

Phytocompound Characterization and Antioxidant Activity of *Urtica massaica* Mildbr. Methanolic Leaf Extract as a Potential Source of Neuroprotective Agents for Cognitive Deficit

Patrick Mukabi^{1*}, Wycliffe Arika Makori¹, Daniel Muthee Gaichu²

¹Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya

²Department of Physical Science, Faculty of Science, Engineering and Technology, Chuka University, Chuka, Kenya
Email: *patrickmukabi1@gmail.com

How to cite this paper: Mukabi, P., Makori, W.A. and Gaichu, D.M. (2026) Phyto-compound Characterization and Antioxidant Activity of *Urtica massaica* Mildbr. Methanolic Leaf Extract as a Potential Source of Neuroprotective Agents for Cognitive Deficit. *Advances in Alzheimer's Disease*, 15, 25-39.

<https://doi.org/10.4236/aad.2026.152002>

Received: May 1, 2026

Accepted: June 27, 2026

Published: June 30, 2026

Copyright © 2026 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Cognitive deficit has been associated with oxidative stress-induced neuronal damage, prompting the need to investigate phytochemicals with antioxidant and potential neuroprotective properties. This study aimed at characterizing the phytochemicals and assessing the *in vitro* antioxidant activity of methanolic leaf extract of *Urtica massaica* Mildbr. Gas chromatography-mass spectrometry (GC-MS) was used to characterize the phytochemicals in the extract. Antioxidant activity was assessed using 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferric Reducing Antioxidant Power (FRAP), superoxide radical scavenging, hydroxyl radical scavenging and lipid peroxidation assays. The extract demonstrated DPPH radical scavenging activity with an IC₅₀ of 0.37 mg/mL and reducing activity in the FRAP assay with an EC₅₀ of 0.62 mg/mL. Superoxide and hydroxyl radical scavenging activities produced IC₅₀ values of 147.2 and 70.79 µg/mL, respectively, while inhibition of lipid peroxidation produced an IC₅₀ of 205.4 µg/mL. *U. massaica* methanolic leaf extract contained diverse phytochemicals and demonstrates antioxidant activity through multiple mechanisms, including radical scavenging, reducing activity and inhibition of lipid peroxidation. These findings provide preliminary biochemical evidence supporting further investigation of *U. massaica* as a potential source of antioxidant compounds relevant to oxidative stress associated with cognitive deficit.

Keywords

Cognitive Deficit, Oxidative Stress, Neuroprotective, Phytochemical,

1. Introduction

Cognitive deficit (CD) is characterized by a decline in cognitive processes such as memory, attention, language, and learning. It can result from neurodegenerative diseases (NDDs), brain injury, substance abuse, and aging [1] [2]. Among NDDs, Alzheimer's Disease (AD) is the most prevalent, accounting for 60%-80% of all cases of CD. Other significant NDDs include Parkinson's Disease, Huntington's Disease and Multiple Sclerosis [3]. The precise pathophysiology of CD is still unclear. Numerous hypotheses exist, such as cholinergic, Oxidative Stress (OS), β -amyloid ($A\beta$) deposition and tau protein hyperphosphorylation [4]. Substantial evidence indicate OS begins before onset of CD symptoms in Alzheimer's disease [5].

Oxidative stress is imbalance between formation of reactive oxygen species (ROS) and the body antioxidant system [6]. The ROS include superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) [7]. The brain is rich in polyunsaturated fatty acids (PUFAs) which are quickly damaged by reactive oxygen species (ROS) and it lacks key antioxidants enzymes [8]. Lipid peroxidation, especially of PUFAs on cell membrane [9], disrupts the integrity of cell membranes, leading to increased membrane permeability, loss of membrane fluidity, and results in cell dysfunction [10]. Medicinal plants, presumed to have antioxidant properties, present a promising avenue for research in the quest to alleviate oxidative stress [11].

Medicinal plants and herbal therapies are increasing popularity as complementary and alternative treatments and they are an important source for phytochemicals, but they have received little scientific scrutiny [12]. These phytochemicals include terpenoids, phenols, flavanols, glycosides, anthocyanins, and steroids, which are responsible for the nootropic and cognitive enhancing effects [13]. These phytochemicals not only work synergistically, they may also affect the action of other constituents from the same or different plant species [14]. Phytochemicals can exert antioxidant effects by either through hydrogen and electron donation or by directly targeting the reactive oxygen species [14] [15]. Therefore, characterization and identification of phytochemicals is important and may aid in the documenting and scientific confirmation of medicinal plants, thereby promoting the conservation and sustainable use of plant resources utilized in traditional medicine [16].

The Maasai stinging nettle (*Urtica massaica* Mildbr.) is the most common nettle species in East Africa, specifically eastern Democratic Republic of the Congo, Uganda, Kenya, Rwanda, Burundi, and Tanzania. The soft leaves are edible and cooked alone or combined with other vegetables and served with Ugali [17]-[19]. The stinging nettle plant has been used traditionally also to manage diabetes, high

blood pressure, arthritis and allergies [20]. It is also used to treat sciatica, incipient wasting, cardiac problems, coughs, palsy, gout, intermittent fever, suppression of menstrual flow and rheumatism [17] [21]. *Urtica massaica* antioxidant profile and phytocompound composition data, particularly in relation to management of cognitive deficits, is scarce.

