



Extract and Fixed Oil of Moroccan *Lavandula dentata* Aerial Parts as Potential Antioxidants

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ABSTRACT

Aromatic and medicinal plants constitute a vast source of bioactive compounds, with remarkable chemical diversity. The aim of the present study is to investigate the antioxidant activity of extracts and fixed oils of *Lavandula dentata* L. aerial parts. Dried powdered aerial parts of *Lavandula dentata* was extracted with methanol by refluxing. Different solvent fractions of aqueous methanol extract of *L. dentata* aerial part were prepared by successive extraction in chloroform, ethyl acetate, and n-butanol in order of increasing polarity. Fixed oil of *L. dentata* aerial parts was obtained by maceration in chloroform at room temperature. Total phenolic and flavonoid contents of the crude methanol extract was determined using standard methods. The antioxidant activity of the crude methanol extract, fractions, and fixed oil was evaluated using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay. Total polyphenolic and flavonoid contents determination revealed that *L. dentata* crude methanol extract have high contents of polyphenols (27.43 mg GAE/g), and flavonoids (20.62 mg QE/g). The ethyl acetate fraction of *L. dentata* exhibited the highest antioxidant activity with IC₅₀ value of 16.41 ± 0.11 µg/mL as measured by the DPPH radical scavenging assay. The n-butanol extract also demonstrated notable antioxidant activity with IC₅₀ value of 42.32 ± 0.86 µg/mL. The fixed oil extracted from *L. dentata* aerial parts demonstrated mild antioxidant activity, with IC₅₀ of 414.2 mg/mL. The findings from this study have shown that *Lavandula dentata* aerial parts contains bioactive compounds with antioxidant potentials.

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Keywords: *Lavandula dentata*, Polyphenols, Flavonoids, Antioxidant, Fixed oil.

Introduction

For several centuries, humans have utilized diverse plants from their surroundings to address their basic needs, and combat a wide array of illnesses. These plants embody an extensive repository of bioactive compounds, credited to secondary metabolites with a wide array of chemical structures, offering a remarkable health benefits.¹ These bioactive compounds like flavonoids and polyphenols exhibit antioxidant properties by effectively scavenging free radicals within the body, thereby mitigating the detrimental consequences of oxidative stress.² Morocco in particular, by virtue of its biogeographical position, is home to a wide diversity of flora and a population with a long history of traditional medical practices.³

As part of the panoply of Moroccan aromatic and medicinal plants, lavender (*Lavandula dentata*) is widely used in Moroccan traditional medicine as sedative, anti-inflammatory, antioxidant, antidepressant, antibacterial, and antifungal agent.^{2,4,5}

Despite the wealth of aromatic and medicinal plants in Morocco, as well as the increasing interest in their use, there has been little effort devoted to the development of therapeutic agents from these plants, the evaluation of which remains a very interesting task and is the subject of many detailed studies on plant resources.⁶ Although, studies have been conducted on the biological properties, notably the antioxidant potential, of various lavender species worldwide, reports on the medicinal properties of fractionated extracts and fixed oils of the Moroccan *Lavandula dentata* species and their chemical constituents are limited.

On the basis of the above, there is the need for the exploration of the chemical composition and the biological properties of *Lavandula dentata* (Lamiaceae), a well-known, commonly used, and highly potent plants in traditional medicine within the Fez region of Morocco.⁴ The aim of the present study is to provide scientific justification for the medicinal use of this plant by assessing the antioxidant properties of its extracts (crude methanol extract and the various flavonoid fractions) and its fixed oils.

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Materials and Methods

Chemicals

Methanol, sodium carbonate, aluminum trichloride, chloroform, hexane, chloroform, ethyl acetate, n-butanol, folin-ciocalteu, 2,2-diphenylpicrylhydrazyl (DPPH), gallic acid, quercetin and ascorbic acid were purchased from Sigma Aldrich (Munich, Germany). All chemical solutions used in this study were of analytical grade.

Collection and identification of plant materials

The aerial parts of *Lavandula dentata* were collected in Imouzzar Kandar City (33°44' N, 5°01' W at 1300 m altitude), Morocco in May, 2020. The plant material was identified by a botanist and a voucher number DL78/24811 was issued. The plant material was dried in the shade for 15 days.

