

Chemical Analysis, Antioxidant, Anti-Alzheimer and Anti-Diabetic Effect of Two Endemic Plants from Algeria: *Lavandula antineae* and *Thymus algeriensis*

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Abstract

The purpose of the present work is searching for new sources of bioactive molecules from plants to use them in treating or controlling some health problems. The methanolic extracts of two endemic species in Algeria *Lavandula antineae* (very few studies on its biological effects) and *Thymus algeriensis* were analyzed by HPLC/UV then tested for their antioxidant effect by the DPPH and ABTS scavenging radical tests, FRAP test and CUPRAC test. The inhibitory power of these same extracts on acetylcholinesterase, butyrylcholinesterase and α -glucosidase was also evaluated. Phenolic acids and flavonoids were found in common in both extracts as 3-hydroxy-4-méthoxycinnamic acid, and quercetin. The results showed considerable antioxidant effects for both plants with minimal IC₅₀ values equal to 10.77±1.14 µg/ml for *L. antineae* and 11.73±0.20 µg/ml for *T. algeriensis*. The minimal value of PR_{0.5} was recorded with *L. antineae* (10.57±0.38 µg/ml) after the BHA. The two species are shown to be effective on acetylcholinesterase especially *T. algeriensis*. *L. antineae* exhibited a high inhibitory power against butyrylcholinesterase with 20.84±9.74 µg/ml IC₅₀ value. The same plant showed more effective than Galantamine in inhibiting α -glucosidase with 168.61±7.60 µg/ml IC₅₀ value. Interesting results were given by methanolic extract of both plants, which can be exploited in medicine and pharmaceutical domains as natural treatments for diseases like Alzheimer and diabetes type 2.

Keywords: *Lavandula antineae*, *Thymus algeriensis*, methanolic extract, HPLC/UV, antioxidant activity, enzymes inhibitory.

1. Introduction

Oxidative stress can be defined as an imbalance between reactive oxygen species (free radicals) and antioxidant systems (Ichai *et al.*, 2011). The uncontrolled formation of reactive oxygen species will often have serious consequences for the body (Pelletier *et al.*, 2004). In several serious diseases, notably those linked to aging, oxidative stress is the original triggering factor; this is the case of cancers, ocular pathologies, diabetes and neurodegenerative diseases like Alzheimer's disease (Favier, 2006). That is why many studies are focusing on searching molecules with antioxidant potential. Indeed, the use of plant extracts and their derived phytochemicals, particularly phenolic compounds, has a probable future for controlling various pathologies. Their capacity to scavenge free radicals can entitle them to promote health effects (Payan, 2004; Subhashini *et al.*, 2011; Mukherjee *et al.*, 2018; Simonovic *et al.*, 2019).

In Algeria, a diverse plant flora can be found, including endemic plants with medicinal proprieties such as *L. antineae* and *T. algeriensis* (Lamiaceae family) (Ozenda, 2004). The genus *Lavandula* is known for its medicinal and ornamental effects; it has a high antioxidant activity (Zuzarte *et al.*, 2011; Nikolic *et al.*, 2014; Ceylan *et al.*,

2015). Thyme has been utilized since ancient times for its pharmacological proprieties (Goatez and Guédira, 2012), especially *Thymus algeriensis* which has antioxidant potential and can act as inhibitors of free radical or scavengers (Delgado *et al.*, 2014; Guesmi *et al.*, 2014).

Our work aims to analyze the chemical composition of the methanolic extracts of *Lavandula antineae* and *Thymus algeriensis* searching for some phenolic compounds, and in order to seek new natural bioactive molecules sources, we have tested the two extracts *in vitro* for their antioxidant activity and their inhibitory capacity against certain enzymes involved in several diseases like acetylcholinesterase, butyrylcholinesterase known by their relation with Alzheimer's disease and the digestive enzyme α -glucosidase linked with diabetes type 2.

2. Material And Methods

2.1. Material

2.1.1. Plant material

Lavandula antineae identification was done in the arid regions scientific and technical research center (CRSTRA)-Biskra, while *Thymus algeriensis* was identified in Bellezma National Park of Batna. Desert lavender was harvested from Biskra during the flowering

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cycle starting at the end of February to early of April. The sampling of *Thymus algeriensis* from Batna was carried out in April. For further preparation of methanolic extracts, aerial sections, precisely leaves and stems, have been dried outdoors and in shade.

2.2. Methods

2.2.1. Methanolic extract preparation

A test sample of 2.5 g of leaf powder was macerated in 25 ml of 80% methanol. Then the macerate was filtered and the solvent was evaporated under reduced pressure at a rotary evaporator at 40-50 °C to dryness. The extract was kept at 4 °C (Falleh *et al.*, 2008).

