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Original article

Antioxidant Capacity of *Equisetum telmateia* and *Urtica dioica*: Secondary Benefits in Renal Health Beyond Crystal Inhibition

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Abstract
Kidney stone disease is a common condition characterized by the formation of crystalline deposits in the kidneys, with calcium oxalate being the primary component of most stones. Oxidative stress plays a significant role in the pathogenesis of kidney stones by promoting inflammation, lipid peroxidation, and renal damage. While current treatments mainly focus on inhibiting crystal formation, there is limited attention given to addressing oxidative stress. This study evaluates the antioxidant capacity of Equisetum telmateia (Giant Horsetail) and Urtica dioica (Nettle) and explores their potential to reduce oxidative stress in renal health. Phytochemical analysis of both plants revealed the presence of key bioactive compounds, including phenolic acids, flavonoids, and fatty acid derivatives, which are known for their antioxidant properties. In vitro assays, including ABTS and DPPH, demonstrated significant radical scavenging activity for both plant extracts, with E. telmateia showing slightly higher inhibition at 500 µg/mL compared to U. dioica. Cytotoxicity testing on zebrafish embryos and Vero cells revealed no significant toxicity at therapeutic doses, confirming the safety of both extracts. These findings suggest that E. telmateia and U. dioica not only possess potent antioxidant activity but may also protect renal tissues from oxidative damage associated with kidney stone formation. The study highlights the therapeutic potential of these plants in kidney stone prevention and management, providing a holistic approach by targeting both crystallization and oxidative stress. Further clinical research is warranted to explore the efficacy of these plants in human kidney stone disease management.
Keywords. Antioxidants, Oxidative Stress, Nephrolithiasis, Phenolic Acids, Renal Protection.

Introduction

Kidney stone disease (nephrolithiasis) is a prevalent condition in the urinary system, affecting a significant portion of the global population. The formation of kidney stones is influenced by various factors such as urinary supersaturation, crystal retention, and oxidative stress. Oxidative stress, resulting from the accumulation of reactive oxygen species (ROS), plays a central role in kidney stone pathogenesis by promoting lipid peroxidation, inflammation, and renal tubular damage. This damage exacerbates stone formation and contributes to the progression of renal dysfunction. However, most current therapeutic strategies focus primarily on inhibiting crystal formation and do not fully address the oxidative damage associated with nephrolithiasis [1,2].

In recent years, there has been growing interest in the potential of medicinal plants to provide dual benefits in kidney stone management by addressing both crystallization and oxidative stress. *Equisetum telmateia* (Giant Horsetail) and *Urtica dioica* (Nettle) are two plants with a long history of use in traditional medicine for treating various renal disorders, including kidney stones. These plants are rich in bioactive compounds with antioxidant, anti-inflammatory, and diuretic properties. *E. telmateia* has been shown to possess significant antioxidant activity, which can potentially protect renal tissues from oxidative damage associated with kidney stones [3]. Similarly, *U. dioica* is known for its diuretic effects, which aid in the prevention of kidney stones by promoting the excretion of excess stone-forming minerals [4] .

Several studies have demonstrated that the antioxidant properties of *E. telmateia* and *U. dioica* are linked to their high content of phenolic acids, flavonoids, and other bioactive compounds. For instance, *E. telmateia* has been found to possess high concentrations of flavonoids and phenolic acids, such as kaempferol and chlorogenic acid, which are known to exhibit strong radical-scavenging activity [5]. *U. dioica* is similarly rich in quercetin derivatives and other polyphenols, contributing to its antioxidant and anti-inflammatory effects [6]. These properties are crucial in mitigating oxidative stress, a major contributing factor in kidney stone formation and renal damage.

Despite the promising therapeutic potential of these plants, research focusing on their combined effects—both in terms of antioxidant activity and their role in preventing stone formation—remains limited. This study aims to evaluate the antioxidant capacity of *E. telmateia* and *U. dioica*, linking their phytochemical composition to their potential to alleviate oxidative stress and protect renal health. By investigating both the antioxidant and anti-inflammatory properties of these plants, this study contributes to a deeper understanding of their potential role in managing kidney stone disease.

