

EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *LAVANDULA ANTINEAE* MAIRE ENDEMIC MEDICINAL PLANT FROM ALGERIA

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**ABSTRACT:**

The present study was designed to investigate the phytochemical screening, total phenolic and flavonoid contents, antioxidant and cytotoxic activities of *Lavandula antineae* Maire for the first time. Phytochemical screening revealed the presence of anthraquinones, triterpenes, saponins, flavonoids, tannins, *O*-heterosides, *C*-heterosides. Total phenolic and flavonoid contents of the extracts varied between 12.49-262.35 mg GAE/g extract and 1.35-4.03 mg QE/g extract, respectively. The antioxidant activity was investigated using DPPH, reducing power and  $\beta$ -carotene/linoleic acid tests. The results indicated that ethyl acetate and diethyl ether fractions exhibited stronger activities than hydromethanolic crude extract and other fractions. In case of cytotoxicity assay, hydromethanolic extract was found to show good toxicity against brine shrimp nauplii (LC<sub>50</sub>= 13.72  $\mu$ g/ml).

**KEYWORDS:** *Lavandula antineae*; Antioxidant activity; Cytotoxic activity; Phytochemical screening.

**INTRODUCTION**

*Lavandula* genus is an important member of Labiatae (Lamiaceae) family. The medicinal importance of *Lavandula* species is well documented and the herbal drugs as well as herbal drug preparations prepared from these plants are registered in many Pharmacopeia<sup>1</sup>. *Lavandula* is employed for its antispasmodic, carminative, antiseptic, analgesic and sedative properties. It has been used traditionally to treat colic, depression, diabetes, epilepsy, migraine, urinal infections, cardiac diseases, eczema and for cleansing wounds<sup>1-3</sup>.

Several essential oils from *Lavandula* have been reported to possess antinociceptive, gastroprotective, anti-inflammatory, analgesic, antiplatelet, antithrombotic, antimicrobial and antifeedant effects<sup>4</sup>. In addition to the effects of essential oils, the neuroprotective, anti-inflammatory, antioxidant, anti-cholinesterase, anticonvulsant, sedative, antidepressant and antispasmodic properties of various *Lavandula* extracts were also demonstrated<sup>2,3</sup>. Phytochemical studies revealed the presence of different secondary metabolites in *Lavandula* species, such as monoterpenes, diterpenes, sesquiterpenes<sup>4,5</sup> and phenolic compounds such as flavonoids, phenolic acids, coumarins, tannins<sup>6</sup>.

*Lavandula antineae* Maire. (Tamahaq name: *tehenok*) is an endemic plant of Central Sahara, where it grows in Algeria, Nigeria, Chad and Sudan<sup>7</sup>. In Algeria, the aerial parts of the plant are used locally in folk medicine for the treatment of chills, bruises, oedema and rheumatism<sup>8,9</sup>. However, there are no experimental studies about biological activities of *Lavandula antineae*, while the only documented phytochemical investigation on *L. antineae* revealed the presence of flavone glycosides<sup>6</sup>.

The objectives of the present research were to perform the preliminary phytochemical screening of the aerial parts of *L. antineae* as well as the total phenolic and flavonoid contents, and to investigate the antioxidant and cytotoxic activities.

**MATERIAL AND METHODS****Plant material**

Aerial parts of *Lavandula antineae* were collected from Hagar, Southern Algeria (Coordinates;UTM: GF96; Latitude: 23°10'0"; Longitude: 5°49'60"), at the flowering stage, in July 2010. Plant identification was carried out by Mme Sahki Boutammine R., botanist, National Institute for Forest Research, Tamanrasset, Algeria and voucher specimen of the plant has been deposited (number LL/2/10). The plant aerial parts were cleaned and air-dried at room temperature in the shade, and then powdered.

**Preparation of extracts**

Powdered plant material (10 g) was extracted for 48 h with 100 ml of methanol–water (70%–30%) at room temperature. The solvent was then removed by filtration and fresh solvent was then added to the residue. The extraction process was third

repeated. The combined filtrates were then concentrated under reduced pressure at 40°C using rotary evaporator to obtain dry extract (1.51 g). The hydromethanolic crude extract was subjected to fractionation using different solvents. The crude extract was first suspended with hot distilled water (100 ml) and kept at room temperature for 12 hours. Then the suspension was defatted using hexane (50 ml, three times) and then successively fractionated with equal volumes of chloroform, diethyl ether, ethyl acetate and n-butanol (50 ml, three times). These fractions were dried over anhydrous sodium sulfate, filtered and concentrated to dryness under vacuum using rotary evaporator. The yields of these fractions were 100 mg, 260 mg, 201 mg and 811 mg respectively. The fractions were then redissolved in methanol at a concentration of 10 mg/ml. All extracts obtained were kept in the dark at +4°C prior to use.