2.2.2. Analysis of the methanolic extract by High Performance Liquid Chromatography (HPLC/UV)

The samples were diluted in methanol and then filtered by 0.45 µm syringe filters. Twenty available standards (phenolic compounds), in fine quantities, have been diluted in methanol. Twenty microliters aliquot of each sample was introduced in the HPLC system combined with a UV-Vis detector at room temperature and with a steady flow rate of 1.0 ml per ml. Compound identification in each sample was established on differences between the retention times of the components determined and the retention times of the standards.

2.2.3. Antioxidant activity in vitro

2.2.3.1. DPPH free radical scavenging test

The antioxidant test by scavenging DPPH radical was conducted in accordance with Bougandoura and Bendimerad (2013) protocol. Fifty microliters of each extract was added to 2 ml DPPH methanolic solution (0.025 g/l). At the same time by combining fifty microliters of the solvent (methanol) with 2 ml of the DPPH methanolic solution, a negative control was prepared. For each concentration, a blank was made and the absorbance was read at 515 nm after 30 min incubation time in the darkness and ambient temperature. BHA and BHT presented the positive control.

2.2.3.2. ABTS free radical scavenging test

ABTS was dissolved in twice-distilled water to obtain a concentration of 7 mM. The cation (ABTS^{•+}) was made by reacting solutions of ABTS stock and K₂S₂O₈ (2.45 mM) in the presence of K₃PO₄ buffer solution. The mixture was incubated in the darkness for 12-16 hours before use at ambient temperature. Absorbance reading was taken at 734 nm. BHT and BHA solutions were prepared at different concentrations and tested as positive controls (Re *et al.*, 1998). The results for DPPH scavenging and ABTS scavenging tests are indicated as an inhibition percentage (I %).

$$I\% = [(Abs\ control - Abs\ test) / Abs\ control] \times 100.$$

2.2.3.3. Iron reduction test: FRAP (Ferric Reducing Antioxidant Power)

Methanolic extract was dissolved in 2.5 ml of Na₃PO₄ buffer at pH 6.6 and 2.5 ml of 1% C₆N₆FeK₃ at different concentrations. The mixture was incubated at 50 °C for twenty minutes. After 2.5 ml of trichloroacetic solution (10%) was put, the mixture underwent centrifugation for 10 min at 3000 g. The supernatant (2.5 ml) was added and agitated with 0.5 ml (0.1 percent) of FeCl₃ and 2.5 ml of distilled water. Absorption was measured at 700 nm. For

BHA and BHT, the same test was performed (Ferreira *et al.*, 2007).

2.2.3.4. Cupric ion reduction CUPRAC (Cupric ion Reducing Antioxidant Capacity)

The method followed was reported by Apak *et al.* (2004), fifty microliters of Cu (II) (10 mM), fifty microliters of the neocuproin (7.5 mM), sixty microliters of the NH₄Ac buffer (1 M, pH = 7), and forty microliters of each plant's methanol at a variety of concentrations. After one hour, absorption was registered at 450 nm.

The reducing power at absorbance value 0.5 (PR0.5) was calculated for both tests FRAP and CUPRAC.

2.2.4. Anti-enzymatic activity in vitro

2.2.4.1. Anti-Alzheimer activity (inhibition of acetylcholinesterase and butyrylcholinesterase)

The spectrophotometric approach was followed by testing extracts' ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes (Ellman *et al.*, 1961). The buffer was made up of 150 µl of Na₃PO₄ at pH 8.0 (100 mM), 10 µl of the solution to test were dissolved into ethanol at various concentration and the amount of 20 µl AChE (5.32 per 10⁻³ U) or BChE (6.85 per 10⁻³ U) was added and incubated at 25 °C for 15 minutes, and then 10 µl DTNB (0.5 mM) had been applied. Then the reaction was initiated by the inclusion of 20 µl of acetylthiocholine iodide at 0.71 mM concentration or butyrylthiocholine chloride at 0.2 mM concentration. Absorbance was read at 412 nm. AChE or BChE inhibition was determined by comparing enzyme activity without extract and its activity in the presence of extract in the following formula:

$$I (\%) = (E - S) / E \times 100$$

E: Enzyme activity without extract S: the enzyme activity in the presence of the extract. The reference compound was galantamine.

2.2.4.2. Inhibition of α-glucosidase

The inhibitory action of α-glucosidase has been carried out respecting Palanisamy *et al.* (2011) method with few modifications. Fifty microliters of the solution to test was mixed with 50 µl of 4-Nitrophenyl α-D-glucopyranoside (5 mM) and 100 µl of the enzyme, the mixture was incubated for 15 minutes at 37 °C. A blank was made for each sample. Absorption was read at 405 nm (0 min and 15 min). Acarbose was used in this experiment as a standard. α-glucosidase's inhibitory function has been demonstrated as follows:

$$\% \text{ inhibition} = (Abs\ extract - Abs\ blank) / Abs\ control \times 100$$

Control: Enzyme + Substrate + Solvent of the extract.