According to the WHO (2023), there is an exponential increase in demand for herbal medicine globally. Plants are a viable substitute due to their affordability, accessibility, and bioactive compounds [22]. This study aimed at investigating the *in vitro* antioxidant activity of the methanolic leaf extract of *Urtica massaica* Mildbr. and characterized its phytocompound using Gas Chromatography-Mass Spectrometry. The finding of this research will add to the expanding corpus of information on alternate therapeutic strategies for the management of cognitive deficit.

2. Materials and Methods

2.1. Plant Collection and Processing

Urtica massaica Mildbr. was collected in its natural habitat at Kaplelach village in Rongai sub-county (latitudes -0.32708 to 0.20146 and longitudes 35.78611 to 36.24127) at an altitude of 1912 meters, Nakuru county, Kenya. It was dried and transported to Biochemistry, Microbiology & Biotechnology laboratory at Kenyatta University. The dried leaves were grounded into a coarse powder with blender and kept in a dry clean container at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), awaiting extraction. The collected plant sample was also taxonomically verified at the National Museum of Kenya and given the accession number K3/H26/2026. Prior to the commencement of the study, approval was applied for from National Commission for Science, Technology and Innovation (NACOSTI); Approval Number: NACOSTI/P/26/4190729.

2.2. Extraction Method

500 g of powdered *U. massaica* M. leaves was added to 2500 ml of absolute methanol and left to macerate for 48 hours on the shaker. A rotary evaporator was used to concentrate the extract in a vacuum at 40°C after filtration with Whatman filter paper No. 1. Extract was then stored at 4°C awaiting analysis [23].

2.3. Experimental Replication

Urtica massaica Mildbr. leaves were collected as a single plant material collection and processed as a single methanolic extraction. The resulting extract was used for the *in vitro* antioxidant assays. For each assay, measurements at each test concentration were performed in triplicate. These triplicate measurements represent repeated assay measurements of the same extract and were therefore treated as technical replicates rather than independent biological replicates. No separate independent plant collections or independent extractions were performed.

2.4. In Vitro Antioxidant Assay

2.4.1. 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

A methanolic dilution of DPPH 0.1 mM was prepared. DPPH methanolic dilution at 2 ml was added to aliquots of extract at 1 ml in the methanol at varying concentrations: 0.0625, 0.1250, 0.2500, 0.5000 and 1.000 mg/mL; triplicates per extract concentration. The mixture's absorbance at 517 nm was determined using a UV-Vis spectrophotometer, after 30 minutes in dark storage at room temperature. Methanol (blank) and standard (Ascorbic acid) served as the controls [24]. Radical scavenging activity percentage was also calculated [25]. Radical scavenging activity percentage was plotted against different concentrations, and the IC₅₀ was graphically calculated.

2.4.2. Ferric Reducing Antioxidant Power (FRAP) Assay

A solution of 10 mM 2,4,6-Tripyridyl-S-triazine (TPTZ) in 40 mM HCl, acetate buffer (300 mM, pH 3.6), and 20 mM FeCl₃ at 1:10:1 (v/v/v) was combined to create the FRAP reagent. Extract solutions at different concentrations (0.0625, 0.125, 0.25, 0.5 and 1.0 mg/mL), with BHT at similar concentration as a positive control, were introduced at 100 µL to 3400 µL of FRAP reagent in each well and mixed carefully. After 30 minutes, absorbance was measured using UV-Vis spectrophotometer at 593 nm. The graph of absorbance at 593 nm against extract concentrations was used to calculate the extract and standard concentrations that can produce an absorbance value of 0.5, which was then regarded as the effective concentration (EC₅₀) [26].

2.4.3. Superoxide Radical Scavenging (SORS) Assay

This activity was assessed by the reduction of nitro blue tetrazolium (NBT) [27]. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system produces superoxide radicals, which transform nitro blue tetrazolium (NBT) into a purple formazan. The 1 ml reaction mixture included phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM), and different doses of *U. massaica* (50, 100, 150, 200, and 250 µg/ml). After 5 minutes of incubation at ambient temperature, the absorbance at 562 nm was measured against a blank to assess the amount of formazan produced. All tests were repeated three times. Quercetin was utilized as a positive control. Radical scavenging activity percentage was also calculated [25]. Radical scavenging activity percentage was plotted against different concentrations, and the IC₅₀ was graphically calculated.

2.4.4. Hydroxyl Radical Scavenging (HORS) Assay

The reaction mixture was prepared by adding ferric chloride (10 mM), 2-deoxyribose (2.8 mM) in 50 mM phosphate buffer (pH 7.4), EDTA (1 mM, 1:1 v/v), and hydrogen peroxide (10 mM) in that order. To start the reaction, 0.1 mL of extract at various concentrations (15.625, 31.25, 62.5, 125, and 250 µg/mL) and gallic acid were added separately. The mixes were incubated at 37°C for one hour. After in-

cubation, 1 mL of 10% (w/v) trichloroacetic acid (TCA) and 1 mL of 0.5% (w/v) Thio-barbituric acid (TBA) produced in 50 mM NaOH were added, and the mixture was heated in a boiling water bath for 15 minutes before cooling to room temperature. The absorbance of the resultant chromogen was measured at 560 nm with a spectrophotometer [28]. The hydroxyl radical scavenging activity was also calculated [25]. Radical scavenging activity percentage was plotted against different concentrations, and the IC₅₀ was graphically calculated.

