



Research paper

Carbohydrates and lipids metabolic enzymes inhibitory, antioxidant, antimicrobial and cytotoxic potentials of *Anchusa ovata* Lehm. from Palestine

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ABSTRACT

Introduction: Throughout history, therapeutically active plant products have received substantial attention due to their valuable role in the discoveries of specific medications. The aim of this study was to assess, for the first time, the antimicrobial, antioxidant, antilipase, anti- α -amylase and cytotoxic properties of four fractions derived from *Anchusa ovata* Lehm. (AO) leaves.

Methods: Antioxidant, antilipase and anti-amylase potentials of (AO) were established using DPPH (1,1-diphenyl-2-picrylhydrazyl), p-nitrophenyl butyrate and dinitro-salicylic acid procedures, respectively, while antimicrobial activity was conducted using broth microdilution assay against eight Gram-positive, Gram-negative bacterial strains in addition to one fungal strain. Moreover, the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] cytotoxic assay was utilized against cervical cancer cells (HeLa).

Results: The methanol fraction of AO showed potential antioxidant, antilipase, and α -amylase inhibitory activities with IC₅₀ values of 9.55 ± 0.13 , 53.7 ± 0.41 and 16.55 ± 1.84 μ g/ml, respectively compared with the positive controls Trolox, Orlistat and Acarbose that had IC₅₀ values of 3.23 ± 0.92 , 12.3 ± 0.35 and 28.18 ± 1.22 μ g/ml, respectively. Moreover, the hexane, acetone, and methanol fractions had wide ranges of antimicrobial potential. In addition, the cytotoxic activity outcomes which showed the best activity was for the aqueous followed by acetone, hexane and methanol fractions with IC₅₀ values of 1.04, 2.72, 3.96 and 17.67 mg/ml, respectively.

Conclusion: Our data demonstrate a wide range of biological characteristics for each AO plant fraction. This profiling information about the methanol fraction provided important data for further research and pharmaceutical applications.

1. Introduction

In the human body, free radicals created by various biochemical processes such as the reduction of molecular oxygen in the aerobic respiration creating hydroxyl and superoxide radicals can be externally formed by splitting water molecules to produce the hydroxyl radicals using the gamma rays of electromagnetic radiation [1,2]. In fact, many studies demonstrated that the presence of uncontrolled free radicals in human body is usually correlated with broad diversity of illnesses such as asthma, atherosclerosis, diabetes, cancer, Alzheimer's disease, senile

dementia, Parkinson's disease and obesity [3,4]. In 2016, approximately two billion adults were classified as overweight and more than 600 million of them were classified as obese [5].

In fact, obesity and being overweight are the major risk factors for many diseases especially cardiovascular diseases which are the leading cause of death around the world, while obesity is considered as one of the major risk factors for insulin resistance and diabetes [6,7].

Diabetes mellitus is considered one of the most dangerous metabolic disorders and a major cause of mortality and morbidity in the world due to its complications in the micro- and macro-vascular systems [8]. The

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number of diabetic people has increased from about 100 million in 1980 to more than 400 million in 2014 [9].

Non-communicable diseases are considered the main leading cause of death worldwide, and cancer occupies the top places in this list. According to the American Institute for Cancer Research, 2018 estimations showed that there are more than 18 million cancer cases around the world [10]. World Health Organization has announced that cancer incidence rate will double in the next two decades unless action is taken [11].

Cancer is a major problem for human life and a public health challenge in the 21st century. The world must direct its resources to find urgent medical treatment and provide strategies for this disease [12,13]. In fact, most of the chemotherapeutic drugs used for the treatment of cancer are extracted from plants such as vinblastine from the Vinca plant and taxol from the Pacific yew bark [14,15].

During the last decade, antimicrobial resistance has started to pose a catastrophic threat to the availability of effective treatments and prevention strategies for a huge range of infectious diseases caused by bacteria, fungi, parasites, and viruses. It is a serious threat to global health systems that requires action across all societies, governmental and non-governmental health sectors, and without sufficient and effective antimicrobial drugs, the success of cancer chemotherapy and surgical operations will be compromised. Global estimations documented in 2016 suggested that about half billion patients developed multi-drug resistance of Tuberculosis only, and antimicrobial resistance is complicating the fight against malaria and HIV, as well [16].

