https://doi.org/10.31925/farmacia.2022.2.5 **ORIGINAL ARTICLE**

PHENOLICS CONTENT, ANTIOXIDANT POTENTIAL, α-GLUCOSIDASE AND α-AMYLASE INHIBITORY ACTIVITIES OF FOUR FOLIAR EXTRACTS FROM *PINUS* SPECIES

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Manuscript received: October 2021

Abstract

The current study focused on determining the contents of total phenolics (TP), flavonoids (FC), condensed tannins (CT), the flavonoid profiles, antioxidant potentials and α-glucosidase and α-amylase inhibitory capacities of the foliar extracts of *Pinus engelmannii* Carr., *Pinus arizonica* Engelm., *Pinus durangensis* Martínez and *Pinus cembroides* Zucc. Specific variations were found in TP, FC, CT, antioxidant potentials and α-glucosidase and α-amylase inhibitory activities. *Pinus durangensis* highlighted for its high level of FC (3.70 mg/g dry extract), high DPPH$^{\bullet}$ scavenging activity, high ABTS$^{\bullet+}$ reducing potential, as well as its high inhibitory activity of α-glucosidase and α-amylase, whereas *Pinus engelmannii* for its high values of TP (340.15 mg/g dry extract) and CT (72.49 mg/g dry extract). Particularly, *Pinus durangensis* could support the development of hypoglycaemic treatments. The flavonoid profiles obtained by HPLC were species-specific, thus may be used for the development of chemical fingerprinting and quality control tools for the botanical authenticity of the bioactive extracts of these species of *Pinus*.

Rezumat

Studiul a avut ca scop determinarea conținutului total de compuși fenolici (TP), flavonoide (FC), taninuri condensate (CT), profilul flavonoidelor, potențialul antioxidant și capacitatea de inhibiție a α-glucozidazei și α-amilazei ale extractelor foliare de *Pinus engelmannii* Carr., *Pinus arizonica* Engelm., *Pinus durangensis* Martínez și *Pinus cembroides* Zucc. *Pinus durangensis* s-a evidențiat printr-un nivel ridicat de FC (3,70 mg/g extract uscat), activitate crescută de captare a DPPH$^{\bullet}$, potențial de reducere a ABTS$^{\bullet+}$, precum și inhibiția α-glucozidazei și α-amilazei. *Pinus engelmannii* s-a evidențiat prin valori ridicate ale TP (340,15 mg/g extract uscat) și CT (72,49 mg/g extract uscat). *Pinus duragensis* prezintă potențial în dezvoltarea unor produse hipoglicemiante. Profilurile flavonoidelor au arătat faptul că ar putea fi utilizate pentru *fingerprinting* chimic și în controlul calității extractelor bioactive din speciile de *Pinus*.

Keywords: antioxidants, flavonoid profiles, hypoglycaemic, *Pinus*

Introduction

Few species of *Pinus*, out of the about 111 recognized in the genus [26], have been analysed for their phenolic compounds; however, the results already published suggest that the species of the genus synthesize and accumulate in their foliar tissues a relevant diversity and abundance of phenolic compounds, such as those found in *Pinus massoniana* Lamb. [29], *Pinus chihuahuana* Engelm., *Pinus leiophylla* Schiede ex Schltdl. & Cham. [1], *Pinus sylvestris* L. [12, 16, 23], *Pinus wallichiana* A. B. Jacks., *Pinus roxburghii* Sarg. [22], *Pinus peuce* Griseb., *Pinus nigra* J. F. Arnold and *Pinus mugo* Turra [16].

Phenolic compounds have been reported to have a wide range of medicinal properties [10, 31, 34]. The abundance and diversity found in the foliar tissues of the previously analysed species of *Pinus* suggest that these compounds may be responsible for several of the medicinal properties recognized for them, such as the antioxidant effects of *Pinus densiflora* Siebold & Zucc. [15] and *P. sylvestris* [12], the expectorant, diuretic and antimicrobial properties of *P. sylvestris* [12] and the vasorelaxant effects of *Pinus morrisonicola* Hayata [8].