2.3. Statistical analysis

Each test was done in triplicate; the comparison of means was carried out by ANOVA one way with the Tukey test where the difference was considered significant to a degree ≤ 0.05. For these purposes, the SPSS Statistics version 25 program was used.

3. Results

3.1. Analysis by HPLC

In a time interval between 3 min and 42 min, peaks were marked on the chromatographic profile of the extract

of *Lavandula antineae* (Figure 01); a dominant component of the plant extract with a percentage of 67.2% was detected at a retention time of 24.4 min, followed by two other components with the following percentages: 8.8% and 4.4%. Their retention times were 32.7 min and 27.4 min, respectively. Many peaks were observed, in a time interval of 3 min to 60 min, on the chromatographic profile of *Thymus algeriensis* extract. Three phenolic components were revealed constituting more than 50% of the total extract, with percentages of 26.4%, 17.3% and 8.2%; their corresponding retention times were, respectively, 32.8 min, 36.4 min and 40.0 min (Figure 2).

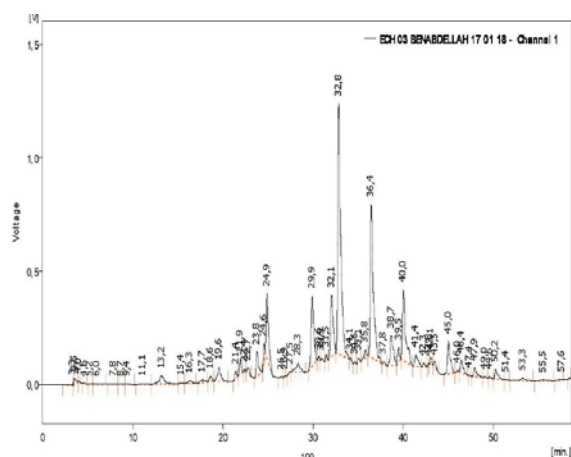


Figure 1. Chromatogram of methanolic extract of *L. antineae*

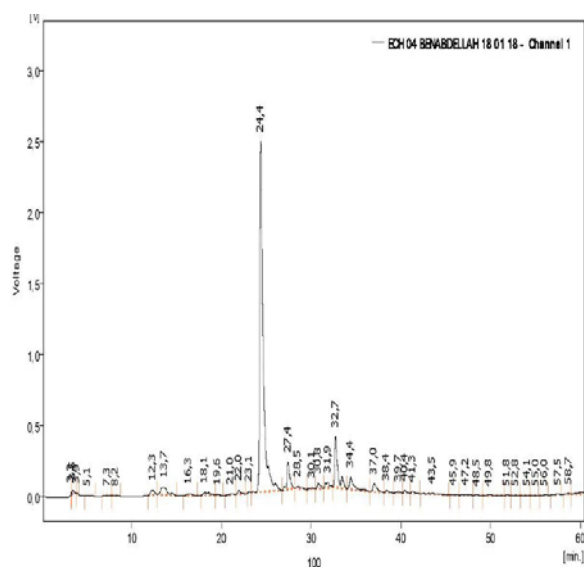


Figure 2. Chromatogram of methanolic extract of *T. algeriensis*

Table 1. Identified components by HPLC/UV in methanolic extract of *L. antineae* and *T. algeriensis*

Plant Component	Retention time (min)	<i>L. antineae</i> (%)	<i>T. algeriensis</i> (%)
3-hydroxy-4-méthoxycinnamic acid	28.287	0.5	1.5
Ferulic acid	26.56	-	0.1
Gallic acid	6.543	0.1	-
Anisic acid	33.037	-	26.4
Salicylic acid	30.747	0.9	0.2
Syringic acid	21.967	0.7	1
Trans-2,3-diméthoxycinnamic acid	39.28	-	0.9
Trans-cinnamic acid	25.173	-	5.4
Vanillic acid	22.623	0.3	0.2
Catechin	21.553	0.7	0.5
Epicatechin	22.503	-	0.1
Euleropein	32.367	-	6.1
Kaempferol	41.103	0.4	1.5
Myricetin	34.27	3	0.2
Quercetin	36.85	1.7	17.3
Rutin	30.687	-	0.2

3.2. Results of the antioxidant activity

3.2.1. Result of DPPH radical scavenging test

An almost similar and more powerful antioxidant power than BHT was noted for *L. antineae* and *T. algeriensis*, their IC_{50} values were 18.59 ± 0.07 and 18.40 ± 0.42 $\mu\text{g/ml}$ respectively (Table 02). The inhibition percentages took their maximum values at the 400 $\mu\text{g/ml}$ concentrations: 90.26 ± 0.99 $\mu\text{g/ml}$ for *T. algeriensis* and 88.54 ± 0.23 $\mu\text{g/ml}$ for *L. antineae*.

