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Phenolic contents, antioxidant, and enzyme inhibitory effects of crude extracts of *Polycarpon polycarpoides* Fiori subsp. *catalaunicum* O. Bolòs & Vigo

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Abstract

The present work aimed to study some pharmacological effects of extracts of the aerial parts of *Polycarpon polycarpoides* Fiori subsp. *catalaunicum* O. Bolòs & Vigo (Caryophyllaceae) widely distributed in the Mediterranean basin. The crude extracts were prepared using four solvents (petroleum ether, ethyl acetate, methanol, and methanol: water 1 : 1, v/v), and then examined for their phenolic contents using spectrophotometric methods and for their *in vitro* antioxidant activity and ability to inhibit urease, acetylcholinesterase, and α-amylase. Low levels of total phenolic were recorded ranging from 14.39 ± 3.40 to 101.84 μg.GAE.mg^{-1} of extract corresponding to the petroleum ether and ethyl acetate extracts, respectively. As for the flavonoids, very low values varying from 0.37 ± 0.13 to 4.22 ± 0.83 μg.GAE.mg^{-1} of extract were obtained. A moderate antioxidant effect was exerted by the extracts most often the methanol and ethyl acetate extracts were the most potent probably due to their polyphenolic content. Remarkable inhibitory effect has been exhibited by the extracts against α-amylase and more specifically, the petroleum ether extract displayed the strongest capacity with a percentage inhibition of 48.19 ± 2.99 % at 400 μg.mL^{-1}. However, all extracts were inactive against urease and acetylcholinesterase. These results could constitute a starting point for carrying out more studies on the plant in order to assess the possibility of valuing it as a source of bioactive compounds.

Introduction

Recently, the awareness of the harmful effects of the excessive use of synthetic products incorporated in pharmaceutical or food formulations for therapeutic, nutritional, organoleptic purposes has led to increased interest in natural products. In addition, the interest of natural products has also arisen from the fact that these compounds can exert multiple effects by their action on various targets providing more beneficial effects (Ayaz *et al*. 2019). Therefore, it is important to research new therapeutic substances, nutraceuticals, or food additives as alternatives to synthetic products (Moldes *et al*. 2017). In this context, plants remain the main source of new therapeutic molecules with high healing potential and less toxic effects (Christaki *et al*. 2012; Gali *et al*. 2020).

Natural antioxidants have received a lot of attention in recent decades for their incorporation into foods as additives to delay the oxidation reactions responsible for the loss of their nutritional and organoleptic qualities (Lourenço *et al.* 2019). In fact, the use of synthetic antioxidants such as butylhydroxyanisol (BHA) and butylhydroxytoluene (BHT) in the food industry has raised concerns about their possible involvement in certain carcinogenesis processes and therefore their replacement by natural compounds with less harmful effects constituted a primary need (Dolatabadi and Kashanian 2010). Antioxidants-based supplements have also been the subject of recent years as there is growing evidence for the involvement of free radicals and other reactive species in the development of certain chronic diseases such as cancer, inflammatory, metabolic, cardiovascular and neurological diseases (Moure *et al.* 2001). Among phytochemicals, polyphenols are recognized as the most potent natural antioxidants found in both edible and non-edible plants due to their structure with hydroxyl groups giving them potential redox properties (Shahidi and Ambigaipalan 2015).

Plants are also considered an important source of therapeutics to treat many diseases due to the presence of various phytochemicals targeting cellular constituents such as enzymes that are central to biochemical reactions. In fact, many therapies used in the management of certain diseases are based on the inhibition of key enzymes as in the case of Alzheimer's disease and type 2 diabetes. The need for new molecules and especially of natural origin comes from the toxicity and/or non-specificity of the available treatments and therefore lower effectiveness of these therapies (Kekuda and Rao 2019).

Caryophyllaceae also known as the rose family is one of the largest families of around 3,000 species generally distributed in the northern hemisphere, particularly in the Mediterranean basin (Derici *et al.* 2021). This family is known for its richness in active metabolites, in particular flavonoids, alkaloids, triterpene saponins, phytoecdysteroids and sterols (Jakimiuk *et al.* 2021). The presence of various active constituents makes the species of this family of medical importance and can be interesting sources of new bioactive constituents of therapeutic or food interests. Caryophyllaceae plants have been reported to exert various biological effects such as antioxidant, antiviral, anticancer, anti-inflammatory and antimicrobial effects. (Chandra and Rawat 2015). *Polycarpon polycarpoides* (Biv.) Fiori subsp. *catalaunicum* O. Bolòs & Vigo is a species of the Caryophyllaceae family that grows in northern Algeria. However, data on its pharmacological effects are poorly provided. Thus, this study is devoted to determining some of the biological effects of this species for its valuation as medicinal or food plant.