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Methods

Plant Material and Extraction

The aerial parts of E. telmateia and the leaves of U. dioica were collected from their natural habitats. The plant materials were carefully cleaned, air-dried, and ground into a fine powder using a mechanical grinder. The powdered plant material (20 g) was subjected to reflux extraction with distilled water (500 mL) for 4 hours. After extraction, the solutions were filtered through Whatman No. 1 filter paper to remove solid residues. The filtrates were then lyophilized to yield dry extracts, which were stored in airtight containers at -20°C until further analysis[7].

Phytochemical Analysis

Qualitative Tests

Standard protocols were used to detect the presence of key phytochemicals in the extracts. Alkaloids were identified using Dragendorff's reagent, flavonoids were screened using a magnesium hydrochloride test, phenolic compounds were detected using the ferric chloride method, tannins were determined by the blue-black precipitate formation with iron salts, and phytosterols were confirmed by the Liebermann-Burchard reaction[8]

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of the extracts was further analyzed using a Thermo Scientific™ TRACE™ 1310 Gas Chromatography-Mass Spectrometry (GC-MS) system. A sample of each extract (1 µL) was injected into the GC-MS system, where compounds were separated on a non-polar column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 60°C to 280°C at a rate of 10°C/min. The compounds were identified by comparing the mass spectra with the National Institute of Standards and Technology (NIST) and Wiley mass spectral libraries[9]

ABTS Radical Scavenging Assay

The antioxidant potential of the extracts was evaluated using the ABTS+ radical cation decolorization assay. The ABTS+ radical was generated by reacting ABTS solution with potassium persulfate and incubating the mixture for 12 hours in the dark. A known volume of extract was added to the ABTS+ solution, and the absorbance was measured at 734 nm. The radical scavenging activity was calculated as the percentage inhibition of the ABTS+ radical[10]

DPPH Assay

The free radical scavenging activity of the extracts was assessed by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The extracts (50 µL) were mixed with a DPPH solution (200 µL, 0.1 mM) in ethanol, and the mixture was incubated in the dark for 30 minutes. The absorbance was measured at 517 nm, and the percentage of DPPH inhibition was calculated [11]

Zebrafish Embryo Toxicity Test

The cytotoxic effects of the extracts were evaluated using zebrafish embryos. The embryos (at the 4–8 cell stage) were exposed to various concentrations of the extracts (200, 400, 600, 800, and 900 µg/mL) for 96 hours. The viability of the embryos was monitored by assessing morphological development and measuring heart rate, which is a well-established indicator of toxicity. Embryo mortality and malformations were also recorded[12]

Vero Cell Sulforhodamine B (SRB) Assay

The cytotoxicity of the extracts was further tested on Vero cells. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and incubated at 37°C in a 5% CO2 atmosphere. After 24 hours of exposure to varying concentrations of the extracts (50–1000 µg/mL), cell viability was measured using the SRB assay. The cells were fixed with 10% trichloroacetic acid, stained with SRB solution, and the absorbance was measured at 570 nm[13]

Statistical Analysis

All experimental data were analyzed for statistical significance using one-way analysis of variance (ANOVA). Results were expressed as mean ± standard deviation (SD) for each group. Statistical significance was determined at $p < 0.05$. Post-hoc tests were performed using Tukey's multiple comparison test, where appropriate, to compare individual groups[14]

Results

The phytochemical analysis of E. telmateia revealed that the extract was predominantly composed of cis-vaccenic acid (11.86%), chlorogenic acid, and trilinolein (11.03%). Notably, phenolic compounds accounted for 50% of the total phytochemicals detected in the extract. On the other hand, U. dioica was found to be

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rich in flavonoids, particularly quercetin derivatives, and phenolic acids, including caffeic acid and chlorogenic acid. Additionally, U. dioica exhibited significant levels of trilinolein (13.93%) and oleic acid derivatives, as shown in the phytochemical composition table.

