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# Investigating the Diverse Therapeutic Potential of *Hypericum triquetrifolium* Aerial Parts in Palestine: Ranging from Examining Metabolic Enzyme Inhibition To Neuroprotective Effects

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## ABSTRACT

The buildup of free radicals and metabolic abnormalities is associated with several human illnesses. The use of antioxidants has the potential to mitigate the detrimental effects of free radicals. At the same time, inhibiting enzymes such as lipase and α-amylase show promise in treating metabolic disorders, including diabetes and obesity. Furthermore, the modulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors is paramount in preserving neuronal integrity and regulating brain processes. Therefore, searching for natural plant inhibitors is of utmost importance. This research aimed to examine the hydrophilic extract derived from the aerial portions of *Hypericum triquetrifolium* to assess its potential as a medicinal agent. The extract's capacity to inhibit α-amylase, lipase, and scavenge free radicals was thoroughly evaluated using established biochemical tests in conjunction with whole-cell patch clamp electrophysiological recordings. The findings of our study indicate that the tested substance had remarkable efficacy, outperforming traditional medications in terms of inhibition. The $IC_{50}$ values for the substance were determined to be 75.85 ± 3.88 µg/ml, 630 ± 2.81 µg/ml, and 6.16 ± 5.69 µg/ml, respectively, for the three different pharmaceuticals. Significantly, our research findings indicate that the extract did not exhibit direct inhibition of AMPA receptor subunits. However, it did have a notable influence on their kinetics, particularly on the rates of desensitization and deactivation. This complex botanical extract shows great promise in treating metabolic disorders, neurodegenerative diseases, and illnesses caused by oxidative stress. Furthermore, its impact on the kinetics of AMPA receptors highlights its ability to protect and maintain the integrity of brain cells.

**Keywords:** α-Amylase, anti-lipase, free radical scavenging, *Hypericum triquetrifolium*, GluA2, AMPA receptor

## INTRODUCTION

Medicinal flora involves studying and using plant species that include bioactive compounds with potential therapeutic properties, either for direct use in medical treatments or as starting materials for synthesizing important pharmaceutical drugs. Many historical traditions, nations, and civilizations have relied heavily on plants and their unprocessed derivatives as the primary therapeutic intervention. This practice has persisted for countless millennia. Species with a high abundance of these vital bio-compounds have remarkable effectiveness in treating many illnesses [1, 2].

Within medicinal plants and their bioactive components, it is crucial to emphasize their fundamental significance in tackling intricate health issues, such as diabetes mellitus [3]. Diabetes mellitus is a medical condition closely associated with the metabolism of carbohydrates and proteins, resulting in elevated blood sugar levels during fasting and after meals. This pathological condition emerges due to impaired insulin action or reduced insulin secretion. The significant increase in morbidity and death rates on a global scale has established diabetes mellitus as a substantial obstacle in pharmaceutical research.

Diabetes mellitus poses a substantial worldwide health issue. As of 2021, the global prevalence of diabetes was estimated to be 6.1%, with over 529 million people affected.

This indicates that more than six out of every 100 people worldwide have diabetes. The data revealed a notable correlation between advancing age and a greater frequency of diabetes, with rates surpassing 20% for those aged 65 to 95. Type 2 diabetes constituted 96.0% of the total number of diabetes cases, hence underscoring its significant prevalence. One notable observation indicated that a high body mass index (BMI) was identified as the predominant risk factor for the development of type 2 diabetes, accounting for more than half (52.2%) of the total disability-adjusted life years (DALYs) attributed to type 2 diabetes worldwide. This highlights the need to tackle obesity and advocate for better lives as essential approaches to mitigate the diabetes pandemic and lessen its impact. The projected figures for the future are cause for concern, as it is expected that the worldwide prevalence of diabetes, adjusted for age, will increase by 59.7% by the year 2050. This spike in prevalence has the potential to impact about 1.31 billion individuals, highlighting the urgent need for comprehensive public health initiatives. The predicted increase in question is mostly ascribed to demographic changes and patterns of obesity, with around 49.6% of the expected growth being linked to the prevalence of obesity. The results underscore the pressing need for efficacious public health initiatives aimed at mitigating obesity and addressing the rising diabetes pandemic [4]. The State of Palestine is now experiencing a very worrisome scenario. The prevalence of obesity is significantly elevated, as shown by the respective rates of 42.0% among adult women and 29.5% among adult males aged 18 years and older who are affected by this condition. The rates in question are above average for women (10.3%) and men (7.5%) in the area. Simultaneously, it is projected that diabetes substantially impacts a considerable proportion of the adult population in Palestine, affecting about 20.7% of adult women and 20.1% of adult males. The coexistence of elevated rates of obesity and diabetes in Palestine underscores the urgent need for thorough study and focused initiatives to tackle these significant health issues [5].

The persistent state of elevated blood glucose levels results in a reduction in the flexibility of blood vessels. It encourages the narrowing of the vascular system, ultimately causing a decrease in the delivery of oxygen and blood. As a result, there is a notable escalation in the likelihood of hypertension, accompanied by the emergence of both macrovascular and microvascular problems such as retinopathy, neuropathy, myocardial infarction, and cerebrovascular events [6]. The α-amylase enzyme is synthesized from human saliva and pancreatic juice, and it serves a crucial function in breaking down glycogen and starch into glucose and maltose, resulting in elevated blood sugar levels. Consequently, inhibiting this enzyme may effectively reduce postprandial hyperglycemia [7].