Preparation of crude methanol extract

One gram (1 g) of the plant powder was dissolved in 90 mL of methanol. After stirring for 10 min, the mixture was heated under reflux for 50 min.⁷ The solution was then filtered through a filter paper under vacuum. The filtrate was made up to 100 mL with methanol and concentrated under vacuum using a Hei-VAP Precision ML G3 rotary evaporator (Heidolph, Germany) at 40°C. The concentrated extract was stored in the freezer until ready for use.

Preparation of fractions

The crushed dried plant material (5 g) was extracted in a methanol/water mixture (85/15 v/v) for 48 h with constant stirring, and solvent replacement after 24 h. The macerates were combined and filtered on filter paper. The filtrate was evaporated to dryness in a rotary evaporator to obtain the crude extract. The crude extract was dissolved in 50 mL distilled water, and allowed to stand overnight. The clear layer was recovered and washed three times with hexane to delipidate extracts and remove impurities. The defatted extract was successively extracted with chloroform, ethyl acetate and n-butanol. For each solvent used, a 30 min contact time was allowed. The organic phases obtained were evaporated in a rotary evaporator, and recovered in 10 mL distilled water, then stored at 4°C.

The yield of each fraction was calculated using the following formula:

$$\text{Yield (\%)} = \frac{M_r}{M_p} \times 100 \quad \text{-----} \quad (1)$$

Where; M_r is the mass (in g) of residue after evaporation of solvent. M_p is the mass (in g) of plant material.

Extraction of fixed oils

A 20 g quantity of *L. dentata* powder was macerated in 100 mL of chloroform with constant stirring for one hour at room temperature. Subsequently, the mixture was filtered, and the resulting filtrate was evaporated in an oven (Heratherm OGS60, Thermo Fisher Scientific, USA) at 40°C to obtain the fixed oil.⁸ The yield of the fixed oil was determined using the following formula:

$$\text{Yield (mL/100g)} = \frac{V}{M_d} \times 100 \quad \text{-----} \quad (2)$$

Where; M_d is the mass (in g) of dry plant. V is the volume of oil recovered (in mL).

Determination of polyphenol content

The polyphenol content was determined following the procedure previously described by El Abdali *et al.* (2021).⁸ Briefly, 0.5 mL of the diluted extract was combined with 3 mL of distilled water and 0.5 mL of 20% Na_2CO_3 . The mixture was thoroughly vortexed, after 3 min, 0.5 mL of Folin-Ciocalteu reagent was added. The reaction mixture was incubated in an oven (Heratherm OGS60, Thermo Fisher Scientific, USA) at 40°C for 30 min, and the absorbance was measured at 760 nm using a UV-Visible spectrophotometer (Selecta, E.U). The total polyphenol content was expressed as milligrams equivalent of gallic acid per gram of dry matter (mg GAE/g DM), using a gallic acid standard curve prepared under the same conditions.

Determination of flavonoid content

The flavonoid content was determined using the aluminum trichloride (AlCl_3) colorimetric method.⁸ Briefly, 2 mL of the methanol extract

was combined with 2 mL of a methanol solution of AlCl_3 (20 g/L). Following a 15-minute incubation period, the absorbance was measured at 425 nm against a blank containing methanol in place of the extract. The total flavonoid content was expressed in milligrams of quercetin equivalent (as standard solution) per gram of dry matter (mg QE/g DM).

Evaluation of antioxidant activity

The antioxidant activity of lavender extract, fractions, and fixed oils was determined using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay according to the method previously described by El Abdali *et al.* (2021).⁸ A volume of 2.5 mL of the plant extracts and fixed oil (with concentrations ranging from 0 to 400 $\mu\text{g/mL}$) was combined with 2.5 mL of a DPPH solution (100 μM) prepared using methanol. The resulting mixture was immediately stirred and then left in the dark for 30 min at room temperature (25°C). Subsequently, the absorbance of the reaction mixture was measured at 517 nm against a blank comprising solely of methanol. The control tube consisted of 2.5 mL DPPH mixed with 2.5 mL methanol. A parallel assay was conducted using ascorbic acid as a reference antioxidant. The results were expressed as the percentage of inhibition calculated based on the reduction in the colour intensity of the mixture according to the following equation:

$$\text{Inhibition (\%)} = \left(1 - \left(\frac{Abs_{test}}{Abs_{control}} \right) \right) \times 100 \quad \text{-----} \quad (3)$$

Where;

$Abs_{control}$ = Absorbance of the control at 517 nm.