Plants have been used as therapeutic agents for the cure of different illnesses and as cosmeceutical products since the prehistoric times [17]. Up to date, phytotherapeutic products are still the mainstay for primary health care of about 80 % of the populations in the developing countries because they have fewer adverse effects, are more compatibility with the human body and have better cultural acceptability [18,19]. Moreover, the last two decades have shown a considerable increase in their usage even in the developed countries. For example, in the United States, botanical medicines and nutraceutical supplements sold currently in health food markets generated about 8 billion dollars of sales in 2017 compared to 4 billion dollars in 1996 [20].

Anchusa genus is represented by fifty species which are distributed in the Irano-Turanian and Mediterranean areas. These plants are utilized widely in the Arabian and Islamic medicine as potent antibacterial agents and used for the treatment of different bacterial infections of the respiratory, gastrointestinal and urinary systems [21–23].

Anchusa ovata Lehm. is a synonym of *Anchusa arvensis* subsp. *orientalis* (L.) Nordh. and *Lycopsis arvensis* L. It belongs to the Boraginaceae family which is commonly named as annual bugloss and small bugloss. It is an annual herbaceous hairy plant, which reaches 50 cm in height and has blue, small, and tubular flowers, with one seed per fruit, while the leaves are very hairy and rough. However, it is widely grown on the sandy heaths, arable lands, bares and waste places throughout the southern regions of Europe and some Mediterranean basin countries [24].

The plant roots contain naphthoquinone pigments of the enantiomeric alkannins and shikonins group, which have antimicrobial, anti-inflammatory and wound-healing potentials. Other investigations have distinguished that these molecules have potential anticancer activity [25]. In addition, the volatile oil of (AO) plant has a moderate anticholinesterase activity [26]. Moreover, the treatment with 100 mg/ml hydroalcoholic extract of (AO) reduced the rate of hemoglobin glycation to 41.90 ± 1.2 % [27].

To the best of the authors' knowledge, no previous studies have been carried out to determine the antimicrobial, cytotoxic, antioxidant, antilipase and anti-amylase activities of AO plant leaves. This investigation aimed to evaluate the antibacterial, antifungal, antioxidant antiobesity, antidiabetic and cytotoxic effects of (AO) aqueous, acetone, hexane, and methanol fractions.

2. Material and methods

2.1. Preparation of plant material

The leaves of AO were collected from the West Bank area of Palestine. Botanical characterization was carried out by a pharmacognosist Dr. Nidal Jaradat at the Pharmacognosy Laboratory at An-Najah National University and stored under voucher specimen number (Pharm-PCT-161).

The separated leaves of AO plants were washed several times with deionized water then dehydrated in the shade for twenty days at controlled humidity (55 ± 5 RH) and temperature (25 ± 2 °C). After their dehydration, the plant materials were pulverized using an electric mill into a fine powder and kept in a paper bag with proper labeling for further use.

2.2. Exhaustive fractionations method

The ground leaves were exhaustively extracted utilizing four solvent exhaustive fractionations assay which was carried out by using different solvents with various degrees of polarities including methanol, water, acetone, and hexane. Briefly, 30 g of the dried plant leaves were taken and placed in a glass bottle then extracted separately with 300 ml of each solvent. Each bottle of plant leaves and the solvent was placed on a shaking machine for 72 h at ambient temperature and was set at 400 rpm. Each solvent was filtered utilizing the suction filtration assay. Then each exhaustive extract was dried in a different way; organic ones were kept in the incubator device at 25 °C until each solvent completely evaporated, while water fraction was dried by using a freeze-dryer for 48 h. Each obtained dried fraction was stored in the refrigerator at a temperature of 2–8 for later use. The obtained percentages yields were 0.71, 1.38, 1.1, and 3.5 %, for the aqueous, methanol, hexane and acetone fractions, respectively.