2.4.5. Lipid Peroxidation (LPO) Antioxidant Assay

A modified Thio-barbituric acid reactive substances (TBARS) method was employed to evaluate the *in vitro* lipid peroxidation inhibitory activity of the methanolic leaf extract of *Urtica massaica*, with ascorbic acid used as the standard reference antioxidant. The reaction mixture consisted of 0.5 mL of linoleic acid emulsion prepared in phosphate buffer (50 mM, pH 7.4) and 0.1 mL of the extract at different concentrations (50, 100, 150, 200, and 250 µg/mL). A parallel set of reactions containing ascorbic acid at corresponding concentrations was prepared as the standard. Lipid peroxidation was initiated by the addition of 0.1 mL of ferrous sulfate (FeSO₄, 0.07 mM) and incubation at 37°C for 1 h. After incubation, 1 mL of trichloroacetic acid (TCA, 10% w/v) and 1 mL of Thio-barbituric acid (TBA, 0.5% w/v in 50 mM NaOH) were added. The mixture was heated in a boiling water bath for 15 min, cooled to room temperature, and centrifuged to remove precipitated material. The absorbance of the resulting pink chromogen was measured at 532 nm using a spectrophotometer. A control containing all reagents except the extract was prepared simultaneously [29]. The percentage inhibition of lipid peroxidation was also calculated [25]. Radical scavenging activity percentage was plotted against different concentrations, and the IC₅₀ was graphically calculated.

2.5. Calculation of Antioxidant Activity and IC₅₀/EC₅₀ Values

Antioxidant activity and half-maximal inhibitory/effective concentrations (IC₅₀/EC₅₀) for the DPPH, hydroxyl, superoxide, LPO, and FRAP assays were determined using concentration-response regression models. For the radical scavenging i.e. DPPH, hydroxyl, superoxide and lipid peroxidation (LPO) assays, percentage inhibition was calculated using the equation [25]:

$$\text{Lipid Peroxidation Inhibition /Radical Scavenging Activity \%} = \frac{A_b - A_s}{A_b} \times 100$$

A_b —absorbance of the blank.

A_s —absorbance of the sample.

To determine IC₅₀ values for these assays, percentage inhibition was plotted against log₁₀-transformed concentrations, and values were derived from the fitted regression equations. For the FRAP assay, the EC₅₀ value was estimated using a quadratic regression model of absorbance against log₁₀-transformed concentration. Precision for all estimated IC₅₀ and EC₅₀ values was expressed using 95% confidence intervals (CI) calculated from the fitted models.

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Assessment

Only 1 µL of the sample was injected. The SH-Rxi-5Sil MS capillary GC column; 30 m, 0.25 mm ID, 0.25 µm film thickness was used for separation. Ultrapure helium was used as the carrier gas at a flow rate of 1.0 ml/min. Injection temperature was set to 280°C. Split Injection mode was used, at a ratio of 10:1. The oven temperature program was set as follows: 50°C (1 minutes); 5°C/min to 150°C (5 min); 10°C/min to 300°C (5 min). Total run time was 46 minutes. The sample was analysed in full scan mode in the range 50 - 700 m/z. Solvent cut – time was set at 3.0 minutes. Ion source temperature was set at 200°C and interface temperature was set at 280°C. Mass spectra of eluted compounds were matched against the NIST 17 and FFNSC-3 Library (Flavours and Fragrances, Natural and Synthetic Compounds Library).

2.7. Data Management and Analysis

Data obtained was first enter into MS excel spread sheet. Descriptive analysis was carried out and shown as mean ± SEM. Inferential analysis was carried out in Minitab statistical software, v19. Significant differences among the group-means were tested by one way ANOVA followed by Tukey's post hoc test. $p < 0.05$ values were regarded as statistically significant. Data were presented using both graphs and tables.

3. Results

3.1. In Vitro Antioxidant Results

3.1.1. 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The *in vitro* DPPH radical scavenging activity of the methanolic leaf extract of *Urtica massaica* demonstrated a concentration dependent decrease in absorbance, as concentration increased from 0.0625 to 1.000 mg/ml. Comparisons revealed that the standard (Ascorbic Acid) exhibited significant DPPH free radical scavenging activity than *U. massaica* at corresponding concentrations. Also, the concentrations required to scavenge 50% of DPPH radicals (IC_{50}) was determined, ascorbic acid demonstrated an IC_{50} value of 0.22 mg/mL compared to *U. massaica* extract of 0.37 mg/mL (Table 1; $p < 0.05$).

3.1.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric reducing antioxidant power activity of the methanolic leaf extract of *Urtica massaica* result demonstrated a concentration dependent increase in absorbance, as concentration increased from 0.0625 to 1.000 mg/ml. Comparisons revealed that the standard (Butylated hydroxytoluene (BHT)) showed a statistically significant difference in reducing activity compared to *U. massaica* at corresponding concentrations. Additionally, the effective concentration required to produce an absorbance value of 0.5 (EC_{50}) was determined, BHT exhibited an EC_{50} value of 0.40 mg/mL compared to that of *U. massaica* extract of 0.62 mg/mL (Ta-

ble 2; $p < 0.05$).

Table 1. Below shows DPPH free radical scavenging assay absorbance values of *Urtica massaica* extract and ascorbic acid at different concentrations.