#### Phytochemical screening

The dried aerial parts of *L. antineae* were subjected to preliminary phytochemical screening to identify the various active chemical constituents present in this species according to standard phytochemical methods as described by Paris & Moysé<sup>10</sup>.

#### Total phenolics content

The Total phenolic content of extracts was determined spectrophotometrically, using the Folin–Ciocalteu assay<sup>11</sup>. Briefly, an aqueous aliquot (0.25 ml) of the extract was added to 3.75 ml of distilled water in a test tube, followed by 0.25 ml of Folin-Ciocalteu's reagent. After 3 min, 0.75 ml of 20% sodium carbonate was added. Tube contents were vortexed and heated at 40°C for 40 min. the blue coloration was read at 760 nm.

#### Total flavonoid contents

Flavonoid contents in the extracts were determined by a colorimetric method described by Lamairson & Carnet<sup>12</sup>. 1.5 ml of 2%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  dissolved in methanol was added to equal volumes of the diluted extract. The mixture was shaken and the absorbance was read at 440 nm after 10 min incubation at room temperature.

#### Determination of antioxidant activity

##### DPPH Radical Scavenging Activity Assay

The method of Braca et al.<sup>13</sup> was used for the determination of the scavenging activity of DPPH free radical. Different methanolic dilutions of extracts were mixed with equal volumes of DPPH methanol solution (0.004% w/v). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated in following way:

$$\% \text{ inhibition} = [(A_C - A_S) / A_C] \times 100$$

Where  $A_C$  is the absorbance of control reaction (containing the equal volumes of DPPH solution and methanol without any sample), and  $A_S$  is the absorbance of the sample (plant extracts and standards).

##### Reducing power assay

The reducing power assay was determined by the method of Oyaizu<sup>14</sup>. Different concentrations of plant extracts in 1ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2M, pH6.6) and potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then; 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and  $\text{FeCl}_3$  (0.5 ml, 0.1%). The absorbance was measured at 700 nm.  $\text{IC}_{50}$  value ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) is the effective concentration at which the absorbance was 0.5 for reducing power.

##### -carotene/linoleic acid bleaching assay

This test was carried out according to a described procedure<sup>15</sup>. 4.8 ml of the obtained emulsion were added to 0.2 ml of extract (2 mg/ml). The absorbance of this mixture was measured at 470 nm every 30 min for 120 min. The bleaching rate (R) of -carotene was calculated according to first-order kinetics, as described in Al-Saikhan et al.<sup>16</sup>:

$$R = \ln (A_{t=0} / A_{t=t}) / t$$

Where,  $\ln$  = natural log,  $t$  is the time in minutes,  $A_{t=0}$  is the initial absorbance of the emulsion immediately after sample preparation ( $t = 0$  min) and  $A_{t=t}$  is the absorbance at time  $t$  (30, 60, 90, and 120 min). The percent of antioxidant activity (AA) was calculated using the equation:

$$AA = (R_{\text{control}} - R_{\text{sample}}) / R_{\text{control}} \times 100$$

Where,  $R_{\text{control}}$  and  $R_{\text{sample}}$  are average bleaching rates of the control (without any sample), plant extract and standards, respectively.

##### Determination of cytotoxic activity

Cytotoxicity of the plant extract was determined by brine shrimp lethality bioassay described by Turker & Camper<sup>17</sup>. Brine Shrimp eggs are hatched to get nauplii. Ten nauplii are taken in tubes containing 2.5 ml of plant extract at different concentration 10000, 1000, 100, 10, 1  $\mu\text{g}/\text{ml}$ . Survivors are counted after 24 hours. The median lethal concentration,  $\text{LC}_{50}$  value of the plant extract was determined.

##### Statistical Analysis

All experiments were carried out in triplicate. Data were expressed as means  $\pm$  S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by a Student's test. Differences were considered significant at  $p < 0.05$ . The correlations between methods were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor.  $\text{LC}_{50}$  value was obtained by a plot of percentage of dead shrimps against the logarithm of the sample concentration using Microsoft Excel.

**RESULTS AND DISCUSSION**

**Phytochemical screening**

Phytochemical constituents in the plants are usually known to be biologically active compounds and responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer<sup>18</sup>. In this study, the preliminary phytochemical screening of aerial parts of *L. antineae* had shown the presence of triterpenes, saponins, flavonoids, tannins, *O*-heterosides and *C*-heterosides. Alkaloids, coumarins and anthraquinones, were not detected; the summary of the results are presented in Table1.