Obesity is one of the most prevalent health problems nowadays, and it is believed to be one of the major causes of tumors and various other health problems. Obesity is often caused by many variables, mainly high caloric food intake and inadequate physical activity, but genetic predisposition can also influence it [8].

Lipase inhibitors reduce the absorption of dietary fat in the body by inhibiting its actions in the gastrointestinal tract lumen, which results in weight loss [9]. Harmful free radicals are essential in significant health issues, such as cardiovascular diseases, cancer, rheumatoid arthritis, Alzheimer's disease, and cataracts [10]. Antioxidant agents are free radical scavenging molecules that neutralize these free radicals before they attack cells, preventing damage to cell lipids, proteins, and carbohydrates. Many natural and synthetic antioxidants have been used to treat human diseases. Such interest in the vital role of antioxidants in human health has permitted research in food science and medicinal herbs, assessing the importance of plants as antioxidants [11].

The flowering plant *Hypericum triquetrifolium* Turra (*H. crispum* L.) is a member of the Hypericaceae family, which includes eight genera and approximately 600 species found worldwide [12]. Over 500 species of small trees, shrubs, and plants comprise *Hypericum's* eight subgenera, the biggest of which is Hypericum [12, 13]. Warm-temperate regions are the native lands of most of the species in this genus, which are

widely distributed worldwide [13, 14]. St. John's wort is a well-known and widely used *Hypericum* medical herb belonging to the *Hypericum* genus of plants (*H. perforatum*). In treating mild to severe depression, it is commonly used as an alternative medication [15]. *H. triquetrifolium* is a plant native to northern Africa, western Asia, and southern and southwest Europe that grows wild in Palestine [16, 17]. It is a perennial herb that grows up to 50 cm high with a dense tangle of thin branches, smooth but dotted with tiny black glands. The leaves are opposite and simple, 5-15 mm long, with wavy ends. Flowers are stalked in groups of 2-5 at the branch end, with five free yellow petals [18].

*H. triquetrifolium* is reported to have a broad spectrum of pharmacological activities, including antioxidant, antibacterial, antinociceptive, anti-inflammatory, and cytotoxic activities [19-26], which could be attributed to its content of secondary metabolites, such as flavonoids, naphthodianthrones, and phloroglucinols [19, 24, 25, 27, 28]. In traditional medicine, the plant is reported to have antispasmodic, astringent, and sedative effects and was used for bile and intestine disorders [29].

Essential oils have long been recognized for their profound influence on the nervous system, modulating various molecular targets to elicit therapeutic effects [2, 30]. Of particular interest are the AMPA receptors, a family of ionotropic glutamate receptors that play a pivotal role in excitatory neurotransmission. AMPA receptors consist of homomeric and heteromeric subunits, collectively governing synaptic plasticity and information processing within the central nervous system [31]. Dysregulation of AMPA receptors is implicated in various neurodegenerative diseases and psychiatric disorders [32]. This study delves into the neuroprotective potential of *H. triquetrifolium* extract, shedding light on its capacity to modulate AMPA receptor activity.

The normal functioning of AMPA receptors is critical for proper synaptic communication, facilitating the rapid transmission of excitatory signals [31, 33]. However, aberrant activation and dysfunction of these receptors have been linked to various neurological disorders, including epilepsy and neurodegenerative conditions. Inhibition of AMPA receptors and regulating their deactivation and desensitization processes have emerged as promising avenues for neuroprotection. This study focuses on *H. triquetrifolium* extract, examining its potential to mitigate AMPA receptor dysfunction and enhance the resilience of the nervous system.

*H. triquetrifolium* has garnered considerable scientific interest as a medicinal plant due to its rich composition of bioactive compounds, thereby presenting several potential pharmacological uses. In most instances, the therapeutic efficacy of medicinal plant extracts is attributed to the collective impact of their many constituents rather than the presence of a single active ingredient. The aqueous extract of *H. triquetrifolium* contains a diverse array of physiologically active constituents. Hence, the present study used a comprehensive approach to examine the hydrophilic extract of this plant for its potential anti-obesity, antidiabetic, neuroprotective, and anti-oxidative properties. The decision has been made to present these results as a cohesive body of work, motivated by our belief in these varied traits' underlying interaction and connectivity. This study aims to emphasize the interconnectedness of these qualities and the overall potential of *H. triquetrifolium* as a therapeutic substance. Our methodology places significant emphasis on the interconnectedness of these discoveries. It presents a consolidated and logical representation of the many therapeutic potentials of this botanical specimen in tackling a range of health-related issues.

## MATERIAL AND METHODS

### *Sample and extraction procedure*

The *H. triquetrifolium* aerial parts (stems, leaves) were collected from the Nablus region of Palestine during the plant's flowering time (June). Pharmacognosist Dr. Nidal Jaradat identified and confirmed this plant in the Herbal Products Laboratory of the Department of Pharmacy at An-Najah National University (Code: Pharm-PCT-1246). The dried plant parts were powdered using a mechanical blender. The extraction of the active substance in our study was based on distilled water. 400 g of dry *H. triquetrifolium*

was weighed and steeped in 4,000 ml of boiling water for an hour. The extract was filtered twice with filter paper and dried in a freeze-drier for 48 hours. Finally, the dried extract was kept in the refrigerator in a closed container at 4 ◦C. The extract yield can be determined using this formula. Yield = (Weight of plant extract/Weight of dry plant X100%), the *H. triquetrifolium* hydrophilic extract yield was 15.37%.