Concentration (mg/ml)	Absorbance	
	<i>U. massaica</i>	Ascorbic Acid
0.0625	0.61 ± 0.0056^A	0.52 ± 0.0018^B
0.1250	0.49 ± 0.0053^A	0.41 ± 0.012^B
0.2500	0.40 ± 0.0087^A	0.34 ± 0.0045^B
0.5000	0.36 ± 0.0040^A	0.27 ± 0.0020^B
1.000	0.21 ± 0.0047^A	0.14 ± 0.0029^B
IC ₅₀	0.37 mg/ml	0.22 mg/mL

Values are expressed as mean $\pm$ SEM; Values with different uppercase superscript letter within the same row and those with the different lowercase subscript letter within the same column are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's test).

Table 2. Below shows Ferric Reducing Antioxidant Power (FRAP) assay absorbance values of *Urtica massaica* extract and BHT at different concentrations.

Concentration (mg/ml)	Absorbance	
	<i>U. massaica</i>	BHT
0.0625	0.13 ± 0.0025^B	0.16 ± 0.0020^A
0.1250	0.20 ± 0.0069^B	0.25 ± 0.0017^A
0.2500	0.29 ± 0.0043^B	0.43 ± 0.012^A
0.5000	0.46 ± 0.0058^B	0.51 ± 0.0028^A
1.0000	0.63 ± 0.015^B	0.75 ± 0.0069^A
EC ₅₀	0.62 mg/ml	0.40 mg/ml

Values are expressed as mean $\pm$ SEM; Values with different uppercase superscript letter within the same row and those with the different lowercase subscript letter within the same column are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's test).

3.1.3. Superoxide Radical Scavenging (SORS) Assay

The *in vitro* superoxide radical scavenging activity of the methanolic leaf extract of *Urtica massaica* results demonstrated a concentration dependent decrease in absorbance, as concentration increased from 50 to 250 μ g/ml. Comparisons revealed that the Quercetin (standard) exhibited significant superoxide radical scavenging activity than *U. massaica* at corresponding concentrations. Additionally, the concentrations required to scavenge 50% of the superoxide radicals (IC₅₀) were determined, Quercetin demonstrated an IC₅₀ value of 89.13 μ g/mL compared to that of *U. massaica* extract of 147.2 μ g/mL (**Table 3**; $p < 0.05$).

3.1.4. Hydroxyl Radical Scavenging (HORS) Assay

The *in vitro* hydroxyl radical scavenging activity of the methanolic leaf extract of

Urtica massaica results demonstrated a concentration dependent decrease in absorbance, as concentration increased from 15.625 to 250 µg/ml. Comparisons revealed that the Gallic acid (standard) exhibited significant hydroxyl radical scavenging activity than *U. massaica* at corresponding concentrations. Additionally, the concentrations required to scavenge 50% of hydroxyl radicals (IC₅₀) was determined, Gallic acid exhibited an IC₅₀ value of 37.15 µg/mL compared to that of *U. massaica* extract of 70.79 µg/mL (Table 4; $p < 0.05$).

Table 3. Below shows superoxide radical scavenging assay absorbance values of *Urtica massaica* extract and Quercetin at different concentrations.

Concentration (µg/ml)	Absorbance	
	<i>U. massaica</i>	Quercetin
50	0.33 ± 0.0032 ^A	0.28 ± 0.0035 ^B
100	0.28 ± 0.0064 ^A	0.22 ± 0.0045 ^B
150	0.21 ± 0.0038 ^A	0.16 ± 0.0028 ^B
200	0.19 ± 0.0029 ^A	0.13 ± 0.0014 ^B
250	0.15 ± 0.0018 ^A	0.10 ± 0.0023 ^B
IC ₅₀	147.2 ug/ml	89.13 ug/ml

Values are expressed as mean ± SEM; Values with different uppercase superscript letter within the same row and those with the different lowercase subscript letter within the same column are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's test).

Table 4. Below shows Hydroxyl radical scavenging assay absorbance values of *Urtica massaica* extract and Gallic acid at different concentrations.

Concentration (µg/ml)	Absorbance	
	<i>U. massaica</i>	Gallic acid
15.625	0.71 ± 0.0064 ^A	0.57 ± 0.0081 ^B
31.25	0.55 ± 0.0064 ^A	0.45 ± 0.0064 ^B
62.5	0.48 ± 0.0042 ^A	0.40 ± 0.002 ^B
125	0.35 ± 0.002 ^A	0.24 ± 0.0029 ^B
250	0.24 ± 0.0036 ^A	0.20 ± 0.0039 ^B
IC ₅₀	70.79 ug/ml	37.15 ug/ml

Values are expressed as mean ± SEM; Values with different uppercase superscript letter within the same row and those with the different lowercase subscript letter within the same column are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's test).

3.1.5. Lipid Peroxidation (LPO) Inhibition Assay

The *in vitro* Lipid peroxidation Inhibition activity of the methanolic leaf extract of *Urtica massaica* results demonstrated a concentration dependent decrease in absorbance, as concentration increases from 50 to 250 µg/ml. Comparisons revealed that the ascorbic acid (standard) exhibited significant lipid peroxidation

inhibition activity than *U. massaica* at corresponding concentrations. Furthermore, the concentrations required to achieve 50% inhibition of lipid peroxidation (IC₅₀) was determined, ascorbic acid demonstrated an IC₅₀ value of 173.2 µg/mL compared to that of *U. massaica* extract of 205.4 µg/mL (**Table 5**; $p < 0.05$).

Table 5. Below shows Lipid peroxidation assay absorbance values of *Urtica massaica* extract and Ascorbic acid at different concentrations.

