SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

NOME E COGNOME: NILOFAR NILOFAR

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TIPOLOGIA DI BORSA RICEVUTA: BORSA SIF PER BREVE PERIODO ALL'ESTERO

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): FINE PERIODO

TITOLO DELLA RELAZIONE: CHEMICAL PROFILING OF NATURAL PRODUCTS FROM SELECTIVE PLANTS FOR THEIR BIOACTIVITY

RELAZIONE:

1. Introduction

The medical literature extensively discusses assertions regarding the disruption of reactive oxygen species (ROS) and free radicals homeostasis. This disruption is often attributed to deficiencies in antioxidant enzymes or non-enzymatic antioxidant networks, resulting in heightened oxidative stress. This stress is implicated in the harmful oxidation and chemical modification of vital biomacromolecules, including DNA, lipids, and proteins. Such modifications play a role in the development of diverse human and animal diseases [1], encompassing conditions such as cancer, atherosclerosis, aging, Alzheimer's, and skin diseases [2, 3]. Moreover, the imbalance of ROS in diabetic mellitus gives rise to complications, including vascular- and neurodegenerative disorders, as well as cardiac dysfunction. Presently, numerous scientific investigations are dedicated to identifying compounds capable of serving as scavengers for free radicals, ROS, and reactive nitrogen species (RNS) in the context of these pathological conditions.

The utilization of herbal medicines is gaining increasing global significance in clinical practices. Plant, are emerging as promising sources of compounds with potential properties including anticancer [4], antibacterial [5], and anti-inflammatory [6] properties. Plant, particularly rich in polyphenolic compounds, demonstrated robust free radical scavenging and antioxidant capabilities [7-9]. Natural products derived from plants also find widespread use as cosmetic or cosmeceutical ingredients due to their ability to decelerate intrinsic skin aging processes and counteract extrinsic factors. Plant anti-aging properties, ascribed to antioxidant metabolites that mitigate free radicals and protect against solar radiation, also involve certain metabolites

influencing enzymes in aging processes, particularly tyrosinase [10], a significant target in cosmetics [11, 12]. Significantly, certain plant metabolite play a pivotal role in the management of Alzheimer's disease (AD) by inhibiting specific enzymes such as acetylcholinesterase (AChE), butyrylcholinesterase (BChE) [13, 14].

Acantholimon, a genus of perennial plants within the Plumbaginaceae family, has been identified for its therapeutic potential. These plants, belonging to the aforementioned genus, were subjected to various tests, including assessments for antimicrobial, antitumor, insecticidal, cytotoxic, and antioxidant activities [15-17]. While pharmacological activities of specific Plumbaginaceae types have been extensively researched, there is a noticeable gap in the literature regarding the biological activities and medical applications of *Acantholimon*, a substantial genus within the family. This study marks the first comprehensive exploration of the chemical composition, antioxidant, and enzyme inhibitory potentials of *Acantholimon acerosum*. Specifically, the research investigates the ethyl acetate (EA), ethanol (EtOH), ethanol-water (50% EtOH), and water (WT) extracts of Acantholimon.

2. Aim of the project

The aim of the project was to analyse the chemical composition and antioxidant activity of the plant *Acantholimon* species extracts. Additionally, this study aimed to investigate specific enzymes highly susceptible to inhibition by plants, including AChE, BChE, α-amylase, α-glucosidase, and tyrosinase. Such inhibition could potentially serve as a therapeutic alternative for certain human diseases, including AD, diabetes, and various skin disorders.

3. Experimental approach

3.1 Total Phenolic and Flavonoids Contents Analysis

The *A. acerosum* extracts phenolic and flavonoid content was assessed using Shakeel et al., method 2023 [18]. For the Folin-Ciocalteu test (phenolic contents), the sample (0.25 mL) was blended with diluted Folin-Ciocalteu reagent (1 mL, 1:9 ratio), stirred, and then after 3 minutes, Na2CO3 solution (1%, 0.75 mL) was added. The solution was incubated at room temperature (RT) for 2 h, the absorbance was measured at 760 nm.