### *Antioxidant capacity assay*

The *H. triquetrifolium* extract solution was gradually diluted to maintain concentrations of 0, 2, 3, 5, 7, 10, 20, 30, 40, 50, and 80 µg/ml using methanol as solvent. Each test tube contained 1 ml of each concentration and was labeled properly. One ml of methanol and one ml of 0.002% methanolic DPPH solution were added to each test tube to prepare 3 ml as the final volume inside each test tube (caution: the preparation steps should be done with minimum light exposure because DPPH is light sensitive). The samples were incubated for 30 min in a dark place, and the spectrophotometer device determined their optical densities at a wavelength of 517 nm. The equation used in this analytical study to calculate the inhibition percentage is shown below:

% DPPH inhibition = (AB−AE)/AB×100%

AB is the recorded absorbance of the blank solution; AE is the recorded absorbance of the *H. triquetrifolium* sample solution.

The plant extract's antioxidant half-maximal inhibitory concentration ($IC_{50}$) was calculated utilizing BioDataFit edition 1.02 [34].

### *α-Amylase Inhibition Assay*

This method was performed by utilizing the assay modified by McCue and Shetty [35]. The following dilutions were prepared: 10, 50, 70, 100, and 500 µg/ml by dissolved plant extract in little milliliters of 10% DMSO and then another dissolved in buffer (0.02 M of $Na_2HPO_4/NaH_2PO_4$, 0.006 M NaCl, at pH 6.9) to give concentrations of 1000 µg/ml. After that, two units/ml of porcine pancreatic α-amylase enzyme solution was freshly prepared in 10% DMSO. A working solution was prepared by mixing 0.2 ml of enzyme solution with 0.2 ml of each hydrophilic extract and then incubated for 10 min at 30 °C. Following the incubation period, 0.2 ml of a freshly prepared 1% aqueous starch solution was added to each working solution. After that, the final solution was incubated for at least 3 min. A 0.2 ml dinitrosalicylic acid (DNSA), a yellow color reagent, was added to stop the reaction. The next step was diluting each working solution with 5 ml of distilled water and then boiling for 10 min in a water bath at 90 °C. The mixture was then cooled to room temperature, and the absorbance was taken at 540 nm. The blank was prepared following the steps above, but the plant extract was replaced with 0.2 ml of the previously described buffer. Acarbose was used as the standard reference, following the same steps used for plant extract.

The following equation was used to calculate the α-amylase inhibitory activity

% α-Amylase inhibitory activity = (AB-AE)/ AB*100%

AB: is the absorbance of blank; AE: is the absorbance of *H. Triquetra folium* hydrophilic extract

### *Porcine pancreatic lipase inhibition assay*

In this study, the porcine pancreatic lipase inhibitory method was used according to [36]. Five solutions were prepared from a 500 µg/ml stock solution from the plant extract in 10% DMSO. The concentrations of these solutions were 0, 10, 50, 100, 500, and 700 µg/ml. Before use, a freshly stock solution was prepared from 1 mg/ml of porcine pancreatic lipase enzyme in Tris–HCl buffer. *P*-nitrophenyl butyrate (PNPB) was prepared by dissolving 20.9 mg in 2 ml of acetonitrile. 0.1 ml of porcine pancreatic lipase and 0.2 ml of each diluted solution series for the plant extract were mixed. Then, a Tri-HCl solution was added to the resulting mixture to get a total volume of 1 ml. After that, the final mixture was incubated at 37 °C for 15 min. The next step was the addition of 0.1 ml of PNPB solution to each test tube and incubated for 30 min at 37 °C. The hydrolysis of the PNPB compound into p-nitrophenolate ions was measured at 410 nm using a UV spectrophotometer to determine the pancreatic lipase activity. Orlistat was used as a positive

control compound by using the same procedure. The following equation was used in this analytical study.

% lipase inhibition = (AB-AE)/AB*100%

Where AB is the recorded absorbance of the blank solution, AE is the recorded absorbance of the sample solution.

The mean values ± SD of standard deviations of all the findings of the *H. triquetrifolium* hydrophilic extract (antioxidant, anti-lipase, and anti-amylase activities) were calculated; results with a p-value of <0.05 were deemed significant.