Experimental

Chemicals

Folin-Ciocalteu, sodium carbonate, vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), neocuproine, AChE type VI-S from electric eel (<1000 U.mg^{-1} solid), urease from *Canavalia ensiformis* (Jack bean, ≥5 U.mg^{-}solid), α-amylase from *Aspergillus Oryzae* (≥150 U/protein) trolox, gallic acid, quercetin, catechin, and other chemicals were purchased from Sigma-Aldrich (Saint-Louis, USA) Acetylthiocholine-iodide was obtained from AppliChem GmbH, (Darmstadt, Germany. All organic solvents and chemicals used in this study were of analytical grade. All the assays were carried out on a 96-well microplate, and the absorbance was recorded using a EnSpire multimode plate reader (PerkinElmer, Waltham, USA).

Plant material

The aerial part of *Polycarpon polycarpoides* was collected in Annaba (northern Algeria) in December 2017. The plant was identified by Dr. Tarek Hamel (Department of Biology, Badji Mokhtar University, Annaba, Algeria). The samples were cleaned and air dried in the dark, and then ground to a fine powder using an electric grinder.

Preparation of crude extracts

The extraction procedure was carried out by soaking 25 g of vegetable powder in 100 mL of the extraction solvent (petroleum ether, ethyl acetate, ethanol and methanol: water (1:1, v/v) for 2 h with continuous stirring (300 rpm) at room temperature, the solution obtained was filtered through a Whatman filter (Whatman filter paper n°4). The marc is extracted twice more to extract the maximum of soluble compounds. The various solvents were then eliminated under reduced pressure at 40 °C using the rotary evaporator Buchi R-215 (Büchi AG, Uster, Switzerland). The residues were stored at 4 °C for further analysis.

Total phenolic contents

Total phenolic content in *Polycarpon polycarpoides* extracts was determined by the Folin-Ciocalteu method on a 96-well microplate as reported by Müller *et al.* (2010). The reaction consisted of mixing 20 µL of samples (1 $mg.mL^{-1}$), 100 µL of Folin-Ciocalteu reagent (diluted in distilled water, 1 : 9, v/v), and 75 µL of sodium carbonate (7.5 %) for 2 h in darkness at room temperature. The absorbance was then read at 765 nm in a microplate reader (Perkin Elmer Enspire, Singapore). Total phenolic contents were expressed as µg gallic acid equivalents per mg of extract ($\mu g.GAE.mg^{-1}$) using the equation of linear regression obtained from the gallic acid standard curve (y = 0.0034 gallic acid + 0.1044, R^2 = 0.9972).

Total flavonoids content

Total flavonoids were quantified by the aluminum chloride ($AlCl_3$) method according to Terkmane *et al.* (2018) with some modifications. A volume of 100 µL of plant extract (1 $mg.mL^{-1}$) and an equal volume (100 µL) of a methanolic solution of aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$, 2 % prepared in methanol) were mixed. The reaction was left for 15 min and the absorbance was then recorded at 415 nm against a blank containing the sample and methanol instead of $AlCl_3$. The results were reported in µg of quercetin equivalent per mg of extract ($\mu g.QE.mg^{-1}$) calculated from the standard curve of quercetin (y = 0.027 quercetin, R^2 = 0.9989).

Condensed tannins content

Condensed tannins contents were investigated following the method reported by Bakhouche *et al.* (2021). In a 96-well microplate, each extract was reacted with hydrochloric acid (30 %) and a methanolic solution of vanillin (4 % in methanol). The absorbance of the red complex flavonols-vanillin was measured at 500 nm. The results were expressed as µg of catechin equivalents (µg CE) per mg of extract calculated from the standard curve of catechin (y = 0.0026 catechin + 0.0301).

