

In-Vitro Assessment of the Antioxidant, Anti-Inflammatory and Antidiabetic Properties of the Ethanolic Leaf Extract of *Urtica dioica* (Stinging Nettle) in Organized Chemical and Cell-Based Models

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ABSTRACT

Various studies have shown that plants are capable of producing diverse chemical substances that perform vital biological roles, including protection from predators like insects, fungi, and plant-eating mammals. This study evaluated the *in-vitro* antioxidant, anti-inflammatory, and antidiabetic activities of *U. dioica* leaf extract. Antioxidant potential was assessed using DPPH, nitric oxide [NO], hydroxyl radical [HO•], and hydrogen peroxide [H₂O₂] scavenging assays. Anti-inflammatory activity was determined by membrane stabilization, protein denaturation inhibition, and protease inhibitory assays. Antidiabetic potential was evaluated using the glucose uptake inhibition assay. Butylated Hydroxytoluene [BHT], Diclofenac sodium, and Metronidazole were used as standards. The extract exhibited significant, dose-dependent activity in all assays. For antioxidant activity, IC₅₀ values were DPPH = 65.81 µg/mL, NO = 34.18 µg/mL, HO• = 18.13 µg/mL, and H₂O₂ = 42.21 µg/mL. For anti-inflammatory activity, IC₅₀ values were: membrane stabilization = 66.41 µg/mL, protein denaturation = 62.89 µg/mL, and protease inhibition = 52.02 µg/mL. The extract also showed glucose uptake inhibitory activity with an IC₅₀ of 96.83 µg/mL. While the standards showed higher potency, the results indicate that *U. dioica* possesses broad-spectrum bioactivity, likely due to its flavonoid and phenolic content. The findings validate the traditional use of *U. dioica* and suggest its potential as a natural source of antioxidant, anti-inflammatory, and antidiabetic agents for therapeutic development.

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INTRODUCTION

Natural plants have played a crucial role in treating and preventing degenerative diseases while promoting health (Enema, *et. al.* 2024). The various beneficial effects of plants and their different components typically arise from the chemicals they possess. Plants produce a diverse range of secondary metabolites that assist in their defense against numerous environmental threats, such as herbivores, pathogens, and UV radiation (Al-Khayri, *et. al.*, 2023). These metabolites comprise alkaloids, flavonoids, terpenoids, glycosides, and phenolic acids, each exhibiting varying degrees of antimicrobial properties. (Divekar, *et. al.*, 2022; Okoroh, *et. al.* 2019) The search for new antimicrobial agents derived from plants is crucial due to the global health threat posed by resistant bacterial strains, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococci (VRE) (Bauer *et. al.*, 2019).

Diabetes mellitus continues to be one of the most significant global metabolic challenges of the 21st century, mainly caused by cellular insulin resistance, widespread inflammation, and intense oxidative stress (Devkota *et. al.*, 2022; Ghorbani *et. al.*, 2022). When a cell experiences prolonged nutrient excess, the ongoing metabolic oxidation of

carbohydrates and lipids initiates an excessive generation of reactive oxygen species (ROS) (Bhattarai *et. al.*, 2023). The gradual buildup of free radicals leads to significant oxidative stress, which then harms macromolecular structures, enhances intracellular pro-inflammatory signaling pathways, and initiates structural deterioration in pancreatic β-cells (Bhattarai *et. al.*, 2023; Ghorbani *et. al.*, 2022). Simultaneously, chronic inflammation disrupts peripheral metabolic processes (Hotamisligil, 2017). Inflammatory signaling directly disrupts the insulin receptor substrate pathway, reducing glucose transporter type 4 (GLUT4) translocation and hindering effective glucose uptake in peripheral skeletal muscles and adipose tissues (Ghorbani *et. al.*, 2022; Somani *et. al.*, 2024). Due to the frequent unwanted side effects of standard synthetic oral hypoglycemic medications and insulin therapies like gastrointestinal issues, rapid weight gain, or sudden hypoglycemic events,—contemporary pharmacological research has increasingly concentrated on multi-targeted natural treatments sourced from ethnobotanical origins (Altamimi *et. al.*, 2022; Devkota *et. al.*, 2022).

Research indicates that extracts of *Urtica dioica* exhibit significant antimicrobial properties against various bacteria,

fungi, and viruses (Semalty *et. al.*, 2017). The clinical adaptability of *U. dioica* leaf extracts arises from its diverse and rich secondary metabolic composition, featuring significant levels of hydrophilic polyphenols, tannins, phenolic acids, and flavonoids including rutin and quercetin (Altamimi *et. al.*, 2022; Somani *et. al.*, 2024). *In vitro* model systems have demonstrated that these localized phytochemical components can directly provide hydrogen atoms to neutralize free radicals, prevent the downstream generation of pro-inflammatory cytokines, and inhibit carbohydrate-digesting enzymes like α -glucosidase (Devkota *et. al.*, 2022; Ghorbani *et. al.*, 2022).