### *Neuroprotective Assessment of the Hypericum triquetrifolium Extract*

#### *DNA Preparation*

The Preparation of high-copy plasmid DNA (up to 20 μg) using the QIAGEN Plasmid Mini Kit involved a meticulously followed protocol. Initially, a selective agar plate was streaked with the plasmid, and from this plate, a single colony was chosen for a starter culture in the Luria-Bertani (LB) medium, which was then carefully inoculated with the appropriate selective antibiotic. After an incubation period of approximately 8 h at 37°C, during which the bacterial culture thrived, the culture was strategically diluted with 3 ml of selective LB medium and subjected to an additional 12–16 h of incubation at the same temperature to allow for optimal growth and plasmid replication. Post-incubation, the bacterial cells were harvested through a precise centrifugation process, resulting in a distinct pellet. This pellet was then meticulously resuspended in 0.3 ml of Buffer P2, and with great care, the mixture was gently inverted to ensure thorough homogenization. Subsequently, the mixture tubes were centrifuged to obtain the supernatant rich in plasmid DNA. In the subsequent step, a QIAGEN-tip 20 was diligently equilibrated with 1 ml of Buffer QBT, and the supernatant containing the precious genetic material was applied to the QIAGEN-tip 20, ensuring a gravity-driven entry into the resin. Buffer QC was employed to wash the QIAGEN-tip, removing impurities and irrelevant substances while retaining the plasmid DNA. The DNA was then eluted with precision and thoroughness using 0.8 ml of buffer QF. Isopropanol was introduced into the eluted solution to induce the precipitation of the DNA. The solution was well mixed and promptly subjected to centrifugation. After this crucial step, the supernatant was meticulously decanted, ensuring no loss of the DNA pellet formed during the process. In the subsequent washing step, ethanol was employed to wash the DNA pellet, following another round of precise centrifugation. Extreme caution was exercised to remove the supernatant carefully without disturbing the pellet containing the coveted plasmid DNA. Finally, the pellet was air-dried under controlled conditions to ensure optimal preservation of the DNA. In the last step of this meticulous process, the DNA pellet was re-dissolved in an appropriate buffer volume, underlining the need for precision and accuracy. The DNA concentration and yield, crucial for the success of subsequent experiments, were determined by spectrophotometric analysis at 260 nm, and quantitative analysis on an agarose gel was carried out, strictly adhering to protocols that ensure readings between 0.1 and 1.0 for reliable DNA quantification. This rigorous and precise protocol guaranteed the production of high-quality, high-copy plasmid DNA essential for the success of our research endeavors.

#### *cDNA Transient Transfection in HEK293t Cells*

The Human Embryonic Kidney 293t (HEK293t) cell line was chosen for its well-established molecular and cellular biology research use. HEK293t cells are easy to culture, transfect, and maintain, making them popular for studying protein expression and function. They exhibit high transfection efficiency, crucial for successfully expressing transfected cDNA constructs. The first step involved preparing a transfection mixture in the cDNA transient transfection process for HEK293t cells. This mixture comprised the desired cDNA plasmids, for instance, the GluA2 isoform or other AMPAR subunits, and a GFP expression vector. The cDNA plasmids and GFP vector were combined in an appropriate ratio, typically using 1 μg of GluA2 and 1 μg of GFP, to create the transfection mixture. After the mixture was prepared, a brief incubation period allowed for

the formation of transfection complexes. The HEK293t cells, which had been cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum and antibiotics, were selected as the host cells for transfection. When the cells reached the desired confluence, the growth medium was aspirated, and the cells were gently washed with phosphate-buffered saline (PBS). The prepared transfection mixture was then added dropwise to the cells, ensuring an even and thorough coverage. The cells and the mixture were gently mixed to facilitate optimal interaction with the transfection reagent. Subsequently, the transfected cells were incubated at 37°C in an atmosphere containing 5% $CO_2$ for a specified duration, typically around 36 h; this incubation period allowed for the successful expression and integration of the transfected cDNA into the host cells. After incubation, the transfected cells were carefully detached and replanted onto coverslips or culture plates pre-coated with Laminin. This replanting step facilitated subsequent experimental procedures, such as electrophysiological recordings. During this process, highly fluorescent cells were identified, indicating successful transfection, and were then selected for further experimental investigations or recordings related to the study of AMPA receptors or other research objectives.

*Cell Culture and Transfection*

HEK293t cells were cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mg/ml streptomycin, and 1 mM sodium pyruvate (Biological Industries; Beit-Haemek, Israel). The cells were incubated at 37°C with 5% CO2 and subcultured biweekly until reaching passage #20. Transfection was performed using jetPRIME (Polyplus: New York, NY) or Lipofectamine 2000 (Invitrogen; San Diego, CA). The selection of transfection reagents, such as jetPRIME and Lipofectamine 2000, is based on their efficiency in delivering cDNA plasmids into the cells. These reagents are optimized for high transfection rates and low cytotoxicity, ensuring a successful transfection process. Post-transfection, cells were incubated for 36 h in 12-well plates before being replated on Laminin-coated coverslips for subsequent electrophysiology recordings.

*Whole-Cell Patch-Clamp Electrophysiology*

-Cell Preparation and Transfection

HEK293t expressing the flip isoform of AMPAR subunits was used for electrophysiological recordings. These cells, obtained from Sigma, Germany, were chosen for their ease of growth, transfection capabilities, and extensive use in cell culture. The AMPAR subunits (GluA1, GluA2, GluA2/3, and GluA1/2) were sub-cloned into pRK plasmids for expression. Transfection involved cotransfecting the AMPAR subunit plasmids along with a GFP-expressing construct. The transfection reagents jetPRIME or Lipofectamine 2000 were used for efficient transfection. Highly fluorescent cells were specifically identified and selected for subsequent electrophysiological recordings.

- Electrophysiology Setup and Data Acquisition

Electrophysiological recordings were conducted 36–48 h post-transfection at a temperature of 22 °C with a membrane potential maintained at -60 mV. A patch was placed before a rapid perfusion system, utilizing a theta tube and a piezoelectric translator for continuous cell washing and extract application. Patch electrodes with 2–4 MΩ resistance were fabricated using borosilicate glass [37].

- Solution Composition

The pipette solution used to fill the patch electrodes consisted of 110 mM CsF, 30 mM CsCl, 4 mM NaCl, 0.5 mM $CaCl_2$, 10 mM trypsin-EDTA solution B (0.25%), 0.05% EDTA, and 10 mM HEPES. The pH was adjusted to 7.2 using CsOH. The extracellular solution, used for bathing the cells, contained 150 mM NaCl, 2.8 mM KCl, 0.5 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM HEPES, with the pH adjusted to 7.4 using NaOH.The composition of the pipette and extracellular solutions is designed to replicate physiological conditions and maintain the ionic balance necessary for the proper functioning of AMPA receptors.