Table 1: Phytochemical Composition of Equisetum telmateia and Urtica dioica

Plant Material	Dominant Compounds	Phenolic Compounds (%)
E. telmateia	Cis-vaccenic acid (11.86%), Chlorogenic acid, Trilinolein (11.03%)	50%
U. dioica	Flavonoids (Quercetin derivatives), Phenolic acids (Caffeic acid, Chlorogenic acid), Trilinolein (13.93%), Oleic acid derivatives	-

In terms of antioxidant activity, the ABTS scavenging assay demonstrated that both E. telmateia and U. dioica were effective in neutralizing the ABTS radical, with the extracts showing 78% and 72% inhibition, respectively, at a concentration of 500 µg/mL. Furthermore, the DPPH assay revealed that the IC50 values for E. telmateia and U. dioica were 89 µg/mL and 102 µg/mL, respectively

Table 2. Antioxidant Activity of Equisetum telmateia and Urtica dioica

Plant Material	ABTS Scavenging (%) at 500 µg/mL	DPPH IC50 (µg/mL)
E. telmateia	78%	89 µg/mL
U. dioica	72%	102 µg/mL

Regarding cytotoxicity, both E. telmateia and U. dioica were evaluated using zebrafish embryos, where both extracts exhibited >90% viability at a concentration of 500 µg/mL, indicating low toxicity. Furthermore, in the Vero cell SRB assay, both extracts showed an IC50 greater than 500 µg/mL, confirming their non-toxic nature. The cytotoxicity results are summarized in the respective table.

Table 3. Cytotoxicity Evaluation of Equisetum telmateia and Urtica dioica

Test	E. telmateia Viability (%)	U. dioica Viability (%)	IC50 (µg/mL)
Zebrafish Embryos	90%	90%	-
Vero Cells	-	-	>500
P-Vlaue	>0.05		

Discussion

The results of this study provide important insights into the antioxidant properties of Equisetum telmateia (Giant Horsetail) and Urtica dioica (Nettle), particularly in the context of their potential role in mitigating oxidative stress in renal health. Both plants exhibited significant antioxidant activity, which supports their traditional use in treating kidney disorders. The antioxidant capacities were evaluated using two commonly employed assays, ABTS and DPPH, which are widely recognized for assessing radical scavenging activity. The findings from these assays confirm that both E. telmateia and U. dioica possess robust antioxidant activities, suggesting their potential to protect renal tissues from oxidative damage, a key factor in the pathogenesis of kidney stones [15,16].

The phytochemical analysis revealed that E. telmateia is rich in phenolic compounds, particularly chlorogenic acid and cis-vaccenic acid, which are well-known for their antioxidant properties. Similarly, U. dioica was found to contain high levels of flavonoids such as quercetin derivatives, along with other phenolic acids, including caffeic acid and chlorogenic acid. These compounds are effective in neutralizing reactive oxygen species (ROS) and have been shown to reduce oxidative stress markers in renal tissues[5] . The ABTS and DPPH assays further demonstrated that the antioxidant activity of E. telmateia (78% inhibition at 500 µg/mL) and U. dioica (72% inhibition at 500 µg/mL) is consistent with the known capabilities of these phytochemicals in scavenging free radicals [10,16]. The higher radical scavenging activity observed in E. telmateia compared to U. dioica can be attributed to its relatively higher content of phenolic acids, which are potent antioxidants. Phenolic compounds, particularly flavonoids like quercetin, play a major role in neutralizing free radicals and reducing oxidative damage [16]. In comparison, studies on U. dioica have highlighted its diuretic effects, which not only help in expelling excess oxalate from the body but also protect against oxidative stress by enhancing antioxidant enzyme activity [4]. These results align with the findings of previous studies, which have shown that U. dioica exhibits significant antioxidant potential that is crucial in protecting renal tissues from oxidative damage associated with kidney stone formation [18].

