions and extended conjugated systems typical of aromatic compounds with antioxidant potential. FTIR spectral analysis further confirmed the presence of carbonyl (C=O), hydroxyl (C–H and COH), and carboxylate (COO^-) functional groups, all of which are characteristic of bioactive plant constituents. Collectively, these findings suggest that water hyacinth roots are a promising source of phytochemicals with significant antifungal and potential medicinal properties, offering a basis for further development of natural antifungal agents.

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Introduction

Medicinal plants have served as foundational pillars for human health, supporting the prevention and treatment of diseases for millennia. According to the World Health Organization, more than 80% of the global population, particularly in developing countries, relies on traditional

plant-based medicines (Abdulhadi et al., 2025). In recent decades, plant extracts have gained increasing scientific interest due to their bioactive compounds, which exhibit diverse pharmacological properties, including antibacterial, antioxidant, anti-inflammatory, and antitumor activities (Netai et al., 2016; Aman and Dipak, 2020; Al-Ibady et al., 2025).

Among the aquatic plants attracting scientific attention is the water hyacinth (*Eichhornia crassipes*), a fast-growing, free-floating species that thrives in warm and humid climates. Although often regarded as one of the world's most invasive aquatic weeds, posing ecological challenges such as obstructing waterways and reducing biodiversity, recent studies have highlighted its rich physiological and phytochemical profile, highlighting its potential pharmaceutical and economic value (Anusiya et al., 2020).

Numerous investigations have reported that *E. crassipes* contains a wide range of bioactive compounds, including flavonoids, phenols, alkaloids, saponins, tannins, and sterols, which exhibit significant antimicrobial and antifungal activities (Annadurai et al., 2013). Research has further revealed a strong correlation between the plant's chemical composition and its ability to inhibit pathogenic microorganisms, positioning it as a promising natural source for developing alternative therapeutic agents (Aswad et al., 2025).

Interest in water hyacinth extends beyond its medicinal potential. It has been widely studied for its role in environmental remediation, particularly in the phytoremediation of heavy metals such as lead, cadmium, and arsenic from contaminated water bodies (Senthil, K., Srisai, S., 2019; Ati et al., 2023). Due to its high organic matter content and adaptability to aquatic environments, it is also utilized in biogas production, composting, and as a component in animal feed (Ati et al., 2024a, b).

The biochemical properties of water hyacinth extracts, particularly those derived from the roots, have been extensively characterized using advanced analytical and spectroscopic techniques. Among these, Gas Chromatography-Mass Spectrometry (GC-MS) has played a crucial role in identifying a diverse array of volatile and semi-volatile phytochemical constituents, such as phenolic acids, esters, fatty acids, terpenoids, and other secondary metabolites with known therapeutic potential. Likewise, Fourier Transform Infrared Spectroscopy (FTIR) has been instrumental in elucidating the functional groups and chemical bonds present in the plant's complex matrix, providing insight into the molecular structure and interactions of its bioactive components. These methods have revealed a biochemical profile that supports the traditional use of the plant in folk medicine and emphasizes its potential for the development of novel pharmacological agents.

Building on these findings, the present study aims to deepen our understanding of the phytochemical and pharmacological potential of water hyacinth, with a particular focus on its antimicrobial and antioxidant properties. By integrating traditional knowledge with modern scientific approaches, this research contributes to the body of evidence supporting the safe and effective medicinal utilization of water hyacinth, a plant often viewed as an ecological nuisance, yet emerging as a promising source of bioactive compounds for therapeutic and industrial applications.

Materials and Methods

Plant collection

Roots of the water hyacinth were randomly collected from the banks of the Tigris River in Baghdad, Iraq. At the time of collection, the river water had a pH of 4.5 and a temperature of 18 °C. The samples were immediately transported to the laboratory. After drying, samples were stored at 4°C for subsequent analyses (Aziz et al., 2023).

Preparation of alcoholic root extracts

Ten grams of air-dried roots were ground and extracted with 200 ml of 70% ethanol. The extraction process involved 10 successive maceration steps under identical conditions. After extraction, the solvent was evaporated using a rotary evaporator, and the resulting crude extract was concentrated and stored at 4°C until further analysis. Four different concentrations (10%, 20%, 30%, and 40% w/v) of the alcoholic extract were prepared for biological and chemical analyses (Bikash et al., 2011; Dagno et al., 2012; Aziz et al., 2023).

pH determination

The pH of the plant extract was measured using a calibrated digital pH meter after mixing the sample with distilled water in a 1:1 ratio (v/v). Each measurement was performed in triplicate to obtain an accurate average (Gabriel et al., 2018).

