Scientific paper

HPLC-DAD profiling and in vitro assessment of antioxidant, anticholinesterase, α-amylase, and α-glucosidase inhibitory activities of *Echium horridum* Batt.

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Received: 11-10-2025

Abstract

The current study evaluated the biochemical activity of *Echium horridum* Batt. acetone extract for the first time. The aerial parts of *Echium horridum* were extracted using acetone and the phenolic profile was characterized via HPLC-DAD. The antioxidant potential was evaluated using *in vitro* assays. The anti-enzymatic activity of the extract was measured using anticholinesterase, α-amylase and α-glucosidase inhibition assays. Seven compounds were identified by HPLC analysis, predominantly phenolic acids, including hydroxybenzoic acid, chlorogenic acid and *trans*-2-hydroxycinnamic acid, alongside the flavonoid quercetin. The extract exhibited significant scavenging activity against DPPH and hydroxyl radicals, though it demonstrated lower potency in the FRAP assay. Conversely, in the ferric thiocyanate and TBARS assays, the extract showed substantial activity, effectively preventing lipid peroxidation. Furthermore, the extract displayed inhibitory potential against α-amylase and α-glucosidase enzymes.

Keywords: *Echium horridum* Batt., Antioxidant, α-amylase, α-glucosidase, Diabetes.

1. Introduction

In recent years, there has been growing interest in biological role of free radicals, given their crucial involvement in various physiological processes and pathological states.[1] Elevated concentrations of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induce oxidative and nitrosative stress, respectively, which can potentially damage biomolecules such as lipids, proteins and DNA.[1–3] Consequently, interest in medicinal plants has increased, as their phytoconstituents serve as natural sources of antioxidant molecules. These compounds possess diverse pharmacological properties that protect human health against various chronic illnesses.[4,5] Plant-derived bioactive substances, primarily polyphenols and flavonoids, have demonstrated significant biological potential, including antioxidant, antidiabetic and anti-Alzheimer activities, with minimal side effects and enhanced tolerance.[6]

A diabetes treatment strategy involves reducing postprandial hyperglycemia. This approach uses natural compounds which inhibit enzymes involved in the breakdown of complex carbohydrates, specifically α-amylase and α-glucosidase. The mechanisms of action for these metabolites are complex and vary depending on the target

tissue. These compounds reduce intestinal glucose absorption, stabilize plasma glucose levels, and preserve β-cell function.[7–9]

Alzheimer's disease is a progressive neurodegenerative disorder that represents a major public health challenge for the elderly population. The therapeutic efficacy of medicinal herbs in this context is closely linked to their antioxidant capacity, as oxidative stress plays a central role in the disease's pathogenesis. Therefore, the integration of plant-derived antioxidants and their bioactive constituents into the diet or as supplements may be a strategic approach to slowing the progression of Alzheimer's disease and mitigating neuronal degeneration.[10] The inhibition of key enzymes, specifically acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), plays a significant role in the management of Alzheimer's disease. These enzymes degrade the neurotransmitter acetylcholine, which is responsible for communication within cholinergic pathways, thereby AChE and BChE inhibitors mitigate the symptoms associated with Alzheimer's disease.[11]

Echium horridum Batt. belongs to the Boraginaceae family and is locally known in Algeria as Horricha. Due to their therapeutic properties, *Echium* species are frequently utilized in traditional Algerian medicine. *Echium* species contain various biologically active compounds, including naphthoquinones, flavonoids, terpenoids, and phenols.[12] Furthermore, the occurrence of antioxidant steroids and the high content of essential polyunsaturated fatty acids, notably α-linoleic acid, highlight the medicinal and nutritional importance of this genus.[12] Experimental studies on bioactive compounds from this species showed numerous activities, including antibacterial, anti-inflammatory, antiproliferative, antidepressant, antioxidant, antiviral, anxiolytic, analgesic and cytotoxic properties.[13,14] Ethnobotanical research on *Echium horridum* Batt. revealed that the local population uses this plant to treat various diseases such as general abrasions, snake bites, and scorpion stings due to their soothing properties. [15] The aim of this study was to evaluate, for the first time, *in vitro* antioxidant, anticholinesterase, α-amylase, and α-glucosidase inhibitory activities of *E. horridum* extract.

