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Effect of polar solvent on the efficiency of phenolic compounds and antioxidant potency of the lavender wild growing in Tunisia

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Abstract

A highest polar solubility of phenolic and flavonoids compounds of *Lavandula stoechas* required the use of appropriate solvent during extraction process. Extraction following an increase ingredient of polarity revealed that the chloroform seems as the best solvent able to release polyphenols followed by the ethyl-acetate. Total phenolic and flavonoids extracted with chloroform were respectively, 282.7 mg Eq GAE/g DW and 125.91 mg Eq QE/g DW. Even for the tree other solvents obtained results showed importance richness of the lavender on polyphenols. Amounts were correlated to the highest antioxidant potency approved. Main functional groups were detected and identified by Fourier transform infrared spectrophotometer (FTIR). Results give to the studied species an important alternative as a promising ecological natural source of bioactive antioxidant compounds and to be incorporated in food, pharmaceutical and cosmetics manufactures.

1. INTRODUCTION

Since middle ages, investigations of medicinal plants have an important interest. The challenge was to maintain as possible the human welfare and healthcare instated of the resort to synthetic drugs that can induce harmful effects on long terms. The growing interest was to identify more and more new active substances extracted from aromatic and medicinal plant growing widely in the nature. Several metabolites contribute to the prevention of different human diseases and they are searched for their insecticidal, molluscicidal, virucidal, fungicidal, antiparasitical, bactericidal and antioxidant properties (Wannes et al., 2009). Mostly widespread on the Mediterranean region, the genus Lavandula with about 28 species is an important component of the Lamiaceae family. As one of the largest families, Lamiacea selected as flowering plants comprises 250 genera and about 7000 species (Napoli et al., 2020). A variety of secondary metabolites as polyphenols, coumarins, triterpenes, sterols and tannins have been found in Lavandula plants. Flowers are rich on phenolic compounds as p-hydroxybenzoic acid, gentisic acid, protocatechuic acid, vanillic acid, rosmarinic acid, caffeic acid, ferulic acid, cinnamic acid, 4-O-caffeoylquinic, lavandufurandiol, lavandunat, lavandupyrones A and B, catechin, vanillin... (Costa et al., 2013; Dvorackova et al., 2014; Turgut et al., 2017; Yadikar et al., 2018). The amount of each component is highly related to the geographical origin of the plant and the climatic environment, the genotype and the growing conditions (Bajalan et al., 2016).

In traditional medicine, Lavandula is used in case of depression and diabetes, superficial wounds, burns and headaches (Gilani et al., 2000). Previous researchers reported that it presented sedative, antibacterial, carminative, antifungal and anti-inflammatory effects (Soheili and Salami, 2019). Essential oil is very effective and therefore it is employed during pharmaceutical and cosmetics preparations and it is also implied in food industry, perfumery and hygiene industry (Canlı et al., 2019; Soheili and Salami, 2019). One of the important lavender, *Lavandula stoechas* is advised to treat colic and chest

ailments as well affections of the respiratory ways (Hassiotis, 2010).

In Tunisia, *Lavandula stoechas* was considered as endangered species. Due to the prolonged drought, harsh climate (climatic and edaphic factors) and the expanse of cleared agricultural fields, the plant growth was restricted (Tofah et al., 2023).

However, given the climatic conditions of oasis areas characterized throughout the year by low air humidity, it would be interesting to introduce certain aromatic and medicinal plants like *Lavandula stoechas*. Undemanding water, the species should be the subject of culture experiments. The development of additional plants growing in the same habitat might promote the creation of more diversified agrosystems while saving water reserves and benefiting from interesting extracted compounds. Oasis could be an excellent appropriate habitat where Lavandula will be also protected from overgrazing and uprooting. In this context, the objective of this study was to identify functional groups of *Lavandula stoechas* extract and to assess the potential impact of solvent extraction on polyphenols and antioxidant power.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

Lavandula stoechas was collected in March from Chebba region (35.201053, 11.028245). The coastal town belongs to subarid climate in the Tunisian Sahel (Weather: 10°C, NW wind at 19 km/h, 58% humidity). Fresh plant was air dried in room temperature and in darkness. Then, it was finely ground using an electric blender and stored until required for use.

2.2. Plant extraction

The plant powder was extracted with 80% ethanol at 40°C during 48 hours and with continuous agitation. Extraction was repeated three times. The collected filtrate was evaporated using a rotary evaporator at 40°C. Residue was partitioned following an increased gradient of polarity, with successively hexane, chloroform, ethyl acetate and n-butanol. Extraction was accomplished by methanol with continuous agitation and during 72 h at 37°C to determine the antioxidant activity. Extract was concentrated on a rotary evaporator under vacuum at 40°C (Lahmar et al., 2017).

