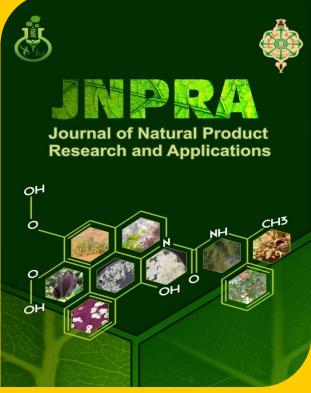
# *In vitro* antioxidant and antidiabetic activities of the hydro-acetone extracts of *Ricinus communis* and *Teucrium polium* from Tlemcen area

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# *In vitro* antioxidant and antidiabetic activities of the hydro-acetone extracts of *Ricinus communis* and *Teucrium polium* from Tlemcen area

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# Highlights

- High contents of polyphenol and flavonoid in *R. communis* than *T. polium*.
- Both plant extracts showed *in vitro* antioxidant and antidiabetic activities.
- Strong anti-radical and iron-reducing power of *R. communis*.
- *T. polium* showed an inhibitory effect on α-amylase activity.

# **Graphical Abstract**



#### Abstract

The aim of the present study was to determine total phenolic and total flavonoid contents in hydro-acetone extracts from the roots of *Ricinus communis* and the aerial part of *Teucrium polium*, and to determine *in vitro* their antioxidant and antidiabetic activities using DPPH, FRAP, and  $\alpha$ -amylase activity assay, respectively. The obtained results reveled that *R. communis* roots hydro-acetone extract contains high contents of polyphenols (499.55 ± 0.02 µg EGA/mg DE) and flavonoids (28.21 ± 0.01 µg eq Cat /mg DE) than *T. polium* aerial part extracts. Both plant extracts showed *in vitro* antioxidant and antidiabetic activities, where *R. communis* showed a potent free radical scavenging activity (DPPH IC<sub>50</sub>=2.01 ± 0.01 µg/mL) and high iron reduction capacity (FRAP EC<sub>50</sub>=41.32 ± 0.01 µg/mL) more than *T. polium* extract (DPPH IC<sub>50</sub>= 7.40 ± 0.01 µg/mL; FRAP EC<sub>50</sub>=74.63 ± 0.01 µg/mL). In addition, in  $\alpha$ -amylase activity assay *T. polium* was more efficient in inhibiting this enzyme (EC<sub>50</sub>= 924 ± 0.19 µg/mL) compared to *R. communis* extract (EC<sub>50</sub>= 1300 ± 0.03 µg/mL).

**Keywords:** *Ricinus communis; Teucrium polium;* total polyphenol contents; DPPH; FRAP; α-amylase.

#### 1. Introduction

The uncontrolled and over-production of free radicals in the human body is currently the main cause of many chronic and neurodegenerative diseases including cancer, diabetes, cardiovascular disease, Alzheimer and autoimmune diseases (Elita et al., 2012). The antioxidants that ensure the absorption and neutralization of free radicals prevent their interaction with the surrounding biomolecules, and prevent the appearance of these diseases or their complications (Kheirabadia & Izadyar, 2016). The side-effects of drugs are worrying users, who are turning to the search for new treatments that are less harmful to the body. Today, the return to the use of medicinal plants is extremely important. Unlocking the secrets of medicinal plants as a rich source of bioactive molecules became the cornerstone of this groundbreaking development, leading to substantial progress in the pharmaceutical field (Iserin, 2001; Jamshidi-Kia et al., 2018). Several scientific studies have been performed to reveal the therapeutic effects of phytochemicals on health by determining their impact on oxidative stress, and assessing their potential to increase enzymatic antioxidants, reduce peroxides, scavenge free radicals, and chelate transition metals (Li et al., 2009). A vast number of medicinal plants used in phytotherapy that have been evaluated for their antibacterial, antidiabetic, and anti-inflammatory effects represent an inexhaustible source of novel therapeutic ingredients (Brewer, 2011; El-Kadi et al., 2021). The beneficial effects of antioxidant phytochemicals on health are related to their direct effect on scavenging free radicals, chelation of transition metals, reduction of peroxides, and stimulation of enzymatic antioxidants (Li et al., 2009). Several studies are focusing on the investigation of medicinal and aromatic plants to identify new natural antioxidants. Ricinus communis (Rhamnaceae) and Teucrium polium (Lamiaceae) are widely used in folk medicine in the treatment of gastrointestinal disorders, inflammations, diabetes, and rheumatism (Bahramikia & Yazdanparast, 2012; Jena and Gupta, 2012; Asghari et al., 2020; Bouafia et al., 2021).