DPPH scavenging activity

The capacity of the *Polycarpon polycarpoides* extracts to trap the DPPH radical was determined according to the method reported by El Aanachi *et al.* (2020). Briefly, 40 µL of extracts or standards (Trolox and ascorbic acid) at different concentrations (6.25 – 800 $\mu g.mL^{-1}$) were reacted with a 160 µL of a freshly prepared solution of DPPH (1 mM in methanol). The residual purple color of the DPPH radical was followed at 517 nm after 15 min of incubation. The results were expressed as percentages of inhibition calculated according to the formula below (Eq. 1):

$$\text{Inhibition } (\%) = ((A_C - A_S)/ A_C) \times 100 \qquad (1)$$

where A_C and A_S are the absorbances of the control (methanol and DPPH solution) and of the sample, respectively. The concentration of the extract inhibiting 50 % of the radical (IC_{50}) was determined from the curve drawn by the percentages of inhibition at different concentrations.

Scavenging activity of $ABTS^{\bullet+}$

The ability of extracts to trap radicals ABTS has been evaluated using the method described by Dudonné et *al.* (2009) with some modifications. The $ABTS^{\bullet+}$ cation solution has been previously prepared by reacting a volume of 7 mM of ABTS with a same volume of 2.5 mM potassium persulfate for 16 hours at room temperature and in

the dark. The resulting solution is diluted with distilled water to obtain a solution having an absorbance of 0.7 at 734 nm. Then, samples (40 µL) at different concentrations were pipetted in a microplate and then 160 µL of a diluted solution of $ABTS^{\bullet +}$ were added and the mixture was allowed to react for 10 min. The absorbance was recorded at 734 nm. The inhibition rates of the radical were determined using the previous formula (1). IC_{50} values were calculated from the equation of the regression curves.

Phenanthroline assay

The method reported by Szydłowska-Czerniak *et al.* (2008) was used to measure the ability of extracts to reduce ferric iron in the phenanthroline-Fe (III) complex. In a 96-well microplate, 10 µL of extract, 50 µL $FeCl_3$ (0.2 %), 30 µL phenanthroline (0.5 % in methanol), and 110 µL methanol were mixed. The reaction was left for 20 min at room temperature and the absorbance was then measured at 510 nm. The results are reported in the form of absorbances. Trolox and ascorbic acid were used as standards.

Cupric Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC test was performed by the method described by Apak *et al.* (2004). In a 96-well microplate, 40 µL of extract, 60 µL of ammonium acetate (1 M), and 50 µL of neocuproine (7.5 mM), and 50 µL of copper (II) chloride (10 mM) are mixed. The mixture is incubated for 1 h, and the absorbance was read at 450 nm. Results were reported as absorbances at different concentrations and were compared to the standards Trolox and ascorbic acid.

α-amylase inhibition

The determination of the inhibitory activity of α-amylase is carried out according to the method of Yang et *al.* (2012) with modifications. In a 96 well-microplate, 25 µL of each extract at different concentrations was mixed with 50 µL of the α- amylase solution (1 $U.mL^{-1}$ in the phosphate buffer saline, 100 mM, pH, 6.9, 6 mM NaCl) for 10 min at 37 °C. The enzymatic reaction was initiated by adding 50 µL of starch (0.1 %). A second incubation for 10 min at 37 °C is carried out and the reaction was stopped by the addition of 25 µL HCl (1 M), and the residual starch is revealed by 100 µL of iodine-potassium iodide solution (IKI). The reading is carried out at 630 nm against a negative control containing all reagents without the extracts. In parallel, controls containing only the starch solution and IKI, or the sample solutions and IKI were performed. Results are reported as percentages of inhibition at different concentrations using the formula below (Eq. 2):

$$\text{Inhibition (\%)} = [1-((A_s-A_b)/(A_e-A_c))]*100 \quad (2)$$

where: A_c is the absorbance of the reaction containing only starch, IKI, and HCl without enzyme and inhibitor solutions, A_e is the absorbance of the control containing all the reagents without inhibitor, A_s is the absorbance of the reaction containing all the reagents in presence of the test sample, A_b is the absorbance of the blank containing only the test sample and IKI.