Additionally, *U. dioica* has demonstrated potential as a natural insulin mimetic or insulin secretagogue, improving metabolic insulin sensitivity and promoting peripheral glucose absorption in insulin-resistant cellular contexts (Ghorbani *et. al.*, 2022; Somani *et. al.*, 2024). This study offers a thorough *in vitro* assessment of the diverse therapeutic benefits of *Urtica dioica* leaf extract through the use of organized chemical and cell-based model setups. It evaluates its direct ability to scavenge free radicals, anti-inflammatory effects, and peripheral glucose absorption capability, aiding in confirming its function as a dietary supplement or safe natural complementary treatment for diabetes management.

MATERIALS AND METHODS

Description of Study Area

This study was carried out at the Chemistry Laboratory – an academic research environment of Kwara State Polytechnic, situated in Ilorin, Kwara State, Nigeria. The location offered an ideal habitat for obtaining fresh *Urtica dioica* leaves, verified by the institution's Department of Environmental Biology.

Study Design

This study is an experimental, in-vitro laboratory analysis aimed at assessing the bioactivities of ethanolic extracts from *Urtica dioica* leaves.

Plant Collection

Fresh leaves of *Urtica dioica* were gathered from the Botanical Garden of Kwara State Polytechnic in Ilorin, Kwara State, Nigeria. The plant was verified by a Botanist from the Department of Environmental Biology at Kwara State Polytechnic, Ilorin. The Leaves were cleaned using distilled water, air-dried at room temperature for 14 days, and ground into coarse powder with a mechanical grinder (Gülçin *et. al.*, 2010).

Chemicals and Reagents

The chemicals and reagents used in this study comprised of methanol, distilled water, ethanol, Folin-Ciocalteu reagent, sodium carbonate, aluminum chloride, quercetin, nitric oxide, DPPH [2,2-diphenyl-1-picrylhydrazyl], hydrogen peroxide, sodium nitroprusside, Griess reagent, egg albumen, phosphate buffer, human blood for HRBC assay, hyposaline, salicylic acid, DMSO, metronidazole, diclofenac sodium, butylated hydroxytoluene (BHT). All reagents utilized were of analytical quality (Sakat *et. al.*, 2010).

Apparatus

UV-Vis spectrophotometer (Systronics AU-2700, India), autoclave (AMB Classic), laminar flow cabinet (Bio II Advance 4, Azbil Telstar S.L.U., Spain), conical flask, beaker, water bath (SubAqua Pro SAP12, United Kingdom),

analytical scale (Quintix 224-1S, Germany) micropipettes, centrifuge (5424 R, Eppendorf SE, Germany).

Methods

Extraction of Plant Material

The powdered leaves (100g) underwent cold maceration in 70% ethanol for 72 hours, shaken periodically. The extract were filtered with Whatman No. 1 filter paper and concentrated under decreased pressure at 80°C using a water bath. The crude extracts were kept at 4°C in dark-colored glass container until needed (Gülçin *et. al.*, 2010).

In-vitro Antioxidant Assays

DPPH Radical Scavenging Assay

The capacity to eliminate DPPH radicals was evaluated. One milliliter (1mL) of extract at 20-100 μ g/mL were combined with 1mL of 0.1 mM DPPH. Following 30 minutes in the darkness, the absorbance was measured at 517 nm. BHT serves as the standard. Percentage (%) inhibition was computed, and IC₅₀ was established (Soobrattee *et. al.*, 2005). % Scavenging = $[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{samples}}) / \text{Abs}_{\text{control}}] \times 100$.

Hydrogen Peroxide (H₂O₂) Scavenging Assay

A 2.5ml of the extract was combined with 0.6 mL of 40 mM H₂O₂ in phosphate buffer. Absorbance was assessed at 230 nm following a 10-minute interval (Ruch *et. al.*, 1989).

Nitric Oxide [NO] Scavenging Assay

The NO scavenging assay evaluates the capacity of plant extracts to compete with oxygen in reacting with nitric oxide produced from sodium nitroprusside, with the quantity of nitrite generated being assessed using Griess reagent. A 2.5ml of the extract was incubated with sodium nitroprusside at 25°C for 150 minutes. Griess reagent was introduced and absorbance measured at 546 nm (Saini *et. al.*, 2015).

Hydroxyl Radical (OH•) Scavenging Assay

The HO• scavenging assay thus assesses how effectively plant extracts can shield biomolecules from oxidative harm by directly neutralizing this radical. The scavenging capability of the extract was evaluated according to the method outlined by Smirnoff and Cumbes (1989) with minor adjustments. Two milliliters of test compounds ranging from 200 to 1000 mg/mL, 0.6 mL of 8 mM ferrous sulfate, 0.5 mL of 20 mM hydrogen peroxide, and 2 mL of 3 mM salicylic acid were combined and incubated at 37°C for 30 minutes.

In-vitro Anti-inflammatory Assays

Inhibition of Protein Denaturation

A reaction mixture with 0.2 mL egg albumin, 2.8 mL phosphate buffer [pH 6.4], and different extract concentrations (7.8 - 1000 μ g/ml) were incubated at 37°C for 15 minutes and subsequently heated at 70°C for 5 minutes. Absorbance was recorded at 660 nm. Diclofenac sodium served as the standard (Sakat *et. al.*, 2010).

HRBC Membrane Stabilization Assay

Human red blood cells were placed in isosaline solution. One millilitre (1mL) of extract were combined with 1mL of HRBC suspension and 2mL of hyposaline. The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2) and the absorbance of the supernatant were measured at 560 nm (Sakat *et. al.*, 2010).