To assess flavonoid content, the extract mixed with 2% aluminium trichloride in methanol. After 10 minutes of incubation at RT, the absorbance at 415 nm was measured. Blank values were subtracted. The results were elucidated using standards, gallic acid (GAE) for phenolics and rutin (RE) for flavonoids.

3.2 *In vitro* Antioxidant Analysis

To evaluate the antioxidant potential of the extract, six complementary *in vitro* spectrophotometric assays were conducted using previously describe method of Sinan et al., [19]. Among these, the ABTS and DPPH assays were employed used to detect the removal capacity of free radicals by plant extracts, gauging its efficacy in combating oxidative stress. Additionally, the FRAP and CUPRAC assays were utilized to examine the extract's reduction capabilities. The antioxidant assessment also included the determination of its metal chelating ability (MCA) and its performance in the phosphomolybdenum (PBD) assay. Through these multifaceted analyses, a thorough understanding of the extract's antioxidant properties was attained.

3.3 Enzyme Inhibition Analysis

The anti-enzymatic properties of the extract were against AChE, BChE, tyrosinase, α-amylase, and α-glucosidase. The enzyme inhibitory assays were conducted in accordance with our previously published data [8]. Inhibition comparisons for AChE and BChE were quantified as mg galanthamine equivalents (GALAE) per gram of extract. Tyrosinase inhibition was expressed in terms of mg kojic acid equivalents (KAE) per gram of extract, while α-amylase and α-glucosidase inhibition were measured as mmol acarbose equivalents (ACAE) per gram of extract.

4. Results and Discussions

4.1 TPC and TFC

Phenolic and flavonoid compounds, possessing various pharmacological benefits, have been derived from the fruits, leaves, and roots of plants for centuries, aiming to address various diseases [20].

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the extracts were presented in Figure 1A and Figure 1B, respectively. The figures indicates that ethanol and 50% ethanol extracts exhibited a higher phenolic content, approximately 100 mg GAE/g. The water extract showed a moderate TPC of 50 mg GAE/g. However, the EA extract gave the lowest TPC among the tested extracts.

Figure 1B showed, EtOH extraction yielded the highest TFC at about 40 mg RE/g, while WT extraction resulted in the lowest TFC at 10.34 mg RE/g. The variations in TPC and TFC among the extracts suggest differences in the concentration of flavonoids depending on the extraction solvent used. A higher TPC and TFC implies a greater concentration of these compounds, suggesting a potentially stronger antioxidant effect in the ethanol extract compared to the water extract. The current findings align with our recently published data, indicating that hydroalcoholic extracts yield higher phenolic and flavonoid contents [21]. Another study conducted by Kiziltas et al. demonstrated that *A. caryophyllaceum* Boiss. aerial part extracts in ethanol and water exhibited elevated phenolic contents [22].

4.2 Antioxidant activity

Figure 2 presents the antioxidant activities of various extracts measured through different assays. Notably, the WT extract consistently exhibited the highest antioxidant activity across assays, indicating its efficacy in neutralizing free radicals and reducing metal ions (Figure 2 A-D), and also showed higher MCA (figure 2E). However, it showed lower antioxidant activity in the PBD assay (figure 2F). In contrast, the EA extract generally demonstrated lower antioxidant activity compared to other extracts. The indicates that choice of solvent played a significant role, with the 50% ethanol extract showing the second-highest removal capacity of free radicals in DPPH (figure 2A) and ABTS assays (figure 2B). The water extract stood out in MCA, suggesting its potential in chelating metal ions. These findings emphasize the importance of considering both the extract type and the assay employed when assessing antioxidant properties. Further exploration of the phytochemical composition of these extracts could provide insights into the specific compounds contributing to their antioxidant activities. Hence, according to Soltanian et al., methanol extracts from three *Acantholimon* species demonstrated robust antioxidant activities [16]. Additionally, the essential oil of *Acantholimon atropatanum* displayed noteworthy free-radical scavenging properties [15].

4.3 Enzyme Inhibition Activity

Figure 3 provides insights into the biological activities of various extracts of *Acantholimon* species, as measured through specific enzyme inhibitory assays. The values are expressed in milligrams of galantamine equivalents per gram (mg GALAE/g) for AChE (figure 3A) and BChE (figure 3B), inhibition assays, millimoles of acarbose equivalents per gram (mmol ACAE/g) for α-amylase(figure 3C) and α-glucosidase inhibition (figure 3D), and milligrams of kojic acid equivalents per gram (mmol ACAE/g) for tyrosinase inhibition (figure 3E).