Phytochemical screening (qualitative analysis)

Qualitative phytochemical screening for the presence of tannins, phenols, flavonoids, saponins, alkaloids, glycosides, carbohydrates, steroids, resins, coumarins, and terpenes was carried out using standard protocols

and reference reagents (Hameed et al., 2020; Hadi et al., 2024).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the alcoholic root extract was performed using a PerkinElmer Clarus 680 GC system coupled with a Clarus 600 EI mass spectrometer. The analysis was conducted at the Faculty of Nanotechnology, Al-Mustansiriyah University, using TurboMass software (version 5.4.2). Compounds were identified by comparing their mass spectra with those in standard reference libraries (Hameed et al., 2020; Ibrahim et al., 2025).

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was conducted to identify functional groups within the alcoholic extract. The spectra were recorded in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}, focusing on the presence of characteristic bands for carboxyl (C=O), hydroxyl (O–H), and other molecular vibrations associated with plant secondary metabolites (Javier et al., 2016).

Preparation of fungal cultures

Six pathogenic fungi viz. *Curvularia* sp., *Fusarium errumense*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, and *Sclerotium rolfsii*, were obtained from the University of Science, Baghdad. These cultures were grown in potato dextrose broth (PDB), with spore concentrations adjusted between 1×10^5 and 5×10^6 cfu/ml (Joshi and Kaur, 2013).

Antifungal activity assay

The antifungal activity of the extracts was evaluated using the well diffusion method on Mueller-Hinton agar plates. Fungal inocula were evenly spread across the agar surface, and wells (6 mm diameter) were filled with different concentrations of the alcoholic extract. Fluconazole served as a positive control, while distilled water was used as a negative control. Plates were incubated at 27°C for 5 days, and the zones of inhibition (ZOIs) were measured in millimeters using a digital caliper. All assays were performed in triplicate (Kumar and Pandey, 2014).

Statistical analysis

All data were statistically analyzed using SAS software version 9.4. One-way analysis of variance (ANOVA) was performed, followed by mean comparison using the least significant difference (LSD) test at a significance level of $p < 0.05$. Results are presented as mean ± standard deviation (SAS Institute Inc., 2012).

Results and Discussion

Phytochemical analysis of alcoholic extracts from water hyacinth roots

The pH of the alcoholic extract was found to be 4.5, indicating moderate acidity. This observation aligns with previous findings on the same species (Pereira et al. 2014). Qualitative phytochemical screening (Table 1) revealed the presence of several important secondary metabolites, including tannins, lysine, flavonoids, saponins, alkaloids, and terpenes. The presence of these compounds is largely attributed to the polarity of the plant material and the efficacy of ethanol as a solvent in extracting semi-polar phytochemicals.

These findings are consistent with earlier studies on the chemical composition of water hyacinth roots and leaves (Preethi et al., 2010; Pereira et al. 2017). However, some discrepancies were noted when compared with studies that reported the absence of certain compounds e.g., tannins, flavonoids, and alkaloids in different plant parts or when non-polar solvents were used (Sadkhan et al., 2025). This highlights the importance of both the selected plant part and the extraction solvent in determining phytochemical yield and profile.

Flavonoids and phenolic compounds are of particular interest due to their strong antioxidant activity and their role in protecting plants from oxidative stress. These compounds have also been shown to prevent oxidative damage in human cells, making them promising candidates for drug development (Saeed et al., 2024). The presence of substantial amounts of flavonoids and phenolics in the root extract enhances its pharmacological potential for use in both preventive and therapeutic applications.

Other detected compounds, such as saponins, steroids, and alkaloids, also contribute to the medicinal value of the extract. Saponins are known to reduce cholesterol levels and stimulate the immune system, while alkaloids possess a wide range of biological activities and are commonly found in traditional remedies for inflammation and neurological disorders. The presence of these compounds, even in moderate concentrations, further supports the potential of water hyacinth roots as a source of bioactive agents.