2.3. Phytochemical analysis

The phytochemical screening tests were established according to the standard analytical methods to identify the presence of primary and secondary metabolic groups including carbohydrates, starch, flavonoids, saponins, glycosides, alkaloid, tannins and phenols, protein and terpenoids [28].

2.4. DPPH radical scavenging activity

The working plant solutions (1 mg/ml) were prepared by mixing 0.1 g of each plant fraction in 0.1 l of methanol then these solutions were diluted by methanol to obtain different concentrations (2, 5, 10, 20, 50 and 100 µg/ml). One ml from each plant fraction working solution and 1 ml of methanol were prepared with 1 ml of DPPH solution and incubated for 30 min in dark place at room temperature. The blank solution was prepared by replacing the plant fraction solution with methanol. Trolox was used as a reference and the absorbance was measured by UV-vis spectrophotometer at 517 nm and compared with the control. The antioxidant activity was calculated by the following equation.

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

Where, I (%), is the percentage of antioxidant activity [29]. However, Trolox which is a vitamin E analog and has powerful antioxidant potential was used as a positive control in the current experiment.

2.5. Porcine pancreatic lipase enzyme inhibitory method

One mg/ml stock plant dilution was made by dissolving 0.1 g from each plant fraction in 100 ml of Dimethyl sulfoxide (DMSO). The

produced solution was diluted several times to obtain different concentrations (50, 100, 200, 300 and 400 µg/ml). In addition, a lipase enzyme stock solution (1 mg/ml) was directly prepared before use by dissolving 25 mg of lipase enzyme powder in 25 ml of 10 % DMSO. The p-nitrophenyl butyrate (PNPB) stock solution was prepared by dissolving 20.9 mg PNPB in 2 ml of acetonitrile. 0.2 ml from each plant fractions was prepared. Serial dilutions were mixed with 0.1 ml of lipase enzyme stock solution and completed with Tris–HCl solution to reach 1 ml of volume. Then incubated for 15 min at 37 °C in water bath and after 15 min, 100 µl of PNPB solution were added and incubated for 30 min at 37 °C. The blank solution was prepared by mixing 100 µl of lipase enzyme (1 mg/ml) solution with Tris–HCl solution up to 1 ml. Orlistat which is a potent antilipase drug was used as a positive control and followed the same previous steps as plant fraction. The absorbance was measured utilizing a spectrophotometer (UV–vis) at 405 nm. However, the lipase enzyme inhibitory potential was measured utilizing the following equation:

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

Where, I (%), is the percentage inhibition of lipase enzyme [30].

2.6. α -Amylase inhibitory method

A plant working solution (1 mg/ml) was prepared by mixing 25 mg of each plant fraction in 10 % of DMSO. This solution was then diluted by the buffer to obtain different dilutions (10, 50, 70, 100, 500 µg/ml). Subsequently, an α -amylases enzyme stock solution (2 units/ml) was prepared by dissolving 12.5 mg of α -amylase enzyme in a minimum amount of 10 % DMSO and the buffer solution were added up to 100 ml. Then corn starch solution was prepared by dissolving 1 g of starch in 100 ml distilled water. A 200 µl from each plant fraction stock solution was mixed with 200 µl of α -amylase stock solution and incubated for 10 min at 30 °C in a water bath. After that, 200 µl of corn starch solution were added and incubated for 3 min at 30 °C. Moreover, 3,5-dinitro salicylic acid was added and boiled in a water bath at 85–90 °C for 10 min and after the solution was cooled, 5 ml of distilled water was added and prepared blank solutions throughout the replacement of the (AO) fractions with 200 µl of buffer solution. In addition, the commercial antidiabetic drug Acarbose was utilized as a positive control. The optical activity of the prepared solutions was assessed at 540 nm utilizing a UV–vis spectrophotometer. The α -amylase inhibitory potential was calculated utilizing the following equation:

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

Where I (%), is the α -amylase inhibitory percentage [31].