Urease inhibition

The inhibitory effect of the extracts on urease was examined using the protocol described by Nabati *et al.* (2012). The extracts (10 µL) were mixed with 25 µL of the urease solution (1 $mg.mL^{-1}$ in 100 mM of phosphate buffer, pH 8.2) and 50 µL of urea (30 mM). The amount of ammonia in the medium was quantified by adding 45 µL of phenol reagent (0.4 g of phenol and 2 mg of sodium nitroprusside in 40 mL of deionized water) and 70 µL of the basic reagent (0.3 g of NaOH and 0.5 mL of hypochlorite in 60 mL of deionized water). The absorbance of the blue color of the complex ammonia-phenol-hypochlorite was recorded at 630 nm. Results are reported in terms of percent inhibition calculated using the following formula (Eq. 3):

$$\text{\% inhibition} = (E-S/E)\times 100 \quad (3)$$

where E represents the absorbance of the control containing the enzyme solution without the tested samples, whereas S is the absorbance of the

reaction medium in the presence of the tested sample.

Acetylcholinesterase inhibition

The inhibition of acetylcholinesterase was examined by the method previously reported by (Cetin Cakmak and Gülçin 2019). Briefly, a volume sample (10 µL) were put in contact with 20 µL of acetylcholinesterase (5.32.10-3 U in 0.1 M phosphate buffer, pH 8) at 25 °C for 15 min. The reaction was started by adding acetylthiocholine iodide (0.71 mM) and the resulting thiocholine was quantified by DTNB (10 µL, 0.5 mM). The absorbance as monitored at 415 nm. The rate of inhibition was determined using the preceding Eq. 3.

Statistical analysis

Data were presented as mean ± standard deviation of three measurements. One-way ANOVA analysis followed by the Tukey's multiple comparison test was adapted to analyse the difference between samples using GraphPad software version 5 (GraphPad Software Inc, California, USA). The *P*-value was set at 0.05.

Results

Phenolic compounds contents

The results of the quantification of the main classes of polyphenols are shown in Table 1. As shown, the total phenolic and flavonoids contents are influenced by the extraction solvent. Ethyl acetate and methanol extracts gave the highest total phenolic contents with values of 108.12 ± 8.15 and 101.84 ± 2.21 $\mu g.GAE.mg^{-1}$ of extract, respectively (the values are not significantly different, $P>0.05$). In another hand, the contents of flavonoids recorded were very weak ranging from 0.37 ± 0.13 (petroleum ether) to 4.22 ± 0.83 $\mu g.GAE.mg^{-1}$ of extract observed in the ethyl acetate extract.

Table 1. Extraction yield, total phenolic, total flavonoids, and condensed tannins contents of *Polycarpon polycarpoides* extracts.

	Total phenolic [$\mu g.GAE.mg^{-1}$]	Total flavonoids [$\mu g.GAE.mg^{-1}$]	Condensed tannins [$\mu g.CE.mg^{-1}$]
Petroleum ether	14.39 ± 3.40^{c}	0.37 ± 0.13^{c}	nd
Ethyl acetate	108.12 ± 8.15^{a}	4.22 ± 0.83^{a}	nd
Methanol	101.84 ± 2.21^{a}	1.43 ± 0.27^{b}	nd
Methanol: water	43.12 ± 1.02^{b}	1.04 ± 0.26^{b}	nd

GAE – gallic acid equivalents, QE – quercetin equivalents, CE – Catechin equivalents. Values bearing different letters in the column are statistically different ($P < 0.05$).

Antioxidant activity

The antioxidant activity of the extracts was assessed using five different methods and the results were reported as percentage of inhibition or absorbances at different concentrations (Fig. 1). The values of IC_{50} obtained in the DPPH and ABTS assays were also reported in Table 2.

Anti-free radical scavenging activity has been studied using free radicals DPPH and ABTS, which are widely used to determine in vitro the ability of extracts or compounds to scavenge free radicals. The extracts showed dose-dependent scavenging activity against both DPPH and ABTS free radicals, as shown in Fig. 1A, B. Comparing the extracts on the basis of their IC_{50} values, the methanol extract showed the most potent scavenging activity against DPPH and ABTS radicals with IC_{50} values of 249.46 ± 11.22 and 73.86 ± 1.89 $\mu g.mL^{-1}$, respectively, followed by ethyl acetate extract, methanol: water and petroleum ether showing the weakest effect against these radicals (IC_{50}>800 $\mu g.mL^{-1}$).

