



Free radicals and enzymes inhibitory potentials of the traditional medicinal plant *Echium angustifolium*

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ARTICLE INFO

Keywords:

Echium angustifolium

Chemical components

Antioxidant

Antiobesity

Antidiabetic

ABSTRACT

Introduction: Herbal medicine has been useful in the treatment of various diseases and has played a critical role in the discovery of new drugs. This study aimed to investigate the phytoconstituents, antioxidant, antidiabetic and antiobesity activities of the traditional medicinal plant, *Echium angustifolium* Mill.

Methods: The constituents of *E.angustifolium* four solvents fractions were established utilizing conventional analytical methods. Antioxidant, antilipase, anti-glucosidase, and anti- α -amylase activities of *E. angustifolium* were conducted using DPPH (1,1-diphenyl-2-picrylhydrazyl α), p-nitrophenyl butyrate, P-NPG (4-nitrophenyl β -D-glucopyranoside), and dinitrosalicylic acid procedures, respectively.

Results: The methanolic fraction of *E. angustifolium* displayed inherent antioxidant properties with an IC_{50} value of 1.95 μ g/mL. Moreover, the hexane fraction presented potential α -amylase inhibitory activity with an IC_{50} value of 92.89 μ g/mL. Additionally, the aqueous fraction had the highest anti-lipase activity with an IC_{50} value of 24.54 μ g/mL.

Conclusion: It can be concluded that *E. angustifolium* has high potential for development as an alternative approach for oxidative stress, diabetes, and obesity.

1. Introduction

Medicinal plants have played a unique role in the treatment and prophylaxis of many diseases and have been used as anticancer, analgesic, antipyretic, antibacterial, anti-inflammatory, and antidiabetic agents [1,2]. Recently, the World Health Organization stated that integrative herbal medicine provided a significant source of primary health care for people living in developing countries [3]. Moreover, various natural herbal extracts containing bioactive ingredients which have generated a major source of pharmaceuticals [4,5].

Reactive oxygen intermediates (ROIs) are the by-products of numerous physiological processes, produced due to the oxidation and reduction of molecules in biological reactions [6]. ROIs are recognized as extremely unstable molecules which can cause unlimited damage to cells, lipids, and DNA proteins [7]. A vast field of research has been dedicated to identifying the harmful effects of ROIs, which has been associated with several diseases such as arteriosclerosis, aging, coronary artery disease, many types of cancer, and different metabolic disorders [8]. Antioxidants are substances that cleanse the body of reactive molecules and are therefore crucial in addressing the numerous

diseases associated with ROIs. Antioxidants are found in many natural substances, and can be concentrated in different herbal extracts.

The prevalence of metabolic disorders, including obesity and overweight, is increasing every year. Diabetes mellitus is a significant global health problem affecting millions of people. Diabetes mellitus and its complications affect patients' day to day activities, reduces overall quality of life and increases mortality. Diabetes mellitus is a chronic metabolic disease resulting from insufficient insulin being produced or cells failing to respond to insulin and leads to high blood glucose levels [9,10]. People with diabetes mellitus have a greater risk of developing severe complications such as atherosclerosis, dyslipidemia, retinopathy, peripheral neuropathy and renal failure. Inhibiting the digestion enzymes such as glucosidase and α -amylase may be an excellent way to reduce the level of glucose in the blood [9], and affect carbohydrate metabolism. Research has revealed that the greatest risk factor is having a high body mass index. Being overweight is associated with various serious diseases, such as diabetes, cancer, chronic renal, and cardiovascular diseases. Therefore, the global health community seeks to identify effective treatments and preventive methods for obesity and overweight [11]. One of the vital policies utilized in the development of

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<https://doi.org/10.1016/j.eujim.2020.101196>

Received 8 June 2020; Received in revised form 24 August 2020; Accepted 25 August 2020

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anti-obesity pharmaceutical preparations is the investigation for potent antilipase agents, especially from herbal extracts due to the global recognition of their safety and potential low-price [9].