2.7. Antimicrobial method

Broth micro-dilution method was used to determine the antimicrobial activity of four solvents fractions of (AO). However, the screened strains including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, and *Candida albicans* were American Type Culture Collection types and have ATCC codes 25922, 13883, 25923, 8427, 700221, 9027, and 90028, respectively in addition to a clinical strain of Methicillin-resistant *Staphylococcus aureus* (MRSA). Each fraction of AO plant was dissolved in DMSO in a concentration of 50 mg/ml for methanol, acetone and water fractions and 25 mg/ml for hexane fraction. Then, two-fold serial dilutions of the antimicrobial agent were prepared in liquid growth medium (Mueller Hinton for the bacteria and RPMI 1640 for the *Candida albicans*) dispensed in 96-well micro-titration plate. After that, each well was inoculated with microbial inoculums, which were prepared in the same medium after dilution of standardized microbial suspension adjusted to 0.5 McFarland scale. Controls were performed on the same plate, including two negative controls that involved; media alone and media with

plant extract serially diluted, and one positive control that contains microbe in addition to the media. After well mixing, the 96-well micro-titration plates were incubated at 37 °C for 18–24 hrs for bacteria and 35 °C for 24–48 hrs for *Candida albicans* [32–34].

2.8. Cytotoxicity procedure

RPMI-1640 medium was utilized to culture the cervical adenocarcinoma (HeLa) cancer cells containing 10 % of fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine. The HeLa cells were grown in a humidified environment at 37 °C degree with 5% CO₂ atmosphere, then was used a 96-well plate to seed the cells at 2.6 *10⁴ cell/well. After 24 h, cells were treated with various concentrations of the tested (AO) fractions. Cell viability was assessed by the Cell-Titer 96® Aqueous One Solution Cell Proliferation (MTS) assay according to the manufacturer's instructions (Promega Corporation, USA). Briefly, at the end of the treatment, 20 µl of MTS solution per 100 µl of media were added to each well and incubated at 37 °C for 2 h. Absorbance was measured using UV–vis-spectrophotometer at wavelength of 490 nm.

2.9. Statistical analysis

All the conducted experiments were determined in triplicate. The results are presented as means ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software version 6.01. Comparisons between three or more groups were performed by one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test.

3. Results

3.1. Preliminary phytochemical analytical tests

The standard analytical tests revealed the presence of various primary and secondary phytochemical metabolites in the hexane, acetone, methanol and aqueous fractions including proteins, carbohydrates, alkaloids, saponins, glycosides, steroids, terpenoids, tannins and phenols as shown in Table 1.

3.2. Antioxidant activity

As depicted in Table 2 and Fig. 1, the free radical scavenging characters' outcomes revealed that the methanol fraction has the most potent antiradical activity followed by the acetone fraction with IC₅₀ values of 9.55 ± 0.13 and 13.18 ± 0.13 µg/ml, respectively.

3.3. Antilipase activity

As illustrated in Table 3 and Fig.2, extracts derived from AO inhibited the activity of pancreatic lipase enzyme in a dose-dependent manner. IC₅₀ values of hexane, acetone, methanol, and aqueous fractions and Orlistat (commercial antiobesity drug) were 501.18 ± 0.65,

Table 1
Standard phytochemical analysis of *Anchusa ovata* (AO) four solvents fractions.

Phytochemical test	Hexane	Acetone	Methanol	Aqueous
Proteins	+	+	+	–
Sugars	–	+	+	+
Carbohydrates	–	+	+	+
Phenols and tannins	–	+	+	+
Flavonoids	–	–	–	–
Alkaloids	–	+	–	+
Saponins	+	–	–	–
Glycosides	+	+	+	+
Killer killani	–	+	+	+
Steroids	–	+	+	+
Terpenoids	–	–	+	–

Table 2
Free radicals' inhibitory activity IC₅₀ values of four Anchusa ovata (AO) fractions and Trolox.