2. Experimental

2.1 Plant material

The aerial parts of *Echium horridum* Batt. were collected from Ouled Addi Guebala in M'sila, Algeria. The plant material was identified by Dr. Djamel Sarri, a botanist at the Department of Nature and Life Sciences, Faculty of Sciences, University of M'sila, Algeria. The specimens were cleaned, shade-dried at room temperature, and ground into a fine powder. The resulting powder was stored in light-protected, moisture-proof glass bottles until further analysis.

2.2 Preparation of plant extract

The extraction process was carried out according to the method described by Dehimi et al.[16] Plant material was first defatted using hexane. After filtration, the plant residues were further extracted by acetone for 48 h at room temperature. The mixture was then filtered through Whatman filter paper and the removal of solvent was performed at reduced pressure; the extract was kept at +4 °C until analysis.

2.3 Phytochemical analyses

2.3.1 Total phenolic content (TPC)

The Folin-Ciocalteu reagent was employed to quantify total phenolic content in EH extract as outlined by Ashhab et al.,[17] with slight modification. Four hundred microliters of plant extract dissolved in acetone at a concentration of 0.5 mg/mL were combined with two milliliters of 1:10 diluted Folin-Ciocalteu reagent and incubated for four minutes at ambient temperature. Subsequently, 1.6 mL of 7.5% sodium carbonate solution was included, and the resultant mixture was incubated for 2h without light. Optical density was assessed at 765 nm with gallic acid as the reference standard.

2.3.2 Total flavonoid content (TFC)

Following the process outlined by Friščić et al.,[18] the samples' total flavonoid content was determined using the aluminum chloride colorimetric technique. Two milliliters of EH extract sample (1 mg/mL) were combined with two milliliters of a 2% aluminum chloride solution. After incubation for 10 minutes, the absorbance was measured at 430 nm using quercetin as a reference.

2.3.3 High-performance liquid chromatography with diode array detection analysis (HPLC-DAD)

The phenolic content of EH extract was analyzed using a Shimadzu (Japan) high-performance liquid chromatography (HPLC) system. This system comprises an LC-20AT solvent delivery pump and an SPD-M20A diode array detector (DAD). All components are operated via the CBM-20A system controller using LC-Solution software. Chromatographic separation was performed using an Inertsil ODS-3 column (4 µm particle size, 4.0 mm × 150 mm) alongside an accompanying guard column of the same type. The column temperature was maintained at 35 °C throughout the analysis. The mobile phase consisted of two solvents: Solvent A: 0.1% acetic acid in water, and Solvent B: 0.1% acetic acid in methanol. A gradient elution program was applied, starting with 2% Solvent B for the first three minutes. The proportion of B was then increased gradually from 2% to 5%

over three minutes, from 5% to 6% over two minutes, and from 6% to 10% over four minutes. This was followed by a one-minute hold at 10%. The gradient then continued from 10% to 25% over five minutes, from 25% to 30% over seven minutes, and from 30% to 40% over five minutes. The percentage of B was then increased from 40% to 42% over six minutes, from 42% to 54% over five minutes, and finally from 54% to 55% over one minute. A further increase was then carried out, raising the percentage of B from 55% to 56% over ten minutes. This was followed by rises to 65% over four minutes, 75% over three minutes, and 85% over two minutes. The percentage of solvent B was then increased to 95% over five minutes and held for two minutes. The gradient then reached 100% over one minute and was held for five minutes, after which it decreased stepwise to 80%, then 50% and finally back to 2% over a total of seven minutes. The flow rate was set to 1.0 mL/min, with a sample injection volume of 20 µL. Detection was conducted using a diode array detector across a wavelength range of 254 nm. All samples and standard solutions were filtered using 0.45 µm PTFE syringe filters (Agilent Technologies) prior to injection, to ensure consistency.[19] The limit of quantification (LOQ) was calculated and found to be 0.01 mg/g.