Concentration (µg/ml)	Absorbance	
	<i>U. massaica</i>	Ascorbic acid
50	0.39 ± 0.0064 ^a	0.33 ± 0.0035 ^B
100	0.35 ± 0.0035 ^b	0.29 ± 0.0029 ^B
150	0.28 ± 0.0075 ^c	0.23 ± 0.0038 ^B
200	0.23 ± 0.0064 ^d	0.19 ± 0.0018 ^B
250	0.14 ± 0.0035 ^e	0.11 ± 0.0075 ^B
IC ₅₀	205.4 µg/ml	173.2 µg/ml

Values are expressed as mean ± SEM; Values with different uppercase superscript letter within the same row and those with the different lowercase subscript letter within the same column are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's test).

3.2. GC-MS Phytocompound Analysis of Methanolic Leaf Extract of *Urtica massaica*

GC-MS analysis of the methanolic leaf extract of *Urtica massaica* Mildbr. revealed a tentatively diverse profile of phytocompounds belonging to different chemical classes. The identified class of compounds included benzofurans, phenolics, saturated and polyunsaturated fatty acids, fatty acid methyl esters, diterpene alcohols, monoacylglycerols, long-chain polyunsaturated diols, Erucamide and tocopherols. **Table 6** below presents selected tentatively identified compounds with sufficiently supported library matches and relevant literature information for biological interpretation. Other chromatographic peaks detected in the analysis were not included because their identities could not be established with sufficient confidence, and some were considered possible analytical artifacts or contaminants.

4. Discussion

The present study has demonstrated that the methanolic leaf extract of *Urtica massaica* Mildbr. possesses measurable *in vitro* antioxidant activity across several complementary assay systems. The extract exhibited radical scavenging activity against DPPH, hydroxyl and superoxide radicals, reducing activity in the FRAP assay, and inhibitory activity against lipid peroxidation. The activity observed across these assays indicates that the extract can interact with different aspects of oxidative processes rather than being restricted to a single antioxidant mechanism. This broad antioxidant profile is of particular interest in the context of cognitive deficit, where oxidative stress has been associated with neuronal damage

Table 6. Below shows GC-MS analysis tentatively identified compounds in the methanolic leaf extract of *Urtica massaica* based on comparison with reference mass spectra in the NIST and FFNSC-3 library.

Tentatively identified compound	Compound Class	Similarity Index	Retention Time	Molecular Weight (g/mol)	Peak Area
Benzofuran, 2,3-dihydro-	Benzofurans	93	15.357	120	0.62
2-Methoxy-4-vinylphenol	Phenolics	93	18.011	150	0.72
n-Hexadecanoic acid	Saturated fatty acid	95	33.759	256	8.67
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty Acid Methyl ester	96	35.29	294	1.15
Linolenate < methyl->	Fatty Acid Methyl ester	95	35.356	292	2.04
Phytol	Diterpene Alcohol	96	35.476	296	1.84
9,12-Octadecadienoic acid (Z,Z)-	Polyunsaturated Fatty acid	96	35.691	280	9.38
Stearic acid	Saturated Fatty Acid	88	35.977	284	2.67
13-Docosenamide, (Z)-	Erucamide	77	37.856	337	0.91
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Monoacylglycerol	89	39.117	330	0.96
6,9-Octadecadienoic acid, methyl ester	Fatty Acid Methyl ester	93	40.528	294	3.12
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Long Chain Polyunsaturated Diol	89	40.553	294	7.92
Vitamin E	Tocopherol	94	44.535	430	5.49

and disruption of cell membrane processes involved in normal cognitive function [5] [9] [30].

The *U. massaica* extract demonstrated a concentration-dependent DPPH radical scavenging activity, with an IC_{50} of 0.37 mg/mL compared with 0.22 mg/mL for ascorbic acid. Although the extract exhibited lower activity than the reference antioxidant, its ability to reduce DPPH radicals indicates the presence of constituents capable of donating hydrogen atoms to stabilize free radicals [15]. Such radical-scavenging capacity may be relevant to oxidative processes associated with neuronal vulnerability, since uncontrolled free-radical reactions can contribute to oxidative modification of cellular components [31]. The observed activity therefore provides preliminary evidence supporting the antioxidant potential of *U. massaica* leaf constituents.

The FRAP assay demonstrated a concentration dependent increase in absorbance for both the *U. massaica* extract and BHT. The concentration corresponding to an absorbance of 0.50 was estimated from quadratic regression of absorbance against log10-transformed concentration. Using this approach, the EC_{50} values were 0.62 mg/mL for *U. massaica* extract and 0.40 mg/mL for BHT. Unlike radical scavenging assays, FRAP primarily reflects the ability of antioxidant constituents to reduce ferric ions through electron transfer mechanisms [26]. The presence of measurable reducing activity therefore complements the radical scavenging effects observed in the DPPH assay and suggests that the extract contains compounds capable of participating in electron transfer reactions. The ability to coun-

ter oxidative reactions through electron donation may contribute to limiting oxidative damage under conditions characterized by increased reactive oxygen species generation [32].

The extract also demonstrated activity against biologically relevant reactive oxygen species, including superoxide and hydroxyl radicals. The IC_{50} values for superoxide and hydroxyl radical scavenging were 147.2 and 70.79 $\mu\text{g/mL}$, respectively, compared with 89.13 and 37.15 $\mu\text{g/mL}$ for quercetin and gallic acid, respectively. Although the reference compounds showed greater activity, the extract demonstrated it is able to scavenge these biologically reactive species. This is relevant because superoxide and hydroxyl radicals can participate in oxidative reactions that damage proteins, lipids and nucleic acids [6] [31]. Consequently, the observed activity against these radicals provides a possible biochemical basis for investigating whether *U. massaica* constituents can attenuate oxidative processes associated with neuronal dysfunction and cognitive deficit.