Echium angustifolium Mill (Boraginaceae family) is a perennial herbaceous plant that grows up to 80 cm. The indumentum of the stems and leaves are heteromorphic, while the leaves have a linear or linear-lanceolate shape. The flower corolla has a narrow trumpet-shape, 15–25 mm, red turning violet after withering (rarely white), densely pubescent, and with longer hairs along the veins. The *E. angustifolium* plant grows in waste land on various soil types, especially in the Eastern regions of the Mediterranean Sea basin [12]. Various *Echium* spp. have been utilized in the ethnomedicine as a sedative, anxiolytic, vulnerary, analgesic, expectorant, febrifuge, diaphoretic, diuretic, and also for the treatment of upper respiratory system infections [13]. Moreover, the extracts of *E. angustifolium* and other species of *Echium* plants have been widely utilized in traditional medicine for several therapeutic purposes, mainly to heal external wounds and ulcers [14,15].

The present study aimed to conduct preliminary phytochemical screening and antioxidant activity of methanol, acetone, aqueous, and hexane fractions of *E. angustifolium*. In addition, this study intended to determine the lipase enzyme inhibitory activity to assess their potential effect against obesity, overweight, and hyperlipidemia. Moreover, the study evaluated the inhibitory effect of the plant fractions against glycosidase and α -amylase enzymes to investigate their antidiabetic potential.

2. Material and methods

2.1. Chemicals

2.1.1. Phytochemical screening

The reagents used for the phytochemical screening were Millon's reagent, Benedict's reagent, and sodium hydroxide, all obtained from IRIS (Germany). From Sigma-Aldrich (France), chloroform and hydrochloric acid were purchased. Iodine solution, sulfuric acid, and Molisch's reagent were purchased from Alfa-Assar (England), Iron (III) chloride from Riedeldehan (Germany), and Ninhydrin reagent was obtained from Alfa Agar (England).

2.1.2. Antioxidant activity

Trolox ((s)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was bought from Sigma-Aldrich (USA). From Sigma-Aldrich (Germany), the 2,2-diphenyl-1-picrylhydrazyl commonly referred to as DPPH reagent was acquired.

2.1.3. Antilipase testing

p-nitrophenyl butyrate (PNPB), Orlistat, and DMSO (Dimethyl Sulfoxide) were bought from Sigma-Aldrich (Schnelldorf, Germany), while porcine pancreatic lipase and the tris-HCl buffer were obtained from Sigma (MO, USA).

2.1.4. α -Amylase testing

Porcine pancreatic α -amylase and DMSO (Dimethyl Sulfoxide) were obtained from Sigma-Aldrich (USA). While Corn starch was purchased from (Alzahraa company, Palestine), and 3,5 Dinitrosalicylic acid was from Sigma (India).

2.1.5. Glucosidase testing

The α -glucosidase enzyme was acquired from Sigma-Aldrich (USA), and P-NPG (4-Nitrophenyl β -D-glucopyranoside) was purchased from Sigma (Germany).

2.2. Equipment

In the current work, the following types of equipment/apparatuses

were used: UV-vis Spectrophotometer from Jenway 7315 (Staffordshire, UK), the balance from Rad-wag (Poland), micropipettes from Finnpiptette (Finland), incubator from Nuve (Turkey), filter papers from Machrery (USA), Grinder from Moulinex (China), microplate reader from Unilab (USA) and microbroth plate from Greiner/BioOne (North America), Shaker apparatus (Memmert shaking incubator, Germany), freeze drier (Mill rock technologyBT85, China)

2.3. Collecting and drying of plant material

The leaves of *E. angustifolium* herb were gathered in April 2018 from the plains of Tubas city, Palestine. The herb was identified and characterized at An-Najah National University in the Herbal Products laboratory and stored under the voucher number (Pharm-PCT-881). The collected leaves were subsequently cleaned by rinsing and then set aside in a shaded area to air dry for two weeks, pulverized using a mechanical mill, and stored in individual well-closed paper bags until the fractionation.