- Recording Procedure and Analysis

A high-speed piezo solution switcher facilitated rapid solution exchange during the recordings, ensuring accurate measurements. The cells were exposed to glutamate and the plant extraction via a double-barrel glass (theta tube). Typical 10%–90% rise times were 200-300 µs, measured from junction potentials at the patch pipette's open tip after recordings.

- Data Analysis and Statistics

Data acquired during the recordings were analyzed using Igor Pro7. Receptor desensitization and deactivation rates were estimated by fitting the current decay curve using appropriate mathematical models. The weighted tau ($\tau_w$) was calculated to obtain a comprehensive measure of decay time. AMPAR-current deactivation ($\tau_w$ deact) and desensitization ($\tau_w$ des) were obtained after applying 10 mM of agonist (glutamate) for 500 ms for desensitization and 1 ms for deactivation, and the weighted tau ($\tau_w$) was calculated as $\tau_w = (\tau f \times af) + (\tau s \times as)$, where af and as are the relative amplitudes of the fast (τf) and slow (τs) exponential component. After fitting the desensitization and deactivation currents, those measurements were taken with two exponentials fitting the current decay starting from 95% of the peak to the baseline current. The double exponential model used two-time constants, τ rise for the rising phase and τ decay for the decay phase of the current. While the decay phase of the current is not always well described using a single exponential, a double exponential is often used to fit the decay phase, and a weighted mean time constant ($\tau_w$) is extracted. Using the weighted mean time constant ($\tau_w$) instead of the single decay time constant is an improved approach. Therefore, an approach based on the use of both the fast (τf) and slow (τs) time constants (τf and τs) is employed. All experiments were repeated in different cells obtained from at least 6 independent transfections (separated in time) at -60 mV potential, pH 7.4, and room temperature (20–23°C). Igor Pro7 (Wave Metrics, Inc) was used for our data analysis. Statistical analyses were performed using GraphPad Prism, employing ANOVA. ANOVA can determine whether there are statistically significant differences in the electrophysiological responses between different experimental groups (e.g., control vs. treated).

## RESULTS

### *α-Amylase suppressant effect*

A concentration of 70 µg/ml of *H. triquetrifolium* hydrophilic extract inhibited the α-amylase activity by 54.11% compared with Acarbose, which in the same concentration inhibited the activity of α-amylase by 66.1%, as shown in Figure 1. However, the α-amylase inhibitory activity $IC_{50}$ values of *H. triquetrifolium* hydrophilic extract and Acarbose were 75.85 ±3.88 and 28.18±1.22 µg/ml, respectively.

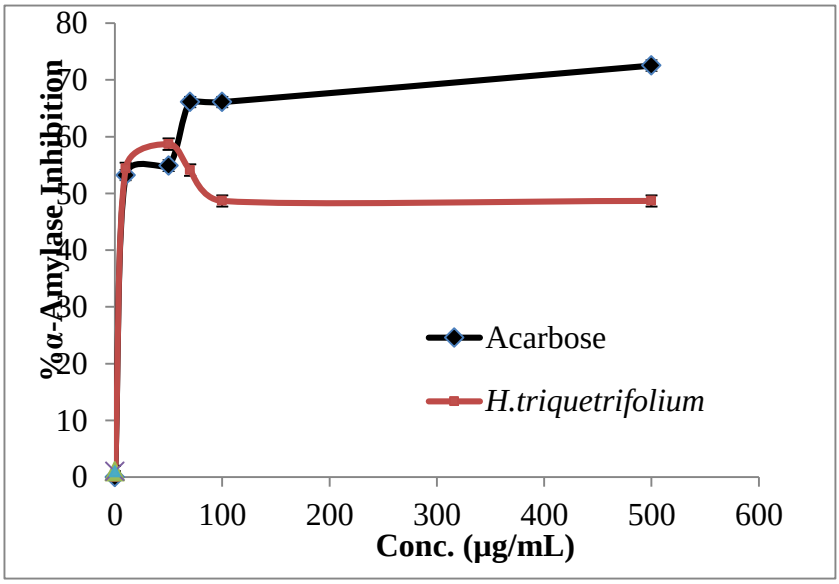


**Figure (1):** α-Amylase inhibitory activity by *H. triquetrifolium* hydrophilic extract and Acarbose.

***Porcine pancreatic lipase inhibitory effect***

Figure 2 depicts that *H. triquetrifolium* hydrophilic extract has an inhibitory effect against porcine pancreatic lipase. At the concentration of 700 µg/ml, the plant extract inhibited 45.71% of the lipase enzyme compared with the potent anti-lipase drug Orlistat, which inhibited at the same concentration of 98.25% of this enzyme. The obtained $IC_{50}$ values were 630 ±2.81 and 12.8 ±0.94 µg/ml, respectively.