In addition, the antioxidant activity of *Polycarpon polycarpoides* extracts was measured by methods based on the reduction of a metal ion associated with a chromophore, which undergoes a color change upon receipt of an electron. The ability of the extracts to reduce iron ions was assessed using the phenanthroline test (Fig. 1C). The extracts can

be classified according to their ability to reduce Fe (III) to Fe (II) reported in terms of absorbances at 200 μg.mL^{-1}in descending order as follows: Methanol: water > ethyl acetate > methanol > ether from petroleum giving the corresponding absorbances 0.65 ± 0.02, 0.58 ± 0.03, 0.50 ± 0.00, 0.35 ± 0.04, respectively. These results were considered low compared to Trolox and ascorbic acid standards giving absorbances of 0.56 ± 0.02 and 0.80 ± 0.0, respectively using a very low concentration (6.25 μg.mL^{-1}). In the reduction of copper ions evaluated by the CUPRAC assay (Fig. 1D), ethyl acetate extract was the most potent providing the highest absorbance at the concentration of 800 μg.mL^{-1} (1.85 ± 0.09). Methanol extract also showed a remarkable ability to reduce copper ions with the absorbance of 1.85 ± 0.09 at the highest concentration used (800 μg.mL^{-1}), followed by ether petroleum extract (1.11 ± 0.02), and finally, aqueous methanol has the lowest reducing capacity with an absorbance of 0.8 ± 0.03. Trolox and ascorbic acid exerted a very potent ability to reduce copper ions with absorbances of 1.34 ± 0.13 and 1.38 ± 0.11, respectively at very low concentrations (25 μg.mL^{-1}).

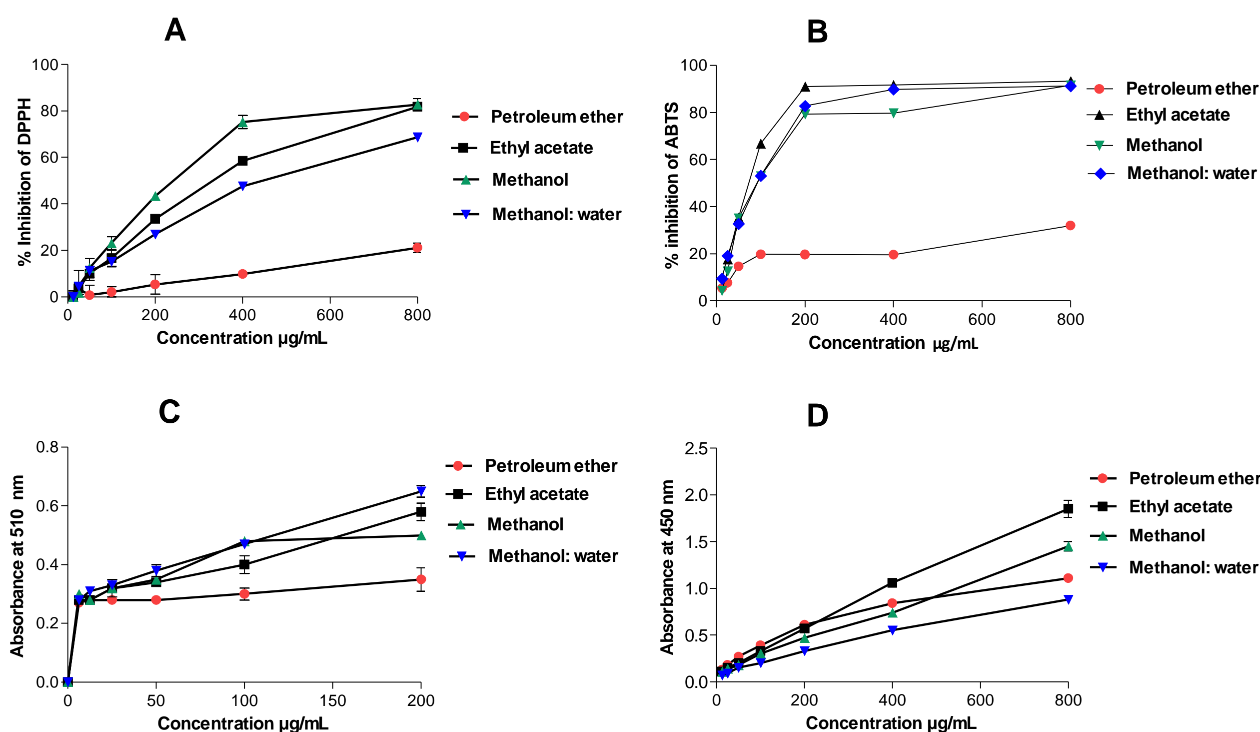

Fig. 1. Dose-dependent effect of the extracts showed in the antioxidant activity determined by different assays. Scavenging effect of the radical DPPH (**A**), ABTS (**B**), reduction of Fe (III)-phenanthroline (**C**), and reduction of copper (**D**). Each point presents the mean ± SD (n = 3).