Diabetes mellitus is a metabolic disorder, in which a hyperglycaemic condition is persistent. It has been estimated that around 336 million people in the world are affected by this disorder [9]. The inhibition of enzymes like α-glucosidase and α-amylase, which generate increases in blood glucose levels, is sought in treatments based on natural products that seek to reduce the side effects caused by the drugs used in the treatments of diabetes [32]. The α-glucosidase and α-amylase inhibitory activities of the needles of *Pinus*

species have been slightly explored, *Pinus tabulaeformis* Carr. from China is one of the few analysed species [35]. Despite the important contributions already done, there is still an important empty of knowledge about the flavonoid diversity and the biological activities of the foliar phenolic compounds of the species of *Pinus*. The current study aimed to determine the flavonoid profiles, contents of phenolic compounds and properties as antioxidants and inhibitors of α-glucosidase and α-amylase of four species of *Pinus* (*Pinus engelmannii* Carr., *Pinus arizonica* Engelm., *Pinus durangensis* Martínez and *Pinus cembroides* Zucc.) from Mexico.

Materials and Methods

Plant material. Needles of adult plants of *P. arizonica*, *P. engelmannii*, *P. durangensis* and *P. cembroides* were collected in Durango, Mexico (Table I). Voucher specimens were deposited at Herbarium CIIDIR. Needles from each species were independently dry at room temperature and ground, and then three pools of sub-samples of each species (triplicate) were formed. Each subsample was analysed separately.

Table I

Collection data for the analysed species of *Pinus* in Durango, Mexico

Species	Curatorial number	Location	Latitude (N)	Longitude (W)	Altitude (m)	Date
Pinus engelmannii	32908	Pueblo Nuevo	24°06'03"	105°30'33"	2300	Sep, 2006
Pinus durangensis	32789	El Mezquital	22°37'54"	104°14'01"	2550	July, 2006
Pinus cembroides	37040	Guadalupe Victoria	24°35'19"	104°16'50"	2930	July, 2008
Pinus arizonica	34767	Topia	25°12'52"	106°30'40"	2414	July, 2007

Preparation of extracts. Phenolic-enriched extracts were prepared according to Barriada-Bernal *et al.* [5]. Two grams of dry and ground material were combined with 40 mL of 50% ethanol (v/v). The samples were sonicated for 30 min, then centrifuged for 10 min at 8000 rpm. The recuperated supernatants were the total extracts. The ethanol was vacuum removed from the total extracts before fractionating with ethyl acetate. The organic fraction was concentrated to dryness and then solubilized in 4 mL of methanol. These were the phenolic-enriched extracts, which were analysed for their phenolic contents and flavonoid profiles.

Total phenolics (TP). TP were assessed using the Folin-Ciocâlteu reagent [20]. TP were estimated from a standard curve of gallic acid (slope = 0.0083, axis crossing point = 0.1013, r = 0.9940) and expressed as micrograms equivalents of gallic acid *per* gram of dry extract (mg GAE/g DE).

Flavonoid contents (FC). FC were determined using an $AlCl_3$ solution [24]. FC were calculated from a standard curve of quercetin (slope = 0.0515, axis crossing point = 0.0216, r = 0.9980) and expressed as milligrams of quercetin equivalents *per* gram of dry extract (mg QE/g DE).

Condensed tannins (CT). The TC contents were determined according to Julkunen-Tiitto R [14]. TC contents were calculated from a standard curve of (-)-epicatechin (slope = 4.8778, axis crossing point = 0.0213, r = 0.9989). The results were expressed as milligrams equivalents of (-)-epicatechin *per* gram of dry extract (mg EE/g DE).

Flavonoid profiles. The foliar phenolic profiles were determined by HPLC-DAD, in a Perkin Elmer Series 200 HPLC system and a Perkin Elmer Brownlee Analytical C18 (4.6 x 250 mm, 5 µm) column, with a gradient method previously described [7]. The UV spectra of the determined compounds were registered between 200 and 400 nm with a DAD (Perkin Elmer Series 200). Structural information was obtained by both interpreting the UV spectra according to the UV theory developed for flavonols, flavones, dihydro-flavonoids and phenolic acids [7], and comparing the retention time (RT) and λ_{max} of the resolved compounds with those of standards. The interpretation of the UV spectra implied considering the λ_{max} of bands I and II of spectra, the split of band II (IIa and IIb), and the presence and position of shoulders. Besides, considering that if spectra fit one to each other, but they have different retention time (RT), then each has a different number or type of glycosides attached. The standards used were: rutin (quercetin-3-*O*-rutinoside; RT: 33.918 min; λ_{max}: 255, 269sh, 301sh, 360), luteolin (5,7,3',4'-tetrahydroxy-flavone; RT: 45.885 min; λ_{max}: 257, 270sh, 292sh, myricetin (3,3',4',5,5',7-hexahydroxyflavone; RT: 30.500 min; λ_{max}: 258, 270sh, 307sh, 373), hyperoside (quercetin-3-D-galactoside; RT: 34.951 min; λ_{max}: 256, 268sh, 304sh, 360), quercitrin (quercetin-3-*O*-rhamnoside; RT: 37.789 min; λ_{max}: 255, 269sh, 303sh, 349), quercetin (RT: 45.95 min; λ_{max}: 265, 295sh, 372) and apigenin (RT: 59.600 min; λ_{max}: 267, 290sh, 335). The phenolic profile of each sample was formed by all compounds in the respective chromatogram.

DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) scavenging activity. The DPPH assay was carried out according to Brand-Williams *et al*. [6]. The amount of extract needed to decrease by 50% the initial DPPH$^{\bullet}$ concentration ($EC_{50(DPPH)}$) was calculated and expressed as micro-grams of extract *per* millilitre (µg/mL). Ascorbic acid was analysed in the same manner as a reference.

Iron-reducing power (IR). The potential of each phenolic-enriched extract (100 µL) to reduce Fe^{3+} to Fe^{2+} was determined according to Siddhuraju and Becker [30]. The formation of Fe^{2+} was assessed by

registering the absorbance at 700 nm. The higher is the A_{700nm}, the higher is the iron reducing power.

$ABTS^{\bullet+}$ (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) reducing capacity. The reduction of $ABTS^{\bullet+}$ was determined according to Re *et al*. [25]. The extract concentration providing 50% inhibition of $ABTS^{\bullet+}$ was calculated ($EC_{50(ABTS)}$) and expressed as micrograms of extract *per* millilitre (µg/mL). Ascorbic acid was analysed in the same manner as a reference.

Inhibition of the α-glucosidase activity. This assay was carried out according to Kim *et al*. [17]. The percentage of inhibition was estimated for five extract concentrations (0.156 - 2.500 mg/mL). The extract concentration, giving 50% of inhibition (IC_{50}), was estimated. Acarbose was analysed in the same manner as a reference.

Inhibition of the α-amylase activity. The α-amylase inhibitory activities were evaluated according to Kim *et al*. [18], using a solution of α-amylase from porcine pancreas (200 µL, 1 U/mL). The percentage of inhibition was estimated for five extract concentrations (1.25 - 10.00 mg/mL). The extract concentration, giving 50% of inhibition (IC_{50}), was estimated. Acarbose was analysed in the same manner as a reference.

Data analysis. Data were subjected to an analysis of variance ($p < 0.05$) and means were discriminated by the Tukey test, using InfoGen. Data correlations were estimated with the Pearson test, using Past 3.0. From the HPLC phenolic profiles, a binary matrix coded by 1 (presence) or 0 (absence) was constructed and subjected to a cluster analysis (similarity Dice index), using Past 3.0.

Results and Discussion

Pinus engelmannii had the highest contents of TP and CT, whereas *P. durangensis* had the highest content of FC (Table II). The TP values of each of the four species of the analysed *Pinus* were higher than those reported for other species of the genus, such as *P. peuce*, *P. nigra*, *P. mugo*, *P. sylvestris* (between 9.8 and 14.0 mg/g dry material) [16], *P. cembra* L. (78.22 mg/g extract) [3] and *Pinus pinaster* knots (20.66 g/kg) [13]. The current results revealed the needles of the four analysed species of *Pinus* as important sources of phenolic compounds, the amounts accumulated being even higher than those of species considered as outstanding for accumulating high quantities of these bioactive compounds, like *Malpighia umbellata* Rose (46.91 mg/g dry extract) [33].

Table II

Foliar contents of total phenolics (TP), flavonoids (FC) and condensed tannins (CT) of four species of *Pinus*

Species	TP (mg GAE/g DE)	FC (mg QE/g DE)	CT (mg EE/g DE)
Pinus engelmannii	340.15 ± 5.90 ^{c}	2.21 ± 0.02 ^{a}	72.49 ± 1.21 ^{d}
Pinus durangensis	327.65 ± 5.52 ^{b}	3.70 ± 0.05 ^{d}	30.91 ± 0.72 ^{c}
Pinus cembroides	271.81 ± 2.87 ^{a}	2.58 ± 0.07 ^{b}	5.28 ± 0.59 ^{a}
Pinus arizonica	262.05 ± 2.19 ^{a}	3.07 ± 0.04 ^{c}	18.72 ± 0.17 ^{b}

The values represent the mean and standard deviation for three independent samples. Different letters in the same column mean significant differences ($p < 0.05$). GAE: Gallic acid equivalents; QE: Quercetin equivalents; EE: (-)-epicatechin equivalents; DE: Dry extract.