*R. communis* is an herbaceous annual or perennial and fast-growing plant that can attain heights of 7 m or more, characterized by large, toothed, palmate, green or reddish leaves with 7 to 9 lobes and 30-60 cm in diameter. The stem is robust, and branched, with a red or green color. The flowers are monoecious, large about 30-60 cm. The fruit is a three-celled thorny capsule containing oval, marbled seeds 8-18 mm long and 4-12 mm broad (Jena and Gupta, 2012). T. polium is a perennial aromatic shrub, 20 to 50 cm high, widely distributed in dry, stony places in the hills and deserts of the Mediterranean region, South-West Asia, Europe, and North Africa. The leaves are sessile, about 3 cm to the long. The flowers are small, in clusters, and range from pink to white (Bahramikia and Yazdanparast, 2012). In the literature, this species has a wide range of beneficial properties, including: antioxidant, antidiabetic, anti-inflammatory, and antimicrobial. These activities are linked to important phytochemical constituents of the plants like: flavonoids, saponins, glycosides, alkaloids, steroids, etc (Bahramikia and Yazdanparast, 2012; Jena and Gupta, 2012; Abdul et al., 2018). The present study aims to determine the polyphenol contents in the hydro-acetone extracts prepared from the roots of R. cumunis and the areal part of T. polium, collected in Tlemcen area, and to investigate, in vitro, their antioxidant and antidiabetic activities.

#### 2. Materials and Methods

#### **2.1 Plant materials**

The plant materials used in this study include the aerial part of *T. polium* (leaves and stems) harvested from Maghnia during April 2021, and the roots of *R. communis* harvested from Tounane (Souahlia) in Tlemcen during February 2023. The botanical identification of samples was carried out in the Laboratory of Ecology and Management of Natural Ecosystems at the University of Tlemcen. The recovered plant material was dried in darkness over one week, cut into small pieces, then stored in the laboratory for further extraction.

#### 2.2 Preparation of hydro-acetone extract

Hydro-acetone extracts of the aerial part of *T. polium* and the roots of *R. communis* were obtained by decoction of samples (25g) in water-acetone mixture (300 mL; 30/70 v/v) for 30 min. The solution was then filtered and the filtrate recovered was evaporated to dryness to obtain a solid crude extract. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at  $+4^{\circ}$ C.

#### 2.3 Total polyphenol contents

Total polyphenol contents of the extracts were determined by using the Folin–Ciocalteu reagent, according to the method of Vermerris et al. (2008) with slight modifications. 100  $\mu$ L of sample extracts (1 mg/mL) were mixed with 2 mL of Na<sub>2</sub>CO<sub>3</sub> (2 %). After 5 min, 0.1 mL of Folin–Ciocalteu reagent (0.2 N) was added to the mixture. The resulting mixture was incubated at room temperature for 30 min before absorbance measurement at 700 nm. Analyses were

carried out in triplicate and results were expressed as  $\mu g$  equivalents gallic acid (EGA) in mg of extract ( $\mu g$  EGA/mg) (Adjdir et al., 2019).