**Table 1: Phytochemicals detected in dried aerial parts of *L.***

Phytochemicals	Results
Anthraquinones	-
Alkaloids	-
Triterpenes	+
Saponins	+
Coumarins	-
Flavonoids	+
Tannins	+
<i>C</i> -heterosides	+
<i>O</i> -heterosides	+

**Total phenolic and flavonoid contents**

Plant phenols are of interest because they are an important group of natural antioxidants and some of them are potent antimicrobial compounds.<sup>18</sup> Therefore, it seems interesting to determine the total amount of phenols and flavonoids in the plant extracts chosen for the study. The total phenolic contents of hydromethanolic extract and its fractions were shown in Table 2. From the results, it appears that the total phenolic contents of *L. antineae* extracts were high and varied significantly. The highest content of phenolics was found in ethyl acetate and diethyl ether fractions (262.35 and 259.45 mg GAE/g, respectively), indicating that phenolic compounds in *L. antinaea* were mainly soluble in ethyl acetate and diethyl ether. The flavonoid contents of *L. antineae* extracts are presented in Table 2. Hydromethanolic crude extract showed the highest flavonoid content, while chloroform fraction showed the lowest one with 4.03 and 1.35 mg QE/g extract, respectively.

**Table 2: Total phenolic and flavonoid contents (mean ± SD) of extracts from *L. antineae***

Extracts	Total phenolic contents <sup>1</sup>	Flavonoids contents <sup>2</sup>
hydromethanolic crude	22.25±2.43	4.03±0.03
chloroform	12.49±1.28	1.35±0.01
diethylether	259.45±12.46	1.73±0.01
ethyl acetate	262.35±6.04	2.51±0.02
n-butanol	16.49±3.66	1.96±0.02

<sup>1</sup>Total phenolic contents was expressed as mg gallic acid equivalents/g dried extract

<sup>2</sup>Total flavonoid contents was expressed as mg quercetin equivalents/g dried extract

**Antioxidant activity**

The antioxidant activity of plant parts is mainly contributed by the active compounds present in them. In this study, the antioxidant activity of *L. antineae* was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power and -carotene–linoleic acid assays.

**DPPH assay**

DPPH is used as a free radical to evaluate antioxidant activity of plant extracts, and the degree of its discoloration is attributed to the hydrogen donating ability of these products, which is indicative of their scavenging potential. As shown in Figure 1, the DPPH radical scavenging activity was increased by increasing the concentration of the samples. IC<sub>50</sub> values were found to be in the following order: ascorbic acid < ethyl acetate = diethyl ether < -tocopherol < n-butanol < hydromethanolic crude < BHT < chloroform [Table 3]. When compared to the standards BHT and -tocopherol, the ethyl acetate and diethyl ether fractions showed significantly (P < 0.05) higher activity. This result suggests that phenolic compounds present in ethyl acetate and diethyl ether fractions play an important role for radical scavenging effects of *L. antinaea*. However, the activity of the extracts exhibited a weak correlation with their phenolic contents. The correlation coefficient (R<sup>2</sup>) was equal to 0.44. Some authors demonstrated that antiradical activity was not solely dependent on phenolic content but it may be due to other phytoconstituents as tannins, triterpenoids or combined effects of them<sup>19,20</sup>.

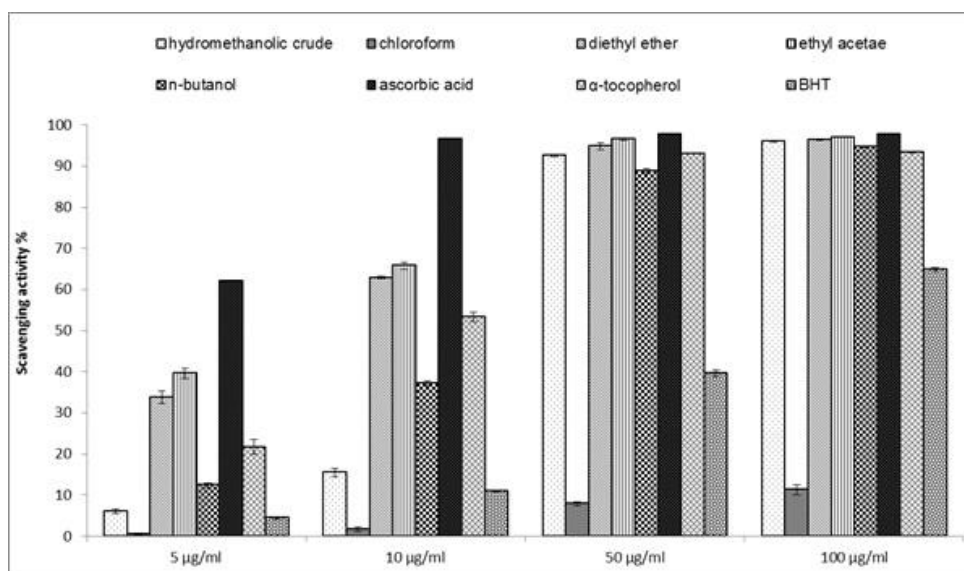
**Table 3: Antioxidant activities of extracts from *L. antineae* and standards measured by different assays**