The FC values varied between 2.21 and 3.70 mg QE/g DE (Table II), representing between 0.64 and 1.12% of the TP. The FC values were similar to those reported for several populations of *P. sylvestris* (between 0.68 and 3.76 mg/g dry matter) [23], *P. roxburghii* Sarg. and *P. wallichiana* A. B. Kacks. (428 and 395 mg/100g dry mass, respectively) [21]. Flavonoids are among the most important phenolic compounds because of their noteworthy beneficial effects on human health [28, 31].

The CT values of the four analysed *Pinus* species are presented in Table II. The contents found for *P. engelmannii*, *P. durangensis* and *P. arizonica* were higher than that reported for the needles of *P. cembra* (12.7 mg/g extract) [3]. The foliar CT values found for *Pinus engelmannii* and *P. durangensis* were higher than that found for the leaves of other species reported as an important source of tannins, like *M. umbellata* (21.16 mg/g dry extract) [33]. Tannins have beneficial effects on human health, as they are antioxidants and blood glucose regulators [19].

The results revealed a total of 24 phenolic compounds. The retention times (RT), λ_{max}, proposed type and the proportion of the flavonoids found for each species of *Pinus* are depicted in Table III.

In the needles of *Pinus cembroides*, three flavonoids were found, representing the simplest profile; in those of *P. engelmannii*, eight flavonoids were found; in those of *P. durangensis*, 12 flavonoids formed its profile; whereas the profile of *P. arizonica* included 14 flavonoids, representing the most complex profile (Table III).

The flavonoid profiles, revealed by the HPLC-DAD analysis, agree with previous reports informing those *Pinus* needles accumulate an important diversity of these compounds [1, 22, 23, 29].

Flavonols were the prevalent flavonoids (23 out of a total of 24) in the four species analysed. According to Campos and Markham [7], the 3',4'-dihydroxyl pattern doublets the band II of the UV spectra of both flavonols (like quercetin glycosides) and flavones (like luteolin glycosides). Compounds **3**, **8**, **16**, **19** and **22**, all found in *P. durangensis* and for which a particular type of flavonol could not be given, had each a doublet band II, indicating the presence of the 3',4'-dihydroxyl pattern in their structures.

Table III

Flavonoids found in the needles of four species of *Pinus*

Number of Compound	RT (min)	$\lambda_{max.}$ (nm)	Proposed type of flavonoid	Found in
1	34.91	256, 268sh, 288sh, 360	Quercetin glycoside	*P. engelmannii* (8444.98) *P. durangensis* (4970.29) *P. arizonica* (5017.24)
2	35.17	256, 268sh, 291sh, 365	Quercetin glycoside	*P. arizonica* (3655.98)
3	35.60	270, 286sh, 358	Flavonol	*P. durangensis* (680.48)
4	36.59	272, 292sh, 355	Herbacetin glycoside	*P. engelmannii* (5679.49) *P. durangensis* (3282.56) *P. arizonica* (6643.82) *P. cembroides* (8545.58)
5	36.71	260, 267sh, 291sh, 355	Flavonol (Possible a Myricetin derivative)	*P. durangensis* (1799.83)
6	37.33	269, 325sh, 355	Herbacetin-3-*O*-glycoside	*P. engelmannii* (10380.04) *P. durangensis* (1755.28) *P. arizonica* (5946.56)
7	37.67	251, 273sh, 292sh, 361	Flavonol	*P. engelmannii* (6637.27) *P. durangensis* (748.10)
8	37.80	267, 285sh, 351	Flavonol	*P. durangensis* (4782.72)
9	38.74	266, 290sh, 352	Kaempferol-3-*O*-glycoside	*P. cembroides* (1569.26)
10	37.51	266, 326sh, 358	Flavonol	*P. arizonica* (3557.26)
11	37.87	254, 269sh, 361	Quercetin glycoside	*P. arizonica* (2734.27)
12	38.48	252, 267sh, 365	Quercetin glycoside	*P. engelmannii* (7784.96) *P. durangensis* (2674.30) *P. arizonica* (1535.15)
13	38.36	256, 269sh, 366	Quercetin glycoside	*P. arizonica* (6775.27)
14	39.35	256, 268sh, 357	Quercetin glycoside	*P. arizonica* (8330.74)
15	39.32	267, 339	Flavone (Apigenin-7-*O*-glycoside)	*P. cembroides* (775.23)
16	39.45	268, 280sh, 360	Flavonol	*P. durangensis* (1556.00)
17	39.45	254, 269sh, 358	Quercetin glycoside	*P. engelmannii* (7137.96)
18	41.08	266, 285sh, 347	Kaempferol glycoside	*P. engelmannii* (5367.34) *P. durangensis* (4565.24) *P. arizonica* (8466.08)
19	41.31	251, 267sh, 287sh, 360	Flavonol	*P. durangensis* (1107.21)
20	41.60	268, 330sh, 357sh.	Flavonol	*P. engelmannii* (7053.84) *P. arizonica* (3556.27)
21	41.78	252sh, 266, 361	Flavonol	*P. arizonica* (9822.06)
22	41.48	251sh, 266, 285sh, 351	Flavonol	*P. durangensis* (1481.10)
23	43.00	268, 332	Flavonol	*P. arizonica* (11920.50)
24	48.31	273, 327sh, 361sh	Flavonol	*P. arizonica* (11150.94)