Determination of Glucose Uptake Capacity

This test was conducted following the established procedure outlined by Cirillo (1962). A 1% suspension was prepared by dissolving commercial baker's yeast in 100ml distilled water. The suspension was stored at room temperature (25°C) overnight. In the following days, a suspension of yeast cells was centrifuged at 4200 rpm for 5 minutes. The procedure was repeated by adding distilled water to the pellet until a transparent supernatant was achieved. Ten parts of the clear supernatant were combined with ninety parts of distilled water to create a 10% v/v suspension of the yeast cells.

Concentration ranges from 7.125–1000 µg/mL of the plant extract was combined with dimethyl sulfoxide (DMSO) until fully dissolved. The solution was subsequently enriched with different concentrations (5, 10, and 25 mM) of 1 mL glucose solution and allowed to incubate for 10 minutes at 37°C. To start the reaction, 100 µL of yeast suspension was added to the glucose and extract mixture, vortexed, and incubated for an additional 60 minutes at 37°C. Following incubation, the tubes were centrifuged for 5 minutes at 3800 rpm, and glucose was measured using a spectrophotometer at 520 nm. The absorbance for the corresponding control was noted at the same wavelength.

Statistical Analysis

All data generated from this study was subjected to statistical analysis using SPSS version 26. All experiments were done in triplicate. Data were expressed as Mean ± SD and analyzed using one-way ANOVA. All graphs were plotted using

Graphpad Prism 5. $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Oxidative stress occurs when there's an imbalance between the generation of reactive oxygen/nitrogen species and the body's inherent antioxidant defenses. Assessing a plant extract through various radical scavenging pathways offers a thorough insight into its total protective potential. In this research, the antioxidant capacity of *Urtica dioica* leaf extract was assessed in vitro through four distinct assays: Hydrogen Peroxide (H_2O_2), DPPH, Nitric Oxide (NO), and Hydroxyl Radical (HO). Butylated Hydroxytoluene (BHT) was used as the reference standard compound. In all four assays, both *U. dioica* and BHT exhibited concentration-dependent radical scavenging activity ranging from 0 µg/mL to 1000 µg/mL.

Antioxidant Assay

According to Table 1 below, *U. dioica* exhibited the most potent activity against $HO^\bullet$ radicals, succeeded by NO, H_2O_2 , and finally DPPH. This implies that the extract might selectively target certain ROS, a phenomenon frequently observed in plant extracts because of the variety of phytochemicals they contain (Soobrattee *et al.*, 2005). The ability of *U. dioica* to scavenge H_2O_2 is due to the presence of flavonoids, phenolic compounds, and ascorbic acid in nettle leaves. This finding correlates the finding of Gülçin *et al.* (2010) and attributed it to the plant's rich phenolic content.

Table 1: The Calculated IC₅₀ Parameters Across all four (4) Antioxidant Assays

Assay	Plant Extract / Standard	IC ₅₀ (µg/mL)	Potency
DPPH	BHT	31.25	Lowest capacity
	<i>U. dioica</i>	65.81	
NO	BHT	15.66	Partially high
	<i>U. dioica</i>	34.18	
$HO^\bullet$	BHT	16.91	Highest capacity
	<i>U. dioica</i>	18.13	
H_2O_2	BHT	14.95	Partially high
	<i>U. dioica</i>	42.21	

DPPH Scavenging Assay

The inhibition percentage (%) of the DPPH radical by *Urtica dioica* leaf extract was compared to the standard antioxidant, Butylated Hydroxytoluene [BHT], at concentrations ranging from 0-1000 µg/mL. *U. dioica* and BHT both showed an increase in DPPH scavenging activity that depended on concentration. At reduced concentrations [0-50 µg/mL], a significant increase in % inhibition was observed for both

samples, suggesting quick transfer of hydrogen atoms to counteract DPPH radicals. As the concentration exceeded 200 µg/mL, the curve started to level off, indicating that the DPPH radicals were saturated. This concentration-dependent pattern aligns with earlier findings on medicinal plants where the antioxidant activity rises with extract concentration because of greater accessibility of flavonoid and phenolic compounds (Gülçin *et al.*, 2010).