2.3. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis with a scanned wave ranging from 4000 to 500cm^{-1} was carried out in order to detect the presence of functional groups in the plant extract which was pressed into pellets. Spectra were recorded on a PerkinElmer 1750 spectrophotometer managed with a diamond attenuated total reflection (ATR) device. The consequent peaks were then compared with previously recorded data of functional groups.

2.4. Total phenolic and flavonoid contents

Total phenolic content was measured according to Folin-Ciocalteu method (Singleton et al., 1999). Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight. Flavonoids content was determined using alimimum chloride method (Lamaison and Carnat, 1991). Results were expressed as mg of quercetin equivalents (QE) per g of dry weight.

2.5. Antioxidant activity assay

Three different methods were followed to evaluate the antioxidant power. DPPH (2-2-diphenyl-1-picrylhydrazyl) scavenging activity (Naik et al., 2003), ferric reducing antioxidant potential (Oyaizu, 1986) and ferrous ion chelating activity activity (Zhao et al., 2008). IC50 was determined and defined as the extract concentration able to scavenge 50% of DPPH and to 50% ferrous ion chelating. However for ferric reducing power, EC50 was calculated by plotting 0.5 of absorbance against the corresponding sample concentration. As IC50 and EC50 are low as they reflected better antioxidant activities.

3. RESULTS AND DISCUSSION

3.1. Morphological analysis

Lavandula stoechas was highly aromatic with very nice calming smell. The plant was growing up to 0.5 m and highly branched. Opposite and grey leaves with 0.8-5.7 cm of length are sessile, lanceolate, oblong and tight. Inflorescence is very compact, sessile and topped with beautiful purple and enlarged floral bracts of 0.6-2.2 cm of length. Flowering period is generally extended between March and May.

3.2. FTIR spectrum analysis

Functional groups peaks ratio of the lavender methanolic extract phytocompounds were showed and identified (Fig. 1). Several peak values proved the presence of a multitude of compounds. Wavenumber between 3000 and 3300 cm^{-1} is due to the hydroxyl group of

alcohols vibrations. Peak at 1650-1760 cm^{-1} indicated the presence of C=O of ester, COOH group and Phenol. Stretching vibrations of C-C, C-O, C-O-C and C-O-P of saccharides were correlated to the wavenumber 1050-1200 cm^{-1}. Lavandufluoren, lavandupyrone A, lavandupyrone B, rosamanic acid, vanilin, caffeic cid, lavandufrandiol, lavandunat are considered among the principal compounds of the studied species.

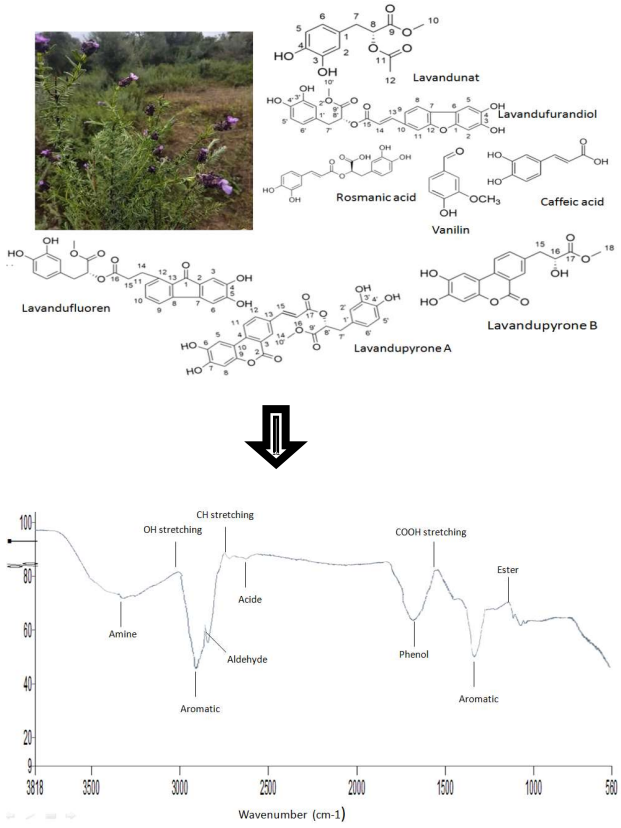

Fig 1. FTIR spectrum of *Lavandula stoechas* extract

3.3. Polyphenols content

During the processing of total phenolic compounds extraction it is very interest to select the best polarity of solvent. Total phenolic extracted with chloroform was the highest with 282.7 mg Eq GAE/g DW (Fig. 2). It was also, even for hexane extract, higher than *Lavandula stoechas* extracted with methanol showing 25.2 mg GAE/g DW (Messsaoud et al., 2012). Ceylana reported also a lowest amount with a concentration of 105.5 mg GAE/g DW (Ceylana et al., 2015). The Tunisian species extracted also with hydro-ethanolic revealed an amount of 130.15 mg GAE/g (Ezzoubi et al., 2020). Obtained results about extraction with an increase gradient of polarity indicated that the chloroform medium lead the effective release of phenolic and flavonoids compounds. Observed differences of secondary metabolites synthesis are may be attributed to the edaphoclimatic conditions, biotic and abiotic stresses, plant physiological stage and extraction process.