Figures in brackets mean peak area (1×10^3, µV x s).

The foliar flavonoid compositions found for each of the four species of *Pinus* analysed were species-specific and contrasting with those reported by other species of the genus, such as *P. densiflora* [15], *P. mugo*, *P. nigra*, *P. peuce* and *P. sylvestris* [16], and *P. leiophylla* and *P. chihuahuana* [1].

The results of the cluster analysis comparing the flavonoid profiles (Figure 1) indicated that these have an important specific discriminatory potential, which may support the development of quality control tools for the botanical authenticity of the flavonoid foliar extracts of *Pinus*.

All extracts displayed different and important anti-oxidant properties, highlighting *Pinus durangensis* as both a potent DPPH$^{\bullet}$ scavenger and an ABTS$^{\bullet+}$ reducer (Table IV). *Pinus engelmannii* showed the highest activity as an iron reducer (Table IV). The four analysed species had higher potentials as DPPH$^{\bullet}$ scavengers and ABTS$^{\bullet+}$ reducers than ascorbic acid here analysed as a reference, which is a well-recognized antioxidant [4], and showed higher activities as DPPH$^{\bullet}$ scavengers than both those of the foliar methanolic extracts of *P. densiflora* (EC_{50} = 32.5 µg/mL) [15] and *P. cembra* (EC_{50} = 186.1 µg/mL) [3]. The ABTS$^{\bullet+}$ reducing power of each of the four analysed species of *Pinus* was higher (Table IV) than that reported for the foliar methanolic extract of *P. cembra* (EC_{50} = 24 µg/mL) [3].

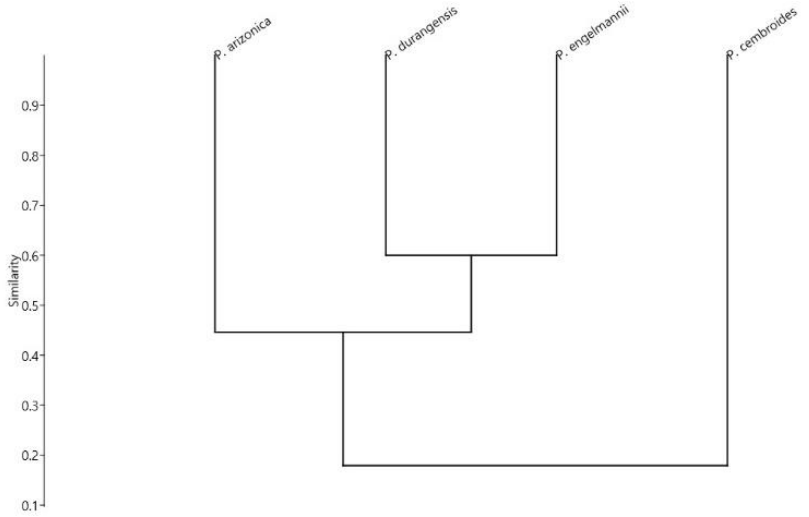

Figure 1.
Results of a principal components analysis based on the foliar flavonoid profiles of four species of *Pinus*