2.4. Solvents exhaustive fractionations method

The method applied for extraction of the dried leaves was the four solvent fractionations technique. This was performed via different solvents characterized by varying degrees of polarities; the solvents used were methanol, water, acetone, and hexane. Roughly, around 2000 mL from each solvent was separated in a bottle, and 200 g leaves of the dried plant were added to every solvent. Both bottles of the leaves and the solvent were positioned on a shaking machine set at 400 rpm for 72 h at ambient temperature. The suction filtration method was implemented for every solvent, after which we dehydrated the yield by different approaches. For the organic solvents, they were dried by leaving the exhaustive extract in the incubator that set at 25 °C for the duration necessary to completely dry each solvent while the utilization of freeze-dryer for water fraction required 48 h wait. Finally, the separate dried fraction obtained was stored at a temperature of 2–8 °C for later use.

2.5. Phytochemical analysis

Following the conventional analytical methods used to detect the incidence of primary and secondary metabolic groups, the phytochemical screening tests were conducted. Starch, carbohydrates, flavonoids, alkaloid, saponins, phenols, tannins glycosides, protein, and terpenoid, were all included in the phytochemical screening analyses [16].

2.6. Antioxidant activity

The preparation of 1 mg/mL of the plant extract solution was obtained by mixing 100 mg of every plant fraction with 100 mL of methanol. The solution proceeded to the dilution process using methanol again to produce the following concentrations: 2, 5, 10, 20, 50, and 100 μ g/mL. Extraction of 1 mL from every plant fraction stock and 1 mL of methanol and 1 mL of DPPH solution was mixed and left to rest at room temperature for 30 min without exposure to light or sun. The blank solution used only methanol without any plant fraction solution in the mixture. The absorbance was measured via a UV-vis spectrophotometer, which was set at 517 nm and equated with the positive control (i.e., Trolox) absorbance. The following equation determined the antioxidant activity:

$$I (\%) = \frac{[ABS_{\text{blank}} - ABS_{\text{rest}}]}{[ABS_{\text{blank}}]} * 100 \%$$

Whereby I (%) represents the antioxidant percentage [17].

2.7. α -Amylase inhibitory method

The preparation of 1 mg/mL of the plant extract solution was obtained by dissolving 25 mg of every fraction in a slight volume of 10 % DMSO and adding a buffer solution so that the total volume was 25 mL. The dilution of the solution to 5, 10, 50, 70, 100, 500 μ g/mL was achieved by adding the buffer correspondingly. The preparation of 2 units/mL of α -amylases enzyme solution was prepared by liquifying 12.5 mg of the enzyme powder with a slight amount of DMSO (10 %) and diluting it with a buffer volume of the solution is 100 mL.

The preparation of the corn starch solution was accomplished by adding 1 g of starch and 100 mL distilled water and mixed. Before the incubation time of 10 min in a water bath set at 30 °C, we added 200 μ l from every plant fraction stock to an equal amount of α -amylase. Once the 10 min duration was completed, corn starch (200 μ l) was added to the solution and left to incubate for an extra 3 min. Before displacing the solution into a boiling water bath at 85–90 °C for 10 min, 3,5-dinitro salicylic acid was added. Finally, after the 10-minute duration, the solution was set to cool down to add 5 mL of DW.

The preparation of the blank solution was completed by exchanging the plant fraction with 200 μ l of a buffer. For the positive control, Acarbose was used to compare the absorbance obtained from a 540 nm UV–vis Spectrophotometer. The following formula estimated the α -amylase inhibitory potential.

$$I (\%) = \frac{[ABS_{\text{blank}} - ABS_{\text{test}}]}{[ABS_{\text{blank}}]} * 100 \%$$

Whereby I (%) represents the percentage of α -amylase inhibition [18].