2.4 *In vitro* antioxidant activity assays

2.4.1 DPPH radical scavenging assay

The DPPH radical scavenging activity of EH extract and quercetin was determined according to the method of Dehimi et al.[20] A volume of 50 µL of extract, at different concentrations, was added to 5 mL of DPPH methanolic solution (0.004%). After incubation for 30 minutes at room temperature, the absorbance was read at 517 nm and the percentage of DPPH free radical inhibition (I %) was calculated using Equation 1.

$$\text{Inhibition \%} = (A_{control} - A_{test}) \times 100 / \text{A control} \quad (1)$$

2.4.2 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of EH extract was assessed using the Fenton reaction method outlined by Geng et al.[21] Briefly, 100 µL of the extract was mixed with 500 µL of $FeSO_4$ (1.5 mM) and 350 µL of hydrogen peroxide (6 mM). After a 5-minute incubation period, 150 µL of salicylic acid (20 mM) was added to the mixture. This was followed by additional 60-minute incubation at 37 °C under the same conditions. The intensity of the resulting blue color was measured at a wavelength of 562 nm. Ascorbic acid was used as a standard. The percentage of radical scavenging was calculated using Equation 2:

$$SA\ \% = (1 - (A_{sample} - A_{self} - A_{blank})/A_{control}) \times 100 \quad (2)$$

Where, SA is the scavenging activity of tested sample (%); A sample is the absorbance of the tested sample; A blank is the absorbance of the blank; A self is the absorbance of the selves and A control is the absorbance of the control.

2.4.3 Ferric reducing antioxidant power assay (FRAP)

This assay was conducted following the method described by Raei et al.,[22] with slight modifications. Briefly, 500 µL of EH extract at different concentrations were mixed with equal volume of phosphate buffer (0.2 M, pH = 6.6) and potassium ferricyanide (1%). The solution was heated at 50 °C for 20 min. The mixture was then added to 500 µL of trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 min. The supernatant was then mixed with 1 mL of distilled water containing 200 µL of 0.1% ferric chloride. After 10 min, the absorbance was measured at 700 nm.

2.4.4 Ferric thiocyanate assay

Linoleic acid peroxidation was determined by the ferric thiocyanate method.[23] The mixture of linoleic acid was prepared by adding 500 µL of EH extract or standard solution (BHT) to 2.5 mL of linoleic acid emulsion and 2.5 mL of phosphate buffer (0.02 M, pH = 7) in a sealed bottle and the samples were incubated at 40 °C. The antioxidant activity was then determined by mixing 100 µL aliquots from the test samples, previously prepared, with 5 mL of ethanol (75%), 100 µL of ammonium thiocyanate (30%, w/v) and 100 µL of 20 mM ferrous chloride prepared in HCl (3.5%). The absorbance of the mixtures was measured at 500 nm after 3 minutes. Lipid peroxidation inhibition (LPI) was calculated up until day four of measurement, using a control containing the reaction mixture without the test sample, according to Equation 3:

$$\text{LPI (\%)} = [1 - (\text{absorbance of sample/absorbance of control})] \times 100 \quad (3)$$

2.4.5 Thiobarbituric acid-reactive substances (TBARS)

The assay is based on the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), the final product of linoleic acid autoxidation after 5 days of incubation under acidic conditions, resulting in the formation of a pink-colored MDA-TBA complex.[24] In brief, 500 µL of the incubated mixture was combined with 1 mL of TBA (0.67%, w/v) and an equal volume of 20% (w/v) trichloroacetic acid (TCA). The mixture underwent incubation in a water bath at 95 °C for 10 minutes, followed by centrifugation at 3000 rpm for 20 minutes. The supernatant's absorbance was recorded at 532 nm.