3.2.2. Result of ABTS radical scavenging test

L. antineae presented inhibition percentage greater than 90% from the concentration of 50 $\mu\text{g/ml}$. Considerable values was recorded for the methanolic extract of *T. algeriensis* from the concentration of 50 $\mu\text{g/ml}$. *T. algeriensis* and *L. antineae* showed close IC_{50} values, 11.73 ± 0.20 and 10.77 ± 1.14 $\mu\text{g/ml}$, respectively (Table 03). Their antioxidant capacity was lower than that of BHT and BHA.

Table 2. Percentages of DPPH inhibition by *L. antineae* and *T. algeriensis* methanolic extracts, BHA, BHT and the corresponding IC_{50}

Plant/Standard	Inhibition %							
	6.25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	IC_{50} $\mu\text{g/ml}$
<i>L. antineae</i> ^a	19.03 \pm 1.86	35.87 \pm 1.18	68.39 \pm 1.32	87.07 \pm 0.13	87.86 \pm 0.07	88.27 \pm 0.20	88.54 \pm 0.23	18.59 \pm 0.07
<i>T. algeriensis</i> ^a	22.23 \pm 2.58	35.50 \pm 1.70	66.92 \pm 3.39	87.71 \pm 0.11	88.42 \pm 0.47	89.66 \pm 0.17	90.26 \pm 0.99	18.40 \pm 0.42
BHA ^a	36.46 \pm 2.45	59.63 \pm 1.50	78.91 \pm 0.77	83.11 \pm 0.46	84.21 \pm 0.50	85.31 \pm 0.35	85.91 \pm 0.50	10.03 \pm 0.84
BHT ^a	18.55 \pm 2.46	32.60 \pm 3.72	53.80 \pm 2.58	74.97 \pm 2.14	83.41 \pm 0.86	84.59 \pm 0.46	85.76 \pm 0.91	23.54 \pm 1.83

Values indicated are means \pm SD of three measurements $p \leq 0.05$. a: subset determined by the tukey test

Table 3. Percentages of ABTS inhibition by *L. antineae* and *T. algeriensis* methanolic extracts BHA, BHT and the corresponding IC₅₀.

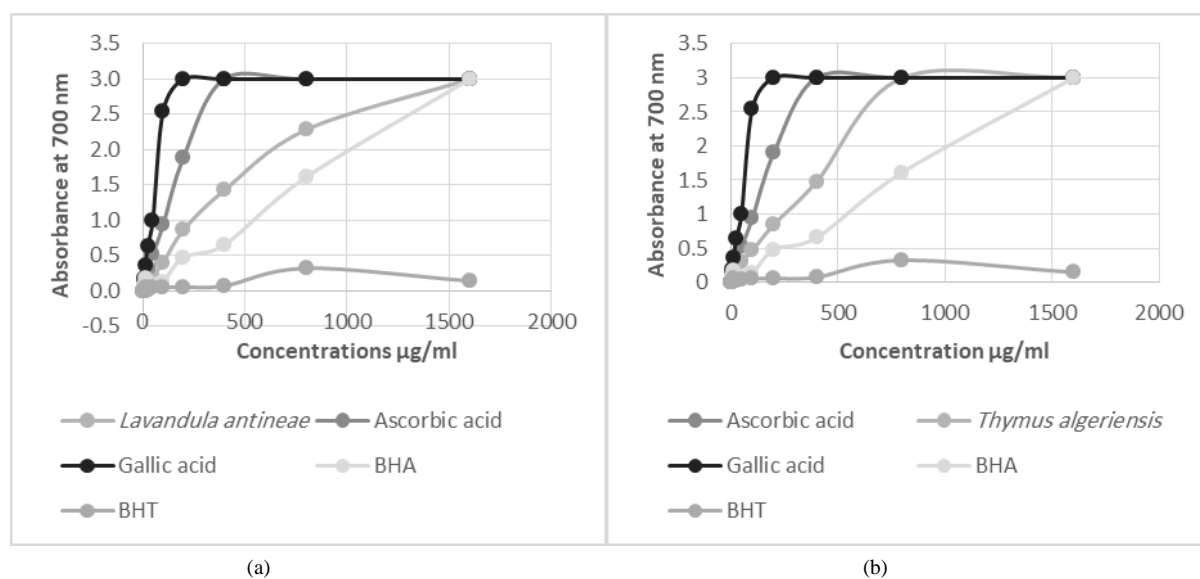
Plant/Standard	Inhibition %							
	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	IC ₅₀ µg/ml
<i>L. antineae</i> ^b	29.73±3.63	60.38±3.78	88.44±0.69	92.01±0.20	92.70±0.10	92.81±0.10	92.87±0.20	10.77±1.14
<i>T. algeriensis</i> ^{ab}	29.96±0.75	55.20±0.85	84.93±1.25	92.01±0.26	92.58±0.35	92.93±0.17	92.98±0.10	11.73±0.20
BHA ^b	93.50±0.09	93.55±0.09	93.60±0.16	93.60±0.95	94.17±0.90	95.37±2.63	95.42±2.69	1.81±0.10
BHT ^{ab}	61.38±0.57	62.02±3.82	76.50±1.40	82.55±1.04	88.60±2.66	90.38±0.67	95.83±0.15	1.29±0.30

Values indicated are means ± SD of three measurements $p \leq 0.05$. a, b: subsets determined by the tukey test

3.2.3. Result of the FRAP test

In regard to *L. antineae* extract, from the 50 µg/ml concentration, the absorbance values were marked by a significant increase, up to the value of 1600 µg/ml where the absorbance approached the value of 3. This increase was greater than that produced by BHA and BHT (Figure 03 a).