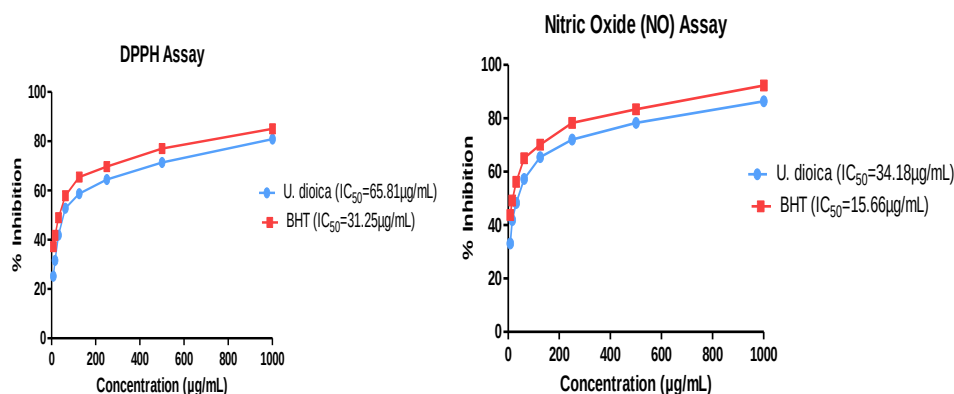


Figure 1: DPPH = 2,2-diphenyl-1-picrylhydrazyl; NO = Nitric oxide

BHT showed markedly greater scavenging activity compared to *U. dioica* across all concentrations tested. At 1000 µg/mL, BHT reached approximately 85% inhibition, whereas *U. dioica* reached about 80% inhibition. The approximately twofold elevated IC_{50} of *U. dioica* indicates that although the plant extract serves as a strong antioxidant, it is not as effective as the synthetic standard antioxidant BHT. Nonetheless, this is anticipated as BHT is a wholly synthetic substance, while *U. dioica* extract is an unrefined blend comprising both active and inactive components. The activity observed in *U. dioica* can be linked to its high levels of phenols, flavonoids, and vitamin C, recognized as DPPH scavengers. Earlier research has indicated IC_{50} values ranging from 45-70 µg/mL for methanol extracts of *U. dioica*, which is consistent with the 65.81 µg/mL found in this study (Gülçin *et al.*, 2010).

The robust DPPH scavenging capability of *U. dioica* indicates its possible role in alleviating oxidative stress. Oxidative stress, resulting from a disparity between ROS and cellular antioxidants, is associated with aging, diabetes, cancer, and inflammatory disorders (Halliwell & Gutteridge, 2015). Natural antioxidants derived from *U. dioica* may act as safer substitutes for synthetic antioxidants such as BHT, which have been associated with possible toxicity concerns with long-term use.

Nitric Oxide Scavenging Assay

Nitric oxide is a reactive free radical produced in biological systems by nitric oxide synthase. An overproduction of NO

interacts with superoxide to create peroxynitrite, an extremely reactive compound associated with inflammation, cancer, and neurodegenerative disorders (Saini *et al.*, 2015). *U. dioica* and BHT exhibited a concentration-dependent rise in NO scavenging activity. There was a sharp rise in inhibition from 0 to 250 µg/mL, followed by a slow leveling off between 500 and 1000 µg/mL. This trend shows that at minimal concentrations, bioactive compounds in the extract successfully neutralize NO radicals, whereas at elevated concentrations, the reaction nears saturation (Soobrattee *et al.*, 2005). At 1000 µg/mL, *U. dioica* reached approximately 86% inhibition, whereas BHT attained around 92% inhibition. This indicates that *U. dioica* has significant anti-nitrosative capabilities.

The comparatively low IC_{50} in relation to the DPPH assay [IC_{50} = 65.81 µg/mL from prior graph] indicates that *U. dioica* might be more potent against nitrogen-centered radicals than against oxygen-centered radicals. This specific activity is crucial since NO is vital in inflammatory processes. *U. dioica*'s capability to scavenge NO might result from directly competing with oxygen for reaction with NO and/or preventing nitrite production (Saini *et al.*, 2015). Flavonoids like quercetin and kaempferol, found in high levels in nettle, are known to suppress NO production by down-regulating iNOS expression and directly quenching radicals. This action endorses the customary application of *U. dioica* for inflammatory issues like arthritis and gout, in which surplus NO generation leads to tissue harm (Gülçin *et al.*, 2010).

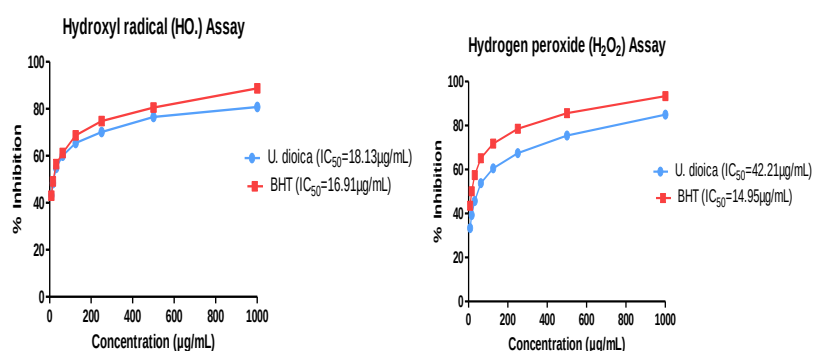


Figure 2: HO = Hydroxyl; H₂O₂ = Hydrogen peroxide

Hydroxyl Radical Scavenging Assay

The hydroxyl radical [HO•] is the most aggressive and harmful among all reactive oxygen species [ROS]. It is produced via the Fenton reaction and can lead to DNA strand breaks, lipid peroxidation, and protein denaturation, all associated with aging, cancer, and inflammatory conditions (Halliwell & Gutteridge, 2015). Both *U. dioica* and BHT showed a concentration-dependent rise in HO• scavenging. A swift rise in % inhibition occurred at low concentrations [0-100 µg/mL], with both extracts surpassing 60% inhibition at 100 µg/mL. Above 250 µg/mL, the curves started to level off, suggesting that the maximum capacity for radical quenching had been reached. At 1000 µg/mL, *U. dioica* attained approximately 80% inhibition, whereas BHT reached around 88% inhibition. This significant activity, even at minimal doses, indicates that *U. dioica* has highly effective HO• scavengers closer to the standard antioxidant BHT.