2.5 *In vitro* enzymatic inhibitory activity

2.5.1 α-Amylase inhibition

The α-amylase inhibitory potential of EH extract was assayed using the Caraway-Somogyi iodine/potassium iodide (IKI) method, with slight modifications.[25] The enzyme α-Amylase (EC 3.2.1.1, from porcine pancreas, Sigma-Aldrich) was employed in this assay. A volume of 25 µL of sample solution or acarbose at different concentrations was mixed with 50 µL of α-amylase solution in phosphate buffer (pH 6.9, 6 mM) in a 96-well microplate, and incubated for 10 min at 37 °C. After pre-incubation, the reaction was initiated by adding 50 µL of starch solution (0.05%) to the previous mixture and incubated again for 10 min at 37 °C. Finally, 25 µL of 1M HCl solution was added to stop the reaction, followed by the addition of 100 µL of iodine potassium iodide solution, and the absorbance was measured at 630 nm. The α-amylase inhibitory activity was calculated using Equation 4:

$$\text{Inhibition \%} = (\text{activity of the enzyme without sample} - \text{activity of the enzyme with sample}) / \text{activity of the enzyme without sample} \times 100 \quad (4)$$

2.5.2 α-Glucosidase inhibition

α-Glucosidase inhibition activity of EH extract was performed according to the previous method.[25] The enzyme α-glucosidase (EC 3.2.1.20, from *Saccharomyces cerevisiae*, Sigma-Aldrich) was used for the experiment. A volume of 50 µL of sample was mixed with equal volumes of glutathione, α-glucosidase solution in phosphate buffer (pH 6.8) and PNPG (4-*N*-trophenyl-α-D-glucopyranoside) in a 96-well microplate, and incubated for 15 min at 37 °C. The reaction was then stopped by adding sodium carbonate (50 µL, 0.2 M). The absorbance was read at 400 nm and the α-glucosidase inhibitory activity was calculated using Equation 4.

2.5.3 Cholinesterase inhibition

The inhibitory activity of acetylcholinesterase (AChE) and butyrylocholinesterase (BChE) was assessed according to a modified version of the method described by Ellman et al.[26] Acetylcholinesterase type VIS, from electric eel 1000 U/mg solid, butyrylcholinesterase from equine serum 100 U/mg solid (Sigma-Aldrich), were used in this study. In a 96-well microplate, 10 µL of the test sample or galantamine (the reference molecule) were added to 20 µL of either AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U), and 150 µL of 100 mM sodium phosphate buffer solution (pH 8.0). After incubating for 15 minutes at 25 °C, 10 µL of DTNB (0.5 mM) and either 10 µL of acetylthiocholine iodide (0.71 mM) or 10 µL of butyrylthiocholine chloride (0.2 mM) were added. After 15 minutes, the absorbance was recorded at 412 nm. A blank (phosphate buffer at pH 8.0 and ethanol) was used to measure the inhibitory activity of AChE or BChE using Equation 4.

2.6 Statistical analysis

Results were presented as means ± standard deviation (SD) of at least three repetitions for each assay. IC_{50} values were obtained from plots of percentage inhibition versus concentration using Graph Pad Prism 8.0.1 and defined as the concentration causing 50% inhibition. Statistical comparisons were conducted utilizing the t-test and one-way analysis of variance (ANOVA), followed by the Tukey test, using the same software. Differences were determined to be significant at $p < 0.05$.

3. Results and discussion

3.1 Phytochemical profiling

The quantification of phenolic compounds (Table 1) revealed that the EH acetone extract contains a considerable amount of polyphenols (226.01 µg GAE/mg) and a moderate amount of flavonoids (20.27 µg QE/mg).

Table 1: Total phenolics and flavonoids contents in *E. horridum* extract.