From the concentration of 25 µg/ml, *T. algeriensis* had absorbance values considerably higher than the values obtained with BHA and BHT (Figure 03 b). A more interesting increase was detected from 400 µg/ml. The PR_{0.5} of the two plants was determined at lower values than the values obtained with BHA and BHT (Table 04).

**Figure 3.** Antioxidant activity of *L. antineae* methanolic extract (a) and *T. algeriensis* methanolic extract (b) obtained by FRAP test**Table 4.** PR_{0.5} obtained by FRAP test for *L. antineae*, *T. algeriensis*, BHA and BHT (µg/ml)

Plant/Standard	PR _{0.5}
<i>L. antineae</i> ^{ab}	155.733±0.196
<i>T. algeriensis</i> ^{ab}	147.44±0.191
BHA ^{a,b}	464±0.007
BHT ^a	7566.66±0.0007

Values indicated are means ± SD of three measurements $p \leq 0.05$.

a: subset determined by the tukey test

3.2.4. Result of copper reduction test (CUPRAC)

The methanolic extract of *L. antineae* had a considerable reducing power (Figure 04 a), the absorbance

values were marked by a strong increase with all the concentrations tested. By comparing it with BHA and BHT, the extract of *L. antineae* was less efficient than BHA, with an absorbance of 2.53 ± 0.19 at 100 µg/ml concentration and more effective than BHT. The PR_{0.5} of the extract was determined to be 10.57 ± 0.3 µg/ml (Table 5).

The methanolic extract of *T. algeriensis* exhibited remarkable reducing power; its effect was more powerful than BHT at concentrations above 200 µg/ml. At concentrations over 400 µg/ml, the plant extract exhibited a performance close to that of BHA (Figure 04 b). The PR_{0.5} of the studied extract was determined to be 25.04 ± 0.86 µg/ml (Table 05).

(a)

(b)

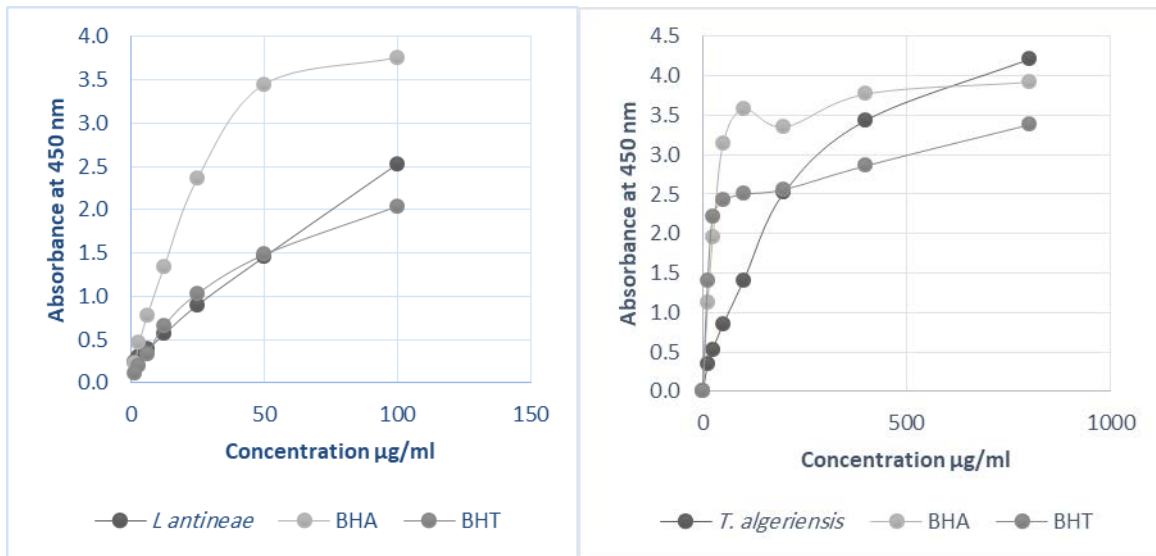


Figure 04 Antioxidant activity of *L. antineae* methanolic extract (a) and *T. algeriensis* methanolic extract (b) obtained by CUPRAC test

Table 5. PR_{0.5} obtained by CURAC test for *L. antineae* and *T. algeriensis* methanolic extracts, BHA and BHT (µg/ml)