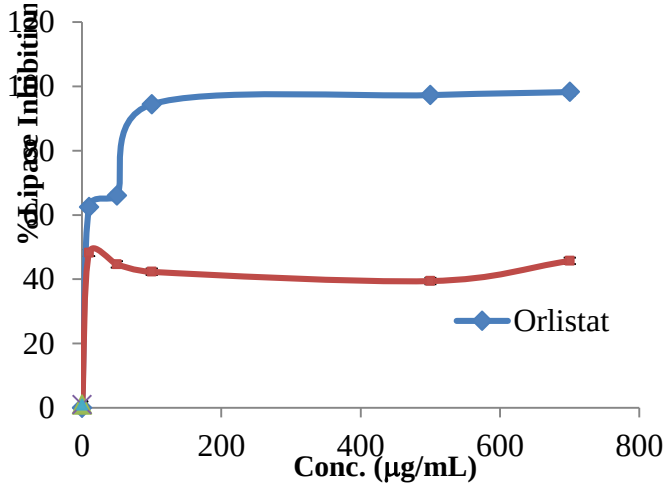


**Figure (2):** Porcine pancreatic lipase inhibitory effects by *H. triquetrifolium* hydrophilic extract and Orlistat.

***Free radicals scavenging effect***

Free radicals have been linked to several harmful pathophysiological processes, including cancer, diabetes, cardiovascular disease, and neurodegenerative disease. Damage caused by free radicals can be prevented and stabilized with the help of antioxidants, which act as electron donors to the damaged cells. They also convert free radicals into waste materials excreted from the body. A diet rich in antioxidant-rich herbs can reduce many diseases caused by free radicals [38]. The outcomes of our research (Fig. 3) revealed that *H. triquetrifolium* hydrophilic extract has a potent free radicals scavenging effect compared with vitamin E analog Trolox. Actually, at the concentration of 80 µg/ml, *H. triquetrifolium* hydrophilic extract notably inhibited the activity of DPPH by 93.21% compared with Trolox, which inhibited DPPH free radicals by 92.65%. However, the obtained $IC_{50}$ values were 6.16 ±5.69 and 2.45±4.23 µg/ml, respectively.

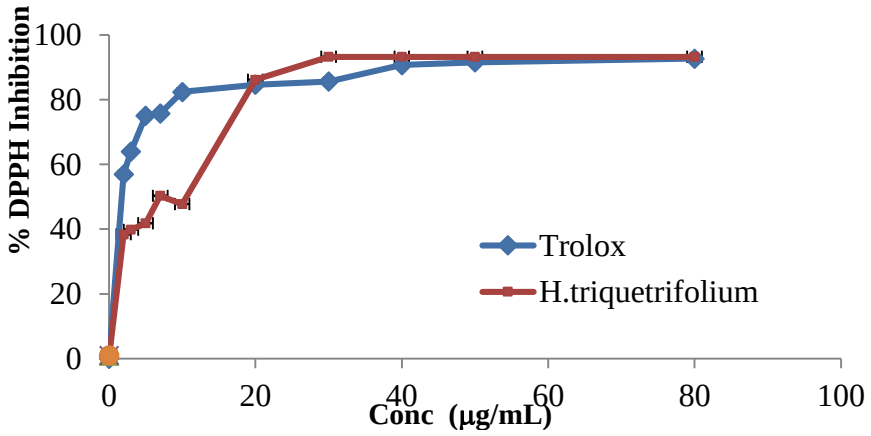


**Figure (3):** DPPH free radical scavenging effect by *H. triquetrifolium* hydrophilic extract and Trolox.

### *Neuroprotective Modulation of AMPA Receptors and Kinetics by Hypericum triquetrifolium Extract*

In our investigation, we sought to elucidate the impact of *H. triquetrifolium* extract on AMPA receptor subunits, including homomeric (GluA1, GluA2) and heteromeric (GluA1/2, GluA2/3) configurations expressed in HEK293t cells. Employing a concentration of 500 μg/ml of the extract, our experimental results, as depicted in Figure 4a, revealed a marginal alteration in the whole-cell current response of these receptor subunits. Specifically, we observed a nearly uniform 1-fold decrease across all subunits. However, it is paramount to underscore that this observed change was statistically nonsignificant. In essence, our findings indicate that *H. triquetrifolium* extract did not exhibit an inhibitory effect on AMPA receptors.

While our study did not unveil a direct inhibition of AMPA receptors by *H. triquetrifolium* extract, it is imperative to recognize the broader context of our investigation. The significance of this plant and other essential oils extends beyond a single target or mechanism of action. These natural compounds often possess a spectrum of bioactive properties, including antioxidant, anti-inflammatory, and neuroprotective attributes. Moreover, their potential therapeutic value might be more pronounced in complex biological systems, such as neuronal networks, where multiple factors contribute to neuroprotection.

Therefore, although our findings do not directly support the inhibition of AMPA receptors by *H. triquetrifolium* extract, they shed light on the intricate nature of botanical compounds. The multifaceted properties of these extracts warrant further exploration, particularly in the context of neuroprotection and the management of nervous system-related disorders. By elucidating the broader effects of such natural substances, we can better understand their potential therapeutic utility in safeguarding the nervous system and overall human health.

To understand the nuanced effects of *H. Triquetrifolium* extract on AMPA receptor subunits, we delved into the kinetics of desensitization and deactivation across homomeric and heteromeric configurations.

The findings of our experiment on the desensitization rates of AMPA receptor subunits following exposure to the extract are shown in Figure 4b. The results of our study indicated that the extract had a differential effect on these rates. The extract had little impact on the desensitization rate of GluA1 subunits, suggesting that this specific subunit exhibited resilience to its action. On the other hand, it was shown that the rate of desensitization of GluA2 subunits was significantly reduced, demonstrating a noteworthy drop of 2-fold. Moreover, when investigating the heteromeric subunits, namely GluA1/2 and GluA2/3, it was revealed that they also exhibited modified rates of desensitization. The former demonstrated a modest reduction of almost one-fold, but the latter showed a more significant drop of around 1.5-fold.