Extract	Total phenolics content (µg GAE/mg)[a]	Flavonoids content (µg QE / mg)[b]
E. horridum acetone extract	226.016 ±0.745	20.275±0.033

[a] micrograms of gallic acid equivalents per milligram of dry extract; [b] micrograms of quercetine equivalents per milligram of dry extract; Values are expressed as mean ± SD (n = 3).

Phenolic substances are one of the most prevalent groups of phytochemicals found in plants. These substances contain at least one aromatically bound hydroxyl group, and they can range from low-molecular-weight molecules to highly complex ones. They typically occur as esters and glycosides rather than free compounds.[27–29] The phytochemical content levels isolated from medicinal plants undergo solvent dependence (polarity degree), extraction and quantification methods, harvest timing, and geographical location.[11] The results of the in-depth chemical characterization of the extract via HPLC-DAD method are presented in table 2.

Among the detected compounds, 4-hydroxybenzoic acid, chlorogenic acid and quercetin are well documented for their roles in combating oxidative stress and protecting against cellular damage associated with chronic diseases such as cancer and diabetes. Notably, quercetin has been shown to increase the expression of key antioxidant enzymes, suggesting its potential contribution to the protective effects of the extract.[30,31]

Table 2: Phenolic compounds identified by HPLC-DAD in the acetone extract of *Echium horridum* Batt.

Compunds (mg/g)	RT	Calib. equation	R^2	Conc.
4-hydroxybenzoic acid	30.867	y = 123758x + 75779	0.9997	0.34
4-hydroxy benzaldehyde	33.367	y = 34376x + 4239.6	0.9996	0.13
Vanillic acid	34.758	y = 66764x + 46508	0.9998	0.01
Chlorogenic acid	40.094	y = 46920x - 36953	0.9995	0.25
p-coumaric acid	40.874	y = 17265x + 343183	0.9951	Tr
trans-2-hydroxycinnamic acid	48.243	/	/	NQ
Quercetin	55.19	y = 89397x – 80467	0.9997	0.25

Tr: Trace amount. NQ: Not quantified.

Furthermore, the analysis identified *trans*-2-hydroxycinnamic acid which possess potent antioxidant and anti-inflammatory properties, primarily due to its ability to neutralize free radicals and activate endogenous cellular defense mechanisms.[30]

3.2 *In vitro* assessment of antioxidant activity of *E. horridum* Batt.

Considerable variability in phytochemical content and oxidative processes necessitated evaluating plant extracts' *in vitro* antioxidant activity using several methodologies. This study demonstrated that the ACEH extract exhibited significant antioxidant properties, as evidenced by its DPPH radical scavenging activity, reducing power, and inhibition of lipid peroxidation.

3.2.1 DPPH radical scavenging assay

The results obtained in the DPPH test are summarized in table 2. EH extract showed a good potential to scavenge the radical DPPH with an IC_{50} of 0.2 mg/mL, while Quercetin used as a standard showed an IC_{50} value of 0.0036 mg/mL.

Antioxidants occurring in medicinal plants extracts can neutralize DPPH by direct reduction via single-electron transfer (SET) or hydrogen atom transfer (HAT).[32] Delocalizing the spare electron throughout the DPPH molecule ensures its stability and gives it a deep violet color with maximum absorption at 520 nm. The presence of an antioxidant reduces this free radical, leading to the decolorization of the solution. Consequently, the decrease in absorbance is proportional to the concentration of antioxidants present in the extract.[28]

3.2.2 Hydroxyl radical scavenging activity

The results demonstrate that the EH extract exhibits effective hydroxyl radical (OH•) scavenging activity, reaching an inhibition rate of 65.93% at 0.90 mg/mL in a dose-dependent manner. In comparison, ascorbic acid displayed superior potency, with 92.43% inhibition at 0.18 mg/mL (Figure 1). The IC_{50} value for the EH extract was determined to be 0.503 ± 0.006 mg/mL. While this indicates a robust scavenging capacity, it remains significantly lower than that of the standard antioxidant, ascorbic acid ($p \leq 0.05$).