Both ethanol (EtOH) and 50% ethanol extracts exhibited elevated inhibitory activity against AChE and BChE, with notable values. These findings suggest the potential of this plant in the treatment of neuromodulator disorders. Conversely, the WT extract exhibited less AChE inhibition and negligible activity against BChE. Regarding the glucose digestive enzyme α-amylase, the EA, 50% EtOH and EtOH extracts showed higher and almost similar α-amylase inhibition activity. However, all the extracts, except EtOH, were inactive against α-glucosidase inhibition. Concerning tyrosinase inhibition, the 50% EtOH and EtOH extracts once again displayed higher inhibition compared to water and EA extracts. These results suggest that ethanol and 50% ethanol extracts exhibited promising inhibitory activities against cholinesterase and α-amylase, coupled with notable antioxidant potential. Conversely, the water extract demonstrated comparatively lower bioactivity in enzyme inhibition assays. Our current findings align with the research conducted by Kiziltas et al., indicating that ethanol extracts from *Acantholimon caryophyllaceum* exhibited a notably strong affinity for both α-amylase and α-glycosidase [22].

5. Conclusion

In conclusion, EtOH and 50% EtOH extracts showed the highest phenolic content, while water extract exhibited moderate levels, and ethyl acetate extract had the lowest. The choice of solvent influenced phenolic and flavonoid content, with ethanol extraction yielding the highest as a result exhibited higher enzymes inhibition activity. Regarding antioxidant activity, the water extract consistently performed well, except in the PBD assay. EA extract generally showed lower antioxidant activity. In enzyme inhibition assays, ethanol and 50% ethanol extracts demonstrated higher activity against cholinesterase and α-amylase, while the water extract showed lower bioactivity. These results suggest the potential health benefits of ethanol and 50% ethanol extracts, emphasizing the importance of solvent choice in extraction processes. Further research is needed to identify specific compounds contributing to these activities.

Figure 1 Total phenolic contents (TPC) and total flavonoids contents (TFC)

Figure 1 A.

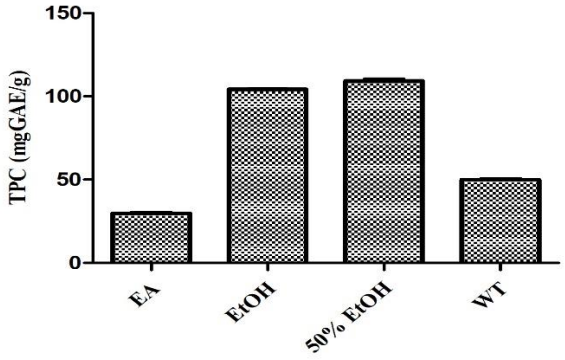


Figure 1 B.

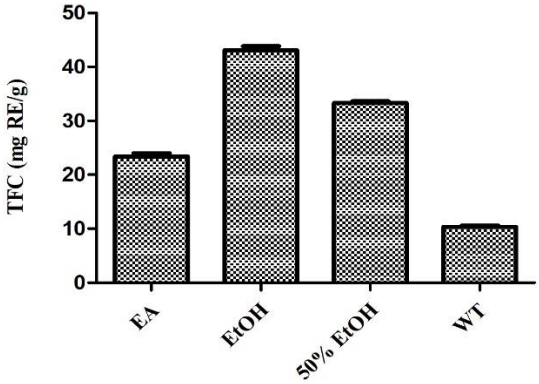


Figure 2 Antioxidant activity of the extracts

Figure 2 A.