The significance of these findings lies in the critical role desensitization plays in regulating the activity of AMPA receptors, particularly the GluA2 subunit. Reduced desensitization rates can lead to prolonged receptor activation, potentially contributing to heightened excitotoxicity in neuronal networks. Given that GluA2-containing AMPA receptors are associated with calcium permeability, mitigating their overactivation is paramount in preserving neuronal integrity.

Moving on to the deactivation rates, as illustrated in Figure 4c, our results offered further insights into the extract's influence on AMPA receptor kinetics. Remarkably, GluA1 subunits exhibited minimal alteration in deactivation rates, demonstrating the relative resilience of this subunit to the extract's effects.

In contrast, the heteromeric subunits GluA1/2 and GluA2/3 exhibited a roughly equivalent increase in their deactivation rates, about doubling in magnitude. The GluA2 subunits had the most pronounced effect, with a remarkable 1.6-fold reduction in deactivation rates. These modifications' significance lies in the deactivation's impact on regulating AMPA receptors. Accelerated

deactivation rates play a crucial role in promoting the rapid termination of receptor channels, resulting in shorter synaptic currents and enhancing the precision of neuronal communication. The phenomena mentioned above play a crucial role in maintaining the temporal accuracy of synaptic transmission, significantly contributing to the precise adjustment of brain circuits.

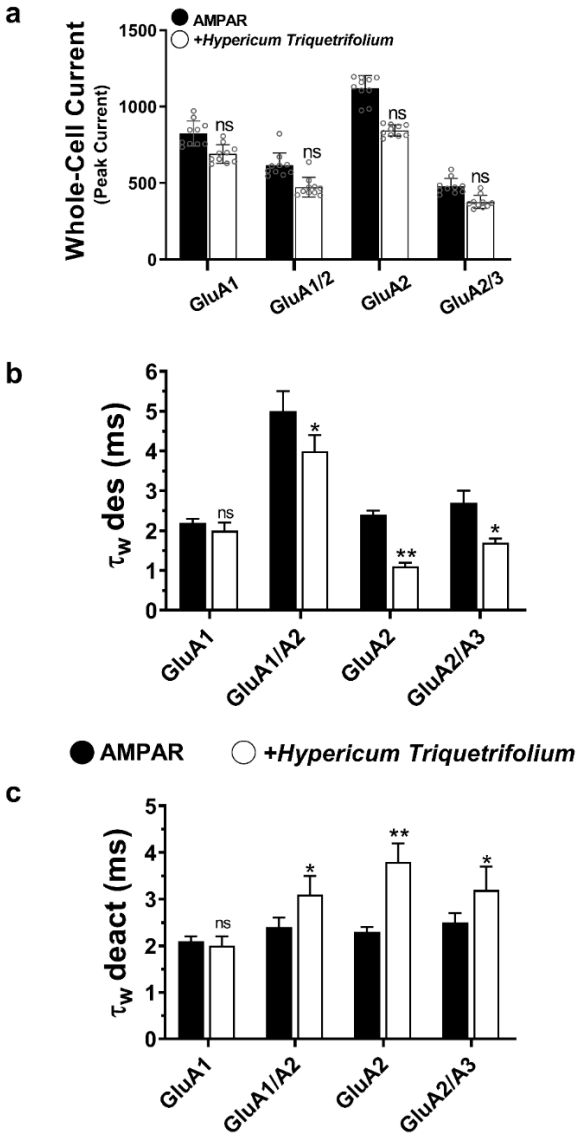


**Figure (4):** Unveiling the Modulatory Effects of *Hypericum triquetrifolium* Extract on AMPA Receptor Subunits and Kinetics. A comprehensive insight into the intriguing influence of *H. triquetrifolium* extract on AMPA receptor subunits expressed in HEK293t cells. Panel **a** showcases the whole-cell current responses (in pA) of these subunits in the presence of glutamate alone (black) and after exposure to 500 µg/ml of *H. triquetrifolium* extract (white). Panels **b** and **c** delve into the tested subunits' kinetics, specifically desensitization rates (τw des) and deactivation rates (τw deact). The traces for all four subunits were meticulously recorded using specialized protocols designed to unveil the intricate dynamics of desensitization and deactivation. Noteworthy, all measurements were meticulously conducted at -60 mV, pH 7.4, and 22°C. Robust statistical analyses employing one-way analysis of variance (ANOVA) reveal the significance levels, with 'ns' denoting non-significance, * $p < 0.05$, and ** $p < 0.01$. Each dataset stems from the meticulous evaluation of 6 cells, and the resulting data is eloquently presented as means ± SEM, with individual data points plotted above each column to illustrate the unique responses of the 6 cells following the extract application.

## DISCUSSION

Plants have a rich history of therapeutic use, and exploring their bioactive compounds continues to be a promising avenue for addressing prevalent global health issues [39]. *H. triquetrifolium*, a native plant of Palestine, has become the focal point of our research, focusing on uncovering its vast therapeutic potential.