Plant Extracts	DPPH <sup>b</sup>	Reducing power <sup>c</sup>	-Carotene/linoleic acid(%)
hydromethanolic extract	23.16±0.28 <sup>a</sup>	201±1.15	20.02±1.83
chloroforme fraction	663.33±10.26	2296±5.7	24.35±1.24
diethyl ether fraction	7.78±0.1	120±1.15	69.88±0.68
ethyl acetate fraction	7.10±0.2	73±1.00	64.13±1.17
n-butanol fraction	16.2±0.6	370±6.00	45.08±0.69
ascorbic acid	04.00±0.1	47±0.28	11.05±1.43
-tocopherol	09.55±0.07	507±4.16	94.95±0.94
BHT	72.16±0.1	633±11.5	96.92±0.51

<sup>a</sup> Each value is presented as mean ± standard deviation (n = 3)

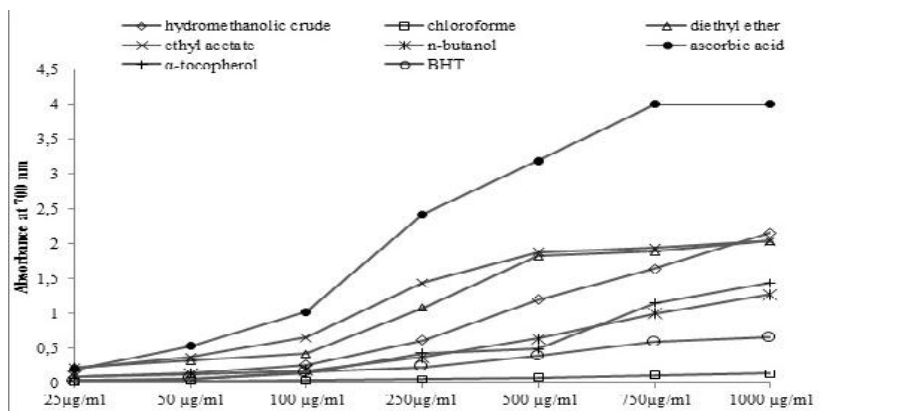
<sup>b</sup> IC<sub>50</sub> in µg/ml

<sup>c</sup> Concentration at which the absorbance was 0.5



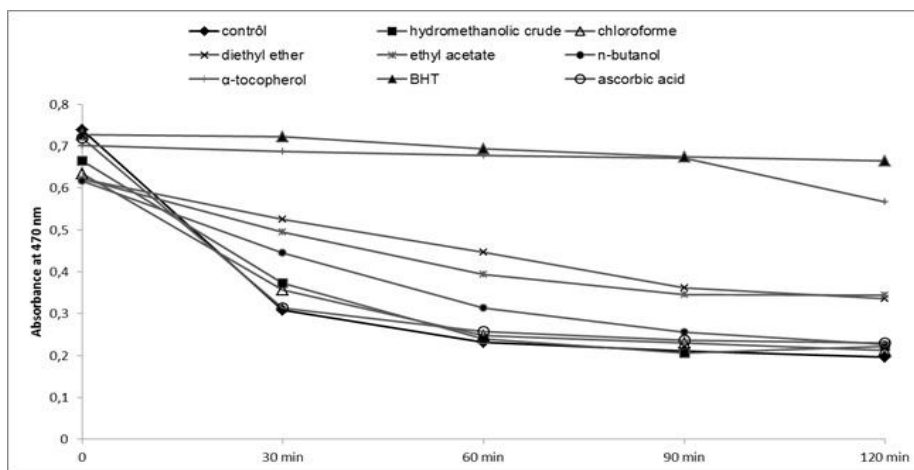
**Fig.1: The DPPH radical scavenging activities of ascorbic acid, -tocopherol, BHT, crude extract and its fractions of *L. antineae*. Reducing power**