The significantly lower IC_{50} in the HO• assay indicates that *U. dioica* is especially potent against hydroxyl radicals. Due to HO• having a half-life of nanoseconds and instantly reacting with surrounding molecules, efficient scavengers need to be available at the production site. The vigorous

activity of *U. dioica* suggests it may provide cellular defense against oxidative DNA damage and lipid peroxidation (Halliwell & Gutteridge, 2015). This endorses the ethnomedicinal application of nettle for chronic inflammatory conditions and wound healing, where HO•-induced tissue damage plays a significant role. The close equivalence to BHT implies that *U. dioica* might be investigated as a natural alternative antioxidant in food and pharmaceutical products.

Hydrogen Peroxide Assay

Hydrogen peroxide is a stable non-radical reactive oxygen species [ROS] capable of crossing biological membranes. It can produce the extremely harmful hydroxyl radical [HO•] through the Fenton reaction, resulting in oxidative harm to DNA, lipids, and proteins (Halliwell & Gutteridge, 2015). Consequently, the capacity of plant extracts to eliminate H₂O₂ serves as a vital sign of their antioxidant and cytoprotective abilities. *U. dioica* and BHT showed an increase in H₂O₂ scavenging that depended on concentration. The inhibition rose significantly from 0-250 µg/mL and then slowly leveled off. At 1000 µg/mL, *U. dioica* reached about 85% inhibition, while BHT reached approximately 93% inhibition.

Excess H_2O_2 leads to oxidative stress in diabetes, heart diseases, and neurodegenerative disorders (Halliwell & Gutteridge, 2015). By scavenging H_2O_2 , *U. dioica* extract could aid in inhibiting the generation of $HO^\bullet$ and ensuing cellular harm. This reinforces the conventional application of nettle as herb for detoxification and anti-inflammation. While BHT exhibited greater effectiveness, worries regarding the toxicity of synthetic antioxidants (Carocho & Ferreira, 2013) position *U. dioica* as a viable natural substitute for nutraceutical and pharmaceutical uses.

Anti-inflammatory Scavenging Assay

The Human Red Blood Cell [HRBC] membrane stabilization test is a commonly utilized *in-vitro* model to assess the anti-

inflammatory properties of plant extracts. Inflammation triggers the release of lysosomal enzymes and reactive species that compromise cell membranes. Substances that stabilize the erythrocyte membrane against lysis caused by hypotonic conditions are deemed to exhibit anti-inflammatory properties, as the stabilization of the lysosomal membrane inhibits the release of inflammatory mediators (Sakat *et al.*, 2010). *U. dioica* demonstrated its highest anti-inflammatory effect in the protease assay compared to the three anti-inflammatory tests. This indicates that inhibiting protease could be a principal way *U. dioica* demonstrates its anti-inflammatory properties.

Table 2: The Calculated IC_{50} Parameters Across all Three (3) Anti-inflammatory Assays

Assay	Plant Extract / Standard	IC_{50} ($\mu g/mL$)	Potency
MD	Diclofenac	43.38	Lowest capacity
	<i>U. dioica</i>	66.41	
PD	Diclofenac	31.03	Partially high
	<i>U. dioica</i>	62.89	
Protease	Diclofenac	12.21	Highest capacity
	<i>U. dioica</i>	52.02	

The figures below display the percentage of inhibition of hemolysis caused by hypotonicity from *Urtica dioica* leaf extract in comparison to the standard anti-inflammatory

medication, Diclofenac sodium, at concentrations ranging from 0 to 1000 $\mu g/mL$.