# 2.4 Total flavonoid contents

AlCl<sub>3</sub> reagent was used to measure flavonoid contents according to Ardestani & Yazdanparast (2007). 0.5 mL of hydro-acetone extracts or catechin (standard) at different concentrations were combined with 150  $\mu$ L of NaNO<sub>2</sub> solution (15%), and 2 mL of distilled water. A subsequent incubation at room temperature for 6 min was followed by the addition of 150  $\mu$ L of AlCl<sub>3</sub> (10%) and 2 mL of NaOH (4%). Following a second 15 min incubation at room temperature, distilled water was added to adjust the total volume to 5 mL, and absorbance was determined at 510 nm. As a result, the data were reported as  $\mu$ g of catechin equivalent/mg dry extract ( $\mu$ g eq Cat /mg) (Terki et al., 2023).

# 2.5 Antioxidant activity

# 2.5.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

*R. communis* and *T. polium* hydro-acetone extracts were investigated for their ability to scavenge free radicals via the DPPH assay according to Atoui et al. (2005). 50  $\mu$ L of extracts solutions at different concentrations were added to 1.95 mL of DPPH solution (25  $\mu$ g/mL). The mixture was incubated for 30 min in the dark at room temperature, then the absorbance was measured at 515 nm (El Haci et al., 2009). The radical scavenging activity of the tested samples was expressed as percentage inhibition of DPPH and calculated as follows;

# *DPPH inhibition* (%) = $[A_0 - A_1/A_0] \times 100$

 $A_0$  and  $A_1$  are the absorbances of the control and the sample, respectively. IC<sub>50</sub> (the half maximal inhibitory concentration) was determined graphically from linear or logarithmic regression curves. Ascorbic acid at different concentrations was used as positive control.

#### 2.5.2 Ferric reducing antioxidant power (FRAP)

The ferric reducing power, based on the reduction of ferric iron to the ferrous form in the presence of the antioxidant components, was evaluated according to the protocol of Karagözler et al. (2008) using the potassium ferricyanide reducing method. 1 mL of each extract was combined with 2.5 mL phosphate buffer (0.05 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After standing at 50°C for 20 min, the mixture was cooled down at room temperature, and the absorbances of the samples were measured at 700 nm. Then, 2.5 mL of trichloroacetic acid solution (10%) was added to the medium and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of FeCl<sub>3</sub> (1%) solution to measure the absorbance at 700 nm (Ferreira et al., 2007). Results were expressed by calculating EC<sub>50</sub> values form linear or logarithmic regression curves. BHA at different concentrations was used as positive control.

# 2.5.3 Anti diabetic activity: a-amylase inhibitory activity

The  $\alpha$ -amylase inhibitory assay adapted from the method of Bernfeld (1955) was evaluated according to the protocol of Laoufi et al., (2017) and Abbou et al., (2022). The mixture containing 200 µL of the tested extracts and 200 µL of 0.02 M sodium phosphate buffer (pH 6.9; 6.7 mM NaCl) containing 1.3 U/mL of porcine pancreatic  $\alpha$ -amylase was pre-incubated at 37 °C for 10 min, and then 200 µL of 0.5 % starch solution were added and incubated at 37 °C for 10 min. 600 µL of DNSA solution was added to the reaction and the tubes were placed in a boiling water bath for 8 min, then cooled down in cold water for 3 min. The reaction mixture was then diluted after adding 1 mL of distilled water and the absorbances were measured at 540 nm. Acarbose was used as a positive control. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula,

# Inhibition of $\alpha$ -amylase activity (%) = $[A_0 - A_1/A_0] \times 100$

 $A_0$  and  $A_1$  are the absorbance of the control and the sample, respectively. IC<sub>50</sub> (the half maximal inhibitory concentration) was determined graphically from linear or logarithmic regression curves.