Conc.	Trolox	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	53.43 ± 3.46	37.69 ± 0.19	38.64 ± 0	20.83 ± 0	13.445 ± 0.185
5	61.51 ± 0.34	34.66 ± 0.095	38.83 ± 0.19	20.83 ± 0	4.735 ± 0.185
10	91.41 ± 0.34	37.31 ± 0.095	44.89 ± 0	50.57 ± 0.19	9.85 ± 0
20	97.29 ± 0.34	37.12 ± 0	44.89 ± 0.19	85.42 ± 0.38	4.735 ± 0.185
50	96.8 ± 0.34	32.005 ± 0.185	63.07 ± 0.19	87.5 ± 0	0.57 ± 0.19
100	97.54 ± 0.69	27.46 ± 0.19	89.21 ± 0.185	91.1 ± 0.19	7.765 ± 0.185
IC ₅₀ (µg/ml), ± SD	3.23 ± 0.92	Inactive	13.18 ± 0.13	9.55 ± 0.13	Inactive

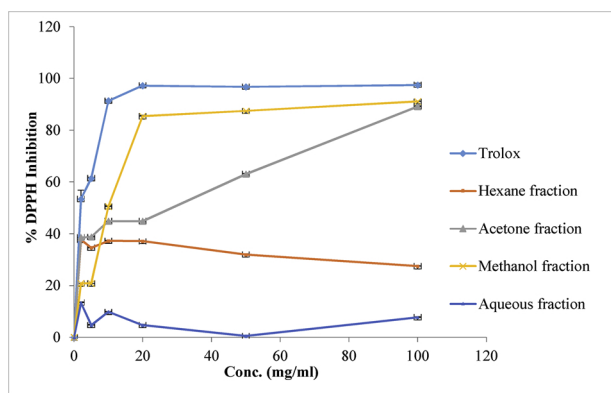


Fig. 1. Free radical inhibitory activity of Anchusa ovata (AO) four fractions and Trolox.

331.1 ± 0.47, 53.7 ± 0.41, 75.85 ± 0.62 and 12.3 ± 0.35 µg/ml, respectively.

3.4. α-Amylase inhibitory activity

As demonstrated in Table 4 and Fig. 3, hexane, acetone, methanol and aqueous fractions obtained from (AO) possessed *in vitro* inhibitory activity against porcine pancreatic α-amylase activity in a concentration-dependent manner with IC₅₀ values 63 ± 1.89, 83.17 ± 0.44, 16.55 ± 1.84 and 72.44 ± 1.86 µg/ml, respectively. While the IC₅₀ of the commercial antidiabetic drug was 28.18 ± 1.22 µg/ml.

3.5. Antimicrobial effects

Broth micro-dilution method was used to determine the susceptibility of microorganisms to antimicrobial drugs, which is the most popular assay in the United States. The outcomes of the current study revealed that the AO plant hexane, acetone, and methanol fractions have various degrees of antimicrobial effects against *S. aureus*, MRSA, *P. vulgaris*, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *E. faecium*, while the aqueous fraction did not show any antimicrobial effect against the selected microbial strains as shown in Table 5. However, the AO plant aqueous, hexane, acetone, and methanol fractions did not reveal any antifungal effects against *C. albicans*.

Table 3
Lipase inhibitory activity IC₅₀ values of four Anchusa ovata (AO) fractions and Orlistat.

Conc.	Orlistat	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
50	91.05 ± 0.77	19.59 ± 0.48	29.05 ± 0.48	53.37 ± 0.46	53.54 ± 0.71
100	93.1 ± 0.42	19.59 ± 0.48	38.34 ± 0.72	57.7 ± 0.47	57.7 ± 0.47
200	94.3 ± 0.42	31.58 ± 0.71	38.34 ± 0.72	57.7 ± 0.47	58.1 ± 0.95
300	97.4 ± 0.12	60.25 ± 0.78	55.5 ± 0.21	57.7 ± 0.47	58.1 ± 0.95
400	97.5 ± 0.00	60.25 ± 0.78	55.5 ± 0.21	94.37 ± 0.17	66.8 ± 0
IC ₅₀ (µg/ml), ± SD	12.3 ± 0.35	501.18 ± 0.65	331.1 ± 0.47	53.7 ± 0.41	75.85 ± 0.62