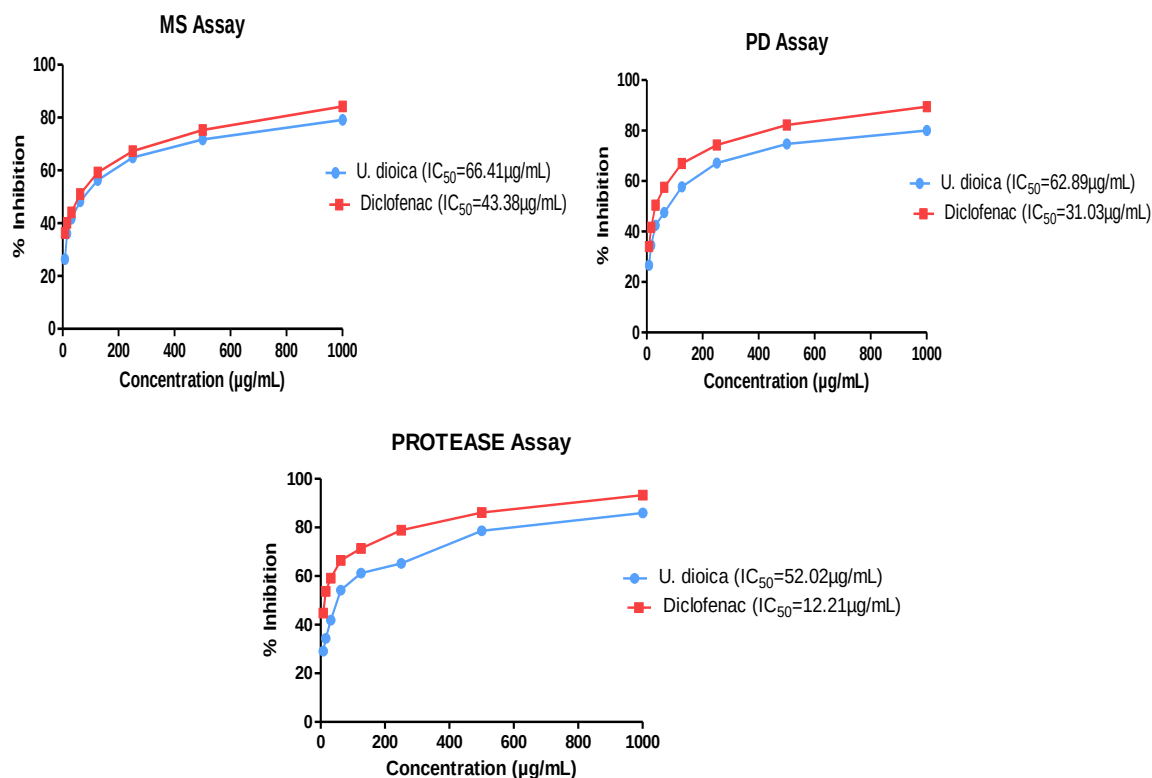


Figure 3: MS = Membrane Stabilization, PD = Protein Denaturation, P = Protease

Membrane Stabilization Assay

U. dioica and Diclofenac both showed an increase in membrane stabilization that depended on their concentrations. A swift rise in % inhibition occurred from 0-250 $\mu g/mL$, followed by a leveling off of the curves. At a concentration of 1000 $\mu g/mL$, *U. dioica* reached approximately 79% inhibition, whereas Diclofenac reached around 84% inhibition. This trend suggests that bioactive components in

U. dioica may interact with the erythrocyte membrane, potentially by attaching to membrane proteins or inhibiting lipid peroxidation, thus preserving membrane integrity (Sakat *et al.*, 2010).

Diclofenac was around 1.5 times stronger than *U. dioica*. Nonetheless, a crude plant extract's IC_{50} of 66.41 $\mu g/mL$ is regarded as noteworthy. The similar IC_{50} values indicate that *U. dioica* exhibits significant anti-inflammatory effects akin

to those of a standard nonsteroidal anti-inflammatory drug (NSAID). The anti-inflammatory properties of *U. dioica* are linked to its phytochemical components, including flavonoids, phenols, and sterols. Gülçin *et al.* (2010) found that *U. dioica* includes quercetin, kaempferol, and derivatives of caffeic acid, which are recognized for their ability to inhibit inflammatory mediators and stabilize cell membranes. Flavonoids are recognized for their ability to reduce the synthesis of prostaglandins and leukotrienes by blocking cyclooxygenase and lipoxygenase enzymes (Rathee, *et al.*, 2009).

Inflammation plays a crucial role in arthritis, asthma, and various other chronic conditions. The stabilization of lysosomal membranes by *U. dioica* suggests it could inhibit the release of proteases and bactericidal enzymes responsible for tissue injury during inflammation (Sakat *et al.*, 2010). This finding backs the conventional use of stinging nettle for rheumatism, joint discomfort, and various inflammatory issues. In contrast to synthetic NSAIDs like diclofenac, known for gastrointestinal and renal side effects, plant-derived anti-inflammatories such as *U. dioica* are frequently better tolerated (Gülçin *et al.*, 2010). Oxidative stress is closely associated with inflammation. The potent antioxidant properties of *U. dioica* noted in DPPH, NO, HO•, and H₂O₂ tests probably play a role in its ability to stabilize membranes.

Protein Denaturation Assay

Protein denaturation (PD) is a well-established contributor to inflammation and tissue injury. In inflammatory states, the denaturation of tissue proteins results in the creation of auto-antigens that may initiate arthritic responses [Sakat *et al.*, 2010]. Agents capable of preventing protein denaturation are thus regarded as having anti-inflammatory potential. The PD assay utilizes egg albumin as a model protein to assess how well plant extracts can safeguard proteins from denaturation caused by heat. *U. dioica* and Diclofenac showed a concentration-dependent rise in the inhibition of protein denaturation. A significant rise was noted between 0-250 µg/mL, with the curves leveling off past 500 µg/mL. At a concentration of 1000 µg/mL, *U. dioica* attained around 80% inhibition, whereas Diclofenac reached approximately 88% inhibition. This suggests that phytochemicals found in *U. dioica* can attach to proteins and inhibit structural unfolding during stress conditions, akin to the action of NSAIDs (Sakat *et al.*, 2010).

The prevention of protein denaturation is significant in conditions like rheumatoid arthritis, where denatured proteins serve as antigens and trigger immune reactions (Sakat *et al.*,

2010). *U. dioica* might aid in decreasing inflammation and pain linked to these conditions by inhibiting denaturation. This finding, along with the data from the Membrane Stabilization (MS) assay (IC₅₀ = 66.41 µg/mL), supports that *U. dioica* has two anti-inflammatory mechanisms: protecting membranes and stabilizing proteins. This combined approach might provide a benefit compared to single-target synthetic medications like diclofenac, commonly linked to side effects such as gastric ulceration.