Antifungal activity

The results presented in Table 2 demonstrated that ethanol root extracts of water hyacinth exhibited significant antifungal potential, particularly at higher concentrations (30% and 40%), when compared to

standard antibiotics such as amoxicillin and gentamicin. The findings indicated that the 30% and 40% ethanol extracts strongly inhibited the growth of *Curvularia* sp., *F. errundifyme*, and *F. moniliforme*. However, no significant inhibitory effects were observed against *F. proliferatum*, *F. oxysporum*, and *S. rolfsii*.

This differential response among fungal species was likely attributed to variations in fungal cell wall composition and the chemical profile of the plant extract, particularly the types and concentrations of phenolic compounds and flavonoids (Preethi et al., 2010; Pereira et al., 2017).

The antifungal activity of the extract was primarily associated with the presence of phenolics and flavonoids, which possessed antioxidant properties that disrupted fungal growth. These compounds were known to inhibit key enzymes such as oxidases, interfere with lipid metabolism, and compromise the integrity of the fungal cell membrane, ultimately leading to ionic imbalance and cell death (Sundari and Ramesh, 2012).

The present findings aligned with previous studies that reported antifungal activity in extracts from other aquatic plants such as *Typha angustifolia* and *Pistia stratiotes*, which also demonstrated increased efficacy with increasing extract concentrations (Thamaraiselvi et al., 2012; Joshi and Kaur, 2013). Similar antifungal effects were reported from alcohol-based leaf extracts of *E. crassipes* (Kumar and Pandey, 2014). The presence of phytochemicals such as flavonoids, tannins, and alkaloids contributed to a wide range of biological activities. Tannins, for instance, were known to precipitate fungal proteins, while alkaloids inhibited key fungal enzymes (Sundari and Ramesh, 2012).

On the other hand, the lack of activity against *F. proliferatum* and *F. oxysporum* could have been due to inherent resistance mechanisms, including protective biofilm formation or reduced membrane permeability to antifungal agents (Sadkhan et al., 2025).

Table 1. Qualitative phytochemical analysis of ethanolic root extracts of water hyacinth.

Phytochemical Compound	Detection Level	Biochemical and Pharmacological Significance
pH	4.5	Moderately acidic environment, supporting compound stability
Tannins	+	Antioxidant, antimicrobial, enzyme inhibitory properties
Carbohydrates	+	Energy source, involved in cellular interactions
Glycosides	–	Typically bioactive but not detected in this extract
Phenols	++	Strong antioxidants, protect against oxidative stress
Resins	+	Antimicrobial and protective effects
Flavonoids	++	Antioxidant, anti-inflammatory, antiviral activities
Saponins	+	Cholesterol-lowering, immunostimulant, antimicrobial properties
Alkaloids	+	Anti-inflammatory, neuroactive, diverse pharmacological effects
Proteins	–	Not detected in this extract
Coumarins	–	Known for anticoagulant activity; absent here
Terpenes	+	Antioxidant, antimicrobial, contribute to aroma and flavor
Steroids	–	Hormonal and pharmacological compounds; not detected

"++" indicates high presence, "+" indicates presence and "–" indicates absence.

Table 2. Antifungal activity of water hyacinth extracts.

Concentration (%)	Zone of Inhibition in mm						Gentamycin	Amoxicillin
	CU	FE	*FM*	FP	*FO*	*SR*		
10	12.0 ±0.59	7.0 ±0.64	9.0 ± 0.73	-	-	-	13.5 ±0.72	4.6 ±0.17
20	13.0 ±0.78	9.0 ±0.72	10.0 ±0.50	-	-	-	20.6 ±1.05	12.5 ±0.48
30	14.0 ±0.85	12.0 ±0.59	10.0 ±0.50	-	-	-	15.7 ±0.75	15.0 ±0.61
40	14.0 ±0.85	14.0 ±0.85	10.0 ±0.50	-	-	-	16.8 ±0.82	16.2 ±0.70
LSD value (P≤0.05)	1.72 *	2.08 *	1.194 NS	-	-	-	2.36 *	2.77 *

CU= *Curvularia* sp., FE= *F. erundiforme*, FM = *F. moniliforme*, FP= *F. proliferatum*, FO= *F. oxysporum,* SR = *Sclerotium rolfsiii*, respectively, and "-" No inhibition of growth.