In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe<sup>+3</sup> to Fe<sup>+2</sup> by donating an electron. The amount of Fe<sup>+2</sup> complex can then be monitored by measuring the formation of Perl's blue at 700 nm<sup>21</sup>. The results showed in the Figure 2 revealed that the reducing power of samples increase with an increase in samples concentration (high absorbance at 700 nm corresponds to high reducing power). According to Table 3, ethyl acetate fraction exhibited stronger reductive potential than hydromethanolic crude or other fractions and these differences were found statistically significant (p<0.05). A moderate correlation between the total phenolic content and the reducing power was also observed (R<sup>2</sup> = 0.51). Our results are in accordance with the previous published data showing the high reductive capability of *Lavandula* species extracts<sup>2,22</sup>.



**Fig.2: Reducing power of various concentrations of hydromethanolic crude extract and its fractions from *L. antineae*. -carotene/linoleic acid bleaching assay**

In the -carotene bleaching assay, oxidation of linoleic acid produces hydroperoxides which attack -carotene molecules and cause a rapid discoloration of the solution, whereas antioxidants prevent -carotene bleaching<sup>23</sup>. As shown in Figure 3, all of investigated samples significantly inhibited bleaching of -carotene in comparison with control. The percentages of antioxidant activities of the hydromethanolic crude and its fractions are given in Table 3. The results showed that diethyl ether fraction exhibited significantly higher antioxidant activity (69.88%) than other fractions followed by ethyl acetate fraction (64.13%). None of extracts was found to be as active as positive controls BHT or -tocopherol. Acid ascorbic, well known as polar antioxidant, remains in aqueous phase and is consequently less efficient in protecting linoleic acid (11.05%). This fact has been previously reported by other investigators<sup>24</sup>. A stronger correlation between the total phenolic content and the antioxidant activity was observed for the -carotene-linoleic acid assay ( $R^2=0.91$ ), which indicates that phenolics significantly contributed to the reduction of the radicals generated by the oxidation of linoleic acid.



**Fig.3: Inhibition of bleaching of -carotene-linoleic acid emulsion by the standards, crude extract and its fractions from *L. antineae*.**

**Cytotoxic activity**

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties<sup>25</sup>. Based on the results, the hydromethanolic extract of *Lavandula antineae* has showed good toxic against brine shrimp nauplii, with  $LC_{50}$  of 13.72 µg/ml. In addition, the degree of lethality was found to be directly proportional to the concentration of the extract (Fig.4). The observed lethality of this plant extract to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components. According to Meyer et al.<sup>25</sup>, a crude plant extract is considered as toxic (active) if it has an  $LC_{50}$  value of less than 1000 µg/ml.

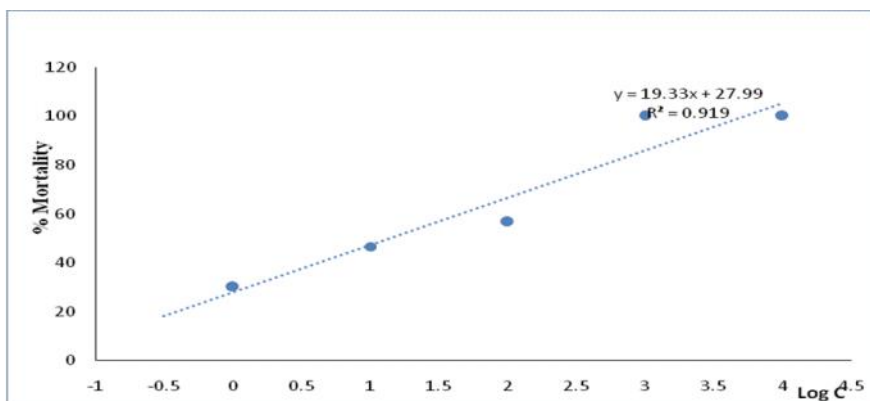


Fig 4: Determination of LC<sub>50</sub> of hydromethanolic extract of *Lavandula antineae* against brine shrimp nauplii.

### CONCLUSION

The present study demonstrated that hydromethanolic crude extract and its fractions from aerial parts of *L. antineae* are different in their antioxidant effects. The results indicate that *L. antineae* possess the potent antioxidant and cytotoxic activities and hence can be a potential natural source in health and medicine. The phytochemical screening revealed chemical constituents that form the foundation of their biological activities observed. The precise composition and chemical characterization of active compounds in *L. antineae* need to be explored further.

### CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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