Plant/Standard	PR _{0.5}
<i>L. antineae</i> ^{ab}	10.57± 0.38
<i>T. algeriensis</i> ^a	25.04± 0.86
BHA ^b	3.64±0.19
BHT ^{ab}	9.62±0.87

Values indicated are means ± SD of three measurements $p \leq 0.05$. a, b: subsets determined by the tukey test

3.3. Result of the anti-enzymatic activity

3.3.1. Result of the anti-Alzheimer's activity

Anticholinesterase: An interesting anti-cholinesterase activity was provided by the methanolic extract of *T. algeriensis*. The increase in inhibition percentages was remarkably noted with the increase in concentrations (Figure 05 b). The percentage of inhibition exceeded 50% and the IC₅₀ of the extract was deduced to a value of 154.47 ± 3.55 µg/ml at the concentration 200 µg/ml (Table 06). *L. antineae* showed an inhibitory effect on acetylcholinesterase which increased slowly by increasing concentrations (Figure 05 a). The inhibition percentages did not exceed the value of 20% until the concentration exceeded the value of 200 µg/ml. The IC₅₀ have been estimated at values greater than 200 µg/ml (Table 6).

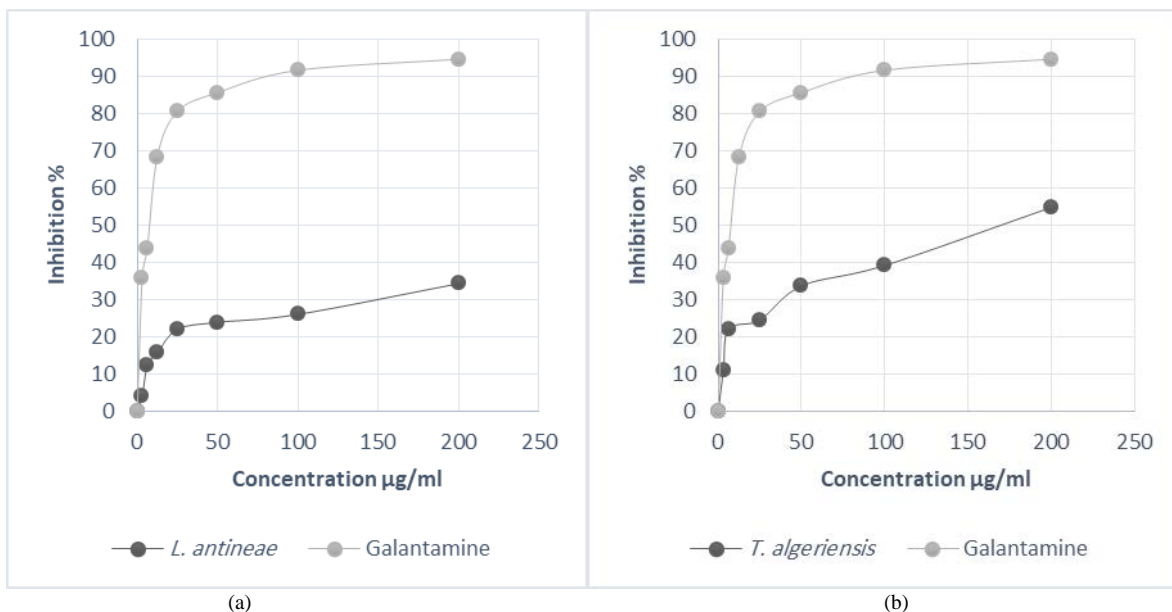


Figure 05. Anticholinesterase activity of *L. antineae* methanolic extract of (a) and *T. algeriensis* methanolic extract (b)

Antibutyrylcholinesterase: A marked increase was recorded in the percentages of inhibition obtained with

methanolic extract of *L. antineae*. Even at low concentrations (3.125, 6.25 and 12.25 µg/ml), this increase

was more considerable than the increase achieved by galantamine. In concentration value equal to 100 $\mu\text{g/ml}$, A small variation was observed between the plant extract and the galantamine performances (Figure 06 a). The IC_{50} was determined to be $20.84 \pm 9.74 \mu\text{g/ml}$, while galantamine was denoted by an IC_{50} equal to $34.75 \pm 1.99 \mu\text{g/ml}$ (Table 07), which may be translated by a promising effectiveness of the plant for the fight against Alzheimer's disease.

The methanolic extract of *T. algeriensis* was characterized by an interesting IC_{50} which equals the value of $161.53 \pm 22.65 \mu\text{g/ml}$ (Table 07). The inhibition percentages increased proportionally with the concentrations. From 25 $\mu\text{g/ml}$ concentration value, the difference in performance of the extract compared to the standard became significantly high (Figure 06 b).