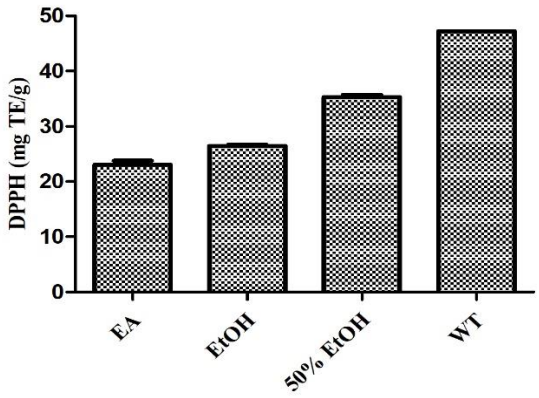


Figure 2 B. ABTS

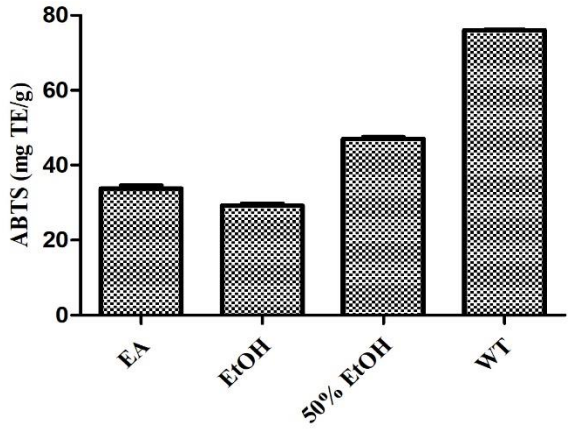


Figure 2 C. CUPRAC

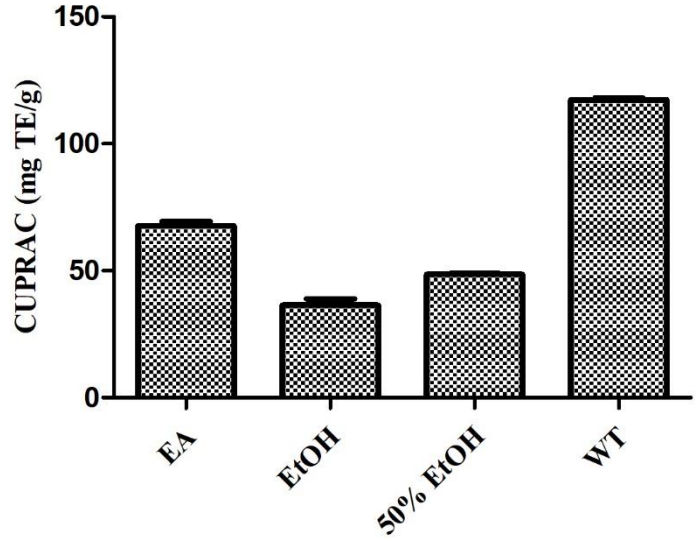


Figure 2 D. FRAP

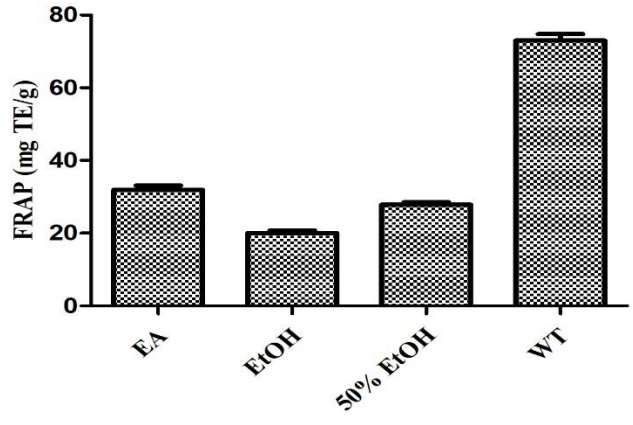


Figure 2 E. MCA

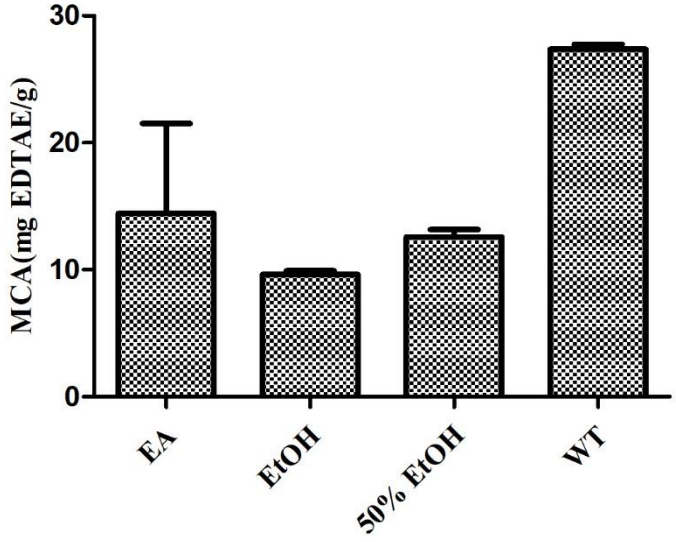