2.8. α -Glucosidase inhibitory activity

The preparation of 1 mg/mL of the plant extract was obtained by mixing 100 mg of every plant fraction with an equal amount of phosphate buffer. The dilution of the solution, achieved by adding more of the buffer resulted in the following concentrations; 100, 200, 300, 400, 500 μ g/mL. Then, 20 μ l from each plant fraction stock solution and α -glucosidase solution (1 unit/mL) was mixed with 50 μ l of phosphate buffer and placed in a water bath for 15 min at a fixed temperature of 37 °C. Subsequently, 20 μ l of P-NPG was added before repositioning the solution in the water bath for an additional 20 min. Finally, to terminate the reaction, 50 μ l of sodium carbonate (0.1 M) was added.

Conversely, the blank solution's preparation was completed without using the plant extract. Instead, the buffer was utilized as a substitute. Acarbose acted as the control in the study, and thus, the absorbance was estimated utilizing UV–vis spectrophotometer at 405 nm to be compared with the Acarbose' absorbance. The α -glucosidase inhibitory activity was estimated using the following formula:

$$I (\%) = \frac{[ABS_{\text{blank}} - ABS_{\text{test}}]}{[ABS_{\text{blank}}]} * 100 \%$$

Defining I (%) as the percentage inhibition of α -glucosidase [19,20].

2.9. Inhibitory method of the porcine pancreatic lipase enzyme

The 100 mg of every plant fraction was dissolved with 100 mL of 10 % DMSO, which yielded 1 mg/mL of the plant extract solution. The solution was then diluted to obtain the following concentrations; 20, 50, 100, 200, 300, and 400 μ g/mL. The preparation of the PNPB, p-nitrophenyl butyrate stock solution was accomplished by dissolving 20.9 mg of PNPB in 2 mL of acetonitrile while the preparation of 1 mg/mL of lipase enzyme stock was performed by liquifying the lipase enzyme powder (25 mg) with a 25 mL of 10 %DMSO. After this, 0.2 mL was extracted from each concentration of every plant fraction and mixed with 0.1 mL of lipase enzyme stock solution and finally adding a Tris–HCl solution so that the total volume is 1 mL. All solutions were then placed in a 37 °C water bath for 15 min, and after adding 100 μ l of PNPB solution, they were placed back for an added 30 min to maintain

the temperature at 37 °C. The control solution was prepared by adding Tris–HCl to 100 μ l of lipase enzyme solution so that the total volume was 1 mL. For the standard reference, Orlistat was used abiding by the same protocol used for plant fractions. Although the absorbance was measured by operating a UV–vis spectrophotometer set at 405 nm, the lipase enzyme inhibiting potential was measured by applying the present equation:

$$I (\%) = \frac{[ABS_{\text{blank}} - ABS_{\text{test}}]}{[ABS_{\text{blank}}]} * 100 \%$$

Defining I (%) as the inhibition percentage of lipase enzyme [21].

2.10. Statistical analysis

Trials were conducted in triplicate for all the experiments, and the mean of each experiment was calculated \pm the standard deviation (\pm SD).

3. Results

3.1. Chemical constituents

Table 1 shows the phytochemical constituents present in *E. angustifolium* aqueous, hexane, methanol, and acetone fractions. The results showed that starch, glycosides, flavonoids, carbohydrates, saponins, and alkaloids were present in all plant fractions, but tannins, phenol, proteins, and terpenoid were present only in the methanol fraction.

3.2. Antioxidant activity

The DPPH method was applied to evaluate the free radical scavenging activity (antioxidant activity) of *E. angustifolium*. Trolox's positive control in this study is acknowledged as a powerful antioxidant and vitamin E analog. Fig. 1 depict the DPPH inhibitory activity by *E. angustifolium* aqueous, methanol, hexane, and acetone fractions. However, results showed that methanol fraction strongly inhibited DPPH free radicals with IC₅₀ of 1.95 μ g/mL followed by the aqueous fraction which has an IC₅₀ value of 29.40 μ g/mL by comparison to Trolox, (Positive control) that corresponded to a value of 1.88 μ g/mL (at an IC₅₀) as presented in Table 2.