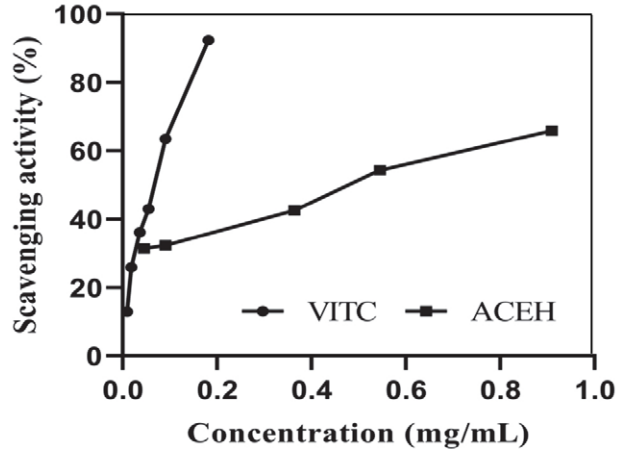

Figure 1: Hydroxyl radical scavenging activity of EH acetone extract.

3.2.3 Ferric reducing antioxidant power

The FRAP assay was employed to measure the electron transfer potential of the EH acetone extract. As shown in table 3, the capacity of the extract to reduce ferric ions (Fe^{3+}) is dose-dependent, with an EC_{50} value of 4.93 mg/mL. However, its effectiveness was significantly lower than that of ascorbic acid, which yielded an EC_{50} value of 0.073 mg/mL.

Table 3: Inhibitory effects of *Echium horridum* acetone extract compared to standard antioxidants.

Sample	IC_{50} value (mg/mL) DPPH	EC_{50} value (mg/mL) FRAP
EH acetone extract	0.20 ± 0.0006****	4,93± 0,0041****
Quercetin	0.0036 ± 0.002	NT
Ascorbic acid	NT	0.073 ± 0.006

Values are presented as mean ± SD (n = 3). NT: not tested. $p < 0.001$.

The FRAP assay is a typical ET-based method that measures the reduction of the ferric (Fe^{3+}) ion–ligand complex to the intensely blue ferrous (Fe^{2+}) complex by antioxidants present in plants extracts. The results indicate that the EH extract demonstrates significant antioxidant activity, partly attributed to its polyphenols and flavonoids contents. These compounds have a high concentration of hydroxyl groups that facilitate the reduction of oxidants and convert them into non-toxic molecules.[33]

3.2.4 Lipid peroxidation inhibition

The ferric thiocyanate assay was used to assess the antioxidant activity of the EH extract. Results after 96 hours of incubation are presented in (Fig. 2A). Compared to the negative control, both the EH extract and the standard BHT significantly inhibited linoleic acid oxidation.