Figure 2 F. PBD

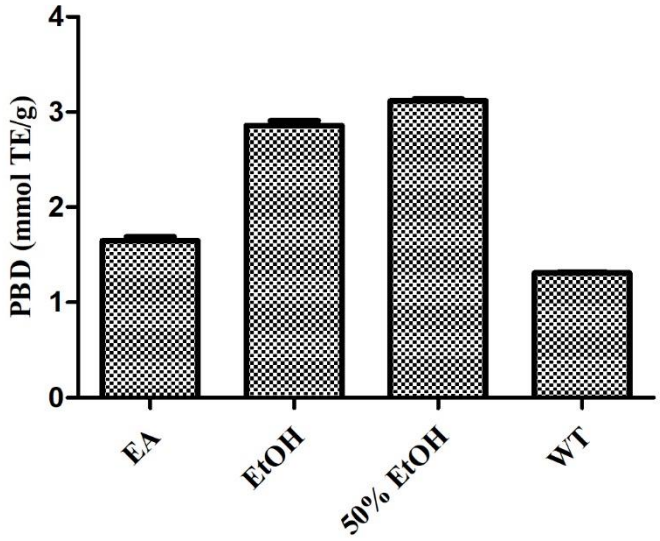


Figure 3 Enzyme inhibitory effects of the tested extracts

Figure 3 A. AChE

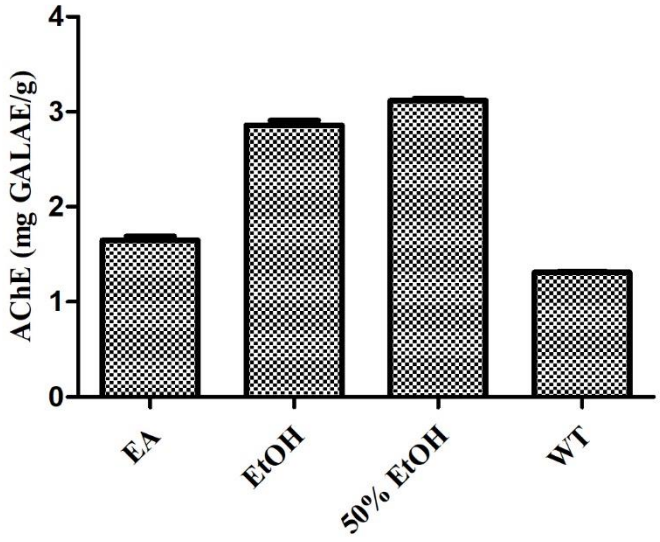


Figure 3 B. BChE

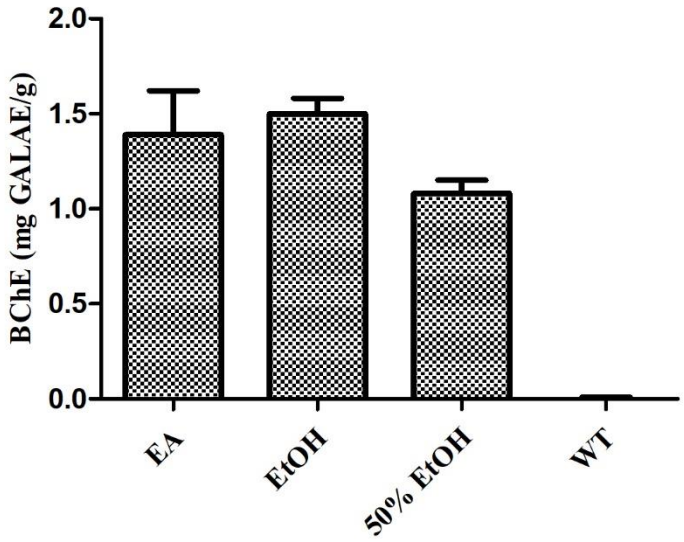


Figure 3 C. α-Amylase

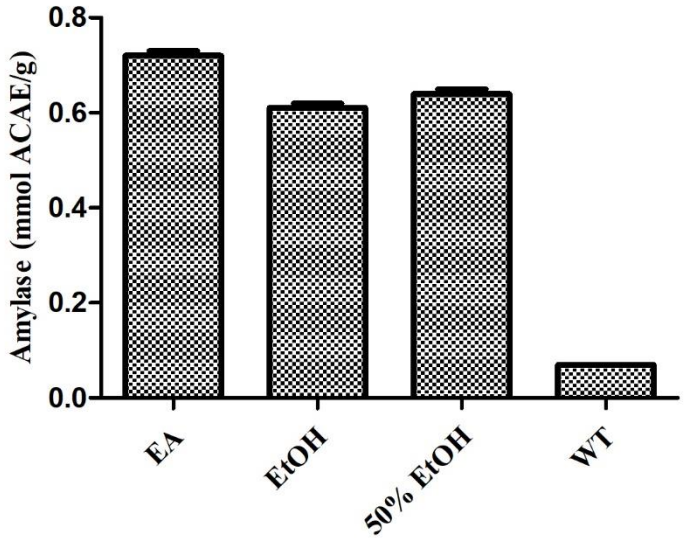