Table 2. IC_{50} values of *Polycarpon polycarpoides* obtained with DPPH and ABTS.

	DPPH IC_{50} [μg.mL^{-1}]	ABTS IC_{50} [μg.mL^{-1}]
Petroleum ether	>800	>800
Ethyl acetate	333.76 ± 1.08^{b}	90.51 ± 2.82^{a}
Methanol	249.46 ± 11.22^{a}	73.86 ± 1.89^{b}
Methanol: water	500,06 ± 3.19^{c}	92.15 ± 1.99^{a}
Trolox	5.12 ± 0.21	3.21 ± 0.06
Ascorbic acid	4.39 ± 0.01	3.04 ± 0.05

Values are reported as means ± SD of three measurements. Values bearing different letters in the same column are significantly different ($P < 0.05$).

Enzyme inhibitory effect

P. polycarpoides extracts have been tested for their ability to inhibit certain enzymes, including α-amylase, urease and acetylcholinesterase and the results were shown in Fig. 2. The results indicated that all the plant extracts exhibited remarkable inhibition of alpha-amylase at a dose-dependent manner (results not shown) and was significantly ($P < 0.05$) influenced by the extraction solvent (Fig. 2A). In this context, the petroleum ether extract was the most potent with a percentage inhibition at 400 µg.mL^{-1} of 48.19 ± 2.99 %.The other extracts also exerted an interesting inhibitory effect and can be classified in ascending order as follows: ethyl acetate > methanol > methanol : water showing percentages of inhibition at the highest concentration (400 µg.mL^{-1}) of 38.89 ± 0.82, 31.74 ± 2.27, and 15.31 ± 2.75 %, respectively. All the extracts were more potent than acarbose, which moderately inhibited the enzyme using high concentrations (62.5 – 4,000 µg.mL^{-1}). At the highest concentration (4,000 µg.mL^{-1}), acarbose inhibited the enzyme with a rate of 53.05 ± 1.59 %. All the extracts were inactive against urease and acetylcholinesterase (Fig. 2B, C, respectively).