Table IV
DPPH• scavenging activity ($EC_{50(DPPH)}$), iron-reducing power (IR) and $ABTS^{\bullet+}$ reducing capacity ($EC_{50(ABTS)}$) of the foliar extracts of four species of *Pinus*

Sample	$EC_{50(DPPH)}$ (µg/mL)	IR (A_{700})	$EC_{50(ABTS)}$ (µg/mL)
Pinus engelmannii	20.06±0.20 [c]	0.511 ± 0.01 [d]	6.06±0.12 [ab]
Pinus durangensis	16.48±0.29 [a]	0.267 ± 0.01 [a]	5.97±0.19 [a]
Pinus cembroides	17.68±0.08 [b]	0.373 ± 0.00 [c]	6.74±0.44 [b]
Pinus arizonica	22.14±0.23 [d]	0.320 ± 0.20 [b]	9.65±0.29 [c]
Ascorbic acid	76.00±0.01 [e]	ND	88.00±0.01 [d]

The values represent the mean and standard deviation for three independent samples. Different letters in the same column mean significant differences ($p < 0.05$). ND: Not determined.

The results of the analysis of correlation revealed a significant association between total phenolics content and $ABTS^{\bullet+}$ reduction (-0.745), which was in agreement with the findings of Dobre *et al*. [11]. Other authors have found a strong association between flavonoid contents and antioxidant activities of plant extracts [2]. However, the current results showed no significant correlation between the flavonoid contents and the antioxidant properties of the four analysed species of *Pinus* (-0.368 with DPPH• scavenging activity, -0.705 with iron-reducing power and 0.084 with $ABTS^{\bullet+}$ reducing capacity), in agreement with Barriada-Bernal *et al*. [5], who also found no correlation between flavonoid content and antioxidant activity. Our results suggest that not only the amounts of flavonoids present in the extracts can define their antioxidant properties, but their particular flavonoid composition may do an important contribution. The chemical structure of the phenolic compounds, particularly of flavonoids, present in each extract could be decisive in conferring the antioxidant potential. Mainly, the disposition of -OH groups is related to the antioxidant activity, orthodi-hydroxyl groups on ring B providing high activities [27]. Particularly for the extract of *P. durangensis*, the high DPPH• and $ABTS^{\bullet+}$ scavenging capacities found could be explained by both its flavonoid concentration (Table II) and its high proportion of flavonols bearing 3',4'-orthodihydroxyl groups (**1**, **3**, **8**, **12**, **16**, **19** and **22**, which represented 58.3% of the total) (Table III). The synergic or antagonistic effects resulting from the interaction among all phenolic compounds present in each foliar extract could also have done some contribution to the antioxidant properties of the analysed species of *Pinus*.

The variations of the inhibition percentage of *α*-glucosidase and *α*-amylase with the increase of extract concentration are shown in Figure 2 and Figure 3, respectively. The extracts of all four analysed species displayed a similar performance, reaching the highest *α*-glucosidase inhibitory activity (between 76.77% for *P. durangensis* and 83.75% for *P. engelmannii*) at the concentration of 1.25 mg/mL. This activity diminished between 1.14 and 1.31 times when the extract concentration increased at 2.50 mg/mL. The four extracts displayed the highest *α*-amylase inhibitory activity (between 57.68% for *P. cembroides* and 66.03% for *P. engelmannii*) at the highest evaluated concentration (10.00 mg/mL).

Significant differences were found in the values of IC_{50} of both inhibitory activities (Table V). The extract of *P. durangensis* had the highest *α*-glucosidase as well as *α*-amylase inhibitory activities.

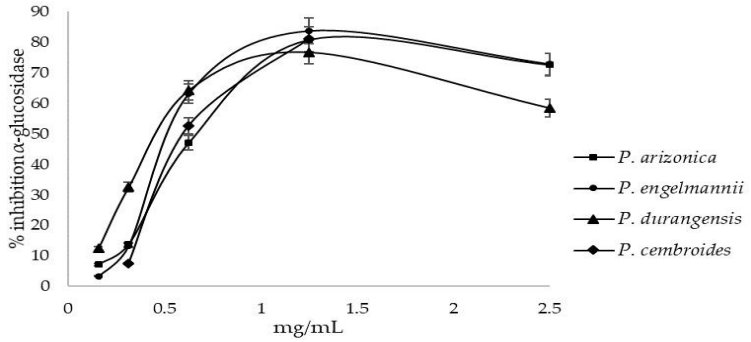

Figure 2.
Changes of the inhibition of α-glucosidase activity according to concentration increases of foliar extracts of four species of *Pinus*