Table 1

Phytochemical screening of *E. Angustifolium* aqueous, methanol, hexane and acetone extracts.

Phytochemical screening test	Test name	Acetone	Water	Methanol	Hexane
Test for proteins	Millon's test	–	–	++	–
	Ninhydrin test	–	–	++	–
Test for carbohydrates	Fehling test	+	++	+	+
	Molisch's test	+	–	+	+
	Iodine test	+	+	+	+
Test for tannins and phenol	Tannins and phenol test	–	–	+++	–
	Flavonoids test	+	+++	+++	+
Saponins test	Saponins test	+++	+++	++	++
Test for glycosides	Liebermann's test	+	+	++	+
	Salkowski test	+	–	++	+
	Keller–Kiliani test	+	–	+	–
	Steroids test	+	+	+++	+++
Test for terpenoids	Terpenoids test	–	–	++	–
	Alkaloids test	+	++	+++	+++

(+++ Designates the incidence of high content, (++) designate the incidence of mild content, (+) designates the incidence of low content and (–) designates the absence of content.

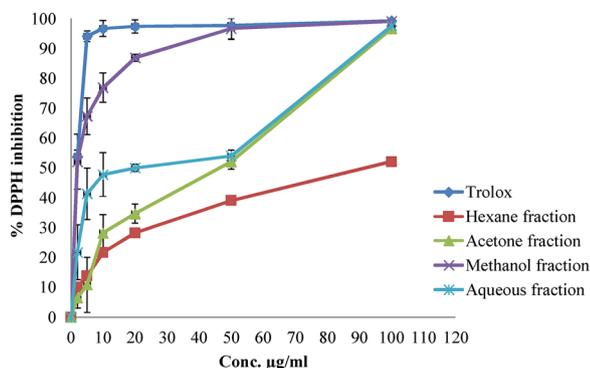


Fig. 1. DPPH inhibitory action by *E. angustifolium* four fractions and positive control (Trolox).

3.3. Antilipase activity

The inhibitory activity of the lipase enzyme was conducted utilizing porcine pancreatic enzyme assay, while the commercial pharmaceutical preparation Orlistat was used as a control (positive). The *E. angustifolium* aqueous, methanol, hexane, and acetone fractions showed lipase enzyme inhibitory activity in dose depending manner, as shown in Fig.2. The obtained results revealed that all the screened plant fractions have potential antilipase activity, as presented with IC50 values in Table 2.

3.4. The inhibitory activity of α-amylase

The inhibitory assay of the porcine pancreatic enzyme was used to evaluate the α-amylase inhibitory activity of *E. angustifolium* aqueous, hexane, methanol, and acetone fractions. Acarbose, the antidiabetic drug commercially available to control and maintain type II diabetes, was used as positive control. The effect of α-amylase on the inhibition of the studied samples is presented in Fig.3. Significantly, the data reveal that the hexane fraction has the highest α-amylase inhibitory activity with an IC50 value of 92.89 µg/mL in comparison with Acarbose, the positive control, which corresponded to the IC50 value of 9.54 µg/mL as presented in Table 2. However at highest concentration (500 µg/mL) the acetone fraction did not reach the 50 % inhibition and because of that IC50 value was not applicable in this fraction.

3.5. Glucosidase inhibitory activity

The P-NPG method was conducted to evaluate the inhibitory effect of α-glucosidase in *E. angustifolium* four extract fractions using Acarbose as a positive control. The glucosidase inhibitory effect of the four fractions of the plant and Acarbose are depicted in Fig. 4. The results showed that the acetone fraction had the highest α-glucosidase inhibitory activity with an IC50 value of 247.52 ± 5.02 µg/mL while the reference compound Acarbose had a value of 37.15 ± 0.33 µg/mL (at IC50) as shown in Table 2.