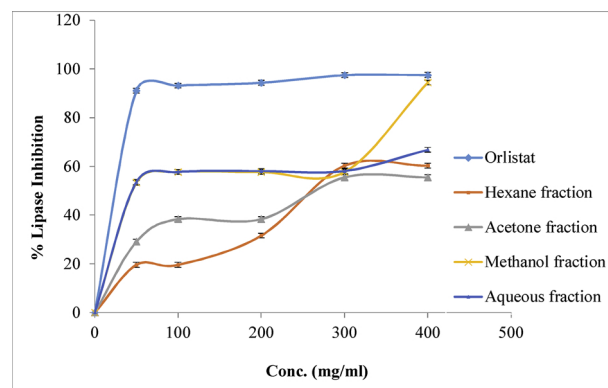


Fig. 2. Porcine pancreatic lipase inhibitory activity of Anchusa ovata (AO) four fractions and Orlistat.

3.6. Cytotoxic activity

As shown in Table 6, extracts derived from AO inhibited the growth of cells in a dose-dependent manner. The IC₅₀ values of methanol, hexane, acetone, and aqueous fractions were 17.67, 2.72, 3.96 and 1.04 mg/ml, respectively.

4. Discussion

No one can deny the great value of the aromatic and medicinal plants in the drug discovery and developments in addition to their important role in the pharmaceutical, cosmeceutical and food industries [35].

4.1. Antioxidant activity

The leaves of the Anchusa ovata Lehm. plant contain cyanidin 3-glucoside and delphinidin 3-glucoside polyphenolic anthocyanin molecules, which have potential antioxidant activity and can protect tissues and cells from the damages caused by the oxidative stress and decrease the risk of cardiovascular various diseases and cancer as well as reduce the complications of diabetes and other metabolic disorders [36]. In a study conducted by Gharib et al. showed that the aqueous and ethanol extracts of AO have an antioxidant effect with an IC₅₀ value of

Table 4
α-Amylase inhibitory activity IC₅₀ values of Anchusa ovata (AO) four fractions and Acarbose.

Conc.	Acarbose	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
10	53.22 ± 1.2	10.8 ± 3.07	42.32 ± 0.13	67.3 ± 9.2	26.41 ± 0.78
50	54.91 ± 0.58	21.7 ± 0	43.61 ± 1.4	67.3 ± 0	35.13 ± 3.8
70	66.1 ± 1.34	28.2 ± 0	51.48 ± 0.27	67.3 ± 0	44.43 ± 4.4
100	66.1 ± 1.62	39.1 ± 6.1	53.43 ± 0.13	67.3 ± 0	70.15 ± 0.16
500	72.54 ± 1.37	52.17 ± 0.3	59.26 ± 0.26	86.9 ± 0	70.15 ± 0.16
IC ₅₀ (µg/ml), ± SD	28.18 ± 1.22	63 ± 1.89	83.17 ± 0.44	16.55 ± 1.84	72.44 ± 1.86

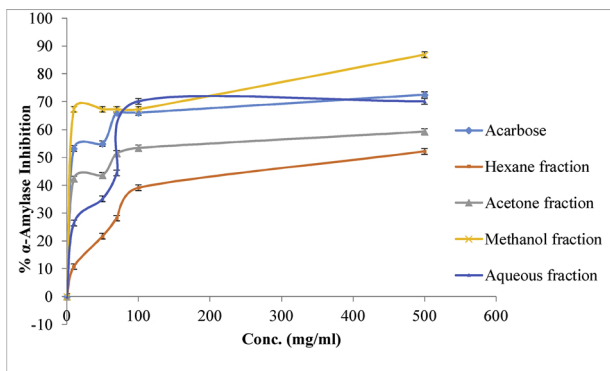


Fig. 3. α-Amylase inhibitory activity of Anchusa ovata (AO) four fractions and Acarbose.