#### 3. Results and discussion

#### 3.1. Polyphenol and flavonoid contents

Table 1 presents the total polyphenol and flavonoid contents in the hydro-acetone extracts of R. communis and T. polium. The obtained results show a high content of polyphenols in the hydro-acetone extract of the roots of R. communis (499.55  $\pm$  0.02 µg EGA/mg) compared to the extract of T. polium which contains  $(117.03 \pm 0.02 \,\mu g \,\text{EGA/mg})$ . For the flavonoid contents, a slightly higher contents were noted in *R. communis* roots extract  $(28.21 \pm 0.01 \ \mu g \ eq \ Cat / mg)$ in comparison with the extract of the areal part from *T. polium* ( $21.55 \pm 0.03 \mu g$  eq Cat /mg). According to the literature, phenolic compound values obtained are important compared to the values determined by Iqbal et al. (2012) in the methanolic extract of the areal part of R. communis and its fractions: n-hexane, chloroform, ethyl acetate and n-butanol, which were ranged from 52 to 98 µg EGA/mg DE. The aqueous-ethanol extract from fresh R. communis leaves contains  $197.43 \pm 2.86 \ \mu g EGA/mg$  of polyphenols and  $68.82 \pm 1.59 \ \mu g$  eq Cat /mg of total flavonoids (Hussain et al., 2022). Likewise, the study of Rampadarath et al., (2014) determined  $632.33 \pm 19.20 \ \mu g EGA/mg FW$  of polyphenols and  $7.61 \pm 1.88 \ \mu g EQ/mg FW$  of flavonoids in the ethyl acetate extract from the fresh leaves of R. communis. According to Ait Chaouche et al., (2018), the contents of polyphenol and flavonoid determined in the methanolic extract prepared from the areal part of T. polium, collected at flowering stage from Oum El Bouaghi (east of Algeria), were estimated at 206.95  $\pm$  1.82 µg EGA/mg DE, and 42.16  $\pm$  0.61 µg eq Cat/mg DE, respectively. Also, El Atki et al. (2019) confirmed that the methanolic extract of the areal part of T. polium collected at flowering stage from Errachidia (Morocco) presented high contents of total phenolics (95.53  $\pm$  1.65µg EGA/mg DE) and flavonoid contents (101.9  $\pm$ 1.97 µg eq Cat/mg DE). The methanolic extracts from different parts of *T. polium*, harvested in Southeast Serbia, mainly from the leaves contained the highest content of polyphenols (157.84

 $\mu$ g EGA/mg) compared to the aqueous and the acetone extracts. However, the acetone extract contained the highest level of flavonoids compared (139.87  $\mu$ g ERut/mg DE) to the other extracts (Stankovic et al., 2012). In addition, the study of Ardestani & Yazdanparast (2007) showed that the aqueous-ethanol extract from the arial part of *T. polium* and its fractions; ethyl acetate and diethyl ether, contained 85.5 to 268.2  $\mu$ g EGA/mg DE of polyphenols and between 21.4 and 197.4  $\mu$ g eq Cat/mg DE of flavonoids. According to the literature, methanol is the most effective solvent in extracting phenolic components from plants in particular lower molecular weight polyphenols, while the higher molecular weight flavanols are better extracted with water acetone mixture (Shi et al., 2005; Stankovic et al., 2012; Chigayo et al., 2016). In this study, we used the mixture aqueous-acetone as extraction solvent, which indicates that extracts obtained from *R. communis* and *T. polium* contained polyphenols in particular high molecular weight flavanols (Chigayo et al., 2016).

**Table 11.** Total polyphenols and flavonoids content in *R. communis* and *T. polium* hydro-acetone extracts.

	TPC (µg EGA/mg DE)	TFC (µg eq Cat /mg DE)		
R. communis	$499.55 \pm 0.02$	$28.21 \pm 0.01$		
T. polium	$117.03\pm0.02$	$21.55\pm0.03$		

TPC: Total phenolic content. TFC: Total flavonoid content. EGA: Gallic acid equivalents. eq Cat: Catechin equivalents. DE: Dried extract. values expressed as means  $\pm$  SD (n=3).