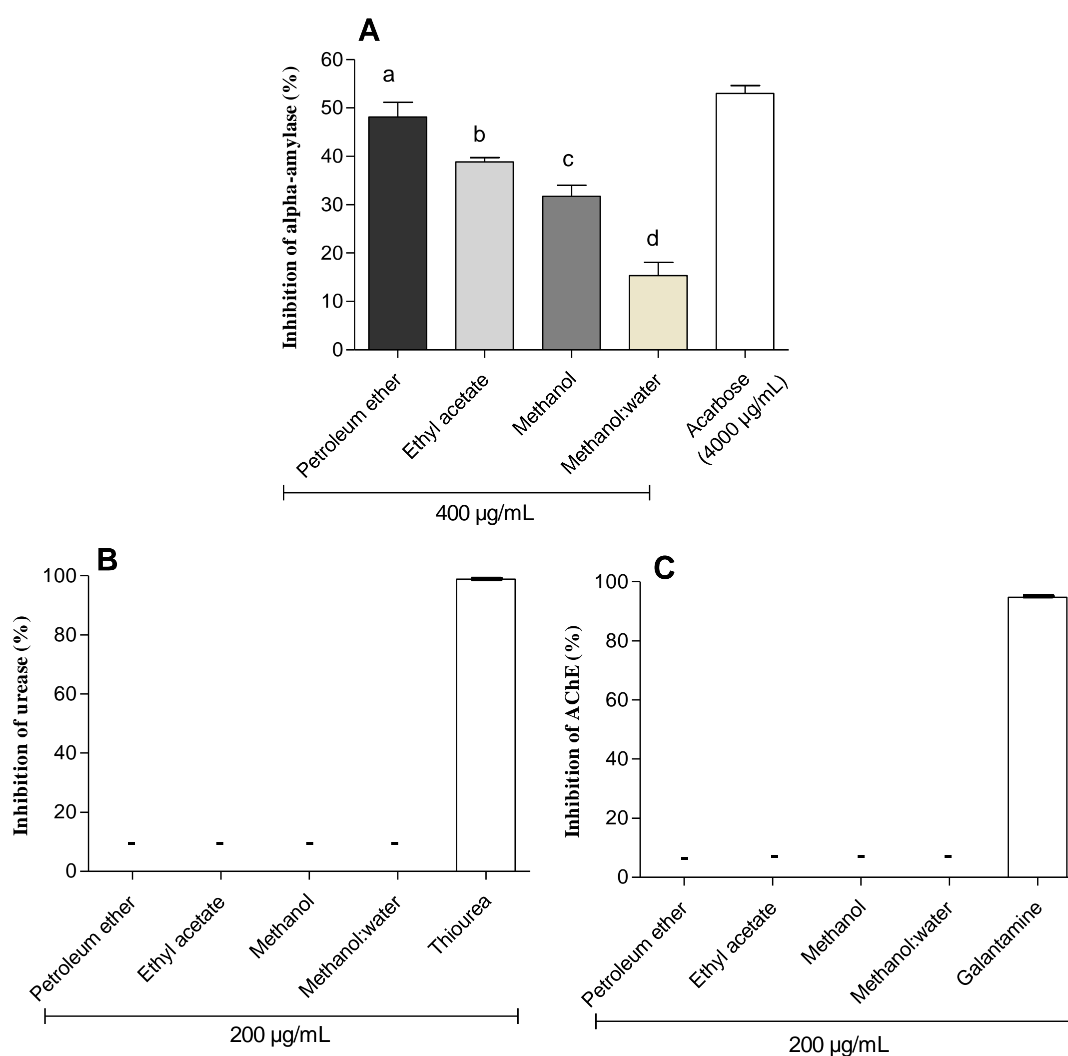

Fig. 2. Enzyme inhibitory effect of *Polycarpon polycarpoides* extracts. Inhibition pecentages of the extracts on α-amylase at 400 µg.mL^{-1} in comparison with acarbose at 4,000 µg.mL^{-1} (**A**). Activity of the extracts and thiourea on urease at 200 µg.mL^{-1} (**B**). Effect of the extracts and galantamine on acetylcholinesterase at 200 µg.mL^{-1} (**C**). Each column presents the mean ± SD of three measurements. Columns bearing different letters are significantly different ($P < 0.05$).

Discussion

Natural bioactive compounds in plants have gained a lot of attention in recent decades as alternatives to synthetics widely used in the pharmaceutical and food industries. Local plants can constitute potential reserves of new bioactive molecules and the study of their bioactivity, and their phytochemical composition represents the starting point of their valuation.

Polyphenols are an important class of molecules of plant origin exhibiting a wide range of pharmacological effects and exerting beneficial effects on human health (Quiñones *et al.* 2013). The phenolic structure of these compounds makes them the most potent antioxidants among other natural phytoconstituents and therefore it is essential to quantify them in plant extracts (Shahidi and Ambigaipalan 2015). The quantity as well as the nature of the compounds extracted strongly depend on the extraction solvent. The highest yield is observed in methanol: water (1 : 1) and this observation could be explained by the fact that Caryophyllaceae plants are particularly rich in glycosylated saponins with high solubility in water. However, phenolic compounds are more concentrated in ethyl acetate and methanol extracts, which is in agreement with the reported literature supporting that these compounds are more soluble in the polar organic solvent than water alone or weakly polar solvents (Babbar *et al.* 2014; Gali and Bedjou 2019; Yakoubi *et al.* 2021)