Table 2 summarizes antioxidant, α-amylase, glucosidase and lipase

Table 2

Results of *E. angustifolium* on free radical, α-amylase, glucosidase and lipase inhibitory activities.

Fractions	Antioxidant activity IC50 (µg/mL), ± SD	α-Amylase inhibitory activity IC50 (µg/mL), ± SD	Glucosidase inhibitory activity IC50 (µg/mL), ± SD	Lipase inhibitory activity IC50 (µg/mL), ± SD
Hexane	91.00 ± 1.23	92.89 ± 0.85	439.67 ± 6.39	29.1 ± 0.62
Acetone	37.77 ± 2.20	N/A	247.52 ± 2.58	26.3 ± 0.23
Methanol	1.95 ± 01.26	255.22 ± 0.59	283.69 ± 5.02	34.67 ± 0.46
Aqueous	29.40 ± 02.14	468.52 ± 0.75	278.37 ± 5.27	24.54 ± 0.66
References	1.88 ± 1.57 (Trolox)	9.54 ± 1.22 (Acarbose)	37.15 ± 0.33 (Acarbose)	12.3 ± 0.35 (Orlistat)

N/A: Not applicable.

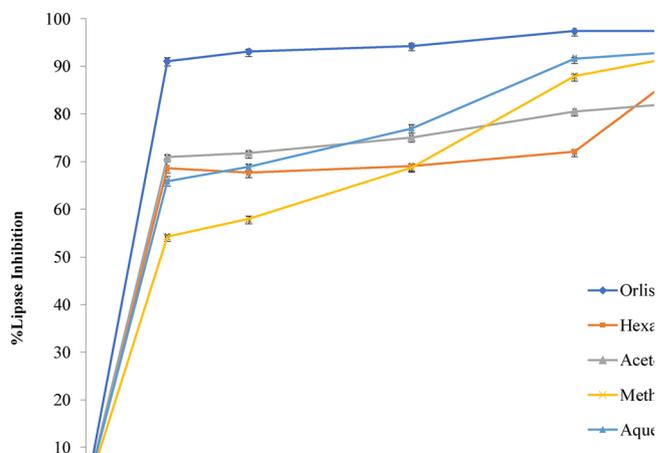


Fig. 2. The inhibitory impact on Porcine pancreatic lipase by *E. angustifolium* four extract fractions and positive control (Orlistat).

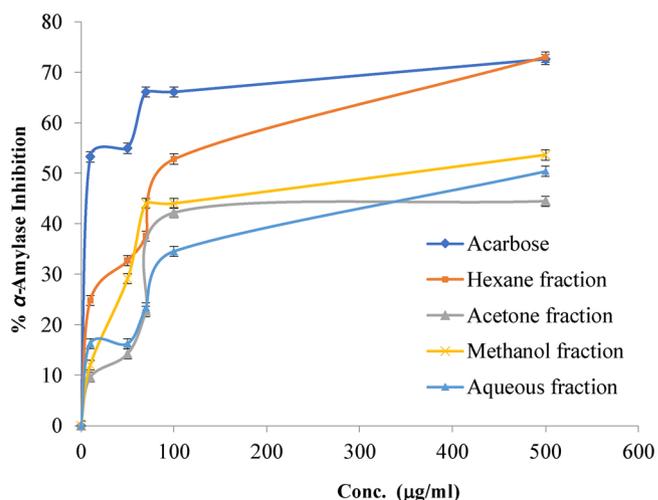


Fig. 3. The inhibitory effect of α-Amylase from *E. angustifolium* four extract fractions and Acarbose.

inhibitory activities of *E. angustifolium*.

4. Discussion

The use of medicinal herbs is as old as humanity itself, and throughout history human beings have searched for medications from nature. Awareness of medicinal herbs has been the result of countless years of struggle against diseases and various medicines have been developed using parts of plant leaves, flowers, fruits, seeds, and roots.. Research and innovations such as new computerized techniques have increased physicians, pharmacists, and chemists' ability to respond to the challenges that have emerged with the spreading of new lethal diseases [22].