#### **3.2.Antioxidant activities**

The scavenging effect of *R. communis* and *T. polium* hydro-acetone extracts compared to the effect of ascorbic acid on the DPPH radical, expressed as percentage of reduction and IC<sub>50</sub> values is reported in Table 2. Dose-dependent antiradical effect was observed for both plant extracts, at low concentrations (0.5 to 3.75  $\mu$ g/mL) *R. communis* extracts provided the highest inhibitory effect on the DPPH radical (43.06 % to 53.92 %) compared to *T. polium* extracts which reduced the DPPH between 51.44 % and 79.83 %, but at higher concentrations from 12.5 to 50  $\mu$ g/mL. Regression equations to derive the IC<sub>50</sub> values showed inverse relationship between IC<sub>50</sub> value and percentage scavenging potential of a sample. The obtained IC<sub>50</sub> values revealed that both plant extracts showed an interesting IC<sub>50</sub> values. *R. communis* root extract showed a comparable IC<sub>50</sub> value (2.01 ± 0.01  $\mu$ g/mL) to that of ascorbic acid (2.47 ± 0.01  $\mu$ g/mL).

However, IC<sub>50</sub> value determined for the aerial part of *T. polium* ( $7.40 \pm 0.01 \mu g/mL$ ) was slightly over than the ascorbic acid value. In FRAP results (Table 3), the hydro-acetone extract of *R. communis* showed a high reducing power for the metallic iron ions. This extract provided an EC<sub>50</sub> value of 41.32 ± 0.01 µg/mL comparable to the value for BHA (reference antioxidant molecule) 43.74 ± 0.025 µg/mL. The *T. polium* extract also showed an EC<sub>50</sub> value of 74.63 ± 0.07 µg/mL, but its iron-reducing capacity was lower than that of *R. communis*.

	Ascorbic Acid (µg/mL)					IC <sub>50</sub> (µg/mL)	
	0.5	1	2	3	4	5	
DPPH radical scavenging (%) of ascorbic acid	13.92 ±0.02	24.16 ±0.01	58.55 ±0.01	66.11 ±0.01	83.08 ±0.01	87.22 ±0.05	2.47 ±0.01
	Hydro-acetone extracts (µg/mL)						
	0.5		1	2.5	3.75		
DPPH radical scavenging (%) of <i>R. communis</i>	43.06 ±0.01		43.26 ±0.01	51.93 ±0.01	53.92 ±0.01		2.01 ±0.01
	12	2.5	25	37.5	5	50	IC <sub>50</sub> (µg/mL)
DPPH radical scavenging (%) of <i>T. polium</i>	-	.44 .02	66.25 ±0.08	76.13 ±0.04		9.83 9.03	7.40 ±0.01

**Table 2.** Antioxidant activity of *R. communis* and *T. polium* evaluated by DPPH radical scavenging (%).

Values are presented as mean±SD (n=3).

**Table 3.** Antioxidant activity of hydro-acetone extracts of *R*. *communis* and *T*. *polium* evaluated by using the ferric reducing power method.

	Concentrations (µg/mL)						
	8	17	33	5	50		
BHA (A 700nm)	0.05 ±0.006	0.15 ±0.037	0.34 ±0.081	0.63±0.10		<b>43.74</b> ± 0.025	
Hydro-acetone extracts (µg/mL)							
	7	13	17	25	42	EC50 (µg/mL)	
R. communis (A 700nm)	0.06 ±0.02	0.13 ±0.01	0.18 ±0.01	0.33 ±0.01	0.51 ±0.01	<b>41.32</b> ±0.01	
	25	50	67	83	167	EC <sub>50</sub> (µg/mL)	
<i>T. polium</i> (A 700nm)	0.16 ±0.04	0.25 ±0.1	0.45 ±0.01	0.66±0.01	1.13 ±0.03	<b>74.63</b> ± 0.07	

Values are presented as mean±SD (n=3). BHA: Butylated HydroxyAnisole. A: absorbance at 700 nm.