Protease Inhibitory Assay

Proteases are enzymes that facilitate the breakdown of proteins through hydrolysis. In the course of inflammatory reactions, lysosomal proteases like elastase and cathepsins are secreted by neutrophils into adjacent tissues. These enzymes lead to tissue injury, break down the extracellular matrix, and encourage inflammation (Sakat *et al.*, 2010). Consequently, blocking proteases is a crucial process for anti-inflammatory drugs. The protease inhibition assay measures how well plant extracts can block proteolytic activity, typically employing casein or bovine serum albumin as a substrate.

U. dioica and Diclofenac both exhibited an increase in protease inhibition that depended on concentration. A swift increase in % inhibition was observed from 0 to 200 µg/mL, then followed by a gradual leveling off. At 1000 µg/mL, *U. dioica* reached approximately 85% inhibition, whereas Diclofenac reached around 92% inhibition. This indicates that the bioactive compounds in *U. dioica* may attach to the active site of proteases or chelate necessary metal ions for protease function, consequently diminishing tissue-degrading capability (Gülçin *et al.*, 2010). Inhibiting protease release plays a crucial role in controlling chronic inflammatory diseases like arthritis, emphysema, and glomerulonephritis, which involve tissue degradation mediated by proteases (Sakat *et al.*, 2010). By inhibiting protease activity, *U. dioica* could assist in minimizing tissue damage and inflammation.

Antidiabetic (Glucose Uptake) Assay

The glucose uptake assay is an *in-vitro* method employed to assess the antidiabetic properties of plant extracts. It evaluates the capacity of substances to increase cellular glucose utilization or to hinder glucose absorption, thus lowering blood glucose levels (Bnouham *et al.*, 2006). Reduced glucose absorption and use is a significant characteristic of Type 2 diabetes mellitus. Agents derived from plants that enhance glucose absorption are viewed as advantageous for managing diabetes.

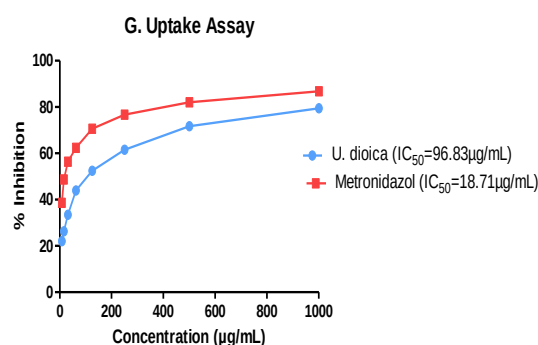


Figure 4: Uptake Assay

The antidiabetic properties of *U. dioica* have been documented earlier and linked to the flavonoids, polysaccharides, and lectins found in the leaves. Gülçin *et al.*

(2010) observed that *U. dioica* can boost insulin secretion and enhance glucose usage. Quercetin and various flavonoids in nettle are recognized for promoting GLUT-4 translocation

and blocking α -glucosidase and α -amylase, which in turn decreases glucose absorption (Sok, et. al. 2021). Both *U. dioica* and Metronidazole showed a concentration-dependent rise in % inhibition. A significant rise was noted from 0-200 $\mu\text{g/mL}$, with the graphs stabilizing past 500 $\mu\text{g/mL}$. At 1000 $\mu\text{g/mL}$, *U. dioica* demonstrated approximately 79% inhibition, whereas Metronidazole showed about 86% inhibition. This suggests that *U. dioica* has bioactive substances that can influence glucose transport.

U. dioica's capacity to block glucose uptake indicates that it could aid in lowering postprandial hyperglycemia by delaying glucose absorption in the intestines (Bnouham et. al., 2006). This mechanism resembles that of acarbose, a common antidiabetic medication.

CONCLUSION

This current research showed that *Urtica dioica* leaf extract has considerable antioxidant, anti-inflammatory, and antidiabetic properties. In antioxidant tests, the extract exhibited significant dose-dependent effects against DPPH, NO, HO $\cdot$, and H $_2$ O $_2$ radicals, with the highest efficacy against hydroxyl radicals (IC $_{50}$ = 18.13 $\mu\text{g/mL}$), similar to BHT. In assessments of anti-inflammatory properties, *U. dioica* successfully stabilized the membranes of human red blood cells (IC $_{50}$ = 66.41 $\mu\text{g/mL}$), inhibited the denaturation of proteins (IC $_{50}$ = 62.89 $\mu\text{g/mL}$), and reduced protease activity (IC $_{50}$ = 52.02 $\mu\text{g/mL}$), suggesting various mechanisms of action. Moreover, the extract demonstrated moderate inhibition of glucose uptake activity (IC $_{50}$ = 96.83 $\mu\text{g/mL}$), reinforcing its antidiabetic potential. While not as effective as the standard BHT, Diclofenac, and Metronidazole, the diverse activity of *U. dioica* indicates potential synergistic effects from its phytochemicals, especially flavonoids and phenolics. These results confirm the conventional application of *U. dioica* and emphasize its promise for advancement as a natural treatment for oxidative stress, inflammation, and diabetes-associated conditions.

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CONFLICT OF INTEREST

There is no conflict of interest.