Abs_{test} = Absorbance of the extract or fixed oil at 517 nm.

The concentration corresponding to 50% inhibition of the DPPH free radical (IC_{50}) was determined from the kinetics of the radical scavenging activity.

Statistical analysis

Data (means and standard deviation) of the various assays were subjected to statistical analysis using "SYSTAT 12" software. Differences between means were analyzed using one way analysis of variance (ANOVA).

Results and Discussion

Percentage yields of different solvent fractions of *Lavandula dentata* aerial parts

Chloroform was employed to extract an organic phase encompassing aglycone and methoxylated aglycone flavonoids. n-butanol was used for the extraction of di-*O*-glycoside, tri-glycoside, and C-glycoside flavonoids. Ethyl acetate was used for the extraction of aglycone flavonoids or flavonoids mono-*O*-glycosides and partially di-*O*-glycosides.¹⁵ Considering the specificity of each solvent used in fractionation, the ethyl acetate and n-butanol fractions containing flavonoid mono-, di-, and tri-glycosides constituted the predominant flavonoid classes gave a significantly higher yield compared to the chloroform fraction (Table 1).

Table 1: Percentage yields of different solvent fractions of *Lavandula dentata* aerial parts

Fraction	Percentage yield (%)
Chloroform	3.20 ± 0.02 ^b
Ethyl acetate	8.00 ± 0.10 ^a
n-Butanol	8.20 ± 0.06 ^a

Values are Mean ± Standard Error of Mean (SEM). Values with different letters are significantly different ($p < 0.05$).

Polyphenol and flavonoid contents of methanol extract of *Lavandula dentata* aerial parts

The polyphenol and flavonoid contents of the crude methanol extract of *L. dentata* are presented in Table 2. Lavender extract contained 27.43 mg GAE/g of polyphenols. Bettaieb *et al.* (2017) observed a higher total polyphenol content in Tunisian *L. dentata* roots (42.57 mg GAE/g) compared to the leaves (39.58 mg GAE/g) and stems (16.17

mg GAE/g).¹⁰ Similarly, an aqueous extract of Moroccan *L. dentata*, as tested by Bachiri *et al.* (2016), exhibited a high total polyphenol content (184.02 mg GAE/g).¹¹ Furthermore, the results of the present study revealed a flavonoid content of 20.62 mg QE/g in *L. dentata* extract. In a similar study, Bachiri *et al.* (2016) reported a high flavonoid content in lavender extract.¹¹ In another study, different flavonoid contents have been found in various parts of *L. dentata*, including the roots (30.06 mg CE/g), the leaves (17.36 mg CE/g), and the stem (10.74 mg CE/g).¹⁰

Polyphenols have attracted the attention of the public and scientific communities due to their diverse biological activities. However, the phenolic composition of a plant depends on various intrinsic (genetic) and extrinsic factors including geographical origin, climatic conditions, cultivation practices, harvest maturity, and storage conditions.¹²

Table 2: Polyphenolic and flavonoid contents of crude methanol extract of *Lavandula dentata* aerial parts

Secondary metabolite	Content
Flavonoids (mg QE/g)	20.62 ± 0.84
Polyphenols (mg GAE/g)	27.43 ± 0.68

Values are Mean ± Standard Error of Mean (SEM).

Antioxidant activity of crude methanol extract of *Lavandula dentata* aerial parts

Antioxidant activity evaluation fall into two main categories: radical scavenging assay and lipid oxidation inhibition assay. Among these, the stable free radical scavenging model is extensively employed to evaluate antioxidant proprieties due to its relatively shorter duration and high reliability, compared to alternative assays.⁸ Several tests are used to measure the antioxidant activity of volatile extracts from aromatic plants. In the present investigation, the free radical scavenging activity of *L. dentata* extracts and fixed oil was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

The results of the free radical scavenging assay, illustrated in Figure 1, revealed that the lavender extract showed a strong free radical scavenging effect depending on the concentration. To better discuss this finding, the concentrations of the test samples necessary to inhibit 50% of the DPPH (IC₅₀) were calculated and presented in Table 3. Considering that the IC₅₀ is inversely proportional to the antiradical potential, the values obtained corroborated the outcomes of the antioxidant activity. Ascorbic acid emerged as an excellent free radical scavenger with IC₅₀ value of 3.12 ± 0.14 µg/mL. The *L. dentata* methanol extract also exhibited a good free-radical scavenging effect, displaying a low inhibitory concentration (IC₅₀ = 233.10 ± 5.23 µg/mL). Similar work on antioxidant activity of lavender showed a significantly high antioxidant propriety with IC₅₀ values ranging between 50.36 µg/mL and 200.80 µg/mL depending on the organ tested.¹⁰