Several studies confirmed the antioxidant properties of *R. communis*, the study of Ahmed et al. (2018) reported the antioxidant effect of the aqueous and ethanolic extracts of bark and roots of *R. communis*, and which showed 50% and 68% radical scavenging activity. Also, Abbas et al. (2018) showed a higher antioxidant activity of leaves and seeds compared to other parts, where the methanol extract showed the highest percentage (95%) followed by acetone (91%), dichloromethane (62%), and *n*-hexane (50%). The antioxidant potent of the *R. communis* aqueous leaves extract achieves DPPH reduction not exceeding 44.49% (Mintiwab and

Jeyaramraja, 2021). In addition, the aqueous-ethanol extract of R. communis of fresh leaves showed a DPPH and iron reduction power with an IC<sub>50</sub> and EC<sub>50</sub> values of 250.10  $\mu$ g/mL and 500 µg/mL (Hussain et al., 2022). According to Iqbal et al., (2012), the butan-1-ol extract from the arial part of *R*. *communis* showed a strong DPPH scavenging effect with IC<sub>50</sub> of  $140 \pm 0.19$ µg/mL. Following the bibliography, our results concerning the free radical scavenging and iron reducing properties of the roots of *R*. *communis* are the most important and the most promising. Regarding the research carried out on the antioxidant power of *T. polium*, rutin and apigenin isolated from the methanol extract of T. polium aerial part extract were found to be the most active fractions as radical-scavengers with IC<sub>50</sub> values of  $23.7 \pm 1.9$ ;  $30.3 \pm 2.1$  and  $20.1 \pm 1.7$ µg/mL, respectively (Sharififar et al., 2009). Also, the methanolic leaves extract showed strong antioxidant activity against DPPH and registered an IC<sub>50</sub> of 26.30 µg/mL (Stankovic et al., 2012). In addition, the methanolic extract of the arial part showed a hight antioxidant activity as measured by DPPH and FRAP assays with IC<sub>50</sub> values of  $410 \pm 0.03 \,\mu$ g/mL and  $210 \pm 0.002$ µg/mL, respectively (El Atki et al., 2019). Ardestani and Yazdanparast (2007), showed that the ethyl acetate fraction required from aqueous-ethanol extract of T. polium possesses a strong antioxidant activity against DPPH with a more interesting IC<sub>50</sub> value (9.8  $\mu$ g/mL). The IC<sub>50</sub> value determined for our hydro-acetone extract of T. polium remains the lowest compared with the values of the published studies on the antioxidant effect of *T. polium*.

It has been reported that polyphenols and flavonoids possess significant antioxidant properties and are often regarded as anticancer, anti-inflammatory, antiviral, and antibacterial agents as a result of their antioxidant and free radical scavenging properties (El Atki et al., 2019). The free radical-scavenging activity of flavonoids is dependent on the presence of free OH groups, especially 3-OH, and to their ability to transfer their hydroxyl groups for neutralizing free radicals and produce FLO•; this effect is primarily attributed to the 3',4'- orthodihydroxy group on the B ring, the 4-carbonyl group on the C ring, and the 5-OH and 3-OH groups on the C ring (Chira et al., 2008; Benariba et al., 2013; El Kadi et al., 2021). *T. polium* is known to contain the flavones eupatorin, cirsimaritin, apigenin-4',7-dimethylether, cirsiliol, as well as rutin, apigenin, and 3,6-dimethoxy-apigenin, 4,7-dimethoxy apigenin, which are antioxidant *in vitro* (Asghari et al., 2020; Sharififar et al., 2009).

# **3.3.Anti diabetic activity:** α-amylase inhibitory activity

The inhibiting effect of hydro-acetone extracts from *R. communis* and *T. polium* is reported in Table 4. Both plant extracts have a dose-dependent inhibitory effect on  $\alpha$ -amylase activity. Hydro-acetone extract concentrations of *R. communis* varying between 0.17 and 3.33 mg/mL showed  $\alpha$ -amylase inhibition percentages varying between 20.94  $\pm$  0.01 and 73.69  $\pm$  0.02 %. whereas *T. polium* extract at low concentrations from 0.16 to 1 mg/mL achieved inhibition percentages between 30.55  $\pm$  0.01 and 51.8  $\pm$  0.19 %. Regarding the IC<sub>50</sub> values determined for each extract, we observed that the hydro-acetone extract of *T. polium* showed a slightly higher value (924.01  $\pm$  0.19  $\mu$ g/mL) than the value determined for the extract of *R. communis* (1300  $\pm$  0.01  $\mu$ g/mL). These values are clearly higher than those of acarbose, the drug for inhibiting  $\alpha$ -amylase (15.01  $\pm$  0.03  $\mu$ g/mL). Few studies have been published on the inhibitory capacity of *T. polium* on  $\alpha$ -amylase, but no scientific work on *R. communis*. According to Dastjerdi et al.