GC-MS analysis

GC-MS analysis identified the major chemical constituents of the ethanolic root extract of water hyacinth. A total of approximately 21 organic compounds were detected (Figure 1). Among these, 14 were low-polarity long-chain aldehydes, which are often used as markers of plant-derived bioactivity. The analysis also revealed the presence of complex ethers and ether-containing compounds, which are known to possess pharmacologically significant properties in medicinal plants. Notably, palmitic acid (n-hexadecanoic acid), a saturated fatty acid with documented antioxidant and antibacterial properties, was also detected (Sadkhan et al., 2025). Previous studies have confirmed the widespread occurrence of this compound in medicinal plants, where it contributes to antimicrobial activity and immunomodulatory effects (Saeed et al., 2024).

The presence of such bioactive constituents, particularly when induced through ethanol extraction, enhances the pharmaceutical value of water hyacinth as a rich source of secondary metabolites. Furthermore, the identification of waxes and volatile compounds suggested the presence of chemical agents capable of interfering with microbial physiology, which likely contributed to the antifungal activity observed in this study (Sundari and Ramesh, 2012; Pereira et al., 2017). Spectroscopic analyses confirmed that the aqueous extract of water hyacinth is rich in biologically active compounds, some of which are well recognized for their therapeutic potential. These findings support the potential use of this plant as a natural alternative to synthetic bioactive agents. Further research is recommended to isolate and quantify the principal active constituents using advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Ion-Associated Molecular Recognition (IAMR) to elucidate their chemical structures and biological activities in greater detail.

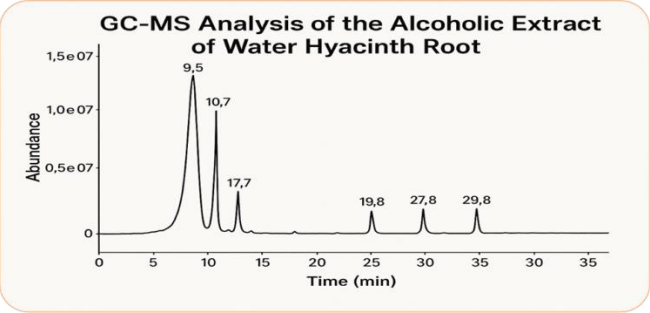

Figure 1. GC-MS analysis of the alcoholic extract of water hyacinth roots.

UV-Visible spectrophotometry

The UV-visible spectrophotometric analysis of the ethanol root extracts of water hyacinth revealed three distinct absorption peaks at 232.5 nm, 287.5 nm, and 322.0 nm, as shown in Figure 2. The absorption maximum at 232.5 nm indicated the presence of π→π* transitions, typically associated with simple aromatic compounds. The additional peaks at 287.5 nm and 322.0 nm suggested the presence of extended conjugated systems or substituted phenolic groups. These features are commonly linked to bioactive aromatic compounds with potential antioxidant properties.

The presence of such compounds supports the hypothesis that the extract contains bioactive constituents capable of interacting with microbial cell lines, acting as free radical scavengers, and disrupting cell membranes. These findings were consistent with previous studies conducted on other aquatic plants, which exhibited similar UV absorption profiles at comparable wavelengths, thereby reinforcing the reliability of the present data (Sundari and Ramesh, 2012).

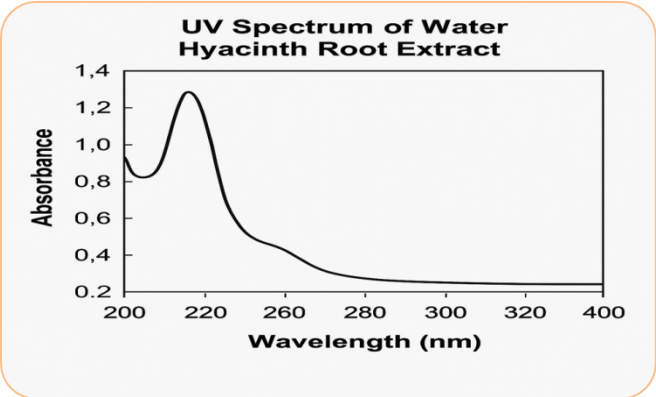

Figure 2. UV spectrum of water hyacinth root extract.