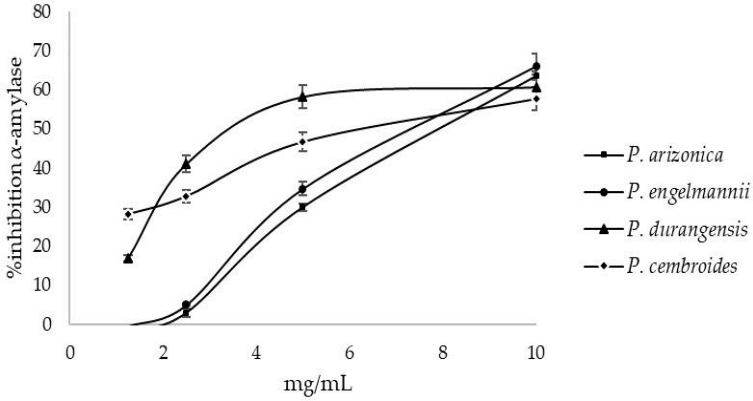

Figure 3.
Changes of the inhibition of α-amylase activity according to concentration increases of foliar extracts of four species of *Pinus*

All the foliar extracts analysed were more active as α-glucosidase inhibitors than acarbose, which is a drug used in antidiabetic treatments [32]. However, all the foliar extracts analysed were less active as inhibitors of α-amylase than acarbose. The % α-amylase inhibition of any of the four analysed species of *Pinus* was higher than those of the bark of *P. koraiensis* Siebold & Zucc. and *P. rigida* Mill. (8.6% and 23.4%, respectively), and similar to that of *P. densiflora* bark (72.7%) [17]. The current results suggest an important hypoglycaemic potential for the foliar extracts of the species of *Pinus* analysed, particularly for that of *P. durangensis*.

Table V
Alpha-glucosidase and α-amylase inhibitory activities of the foliar extracts of four species of *Pinus* and acarbose

Sample	$IC_{50(\alpha\text{-glucosidase})}$ (mg/mL)	$IC_{50(\alpha\text{-amylase})}$ (mg/mL)
P. engelmannii	0.50 ± 0.00 ^{a}	6.93 ± 0.01 ^{d}
P. durangensis	0.44 ± 0.02 ^{a}	3.46 ± 0.05 ^{b}
P. cembroides	0.55 ± 0.02 ^{ab}	5.96 ± 0.05 ^{c}
P. arizonica	0.64 ± 0.02 ^{b}	8.00 ± 0.06 ^{e}
Acarbose	6.64 ± 0.06 ^{c}	0.042 ± 0.00 ^{a}

The values represent the mean and standard deviation for three independent samples. Different letters in the same column mean significant differences ($p < 0.05$).

Besides the concentration and synergic or antagonistic effects, the structure of flavonoids in each plant extract also can determine the α-amylase and α-glucosidase inhibitory activities because different hydroxylation and methylation patterns confer variable inhibitory potential. This potential is the result of the interaction of the hydroxyl and methoxyl groups of the flavonoids in an extract, which interact with both the enzymes and starch, altering the active sites in the first ones and the functional properties of the second one [32]. Thus, the different phenolic compositions revealed in the current study for the foliar extracts of *Pinus* analysed could explain the variation found in the inhibitory activity against α-amylase and α-glucosidase. However, the high and significant correlation (0.798) found between CT and α-amylase inhibitory activity indicates a relevant participation of this kind of phenolics to confer the α-amylase inhibitory activity

to the foliar extracts of the four species of *Pinus* analysed. The current results is in agreement with those of Kumari *et al*. [19], who reported that tannins have an important activity as blood glucose regulators.

Conclusions

Although with variable contents, the four analysed species of *Pinus* represent important sources of total phenolics, flavonoids and tannins, which have valuable antioxidant potentials, as well as *α*-glucosidase and *α*-amylase inhibitory activities, particularly *P. durangensis* could support the development of hypoglycaemic treatments. The species-specific foliar flavonoid profiles, obtained by HPLC-DAD, could support the development of chemical fingerprinting and quality control tools for the botanical authenticity of the bioactive extracts of these species of *Pinus*.

Conflict of interest

The authors declare no conflict of interest.

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