(2015), the hydroalcoholic extract from *T. polium* arial part showed inhibition of  $\alpha$ -amylase with an IC<sub>50</sub> value of 3630 µg/mL against 37 µg/mL of acarbose. In addition, Salehi et al. (2013) confirmed that *T. polium* methanolic extract exhibited  $\alpha$ -glucosidase inhibitory properties with an IC<sub>50</sub> value of 10.2 ± 0.4 µg/mL. However, the methanolic and aqueous extracts of *T. polium* provided a low inhibition percentage of  $\alpha$ -glucosidase 7 % and 1 %, respectively (Gholam et al., 2008). The inhibitory effect of our extracts on  $\alpha$ -amylase activity is mainly related to the high polyphenol and flavonoid contents of these extracts. According to the bibliography, phenolic compounds, mainly flavonoids, are the best inhibitors of digestive enzymes. The chemical structure of flavonoids, the number of rings and the position of their hydroxyl groups in the molecule inhibit these enzymes by the formation of non-covalent hydrogen and hydrophobic bonds with carbohydrate polymers, where the -OH groups can interact with the side chains of amino acids of the active site of the enzyme, such as Asp197 and Glu233 (Xiao et al., 2013; Abdelli et al., 2020).

	Concentration (µg/mL)				IC50	
	3	7	13	53	107	(μg/mL)
Acarbose	14.98	34.25	45.87	81.65	90.83	15.01
(% inhibition)	±0.04	±0.01	±0.03	±0.03	±0.03	±0.03
		<b>IC</b> 50				
	170	333	1330	2000	3333	(μg/mL)
R. communis	20.94	30.45	51.98	62.27	73.69	1300
(% inhibition)	±0.01	±0.01	±0.01	±0.02	±0.02	$\pm 0.01$
	167	333	500	667	1000	IC50 (µg/mL)
T. polium	30.55	35.41	41.66	44.16	51.8	924.01
(% inhibition)	±0.01	±0.26	$\pm 0.04$	±0.22	±0.20	$\pm 0.19$

**Table 4.** *In vitro* percentage of inhibitory activity of *R. communis* and *T. polium* extracts against porcine pancreatic  $\alpha$ -amylase.

Values are presented as mean  $\pm$  SD (n=3).

# 4. Conclusion

The obtained results indicated that the hydro-acetone (30/70, v/v) mixture ensured the extraction of a high level of phenolic compounds from the roots of *R. communis* extract more than the aerial part of *T. polium*. The high content of phenolic compounds in the hydro-acetone extract of *R. communis* ensured its anti-free radical and iron-reducing power, while the hydro-acetone extract of *T. polium* was highlighted in comparison with the extract of *R. communis* by its inhibitory effect on  $\alpha$ -amylase activity. Therefore, *R. communis* and *T. polium* require further studies to identify the phyto-constituents which are responsible for their antioxidant and anti-diabetic properties, and allow them to be investigated in the food industry and in the treatment of oxidative stress-related diseases and diabetes mellitus.

# **Conflict of interest**

Authors declare that there is no conflict of interest.

# **Author Contribution Statement**

Nabila Benariba supervised the findings of this work; Zohra FEKHIKHER, Radia BRIXI-GORMAT, Hanane BENRAMDANE, Houda YOUBI, Ibtissam KOUAR, Hanane MILOUDI, Soumia MEHARRAR: Carried out the survey; Imad Abdelhamid EL HACI, Houria MEDJDOUB, Rachid AZZI: discussed the results and contributed to the final manuscript.

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