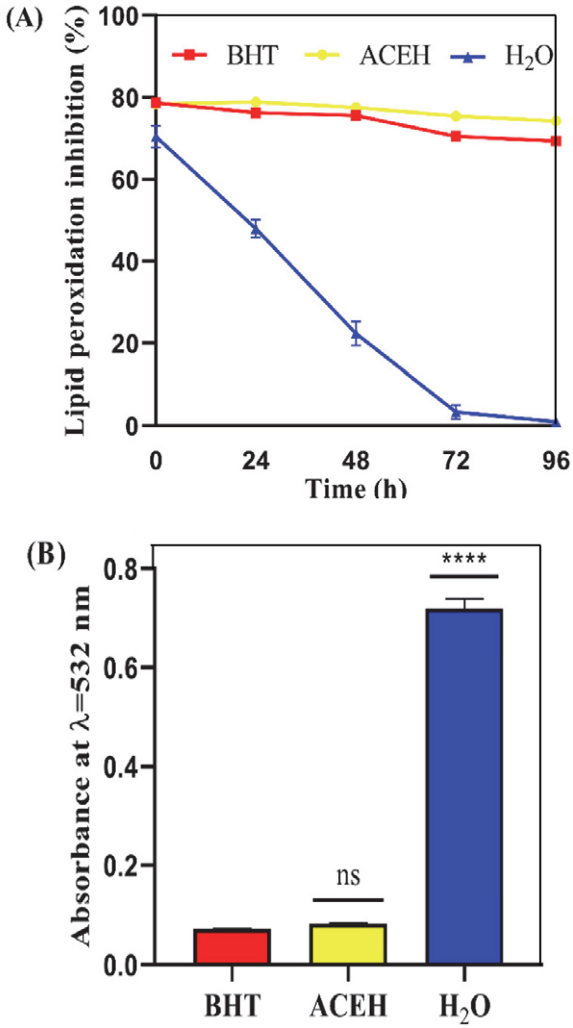

Figure 2: Lipid peroxidation inhibition of *E. horridum* Batt. acetone extract (ACEH). (A): ferric thiocyanate method. (B): TBARS method. Values are expressed as mean ± SD (n = 3). ns: $p > 0.05$ and ****shows statistically significant differences at $p \leq 0.0001$ compared to BHT.

The EH acetone extract exhibited a higher inhibition rate of 75.46 ± 0.34%, compared to BHT, which demonstrated an inhibition rate of 69.41 ± 0.27%

The TBARS assay revealed that the EH extract, at a concentration of 2 mg/mL, yielded an absorbance of 0.083, indicating low levels of MDA. This value did not differ significantly ($p > 0.05$) from the standard BHT, which recorded an absorbance of 0.071 (Fig. 2B). Consequently, the results from both lipid peroxidation assays suggest that the inhibitory activity of the extract is comparable to that of the synthetic antioxidant BHT.

Lipid peroxidation refers to the oxidative degradation of lipids, mainly unsaturated fatty acids, inside cellular membranes, driven by oxidative stress in cells.[34] The efficacy of natural extracts in the prevention or retardation of oxidation is based on the idea that linoleic acid undergoes oxidation by ROS generated within the reaction system. The main end product of the oxidative breakdown of unsaturated lipids is malondialdehyde (MDA), which interacts with thiobarbituric acid (TBA) to form a pink-colored adduct (MDA2(TBA).[28,32] The data suggest that the EH acetone extract not only prevents the initiation of lipid oxidation but also effectively mitigates oxidative damage during its propagation stages. The antioxidant activity observed is probably due to the extract's high concentration of bioactive substances. The decrease in color intensity of the resulting chromophore may be due to the stabilization and neutralization of peroxyl radicals produced by linoleic acid through polyphenols, whereby electrons are donated, free radicals are scavenged and transformed into more stable molecules.[11] The alignment of the extract's chemical composition with its antioxidant performance highlights the potential for synergistic effects among the phenolic constituents, regardless of their individual concentrations. Furthermore, the extract demonstrated a potent inhibitory effect on lipid peroxidation, suggesting its ability to safeguard cell membranes against oxidative damage. This finding lends weight to the traditional use of the plant in folk medicine as an anti-inflammatory agent.

3.3 *In vitro* enzyme-inhibitory activity of *E. Horridum* Batt.

In vitro assays for antidiabetic potential indicated that the EH acetone extract exhibited inhibitory activity against α-amylase, with an IC_{50} value of 329.58 µg/mL, whereas acarbose showed an IC_{50} of 36.74 µg/mL. Similarly, in the α-glucosidase inhibition test, the studied extract displayed inhibitory activity against the enzyme with an IC_{50} of 304.32 µg/mL; this potency was lower than that of acarbose (IC_{50} = 22.28 µg/mL). On the other hand, the EH extract showed no detectable activity against the AChE and BChE enzymes (Table 4).

Medicinal plants exhibiting anti-diabetic properties represent a potential source for developing safer and more

Table 4: α-Amylase, α-glucosidase and cholinesterase inhibitory activity of *Echium horridum* extract.