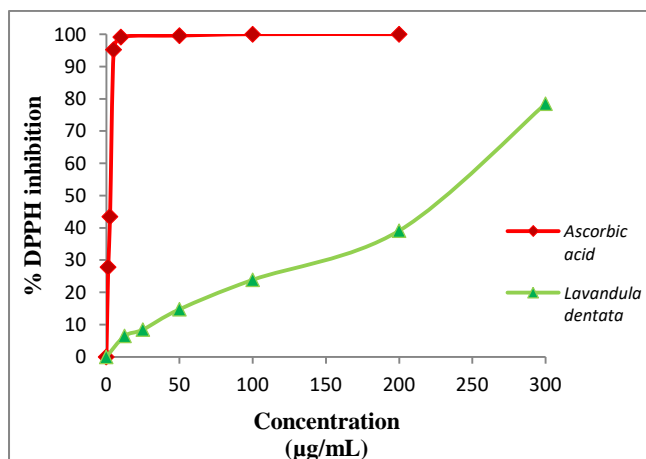


Figure 1: DPPH radical scavenging activity of ascorbic acid and methanol extract of *Lavandula dentata* aerial parts

Indeed, the antioxidant activity obtained in the plants studied is probably explained by their high content of free radical scavenging compounds, particularly polyphenols, which are present in *L. dentata*. In general, the free radical scavenging effect of plant extracts increases with increasing polyphenol concentration. Falleh *et al.* (2008)⁷ reported a highly significant correlation between polyphenol content (total polyphenols and flavonoids) and the free radical scavenging activity against DPPH radicals of the methanol extract of *Cynara cardunculus*, a plant of the Asteraceae family.⁷ Additionally, it is important to note that the antioxidant effect of an extract may vary depending on the quality of the polyphenols present, such as flavonoids, which have demonstrated their own distinct antioxidant activity.¹³ Furthermore, recent studies indicated that the enzyme-mediated hydrolysis of food proteins generates certain specific peptides possessing antioxidant properties. Notably, this phenomenon has been observed in proteins present in oilseeds.¹⁴

Table 3: Antioxidant activity of ascorbic acid, methanol extract, fractions, and fixed oil of *Lavandula dentata* aerial parts

Sample	Antioxidant activity (IC ₅₀ in µg/mL)
Ascorbic acid	3.12 ± 0.14 ^a
MELD	233.10 ± 5.23 ^d
CFLD	2050.12 ± 10.33 ^e
EFLD	16.41 ± 0.11 ^b
BFLD	42.32 ± 0.86 ^c
Fixed oil	414.20

Values are Mean ± Standard Error of Mean (SEM). Values with different letters are significantly different ($p < 0.05$). MELD = Methanol extract of *Lavandula dentata*, CFLD = Chloroform fraction of *Lavandula dentata*, EFLD = Ethyl acetate fraction of *Lavandula dentata*, BFLD = n-Butanol fraction of *Lavandula dentata*.

Antioxidant activity of different solvent fractions of *Lavandula dentata* aerial parts

The different fractions obtained through the liquid-liquid extraction of *Lavandula dentata* were subjected to the DPPH radical scavenging test. As illustrated in Figure 2, among the fractions, ethyl acetate exhibited the highest antioxidant activity for *L. dentata*, followed by n-butanol and, lastly, chloroform. The antioxidant potential of each fraction increased with increasing concentration. These findings were corroborated by the IC₅₀ values. As indicated in Table 3, the ethyl acetate fraction of *L. dentata* exhibited the lowest IC₅₀ value of 16.41 µg/mL. In contrast, the chloroform fraction demonstrated a notably higher IC₅₀ value of 2050.12 ± 10.33 µg/mL.

On the other hand, the phenolic fraction does not incorporate all antioxidants, and the synergistic interactions between antioxidants in a mixture mean that antioxidant activity is reliant not only on concentration but also on the composition and nature of the antioxidants.⁷ This aspect should be taken into consideration in assessing the biological activity.