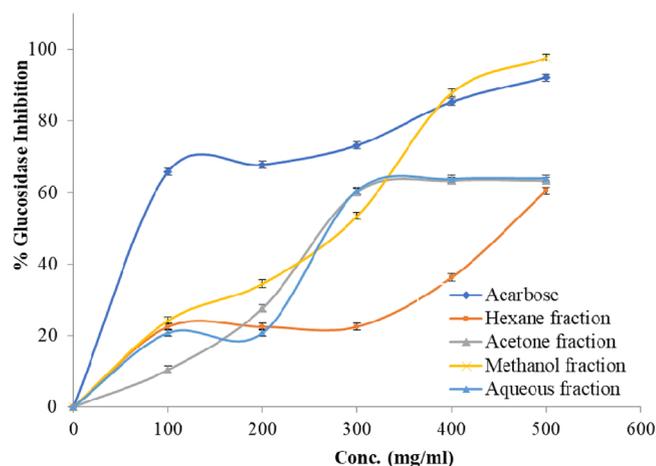


Fig. 4. Glucosidase inhibitory activity *E. angustifolium* aqueous, hexane, methanol and acetone fractions and Acarbose.

Antioxidants are essential molecules for the human body that counteract the oxidation process, which lead to cell damage and can result in harmful diseases such as cancer, atherosclerosis, Parkinson's disease, neurodegenerative disorders and many other diseases [23]. Studies have shown that some herbal contents mimic the action of endogenous antioxidants, and many of the modern health care systems advise people to take supplements to reduce oxidative stress in order to decrease or prevent many common diseases [24]. In this study the antioxidant activity of *E. angustifolium* was estimated using the DPPH method, which is usually used to investigate the free radical scavenging ability of plant extracts. It is widely used in scientific literature due to its simplicity and accuracy.

The results in the current study indicated that this plant species has potential antioxidant activity. However, the highest antioxidant potential was observed clearly in the methanol, and aqueous fractions with IC_{50} doses of $1.95 \mu\text{g/mL}$ and $29.40 \mu\text{g/mL}$, respectively, in comparison to the IC_{50} value of $1.88 \pm 1.57 \mu\text{g/mL}$ obtained from Trolox which acted as a positive control. A study conducted by Eruyur et al., on *E. angustifolium* from Turkey found that it also had an antioxidant potential with IC_{50} of $5.45 \pm 0.017 \mu\text{g/mL}$ [14]. According to our phytochemical screening, the polar fractions (methanol and aqueous) of this plant species is also rich in phenols and flavonoids, and these two classes of natural products have potent antioxidant activity due to presence of many hydroxyl groups in their structure which can remove the free harmful oxygen radicals.

Obesity and overweight are considered a significant public health problem at the global level due to their relationship with various diseases including inflammation, cancer, diabetes, immunodeficiency, and many cardiovascular diseases. Numerous drugs have been used in an attempt to reduce body mass index, but they have many miserable side and adverse effects. Natural sources may be helpful in the search for anti-obesity drugs especially if they can decrease lipid absorption resulting in a decrease in body mass.

Another promising finding was that all the obtained fractions of *E. angustifolium* plant demonstrated potent activity against porcine pancreatic lipase enzyme compared with the positive control antiobesity drug Orlistat. However, the aqueous fraction possessed the highest lipase inhibitory effect [IC_{50} $24.54 \pm 0.6 \mu\text{g/mL}$] followed by acetone [IC_{50} $26.3 \pm 0.23 \mu\text{g/mL}$] then hexane [IC_{50} $29.1 \pm 0.62 \mu\text{g/mL}$] and methanol [IC_{50} $34.67 \pm 0.46 \mu\text{g/mL}$].