The study highlighted the remarkable capacity of *H. triquetrifolium* to effectively suppress α-amylase, a pivotal enzyme that plays a central role in carbohydrate metabolism. This inhibition, while not reaching the potency of Acarbose, introduces a compelling avenue for addressing the complex challenges of diabetes and obesity. By impeding α-amylase, the plant's extract showcases its potential to modulate blood sugar levels and carbohydrate digestion, making it a noteworthy contender in the quest for natural solutions to metabolic disorders.

What further accentuates *H. triquetrifolium's* significance is its substantial inhibitory effect on porcine pancreatic lipase, an enzyme vital in the intricate web of obesity control by limiting the absorption of dietary fats. This finding underscores the plant's potential as a natural anti-obesity agent with a multifaceted approach. By curbing both carbohydrate and fat metabolism, it possesses a unique dual action, offering the promise of a holistic strategy in tackling the pressing global health concern of obesity. These findings offer insights into the plant's therapeutic potential and open up new possibilities for developing novel natural remedies for complex metabolic conditions.

*H. triquetrifolium's* impressive capability for free radical scavenging is equally deserving of our attention. This trait holds paramount importance in the realm of health, particularly in the context of conditions strongly associated with oxidative stress. Diseases such as cancer, diabetes, cardiovascular ailments, and neurodegenerative disorders are all characterized by the excessive generation of harmful free radicals, which can wreak havoc on cells and tissues.

The robust antioxidant potential displayed by *H. triquetrifolium* extract raises it to the forefront as a promising candidate in the domain of natural antioxidants. This is of profound significance, as antioxidants act as frontline defenders against the pernicious effects of oxidative stress. Donating electrons to unstable free radicals stabilizes these rogue molecules, preventing them from inflicting cellular damage and adverse health outcomes.

In a world where the prevalence of oxidative stress-related diseases continues to rise, discovering a potent antioxidant source like *H. triquetrifolium* offers hope. It may shield against oxidative stress's multifaceted and destructive health effects, providing a potential avenue for prevention and therapeutic intervention. This underscores the plant's value as a bioresource and highlights its role in the ongoing quest to combat a range of debilitating and prevalent health conditions.

Intriguingly, the study extended its exploration into the modulation of AMPA receptors by *H. triquetrifolium* extract, hinting at its potential for neuroprotection. While the study did not conclusively demonstrate the direct inhibition of AMPA receptors, it illuminated the intricate nature of botanical compounds. These natural substances often transcend a single mechanism of action, encompassing a spectrum of bioactive properties, including neuroprotective attributes.

The nuances of AMPA receptor kinetics unveiled in the study provide a unique perspective on the potential impact of *H. triquetrifolium* extract on neuronal network activity. This suggests the plant's role in preserving neuronal integrity and fine-tuning the precision of synaptic transmission—a key element in maintaining the brain's overall functionality.

In summary, these findings shed light on the multifaceted nature of *H. triquetrifolium* and its potential contributions to natural therapeutics. From its effects on α-amylase and porcine pancreatic lipase, which bear promise for managing diabetes and obesity, to its potent antioxidant capacity with implications for various health conditions, the

plant exhibits remarkable versatility. The study's insights into the modulation of AMPA receptors underscore botanical compounds' intricate and nuanced nature, promising further exploration in the context of neuroprotection and nervous system-related disorders. This multifaceted plant merits further examination, offering a potential treasure trove of human health and well-being benefits.

## CONCLUSION

Based on other studies that have shown the presence of secondary metabolites, including flavonoids, naphthodianthrones, and phloroglucinols, in *H. triquetrifolium*, our study was centered on exploring the neuroprotective properties of the extracted plant material. The present work investigated the hydrophilic extract's anti-amylase, anti-lipase, and antioxidant properties derived from *H. triquetrifolium*. Based on our analysis, it is hypothesized that the observed effects may be primarily ascribed to the presence of polyphenolic compounds within the extract. The polyphenols have shown the ability to inhibit the enzymes α-amylase and lipase and scavenge free radicals. Significantly, our findings have shown the capacity of the hydrophilic extract derived from *H. triquetrifolium* to impede the kinetics of AMPA receptors and regulate the rates of desensitization and deactivation. The results of this study indicate that *H. triquetrifolium* has potential therapeutic effects for neurodegenerative illnesses, as well as further substantiating its potential for managing diabetes, obesity, and disorders associated with oxidative stress. However, to maximize the therapeutic capabilities of this botanical extract, future research endeavors must prioritize the isolation and characterization of its bioactive constituents and the evaluation of its toxicological and pharmacological impacts on various species.

## Declaration

### Ethics approval and consent to participate

Not applicable.

### Funding

None

### Competing interest

The authors declare that they have no competing interests.

### Consent for publication

All authors have given their consent for publication.

### Author Contributions

Conceptualization, **NJ and MQ**; Methodology, **NJ, MQ, MH, FL, RG, BG, and AJ**; Software, **NJ and MQ**; Formal analysis, **NJ** and **MQ**; Validation, **NJ, MQ, MH, FL, RG, BG, and SB**; Investigation, **NJ, MQ, MH, FL, RG, BG, and AJ**; Resources, **NJ and MQ**; Data Curation**, NJ and MQ**; Characterization, **NJ, MQ, and MH**; Supervision, **NJ**, and **MQ**; Writing – original draft, **NJ and MQ**; Writing - review & editing, **NJ, MQ, MH, FL, RG, BG, and SB.** All of the research findings presented in this study are derived exclusively from the projects conducted by students, except for the electrophysiological experiments.

### Availability of data and materials

All data generated or analyzed during this study are available.

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