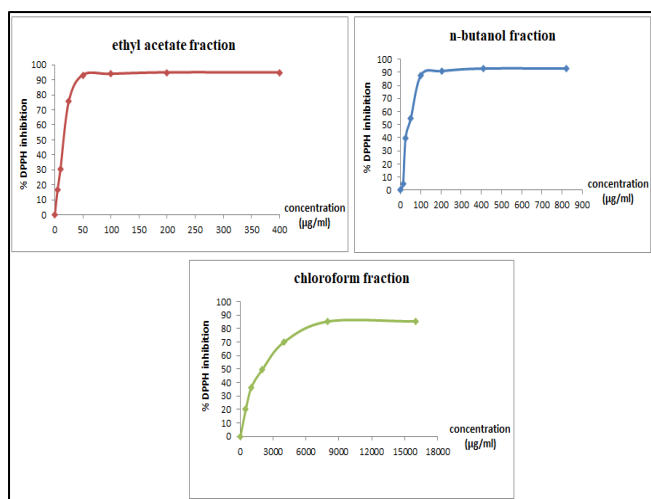


Figure 2: DPPH radical scavenging activity of different solvent fractions of *Lavandula dentata* aerial parts

Antioxidant activity of *Lavandula dentata* fixed oils

The fixed oils extracted from lavender, with a yield of 8%, were assessed for their ability to scavenge the DPPH free radical. According to the results as shown in Figure 3, the fixed oils of *L. dentata* exhibited a dose-dependent free radical scavenging activity. This antioxidant effect was reflected by an IC_{50} value of 414.20 $\mu\text{g/mL}$ (Table 3). Using the same test, a recent study revealed that the fixed oils of linseed displayed an antioxidant effect, with a low IC_{50} value of 39.70 mg/mL .⁸ Similarly, an investigation conducted on the fixed oil of *Pistacia lentiscus* demonstrated strong antioxidant activity, with an IC_{50} value of 20.61 $\mu\text{g/mL}$ in the DPPH scavenging test.¹⁶ The low antioxidant activity of fixed oils can be attributed to their rapid oxidation post-extraction, resulting in the loss of their protective antioxidants, primarily hydrophilic compounds that do not coexist with the extracted oils.¹⁷

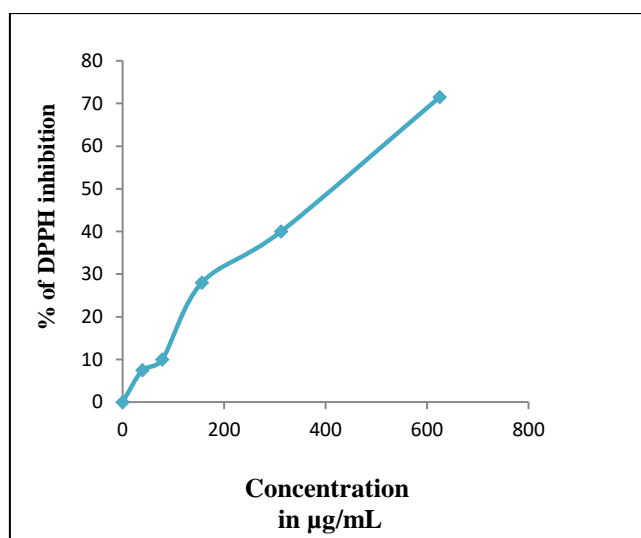


Figure 3: DPPH radical scavenging activity of fixed oil of *Lavandula dentata* aerial parts

Conclusion

Lavandula dentata extracts have demonstrated significant antioxidant activity attributed to the presence of polyphenolic compounds. The fractionation of flavonoids, extracted using solvents of varying polarity, highlights the effectiveness of aglycone flavonoids or those containing mono-*O*-glycosides and partial di-*O*-glycosides in terms of their antioxidant activity. Additionally, the fixed oils derived from this plant contain antioxidant agents. This study underscores the

therapeutic benefits of the bioactive compounds and free radical inhibitors in *Lavandula dentata*, confirming its use in Moroccan traditional medicine. Nonetheless, further research is essential to pinpoint and validate the antioxidant capacity of specific bioactive molecules through *in vivo* studies, thereby substantiating their therapeutic importance.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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