All the studied plant fractions contain high amounts of saponins and, as manifested previously in other studies, that this class of natural compound has potent antilipase activity such as in Ginseng roots and Fenugreek seeds and many other plants containing saponins [25–27]. A study conducted by Marrelli et al. found that another species of *Echium* plant genus (*vulgare*) has a potent activity with 41 % of lipase inhibition

compared with Orlistat [28].

The present study evaluated antidiabetic activity by measuring the inhibitory effect of *E. angustifolium* plant four fractions against glycosidase and α -amylase carbohydrates metabolic enzymes. The displayed data reveal that hexane fraction has the highest α -amylase inhibitory activity with a value of [IC_{50} $92.98 \pm 0.85 \mu\text{g/mL}$]. In contrast with, Acarbose, the positive control, that had a value of [IC_{50} $9.54 \pm 1.22 \mu\text{g/mL}$], followed by methanol fraction [IC_{50} $255.22 \pm 0.59 \mu\text{g/mL}$], and aqueous fraction [IC_{50} $468.52 \pm 0.75 \mu\text{g/mL}$], while the IC_{50} value of acetone fraction was not applicable because the highest concentration could not reach 50 % inhibition.

Moreover, the acetone *E. angustifolium* fraction possessed the highest glycosidase inhibitory activity with an IC_{50} of $247.52 \pm 5.02 \mu\text{g/mL}$ in comparison to the reference (Acarbose), which had an IC_{50} value of $37.15 \pm 0.33 \mu\text{g/mL}$, followed by methanol fraction [IC_{50} $283.69 \pm 2.58 \mu\text{g/mL}$] and aqueous fraction [IC_{50} $278.37 \pm 5.27 \mu\text{g/mL}$]. Hexane fraction possessed the lowest α -glycosidase inhibitory effect [IC_{50} $439.67 \pm 6.39 \mu\text{g/mL}$].

Marrelli et al., showed that *E. vulgare* hydroethanolic extract had anti-amylase activity with an IC_{50} value of $69.18 \pm 1.3 \mu\text{g mL}^{-1}$ compared with acarbose which had an anti- α -amylase IC_{50} value of $50 \pm 0.9 \mu\text{g mL}^{-1}$ [28].

The phytochemical screening in this study revealed that the hexane and methanolic fractions of *E. angustifolium* plants are rich in alkaloids and steroids, which were previously reported in the literature to have powerful anti-glycosidase and anti- α -amylase activities [29–32]. The study is the first to investigate the biological potential of *E. angustifolium* plant. Specifically, no previous studies investigated anti-glycosidase and anti- α -amylase activities.

5. Limitations

Further phytochemical and pharmacological studies on *E. angustifolium* are required to isolate the bioactive compounds from this plant, elucidate their chemical structures and estimate their therapeutic activity in vivo.

6. Conclusion

The outcomes of the current investigation showed that *E. angustifolium* aqueous, methanol, hexane, and acetone fractions have a powerful antilipase effect, while the methanol fraction has a potential antioxidant effect. Also, the hexane fraction showed potent α -amylase activity. Briefly, the biological potential of *E. angustifolium* can be explained by the methanol fraction's inhibitory activity on free radicals, the high concentration of α -amylase, and glucosidase enzymes that have significant antilipase activity. Thus, the obtained fractions could be utilized in the future to manufacture pharmaceutical formulations or be used as herbal supplements to combat certain diseases.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical approval

Not applicable.

Financial support

No funding was received for the conduct of this study

CRediT authorship contribution statement

Fuad Al-Rimawi: Conceptualization, Methodology, Validation,

Writing - original draft, Writing - review & editing, Supervision. **Nidal Jaradat:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Mohammad Qneibi:** Methodology, Validation, Data curation, Writing - review & editing. **Mohammed Hawash:** Methodology, Validation, Formal analysis, Data curation, Writing - review & editing. **Nour Emwas:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest to declare. Nidal Jaradat is a member of the Editorial board of the European Journal of Integrative Medicine.

Acknowledgment

The authors wish to thank An-Najah National University and Al-Quds Universities for their support.

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