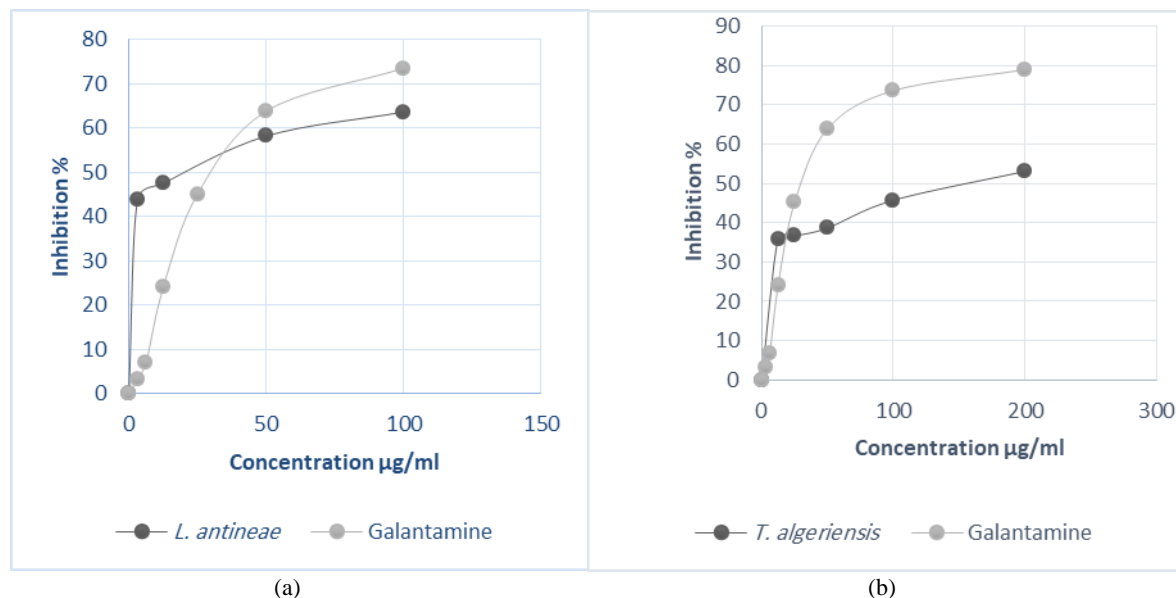


Figure 6. Antibutyrylcholinesterase activity of *L. antineae* methanolic extract (a) and *T. algeriensis* methanolic extract (b)

Table 6. IC_{50} of anticholinesterase activity of *L. antineae*, *T. algeriensis*, and galantamine.

Plant/Standard	IC_{50} ($\mu\text{g/ml}$)
<i>L. antineae</i> ^{ab}	>200
<i>T. algeriensis</i> ^b	154.47 ± 3.55
Galantamine ^c	6.27 ± 1.15

Values indicated are means \pm SD of three measurements $p \leq 0.05$.
a, b, c: subsets determined by the tukey test

Table 7. IC_{50} of ntbutyrylcholinesterase activity of *L. antineae*, *T. algeriensis* and galantamine

Plant/Standard	IC_{50} ($\mu\text{g/ml}$)
<i>L. antineae</i> ^a	20.84 ± 9.74
<i>T. algeriensis</i> ^a	161.53 ± 22.65
Galantamine ^a	34.75 ± 1.99

Values indicated are means \pm SD of three measurements $p \leq 0.05$.
a: subset determined by the tukey test

3.3.2. Result of the α -glucosidase inhibition test

The methanolic extract of *L. antineae* showed significant inhibitory activity against α -glucosidase enzyme; the IC_{50} was estimated at a value equal to $168.61 \pm 7.60 \mu\text{g/ml}$ (Table 08). Galantamine gave an IC_{50} value equal to $275.43 \pm 1.59 \mu\text{g/ml}$; therefore, *L. antineae* gave promising effect for the inhibition of one of enzymes involved in diabetes types 2 disease. The methanolic extract of *T. algeriensis* did not show any inhibitory effect on α -glucosidase for all the tested concentrations.

Table 8. Inhibition activity of α -glucosidase by methanolic extracts of *L. antineae*, *T. algeriensis* and acarbose

Plant/Standard	Inhibition %								IC_{50} ($\mu\text{g/ml}$)
	3.125 $\mu\text{g/ml}$	6.25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	800 $\mu\text{g/ml}$	
<i>L. antineae</i> ^a	NT	NT	0.00 ± 0.0	0.00 ± 0.0	12.815 ± 3.41	48.079 ± 1.62	53.899 ± 2.21	63.702 ± 0.0	168.61 ± 7.60
<i>T. algeriensis</i>	No activity								
Acarbose ^a	78.125 $\mu\text{g/ml}$	156.5 $\mu\text{g/ml}$	312.5 $\mu\text{g/ml}$	625 $\mu\text{g/ml}$	1250 $\mu\text{g/ml}$	2500 $\mu\text{g/ml}$	5000 $\mu\text{g/ml}$	IC_{50} ($\mu\text{g/ml}$)	
	27.43 ± 2.18	38.91 ± 3.20	54.86 ± 1.79	67.29 ± 2.63	80.19 ± 1.66	85.54 ± 0.45	91.05 ± 0.72	275.43 ± 1.59	

NT: Not tested. Values expressed are means \pm SD of three measurements $p \leq 0.05$. a: subset determined by the tukey test.