The antioxidant properties of plant extracts or natural ingredients have prompted manufacturers to incorporate them as natural alternative additives in foodstuffs to preserve their nutritional and organoleptic quality or for their use for therapeutic purposes in the form of supplements in order to prevent the deleterious actions of reactive species on biological molecules (Moure *et al.* 2001). *Polycarpon polycarpoides* extracts have shown moderate antioxidant activity as studied by free radical scavenging methods (DPPH and ABTS) and by the reduction of iron and copper ions indicating that the various constituents of the extracts can act as free radical scavengers or reducing agents. Although various phytoconstituents have an antioxidant effect, phenolic compounds are to date the most powerful natural antioxidant due to their structure bearing hydroxyl groups allowing them to transfer electrons and/or hydrogen (Shahidi and Ambigaipalan 2015). This fact can explain the high antioxidant effect exhibited by the ethyl acetate, methanol, and methanol: water extracts with the highest phenolic levels in comparison with petroleum ether extract having the lowest contents. Indeed, several studies have reported a tight relationship between the phenolic content of different materials (fruits, vegetables, and medicinal plants, and algae) and their antioxidant capacity (Abdullah *et al.* 2013; Odabasoglu *et al.* 2004; Ye *et al.* 2015)

Besides the antioxidant action, *P. polycarpoides* showed an interesting effect of inhibiting α-amylase, a key enzyme involved in the degradation of polysaccharides and therefore preventing their absorption. This approach is widely adopted to control postprandial hyperglycemia and prevent the complications characteristic of type 2 diabetes (Bourebaba *et al.* 2016). Indeed, herbal extracts have been reported to exert a better effect than the most widely used drugs like acarbose and miglitol and therefore can be used as alternatives. Especially, the species belonging to the Caryophyllaceae family are known to exert antidiabetic effects through their ability to inhibit carbohydrates hydrolysing enzymes including α-glucosidase and α-amylase (Patra *et al.* 2020; Derici *et al.* 2021).In contrast to the antioxidant effect of *P. polycarpoides*, the petroleum ether extract with low content of phenolic compounds exhibited the highest inhibitory effect on α-amylase compared to other extracts which suggests that lipophilic molecules can exert a more effective action than hydrophilic molecules. The effect of extraction solvent on decreasing α-amylase activity was also observed in extracts of *Swertia chirata* where the inhibitory effect diminished with increasing solvent polarity to be totally inactive using water (Dutta *et al.* 2012).

On another hand, *P. polycarpoides* was inactive against urease and acetylcholinesterase. Urease is involved in the survival mechanisms of *Helicobacter pylori* in the acid environment of the stomach by producing ammonia. The action of plants or drugs on urease may be of therapeutic interest in combating the development of *H. pylori*

since it is believed that most gastric and duodenal ulcers are due to this bacterium (Amtul *et al.* 2012). While acetylcholinesterase is the principal target for Alzheimer's disease treatments that aim to increase the concentrations of acetylcholine in the synapsis clefts to replace the lack of the secretion of this neurotransmitter due to the degeneration of neurons and therefore restore the cholinergic transmission directly associated with the cognitive capabilities (Gali and Bedjou 2019). By referring to literature, only a small number of species belonging to the Caryophyllaceae family have been reported for their inhibitory effect on urease. *Saponaria officinalis L.* has been reported to inhibit jack bean urease with an IC_{50} value of 607.80 µg/mL, which is very low activity compared to standard hydroxyurea (IC_{50} = 37 µg.mL^{-1}) (Mahernia *et al.* 2015). Similarly, the anticholinesterase effect of Caryophyllaceae species has been rarely studied. Recently, Mamadalieva *et al.* (2019) reported slight activity against acetylcholinesterase and butyrylcholinesterase from a methanolic extract as well as some ecdysteroids isolated from *Silene viridiflora.* Since ethyl acetate extract gave significant antioxidant activity and inhibitory effect on α-amylase, one would conclude that ethyl acetate is the best solvent to extract bioactives of therapeutic interest from *P. polycarpoides.* This solvent exhibited some degree of hydrophobicity to extract low polar molecules with inhibitory effects on α-amylase and was able to extract aglycone phenolic compounds with antioxidant properties.

Conclusion

Throughout the paper, some biological effects (antioxidant activity and the inhibition of α-amylase, urease, and acetylcholinesterase) of the endemic plant *Polycarpon polycarpoides* were examined to investigate its possible therapeutic potential or its use as a source of bioactive compounds. Following the various results showing the low contents of polyphenols explaining the moderate antioxidant activity of the plant, and no activity against urease and acetylcholinesterase but remarkably active against alpha-amylase, the plant can be valued in the management of type 2 diabetes. However, as this work represents the first study on the biological actions of *P. polycarpoides*, other biological effects must be studied to take into account the possible presence of compounds that may exert other effects to conclude on the usefulness of the plant as a remedy or as a source of bioactive ingredients.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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