In addition to direct radical scavenging activity, *U. massaica* extract demonstrated an ability to inhibit lipid peroxidation, with an IC_{50} of 205.4 $\mu\text{g/mL}$ compared with 173.2 $\mu\text{g/mL}$ for ascorbic acid. Lipid peroxidation is an important consequence of oxidative stress because oxidation of membrane lipids can alter membrane integrity and generate secondary reactive products capable of modifying cellular proteins and nucleic acid [5] [8]. The inhibition observed in the present study therefore extends the antioxidant profile of the extract to protection against oxidative lipid damage. This finding is particularly relevant to neuronal systems because membrane lipid integrity is important for cellular signaling and normal neuronal function [9].

GC-MS analysis produced library matches consistent with the tentative presence of several phytochemicals, including Benzofuran, 2,3-dihydro-, 2-Methoxy-4-vinylphenol, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z, Z)-, methyl ester, Linolenate < methyl->, Phytol, 9,12-Octadecadienoic acid (Z, Z)-, Stearic acid, 13-Docosamide, (Z)-, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 6,9-Octadecadienoic acid, methyl ester, E, E,Z-1,3,12-Nonadecatriene-5,14-diol and Vitamin E. The detection of compounds such as Benzofuran, 2,3-dihydro-, 2-Methoxy-4-vinylphenol, Phytol and Vitamin E is of particular interest because compounds have been associated with antioxidant activity [33]-[36] and may not only work synergistically, but also affect the action of each other [13]. Also, Erucamide identified as 13-Docosamide, (Z)-, has been reported to possess neurotrophic activity and to promote neuronal and vascular rescue in experimental models of retinal degeneration [37].

The antioxidant activity observed in the present study provides preliminary biochemical evidence that the extract contains phytochemical constituents capable of interacting with oxidative processes that may be relevant to neuronal vulnerability. However, these findings should not be interpreted as direct evidence of neuroprotection. The present *in vitro* assays do not establish protection of neuronal cells or improvement of cognitive functions. Similarly, the GC-MS results

represent tentative library-based compound identifications and do not establish that individual constituents are responsible for the observed antioxidant effects. The observed antioxidant profile therefore provides a rationale for further investigation of *U. massaica* in cellular and *in vivo* models of neuronal oxidative stress and cognitive deficit.

5. Conclusion

The methanolic leaf extract of *Urtica massaica* Mildbr. demonstrated measurable *in vitro* antioxidant activity across several complementary assay systems, including DPPH, FRAP, superoxide and hydroxyl radical scavenging, and inhibition of lipid peroxidation. GC-MS analysis also revealed a diverse phytochemical profile, with several compounds tentatively identified through spectral-library matching. These findings indicate that *U. massaica* leaf extract contains constituents with antioxidant properties that may be relevant to oxidative processes associated with cognitive deficit. However, the present study does not establish direct neuroprotective activity or cognitive enhancement. Further cellular, *ex vivo* and *in vivo* studies, together with isolation and characterization of individual phytochemical constituents, are required to establish the neurobiological relevance and mechanisms of action of the extract.

Acknowledgements

The authors sincerely acknowledge Kenyatta University for providing the facilities and academic environment necessary for the successful completion of this research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Dhakal, A. and Bobrin, B.D. (2020) Cognitive Deficits. StatPearls Publishing.
- [2] Viola, T.W., Orso, R., Florian, L.F., Garcia, M.G., Gomes, M.G.S., Mardini, E.M., *et al.* (2023) Effects of Substance Use Disorder on Oxidative and Antioxidative Stress Markers: A Systematic Review and Meta-Analysis. *Addiction Biology*, **28**, e13254. <https://doi.org/10.1111/adb.13254>
- [3] Barnes, D.E. and Yaffe, K. (2011) The Projected Effect of Risk Factor Reduction on Alzheimer's Disease Prevalence. *The Lancet Neurology*, **10**, 819-828. [https://doi.org/10.1016/s1474-4422\(11\)70072-2](https://doi.org/10.1016/s1474-4422(11)70072-2)
- [4] Ferrari, C. and Sorbi, S. (2021) The Complexity of Alzheimer's Disease: An Evolving Puzzle. *Physiological Reviews*, **101**, 1047-1081. <https://doi.org/10.1152/physrev.00015.2020>
- [5] Roy, R.G., Mandal, P.K. and Maroon, J.C. (2023) Oxidative Stress Occurs Prior to Amyloid A β Plaque Formation and Tau Phosphorylation in Alzheimer's Disease: Role of Glutathione and Metal Ions. *ACS Chemical Neuroscience*, **14**, 2944-2954. <https://doi.org/10.1021/acscchemneuro.3c00486>