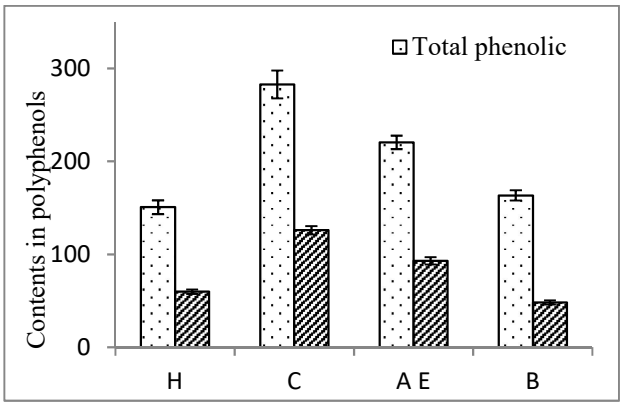

Fig 2. Total phenolic (mg Eq GAE/g DW) and flavonoid (mg Eq QE/g DW) contents of different extracts of *Lavandula stoechas*. H: hexane; C: chloroform; A E: ethyl acetate; B: n-butanol. Total phenolic expressed on mg Eq GAE/g DW; mg of gallic acid equivalents per g of dry weight. Flavonoids expressed on mg Eq QE/g DW; mg of quercetin equivalent (QE) per g of dry weight. The values are the mean of three determinations ± standard error.

Polyphenols of the Tunisian *Pergularia tomentosa* subjected to the same extraction process as *Lavandula stoechas* presented the best concentrations with chloroform and ethyl acetate due to their highest solubility (Lahmar et al., 2017). In fact, some researchers reported that methanol and ethanol were considered as the best polar solvents to extract phenolic and flavonoid contents (Khlif et al., 2015). Phenolic compounds extracted from the Tunisian lavender wild growing could be implied during the food formulation and in medical industries due to their beneficial health effects.

3.4. Antioxidant potency

Phenolic compounds extracted from *Lavandula stoechas* has been tested with several assays of antioxidant activity. Methanolic extract showed a significantly considerable scavenging activity with IC_{50} of 32.5 µg/ml compared to BHT showing IC_{50} of 57.4 µg/ml. Our result was higher to those found by an extraction with ethanol which let to have IC_{50} equal to 55 µg/ml (Sriti et al., 2022). In the same way, Lavandula exhibited a ferric reducing antioxidant potential with EC_{50} of 88.24 µg/ml. Chelating power with

EC_{50} of 2.08 µg/ml was also important to be considered.

However, the extraction method and the protocol followed to determine the antioxidant activity can explain variances. Antioxidant activity is mainly correlated to the presence of phenolic and flavonoids compounds at higher concentrations and specifically to caffeic acid, carvacrol, thymol methyl ether and rutitin (Ceylan et al., 2015). Chemical structure of the present components in the plant extract, as well as their synergistic or antagonistic effect can explain difference on the antioxidant activity. The physiological stage of the plant at the moment of collection has to be examined also, yet extraction during the vegetative stage exhibited a lowest IC50 related to a greater antioxidant activity. In fact, polyphenols synthesis decreased during flowering and fructification (Sriti et al., 2022). A positive correlation between polyphenols concentration and antioxidant potency was also established for other medicinal plants (Lahmar et al., 2017, Lahmar et al., 2022).

4. CONCLUSION

The present study revealed the solvent extraction significantly affected total phenolic and flavonoids content of *Lavandula stoechas* growing in Tunisia. In addition, the variance of the antioxidant activity depending on the reagent during the followed experimental method was showed. The findings proved high amounts on polyphenols. Carried FTIR analysis proved the presence of phenolic compounds mainly of aromatic, phenols and amine structures. A strong positive correlation was established between total phenolic amount and antioxidant power. Extracted compounds were searched for pharmaceutical and industries applications. In addition, the potential of this plant encouraged its implication in arid and semi-arid habitats and principally in oasis area. Lavandula will lead to enrich agricultural products.

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