Sample	Antidiabetic inhibitory activity		Anticholinesterase activity	
	α-Amylase inhibitory activity	α-Glucosidase inhibitory activity	AChE	BChE
	IC_{50} (µg/mL) 50–400 µg/mL	IC_{50} (µg/mL) 25–200 µg/mL	IC_{50} (µg/mL) 25–200 µg/mL	
EH extract	329.58±0.76	304.32±2.99	NA	NA
Acarbose	36.74±4.50	22.28±2.03	NT	NT
galantamine	NT	NT	4.44±1.57	34.58±0.60

Values expressed herein are mean ± SEM of three parallel measurements; NA: not active; NT: not tested

cost-effective anti-diabetic medications. Anti-diabetic agents operate by inhibiting the enzymes α-amylase and α-glucosidase, essential in breaking dietary carbohydrates into glucose.[8] The EH extract's inhibition of these enzymes demonstrates its ability to postpone the degradation of carbohydrates into oligosaccharides. These activities can be attributed to the metabolites, phenolics, and flavonoids identified as constituents of herbal extracts possessing anti-diabetic properties. These compounds inhibit α-amylase and α-glucosidase by forming hydrogen bonds between their hydroxyl groups and the residues of the active site. This interaction may induce conformational changes at the enzyme's active site or may involve compounds that act as substrate analogs, thereby competing for the active site of these enzymes.[7,35,36] In a study of the hydroalcoholic extract from *Echium trygorrhizum* roots, another Algerian species within the same genus, the extract exhibited an activity against α-amylase with an IC_{50} of 0.56 mg/ml, indicating that the species *E. horridum* may possess more potent anti-diabetic effects.[7] It is likely that quercetin, chlorogenic acid, and related phenolics contributed to this inhibitory activity, as previous studies have reported their ability to modulate glucose metabolism by targeting these digestive enzymes.[37,38]

4. Conclusion

This study demonstrates that the acetone extract of *Echium horridum* Batt. is rich in phenolic compounds characterized by potent antioxidant activity. The extract effectively scavenges DPPH and hydroxyl radicals, exhibits strong ferric reducing power, and significantly inhibits linoleic acid peroxidation. These findings suggest that *E. horridum* may serve as an effective agent in preventing and controlling free radical-induced oxidative stress. Furthermore, this study reports, for the first time, the inhibitory effects of the extract on α-amylase, α-glucosidase, AChE, and BChE. These results highlight a significant antidiabetic potential, likely attributable to its specific phytochemical constituents. However, further research is required to isolate the bioactive compounds and elucidate their precise mechanisms of action.

Acknowledgement

The authors are thankful to Dr. Sarri Djamel from the department of Life and Nature Sciences in the University of M'sila-Algeria, for the identification of the studied plant.

Conflict of interest

The authors declare no conflict of interest.

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Povzetek

Trenutna študija je prvič ovrednotila biokemijsko aktivnost acetonskega ekstrakta vrste *Echium horridum* Batt. Zračne dele rastline *Echium horridum* so ekstrahirali z acetonom, fenolni profil pa so karakterizirali z metodo HPLC-DAD. Antioksidativni potencial so ocenili z uporabo *in vitro* testov. Proti encimsko aktivnost ekstrakta so določili z inhibicijskimi testi za antiholinesterazo, α-amilazo in α-glukozidazo. Z analizo HPLC so identificirali sedem spojin, pretežno fenolne kisline, vključno s hidroksibenzojsko kislino, klorogensko kislino in *trans*-2-hidroksicinamično kislino, ter flavonoid kvercetin. Ekstrakt je pokazal pomembno lovilno aktivnost proti radikalom DPPH in hidroksilnim radikalom, vendar nižjo učinkovitost v testu FRAP. Nasprotno pa je v testih feri-tiocianata in TBARS izkazal izrazito aktivnost ter učinkovito preprečeval lipidno peroksidacijo. Poleg tega je ekstrakt pokazal tudi inhibitorni potencial proti encimoma α-amilazi in α-glukozidazi.