In this study, both E. telmateia and U. dioica demonstrated low cytotoxicity, as evidenced by the >90% viability of zebrafish embryos exposed to 500 µg/mL of the extracts. The Vero cell SRB assay further confirmed that both extracts exhibited an IC50 greater than 500 µg/mL, indicating a lack of significant toxicity. This finding is in line with other studies that have assessed the safety of E. telmateia and U. dioica.

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For example, E. telmateia extracts have been shown to have a safe toxicity profile in vitro, with no adverse effects observed in cell cultures [15]. Similarly, U. dioica has been widely regarded as safe in both human and animal models, with no significant toxicity observed at therapeutic doses [4].

The non-toxic nature of these extracts at the tested concentrations is a critical factor, as it underscores their potential for therapeutic use in managing kidney stone disease. The low cytotoxicity of both plants makes them promising candidates for further clinical investigations, especially considering their antioxidant and anti-inflammatory properties, which are essential in addressing both the primary and secondary causes of kidney stone formation [1,3].

When compared to synthetic antioxidants such as vitamin C, E. telmateia and U. dioica offer a more holistic approach to kidney stone prevention. While synthetic antioxidants are effective in reducing oxidative damage, they typically do not address the multifaceted nature of kidney stone formation, which involves not only oxidative stress but also crystal formation, inflammation, and urinary supersaturation. In contrast, the phytochemicals present in E. telmateia and U. dioica provide a broad spectrum of activity, including antioxidant, anti-inflammatory, and crystallization-inhibitory effects. For example, E. telmateia has been shown to inhibit the crystallization of calcium oxalate, a major component of kidney stones, through its antioxidant activity and phenolic content [15].

In a similar vein, U. dioica has been demonstrated to exhibit diuretic effects that help reduce the concentration of stone-forming ions in the urine, thus preventing crystallization. Additionally, U. dioica's quercetin derivatives have been found to reduce oxidative stress markers and prevent renal inflammation, which are key contributors to kidney stone formation [16]. These findings suggest that the combination of antioxidant and anti-inflammatory properties in both E. telmateia and U. dioica could provide a more comprehensive solution to kidney stone management than synthetic antioxidants alone.

The antioxidant and cytoprotective effects observed in this study suggest that E. telmateia and U. dioica could serve as effective adjuncts in the treatment and prevention of kidney stones, especially when used in combination with conventional therapies. Their ability to reduce oxidative stress and inflammation while inhibiting crystallization positions them as promising candidates for further clinical trials. Moreover, the low toxicity observed in both plants makes them suitable for long-term use in renal health management.

Further studies are needed to explore the synergistic effects of these plants when used in combination with other kidney stone management strategies, such as dietary modifications or pharmacological agents. Clinical trials focusing on the bioavailability and efficacy of the bioactive compounds in E. telmateia and U. dioica will be essential to confirm their therapeutic potential and establish optimal dosages for human use.

Conclusion

This study highlights the significant antioxidant and cytoprotective potential of Equisetum telmateia and Urtica dioica in mitigating oxidative stress associated with kidney stone formation. Both plants demonstrated strong radical-scavenging activity, supporting their traditional use in managing renal disorders. Additionally, the low cytotoxicity observed in both E. telmateia and U. dioica extracts further underscores their safety for therapeutic use. These findings suggest that beyond their crystal-inhibiting effects, both plants may offer a protective role against renal oxidative damage, providing a holistic approach to kidney stone prevention and treatment. Given their promising results, further clinical studies are essential to validate their efficacy and establish optimal dosages for human use, particularly in the management of nephrolithiasis. With their multifaceted biological activities, E. telmateia and U. dioica have the potential to be valuable adjuncts in kidney stone management and broader renal health.

Conflicts of Interest

The authors declare no conflicts of interest.

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