4. Discussion

The chemical composition of *L. antineae* extracts has not been studied before. The majority of research was carried out on other species of the same genus, notably *L. angustifolia*. Extracts from the latter were shown to be rich in caffeic acid, rosmarinic acid, and 4-hydroxybenzoic acid (Turgut *et al.*, 2017); we did not mark their presence in our extract (Table 1). A study by Boutaoui *et al.* (2018) has demonstrated the presence of ferulic acid and catechin in the ethanolic extract of *T. algeriensis*. We have also found the same components but in few percentages (Table 01).

L. antineae extract has provided an antioxidant effect greater than *L. dentatae* (Bouzidi *et al.*, 2018); the latest one was marked by IC₅₀ values ranging from 0.33 to 1.84 mg/ml which were obtained by methanolic extracts from various plant parts. Most studies of antioxidant activity by the DPPH scavenging test, which are carried out on species of the genus *Lavandula*, are carried out on essential oils. Their results show a strong antioxidant activity (Mohammedi and Atik, 2012; Bettaieb Rebey *et al.*, 2012; El Hamdaoui *et al.*, 2018). By comparing the results of these studies and the result in our work, we can attribute to the methanolic extract of *L. antineae* a remarkable antioxidant capacity. Khled Khoudja *et al.* (2014) found an IC₅₀ of the methanolic extract of *T. algeriensis* equal to 179 ± 0.012 µg/ml, a value significantly higher than that obtained in our results. In another study carried out on methanolic extract of the same plant, IC₅₀ was estimated at a value of 7 ± 0.02 µg/ml (Megdiche-Ksouri *et al.*, 2015). Our results do not agree with the results obtained by Guesmi *et al.* (2014) who found that BHT exerted a more powerful antioxidant activity than methanolic extract of *T. algeriensis*. This difference in anti-free radical power within the same species can be attributed to several factors. Several studies have shown that water addition at low rates to the solvent ameliorates the extraction of powerful antioxidants (Turkmen *et al.*, 2006; Zhao and Zhao, 2013). Different origins of the same species can also influence antioxidant potential (Bettaieb Rebey *et al.*, 2012).

In our study, the methanolic extract of *T. algeriensis* exhibited a greater AChE inhibitory effect than the effect which was given by the ethanolic extracts of six other species of *Thymus*, namely *T. longicaulis*, *T. serpyllum* subsp. *Serpyllum*, *T. pulegioides*, *T. striatus*, *T. praecox* subsp. *polytrichus* and *T. vulgaris* where the IC₅₀ values took between 656.06 and 837.96 µg/ml (Kindl *et al.*, 2015). Our extract seems to be more effective than the leaves essential oils of the same plant which provided an IC₅₀ value equal to 98.84 ± 1.81 µg/ml (Bendjabeur *et al.*, 2018). A study by Bendjabeur *et al.* (2018), which was done to evaluate the inhibitory effect of *T. algeriensis* against butyrylcholinesterase, has found that essential oils extracted from the leaves provided a slightly lower IC₅₀ value than our extract and which equal to 124.09 ± 2.84 µg/ml. Even at a concentration equivalent to 1 mg/ml, ethanolic extracts of *L. angustifolia* and *L. pedunculata* established an inhibition of AChE less than the inhibition provided by *L. antineae*, with inhibition percentages equal to 28.4 ± 3.8 and 42.0 ± 16.8% (Ferreira *et al.*, 2007). Plants can be regarded as good bioactive compound

sources with an ability of inhibiting enzymes such as AChE and BChE (Murray *et al.*, 2013). Many secondary metabolites as terpenes, quinones, alkaloids and phenols were shown very effective to inhibit α-glucosidase enzyme and can be clinically developed for treating diabetes type 2 (Yin *et al.*, 2014).

5. Conclusion

HPLC/UV analysis revealed the common existence of quercetin, 3-hydroxy-4-methoxycinnamic acid, salicylic acid, syringic acid, Kaempferol and myricetin in the methanolic extract of the two plants. *L. antineae* presented an interesting antioxidant activity and a very promising inhibitory power of butyrylcholinesterase and α-glucosidase which is more effective than the standards used, hence the possibility of its use for the treatment of Alzheimer's disease and type 2 diabetes. *T. algeriensis* was also marked by an appreciable antioxidant activity and an ability to inhibit cholinesterase and butyrylcholinesterase. The potential involvement of natural antioxidants in the replacement of conventional treatments for several diseases, such as age-related diseases, could be significant and should be elucidated in long-term clinical trials.

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Conflict Of Interest

The authors proclaim no conflict of interest.

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