- [6] Aranda-Rivera, A.K., Cruz-Gregorio, A., Arancibia-Hernández, Y.L., Hernández-Cruz, E.Y. and Pedraza-Chaverri, J. (2022) RONS and Oxidative Stress: An Overview of Basic Concepts. *Oxygen*, **2**, 437-478. <https://doi.org/10.3390/oxygen2040030>
- [7] Collins, A.E., Saleh, T.M. and Kalisch, B.E. (2022) Naturally Occurring Antioxidant Therapy in Alzheimer's Disease. *Antioxidants*, **11**, Article 213. <https://doi.org/10.3390/antiox11020213>
- [8] Su, L., Zhang, J., Gomez, H., Murugan, R., Hong, X., Xu, D., et al. (2019) Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. *Oxidative Medicine and Cellular Longevity*, **2019**, Article ID: 5080843. <https://doi.org/10.1155/2019/5080843>
- [9] Van der Paal, J., Neyts, E.C., Verlackt, C.C.W. and Bogaerts, A. (2016) Effect of Lipid Peroxidation on Membrane Permeability of Cancer and Normal Cells Subjected to Oxidative Stress. *Chemical Science*, **7**, 489-498. <https://doi.org/10.1039/c5sc02311d>
- [10] Ashraf, M.V., Khan, S., Misri, S., Gaira, K.S., Rawat, S., Rawat, B., et al. (2024) High-Altitude Medicinal Plants as Promising Source of Phytochemical Antioxidants to Combat Lifestyle-Associated Oxidative Stress-Induced Disorders. *Pharmaceuticals*, **17**, Article 975. <https://doi.org/10.3390/ph17080975>
- [11] Komariah, M., Amirah, S., Maulana, S., Abdurrahman, M.F., Ibrahim, K., Platini, H., et al. (2023) The Efficacy of Herbs as Complementary and Alternative Therapy in Recovery and Clinical Outcome among People with COVID-19: A Systematic Review, Meta-Analysis, and Meta-Regression. *Therapeutics and Clinical Risk Management*, **19**, 611-627. <https://doi.org/10.2147/tcrm.s405507>
- [12] Sharma, D., Tyagi, S., Dan, S., Pant, M. and Kumar, A. (2021) Indigenous Therapeutics of Alzheimer: A Review of Ayurvedic Herbs from Its Ethnobotany to Phytotherapy. *Letters in Applied NanoBioScience*, **11**, 4178-4191. <https://doi.org/10.33263/LIANBS114.41784191>
- [13] Gregory, J., Vengalasetti, Y.V., Bredesen, D.E. and Rao, R.V. (2021) Neuroprotective Herbs for the Management of Alzheimer's Disease. *Biomolecules*, **11**, Article 543. <https://doi.org/10.3390/biom11040543>
- [14] Gulcin, İ. and Alwasel, S.H. (2025) Fe³⁺ Reducing Power as the Most Common Assay for Understanding the Biological Functions of Antioxidants. *Processes*, **13**, Article 1296. <https://doi.org/10.3390/pr13051296>
- [15] Jafri, S.A.A., Khalid, Z.M., Khan, M.Z. and Jomezai, N. (2022) Evaluation of Phytochemical and Antioxidant Potential of Various Extracts from Traditionally Used Medicinal Plants of Pakistan. *Open Chemistry*, **20**, 1337-1356. <https://doi.org/10.1515/chem-2022-0242>
- [16] Ghosh, S., Singha, P.S. and Ghosh, D. (2023) Neuroprotective Compounds from Three Common Medicinal Plants of West Bengal, India: A Mini Review. *Exploration of Neuroscience*, **2**, 307-317. <https://doi.org/10.37349/en.2023.00030>
- [17] Nduwamungu, J., Ugirabe, M.A., Mahoro, J., Dusingize, M.C., Kabarungi, M., Irimaso, E., et al. (2024) New Insights into the Indigenous Knowledge of the Uses of the Common Stinging Nettle (*Urtica massaica* Mildbr.) in Rwanda. *Cogent Food & Agriculture*, **10**, Article ID: 2306722. <https://doi.org/10.1080/23311932.2024.2306722>
- [18] Fern, K. (2026). *Urtica Massaica*. Useful Tropical Plants Database. Retrieved from Useful Tropical Plants Database—Urtica Massaica. <https://tropical.theferns.info/viewtropical.php?id=Urtica+massaica>
- [19] Workneh, T.S., Mbugue, D.O. and Bultosa, G. (2026) Processing, Nutrition and Value Addition Principles of Neglected and Underutilized African Foods. Academic Press.