16.44 ± 2.80 and 14.12 ± 2.60 µg/ml, respectively [37]. However, the current study revealed that the methanol fraction has the most potent antioxidant action followed by the acetone fraction with IC₅₀ values of 9.55 ± 0.13 and 13.18 ± 0.13 µg/ml, respectively comparing with Trolox, which has an IC₅₀ value of 3.23 ± 0.92 µg/ml. On the other hand, hexane and aqueous fractions were inactive.

4.2. Porcine pancreatic lipase inhibitory activity

In an investigation established by Conforti et al., they found that the hydroalcoholic extract of *Anchusa azurea* flowers has antilipase effect with an IC₅₀ value more than 10,000 µg/ml [38]. While the current investigation results showed that the methanol fraction of (AO) plant has potential antilipase activity followed by an aqueous fraction with IC₅₀ doses of 53.7 ± 0.41 and 75.85 ± 0.62 µg/ml, respectively in comparison with the commercial antilipase drug Orlistat which has a lipase inhibitory activity with an IC₅₀ value of 12.3 ± 0.35 µg/ml.

4.3. α-Amylase inhibitory activity

α-Amylase and α-glucosidase are the metabolic enzymes which are charged in the hydrolysis of carbohydrates in the gastrointestinal tract, also they are responsible for the metabolic disorder that causes postprandial hyperglycemia [39]. However, α-amylase hydrolyzes the starch (amylum) and glycogen by the cleavage of α-1,4-glycosidic bonds between the polysaccharide units of polysaccharides while the α-glucosidase hydrolyzes the disaccharide to monosaccharide units,

Table 5
Antibacterial activity MIC values (mg/ml) of Anchusa ovata (AO) four fractions.

Fractions	MRSA	<i>E. faecium</i>	<i>E. coli</i>	<i>k. pneumoniae</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>S. aureus</i>
Hexane	R	R	R	R	6.25	R	6.25
Methanol	R	R	R	R	12.5	12.5	6.25
Acetone	12.5	12.5	12.5	12.5	12.5	R	12.5
Aqueous	No antibacterial activity						

R = Resistant.

Table 6
The cytotoxic activity of four Anchusa ovata (AO) solvent fractions.

Conc.	Methanol fraction Relative quantity to control	Acetone fraction	Hexane fraction	Aqueous fraction
0.3125	0.989882	1.155529	0.818118	0.981176
0.625	0.922588	1.065412	0.762824	0.923529
1.25	0.930353	1.061412	0.872235	0.386824
2.5	0.931765	0.834353	0.964941	0.143529
5	0.796706	0.118824	0.107529	0.150588
10	0.66147	0.133176	0.106118	0.187059
IC ₅₀ (mg/ml)	17.67	2.72	3.96	1.04

which is the main cause of the postprandial hyperglycemia. Therefore, the inhibitors of α-glucosidase and α-amylase enzymes are considered among other effective agents that can regulate postprandial hyperglycemia and control type II of diabetes. It has been well acknowledged that phytochemicals and plants extracts are potential alternatives to chemical inhibitors against α-amylase and α-glucosidase [40].

In a study conducted by Sarikurkcu1 et al. found that the methanol extract of *Anchusa undulata* from Turkey has an α-amylase inhibitory activity with an IC₅₀ value of 0.193 ± 0.006 mmol ACEs/g extract [41]. However, the results of the current study showed that the methanol fraction of AO plant had powerful α-amylase inhibitory activity even stronger than the commercial α-amylase inhibitor Acarbose followed by the aqueous and acetone fractions. The methanol fraction of our plant has α-amylase inhibitory activity with IC₅₀ dose of 16.55 ± 1.84 µg/ml, which is much higher than the commercial antidiabetic drug (Acarbose) which has an IC₅₀ value of 28.18 ± 1.22 µg/ml.

4.4. Antimicrobial effects

Each year in the U.S.A., about 2 million patients are infected with antibiotic-resistant bacterial pathogens, and about 23,000 people die as a result. In this case, the world health care systems urgently need to adjust the ways of using and prescribing antibiotics. Even more novel antibiotics are discovered, without behavior changes, the resistance against antibiotic will remain the major life-threat issue and these changes also must include more work to decrease the spread of these infections throughout the development of vaccines, body and food hygiene [42].