FTIR spectroscopy analysis

FTIR spectroscopy was employed to analyze the pan-extracts of water hyacinth root extracts within the wavelength range of 4000 to 400 cm-1, using an analytical resolution of 4 cm^{-1}. Five consistent scans were conducted to ensure the spectral stability of the samples (Javier et al., 2016).

The results revealed a prominent absorption band at approximately 1600.1 cm^{-1}, attributed to the stretching vibrations of the carbonyl group (C=O), characteristic of compounds such as ketones and aldehydes (Figure 3). Another significant peak appeared at 2926.5 cm^{-1},

corresponding to C–H stretching vibrations, particularly associated with CH_3 groups bound to hydroxyl functionalities in alcohols (Thamaraiselvi et al., 2012).

A distinct peak at 1402.29 cm^{-1} was likely related to bending vibrations of COH groups, while a strong band at 1056.27 cm^{-1} was attributed to asymmetric stretching vibrations of the carboxylate group (COO^-). Moreover, a sharp peak observed near 1750 cm^{-1} further supported the presence of carbonyl-containing functional groups.

These spectral features were consistent with previous findings on aquatic plant extracts. The absorption band near 1600 cm^{-1} has been recognized as a reliable indicator of heavy metal absorption from soil or water, supporting the potential use of aquatic plants like water hyacinth in biosorption applications (Sundari and Ramesh, 2012). Other studies have also reported absorbance bands in the 1050-1100 cm^{-1} range, corresponding to carboxylic and phenolic groups.

In conjunction with UV-visible spectroscopic results, the FTIR spectra provided a comprehensive chemical profile of the root extract. The observed peaks supported the presence of bioactive aromatic and phenolic compounds, including flavonoids, saturated fatty acids, and alcohols.

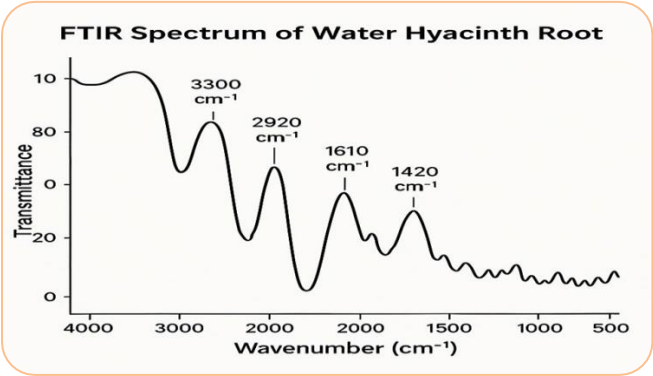

Figure 3. FTIR spectra of water hyacinth root recorded at a constant time.

Conclusion

Water hyacinth is a widely distributed invasive aquatic plant that serves as a rich source of bioactive phytochemicals with notable medicinal potential. Phytochemical screening revealed the presence of tannins, resins, flavonoids, saponins, alkaloids, and terpenes. Numerous studies have documented that these secondary metabolites possess significant pharmacological properties, although the medicinal potential of *E. crassipes* remains largely underexploited.

The antifungal activity of water hyacinth extracts demonstrated statistically significant differences ($p \leq 0.05$) against *Curvularia* sp., *F. errundifyme*, and *F. moniliforme*. GC-MS analysis confirmed the presence of several volatile organic compounds, including sterols, aldehydes, ethers, and important esters. UV-visible and FTIR spectral analyses further supported the presence of diverse functional groups and bioactive molecules, contributing to therapeutic potential of the plant.

In summary, this study supports the potential of water hyacinth as a sustainable and economically viable source of bioactive compounds for use in pharmaceuticals and antifungal therapies. Its antimicrobial properties against pathogenic fungi reinforce its value in the treatment of various infections. Further research is recommended to explore the long-term toxicity and to evaluate its efficacy and safety for wider industrial and medicinal applications.

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Authors' Contributions

HFR and ZAA designed the study; SH prepared the materials, collected and analyzed the data; HFR helped in disease scoring. ZAA and SH supervised the studies; SH and ZAA wrote the manuscript; All the authors proofread and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 3: Good Health and Well-being
SDG 6: Clean Water and Sanitation
SDG 12: Responsible Consumption and Production

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