Figure 3 D. α-Glucosidase

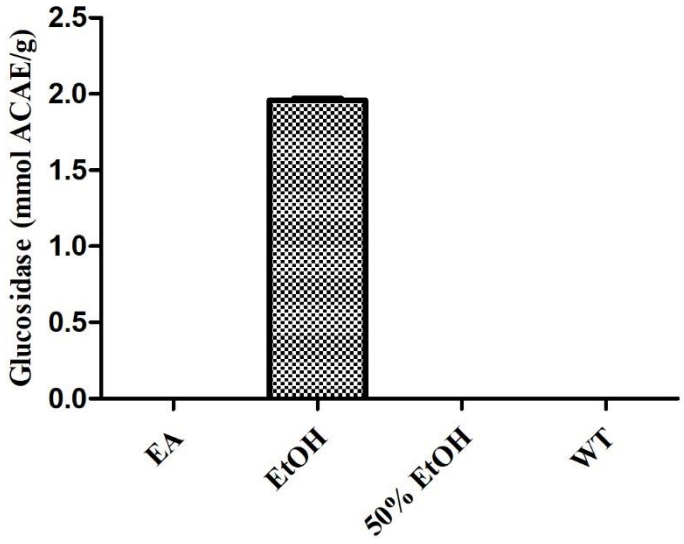
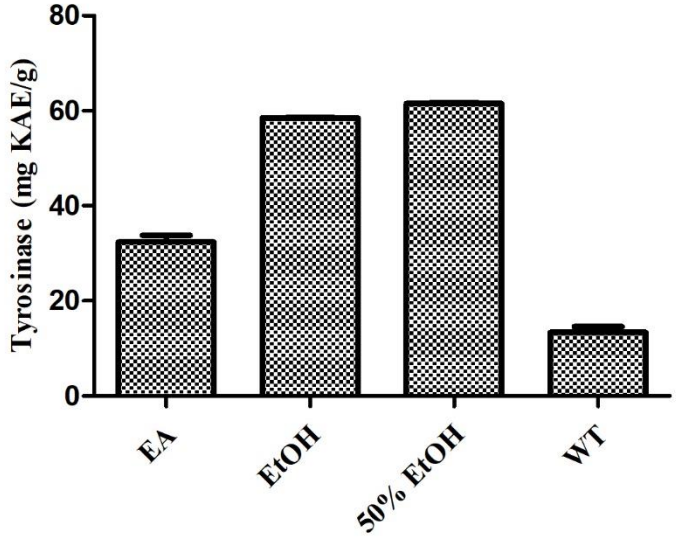


Figure 3 E. Tyrosinase



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Data 26/12/2023 Firma _____ _____

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