The results revealed that the hexane, methanol, acetone fractions have antibacterial activity against *S. aureus* with MIC values of 6.25, 6.25, 12.5 mg/ml, respectively, while the methanol fraction was the

only one which affected the growth of *P. vulgaris* with MIC dose of 12.5 mg/ml. Moreover, the hexane, methanol, acetone fractions inhibited the growth of *P. aeruginosa* with MIC doses of 6.25, 12.5, 12.5 mg/ml, respectively, while the acetone fraction was the least that affected *K. pneumoniae*, *E. coli*, *E. faecium* and MRSA with MIC values of 12.5 mg/ml, respectively. Evidently, the hexane, methanol, acetone and aqueous fractions of AO plant were inactive against the screened *C. albicans* strain and the aqueous fraction was an inert fraction against all of the screened pathogens.

A study conducted by Shtayeh et al. from Palestine found that the aqueous extract of *Anchusa strigosa* has inhibition zone diameter against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans* of 6.0 mm, respectively, while the ethanol extract has an inhibition zone diameter of 14.7, 6.0, 6.0, 12.6, 6.0, and 10.6 mm, respectively [43]. Another study conducted by Miri et al. from Iran found that the ethanol leaf extract of *A. ovata* has inhibition zone diameter against *K. pneumoniae*, *B. subtilis*, *E. coli*, *S. aureus*, and *P. aeruginosa* were 9, 4, 9, 11 and 16 mm, respectively. While the inhibition zones of the aqueous extract were 6, 2, 8, 8 and 19 mm, respectively [44].

4.5. Cytotoxic activity

The cytotoxic results showed that AO methanol, hexane, acetone, and aqueous fractions have a little to moderate cytotoxic effect. The cytotoxic activity of AO in different solvent fractions showed that the highest activity was for aqueous followed by acetone, hexane and methanol fractions with IC50 values of 1, 3, 4 and 17 mg/ml, respectively. This is less strong than previous reports by Boskovic et al., where chloroform, ethyl acetate, ethanol, acetone, and petroleum extracts derived from *A. officinalis* plant have shown to possess cytotoxic activity against Hep 2 cancer cells with IC50 0.14 ± 2.12 , 0.17 ± 0.2 , 0.14 ± 0.87 , 0.14 ± 1.30 and 0.18 ± 2.26 mg/ml, respectively [45]. Our data suggest that fractions derived from AO, especially methanol, have little cytotoxic effect and it could be considered safe, however, this needs further in vivo validation.

This is the first report of antioxidant, antibacterial, antiamylase antilipase and cytotoxic effects of AO plant four solvents fractions from Palestine. The authors suggest that AO plant screened in this study could be utilized to investigate pharmacologically active natural agents that may lead to the discovery of new pharmaceutical entity.

However, further isolation and purification of potentially active compounds and ultimately in-vivo screening are required to determine the side effects, toxicological characteristics, pharmacokinetics and pharmacodynamic effects of the AO plant active ingredients.

5. Conclusion

In comparison with the positive controls Trolox, Orlistat and Acarbose, the methanol fraction of (AO) plant revealed potential antioxidant, antilipase, and α -amylase inhibitory activities. Moreover, antimicrobial tests showed that the hexane, acetone, and methanol fractions have a wide range of potential. In addition, the cytotoxic analysis of the tested fractions revealed little to moderate cytotoxic activity, suggesting a safe source of potential pharmacological compounds. It is clearly shown that the methanol AO fraction should be tested further as effective chemical against oxidative stress, hyperlipidemia, hyperglycemia, and safety to serve for replacement therapy of chemical drugs or as natural supplement to help in decreasing the complications caused by these lethal diseases.

Authors' contributions

All research done by the authors.

Financial support

None.

Ethical approval

None.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.eujim.2020.101066>.

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