- [20] Kamicha, W., N. Karanja, R. and W. Ngaruiya., G. (2023) Indigenous Use and Commercialization of *Urtica dioica* L. from Local Communities of Kieni West Sub County, Nyeri County, Kenya. *Journal of Environmental Science and Public Health*, **7**, 25-30. <https://doi.org/10.26502/jesph.96120183>
- [21] Devkota, H.P., Paudel, K.R., Khanal, S., Baral, A., Panth, N., Adhikari-Devkota, A., et al. (2022) Stinging Nettle (*Urtica dioica* L.): Nutritional Composition, Bioactive Compounds, and Food Functional Properties. *Molecules*, **27**, Article 5219. <https://doi.org/10.3390/molecules27165219>
- [22] El-Saadony, M.T., Saad, A.M., Mohammed, D.M., Korma, S.A., Alshahrani, M.Y., Ahmed, A.E., et al. (2025) Medicinal Plants: Bioactive Compounds, Biological Activities, Combating Multidrug-Resistant Microorganisms, and Human Health Benefits—A Comprehensive Review. *Frontiers in Immunology*, **16**, Article 1491777. <https://doi.org/10.3389/fimmu.2025.1491777>
- [23] Moriasi, G., Ireri, A. and Ngugi, M. (2020) Cognitive-Enhancing, *ex Vivo* Antilipid Peroxidation and Qualitative Phytochemical Evaluation of the Aqueous and Methanolic Stem Bark Extracts of *Lonchocarpus eriocalyx* (Harms.). *Biochemistry Research International*, **2020**, Article ID: 8819045. <https://doi.org/10.1155/2020/8819045>
- [24] Huang, J.J., Xu, W., Lin, S. and Cheung, P.C. (2016) Phytochemical Profiles of Marine Phytoplanktons: An Evaluation of Their *in Vitro* Antioxidant and Anti-Proliferative Activities. *Food & Function*, **7**, 5002-5017. <https://doi.org/10.1039/c6fo01033d>
- [25] Arika, W., Kibiti, C.M., Njagi, J.M. and Ngugi, M.P. (2019) *In Vitro* Antioxidant Properties of Dichloromethanolic Leaf Extract of *Gnidia glauca* (Fresen) as a Promising Antiobesity Drug. *Journal of Evidence-Based Integrative Medicine*, **24**, 1-17. <https://doi.org/10.1177/2515690x19883258>
- [26] Fernandes, R.P.P., Trindade, M.A., Tonin, F.G., Lima, C.G., Pugine, S.M.P., Munekata, P.E.S., et al. (2016) Evaluation of Antioxidant Capacity of 13 Plant Extracts by Three Different Methods: Cluster Analyses Applied for Selection of the Natural Extracts with Higher Antioxidant Capacity to Replace Synthetic Antioxidant in Lamb Burgers. *Journal of Food Science and Technology*, **53**, 451-460. <https://doi.org/10.1007/s13197-015-1994-x>
- [27] Hazra, B., Biswas, S. and Mandal, N. (2008) Antioxidant and Free Radical Scavenging Activity of *Spondias pinnata*. *BMC Complementary and Alternative Medicine*, **8**, Article No. 63. <https://doi.org/10.1186/1472-6882-8-63>
- [28] Shabbir, M., Khan, M.R. and Saeed, N. (2013) Assessment of Phytochemicals, Antioxidant, Anti-Lipid Peroxidation and Anti-Hemolytic Activity of Extract and Various Fractions of *Maytenus royleanus* Leaves. *BMC Complementary and Alternative Medicine*, **13**, Article No. 143. <https://doi.org/10.1186/1472-6882-13-143>
- [29] Kim, J. (2018) Evaluation of *in Vitro* Antioxidant Activity of the Water Extract Obtained from Dried Pine Needle (*Pinus densiflora*). *Preventive Nutrition and Food Science*, **23**, 134-143. <https://doi.org/10.3746/pnf.2018.23.2.134>
- [30] Abyadeh, M., Gupta, V., Paulo, J.A., Mahmoudabad, A.G., Shadfar, S., Mirshahvaladi, S., et al. (2023) Amyloid- β and τ Protein beyond Alzheimer's Disease. *Neural Regeneration Research*, **19**, 1262-1276. <https://doi.org/10.4103/1673-5374.386406>
- [31] Rossmann, M.P., Dubois, S.M., Agarwal, S. and Zon, L.I. (2021) Mitochondrial Function in Development and Disease. *Disease Models & Mechanisms*, **14**, dmm048912. <https://doi.org/10.1242/dmm.048912>
- [32] Ying, J.F., Lu, Z.B., Fu, L.Q., Tong, Y., Wang, Z., Li, W.F. and Mou, X.Z. (2021) The Role of Iron Homeostasis and Iron-Mediated ROS in Cancer. *American Journal of*

Cancer Research, **11**, 1895-1912.

- [33] Sinha, A., Siddiqui, A., Tripathi, V., Yadav, A., Singh, G., Fatima, N., *et al.* (2025) Green Synthetic Strategies and Medicinal Applications of Benzofuran: A Review. *Proceedings of the International Conference on Sustainable Science and Technology for Tomorrow (SciTech 2024)*, Hyderabad, 16-20 September 2024, 236-254. https://doi.org/10.2991/978-94-6463-876-9_18
- [34] Bustanussalam, B., Hapsari, Y., Rachman, F. and Septiana, E. (2023) Identification of Antioxidant Active Compounds from Watercress (*Nasturtium officinale* R.Br). *Proceedings of the 1st International Conference for Health Research-BRIN (ICHR 2022)*, 23, Jakarta, 23-24 November 2022, 374-384. https://www.researchgate.net/publication/368976127_Identification_of_Antioxidant_Active_Compounds_from_Watercress_Nasturtium_officinale_RBr
- [35] Okpala, E.O., Onocha, P.A. and Ali, M.S. (2022) Antioxidant Activity of Phytol Dominated Stem Bark and Leaf Essential Oils of *Celtis zenkeri* Engl. *Trends in Phytochemical Research*, **6**, 137-144.
- [36] Chen, T.H., Wang, H.C., Chang, C.J. and Lee, S.Y. (2024) Mitochondrial Glutathione in Cellular Redox Homeostasis and Disease Manifestation. *International Journal of Molecular Sciences*, **25**, Article 1314. <https://doi.org/10.3390/ijms25021314>
- [37] Wei, G., Chatterjee, S., Yang, Q., Vijayakumar, S., Ogasawara, D., Giles, S., *et al.* (2026) A Fatty Acid Amide Activates Myeloid Cells and Improves Neurovascular Outcomes in Retinal Degeneration. *Nature Neuroscience*, **29**, 1801-1813. <https://doi.org/10.1038/s41593-026-02341-w>