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REFERENCES

Al-Khayri, J. M., Rashmi, R., Toppo, V., Chole, P. B., Banadka, A., Sudheer, W. N. & Rezk, A. A. S. (2023). Plant

secondary metabolites: the weapons for biotic stress management. *Metabolites*, 13(6), 716.

Altamimi, J. Z., Alfari, N. A., Alshammari, G. M., Algalil, F. M., Al-Sabhan, F. A., Yahya, M. A., & Al-Badr, N. A. (2022). Hydroethanolic extract of *Urtica dioica* L. (stinging nettle) leaves as disaccharidase inhibitor and glucose transport in Caco-2 hinderer. *Molecules*, 27(24), 8872. <https://doi.org/10.3390/molecules27248872>

Bauer, L. S., et. al. (2019). The Role of Natural Products in the Development of Novel Antimicrobials. *Future Microbiology*, 14(4), 435-451.

Bhattarai, S., Shrestha, S., Marasini, B. P., & Chaudhary, R. (2023). Antioxidant activity of *Urtica dioica*: An important property contributing to multiple biological activities. *Plants*, 12(1), 101. <https://doi.org/10.3390/plants12010101>

Bnouham, M., Ziyat, A., Mekhfi, H., Tahri, A., & Legssyer, A. (2006). Medicinal plants with potential antidiabetic activity - A review of ten years of herbal drug research. *International Journal of Diabetes and Metabolism*, 14, 1–25.

Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and chemical toxicology*, 51, 15-25.

Cirillo, V. P. (1962). Mechanism of glucose transport across the yeast cell membrane. *Journal of bacteriology*, 84(3), 485-491.

Devkota, H. P., Paudel, K. R., Khanal, S., Baral, A., Panth, N., Adhikari-Devkota, A., Jha, N. K., Das, N., Singh, S. K., Chellappan, D. K., Dua, K., & Hansbro, P. M. (2022). Stinging nettle (*Urtica dioica* L.): Nutritional composition, bioactive compounds, and food functional properties. *Molecules*, 27(16), 5219. <https://doi.org/10.3390/molecules27165219>

Divekar, P.A.; Narayana, S.; Divekar, B.A.; Kumar, R. et. al. (2022) Plant Secondary Metabolites as Defense Tools against Herbivores for Sustainable Crop Protection. *International Journal of Molecular Sciences*, 23(5): 2690.

Enema, O.J., Umoh, U.F. & Johnny, I.I. (2024). GC-MS Analysis, Quantitative Phytochemical Profile and In-vitro Antioxidant Studies of the Stem back of *Dennettia tripetala*. *European Journal of Med. Plants*. 35(4), 30-41.

Ghorbani, Z., Samavati, M., Hedayati, M., & Elahi, S. (2022). Anti-diabetic potential of *Urtica dioica*: Current knowledge and future direction. *Journal of Diabetes & Metabolic Disorders*, 21(1), 1033–1043. <https://doi.org/10.1007/s40200-022-01021-3>

Gülçin, I., Küfrevioğlu, O. I., Oktay, M., & Büyükkokuroğlu, M. E. (2010). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of Ethnopharmacology*, 90, 205–215. <https://doi.org/10.1016/j.jep.2003.09.028>

Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press, New York.

- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
- Hotamisligil, G. S. (2017). Inflammation, metaflammation and immunometabolic disorders. *Nature*, 542(7640), 177-185.
- Okoroh P.N, Duru M.K.C, Onuoha S.C. & madi B.A. (2019) Proximate composition, phytochemical and mineral analysis of the fruit of *Ficus capensis*. *International Journal of Innovative Research and Development*, 8(8): 84-88.
- Pietta, P. G. (2010). Flavonoids as antioxidants. *Journal of Natural Products*, 63, 1035–1042. <https://doi.org/10.1021/np9904509>
- Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., & Kohli, K. (2009). Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy) (Discontinued)*, 8(3), 229-235.
- Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10, 1003–1008. <https://doi.org/10.1093/carcin/10.6.1003>
- Saini, R., Garg, V., Dangwal, K., & Garg, M. (2015). Evaluation of antioxidant and anti-inflammatory activity of *Cassia fistula* bark extract. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7, 321–325.
- Sakat, S., Juvekar, A. R., & Gambhire, M. N. (2010). In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2, 146–155.
- Semalty, M., Adhikari, L., Semwal, D., Chauhan, A., Mishra, A., Kotiyal, R., & Semalty, A. (2017). A comprehensive review on phytochemistry and pharmacological effects of stinging nettle (*Urtica dioica*). *Current Traditional Medicine*, 3(3), 156-167.
- Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, 28(4), 1057-1060.
- Sok Yen, F., Shu Qin, C., Tan Shi Xuan, S., Jia Ying, P., Yi Le, H., Darmarajan, T., ... & Salvamani, S. (2021). Hypoglycemic effects of plant flavonoids: a review. *Evidence-Based Complementary and Alternative Medicine*, 2021(1), 2057333.
- Somani, R., Yadav, A., Singh, R., & Sharma, K. (2024). Potential benefits of *Urtica dioica* and its combination with vitamin E in experimental diabetes. *Journal of Medicinal and Pharmaceutical Clinical Research*, 4(2), 114–125.
- Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., & Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 579[1–2], 200–213. <https://doi.org/10.1016/j.mrfmmm.2005.03.023>

