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Ethnobotanical study of antidiabetic plants used in Souk Ahras, Algeria and assessment of the antioxidant activity of Olea europaea L

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ABSTRACT
The present study aimed to conduct an ethnobiological survey on the use of antidiabetic plants in the Souk-Ahras region of Algeria, while also investigating the antioxidant activity of Oleaeuropaea L. leaves. An ethnobiological survey was conducted using a semi-structured questionnaire directed at diabetic patients, traditional healers, and herbalists. The polyphenol and flavonoid contents of various extractsof Oleaeuropaea L. leaves were determined through spectrophotometric methods, and the antioxidant activity was assessed using the DPPH radical scavenging assay.

A total of 200 participants were surveyed, comprising 52% women and 48% men, with the majority (31%) falling within the age group of 50 to 60 years. Notably, 55% of those utilizing traditional medicine were illiterate. The survey identified 23 species of medicinal plants belonging to 16 different families, with the Lamiaceae family being the most represented (3 species). The most frequently mentioned species included Olea europaea L. (18%), Artemisia herba alba Asso(14.5%), Rosmarinus officinalis L. (10.5%), and Eucalyptus globulus Labill (10%). The leaves were the most commonly used part of the plants (42 %), and infusions were the preferred method of preparation (39%).

Furthermore, the study revealed that the infusion extract of Oleaeuropaea L. exhibited the highest polyphenol content (161.45±0.03 µg GAE/mg) and demonstrated the strongest antioxidant activity, while the decoction extract showed the highest flavonoid content (94.2±0.032 mg QE/mg). These findings underscore the significance of traditional medicinal plants in local healthcare and their potential benefits, particularly in the management of diabetes.

Keywords: Ethnobotanical study, Diabetes, Polyphenols, Flavonoid,Antioxidant activity.

INTRODUCTION

Algeria is characterized by a very rich and highly diversified flora due to its geographical location and diverse climatic and topographic conditions, This biodiversity is not only a testament to the country's natural heritage but also serves as a valuable resource for traditional medicine (Azzi et al., 2012; Berrabah et al., 2019; Boussaid et al., 2018; Makhloufi et al., 2021; Makhloufi et al., 2020).In recent years, ethnobotanical studies have garnered considerable attention for their potential to reveal traditional knowledge regarding medicinal plants and their applications in modernhealthcare. A particularly intriguing area of research is the use of plants in the treatment of diabetes, a chronic condition that affects millions of people worldwide. Recent studies have identified various plant species with antidiabetic properties, largely attributed to their bioactive compounds, which can aid in regulating blood sugar levels and enhancing overall metabolic health(Si Yuanet al., 2013; Idm'hand et al., 2020). Furthermore, many of these plants exhibit antioxidant activity, which is essential for combating oxidative stress linked to diabetes and its complications (Krishnaiah et al., 2011). The methods used to extract these bioactive compounds are crucial in determining their effectiveness. Traditional techniques such as maceration, infusion, and decoction are commonly employed to extract beneficial constituents from plant materials. Maceration involves soaking plant parts in a solvent to facilitate the release of compounds, while infusion and decoction entail boiling or steeping the plant material to extract active ingredients (Ingleet al., 2017).In this context,the present study aimed to investigate the ethnobotanical significance of selected plants used in diabetes treatment, assess their antioxidant activity, and compare the effectiveness of various extraction methods in obtaining bioactive compounds. By integrating traditional knowledge with scientific research, we aspire to contribute to the development of natural therapeutic options for diabetes management.

MATERIALS AND METHODS

Description of the study area

The Souk-Ahras region is situated in northeastern Algeria. The geographic coordinates are 36°17'11"north and 7°57'4" east. It is characterized by two types of climate sub humid in the north and semi-arid in the south.The northern part is located in mountainous part of the Tell Atlas and the southern part is located on the high plains.

Ethnobotanical survey

The ethnobiological survey was carried out from December 2022 to March 2023, utilizing a semi-structured questionnaire directed at diabetic patients, traditional healers, and herbalists.Questionnaires were included personal information, plant local name, plant parts used, method of preparation and traditional use. Questionnaires data were then transferred to Microsoft Excel 2007 and processed.

Plant material and preparation of extract

The plant material used in this study consists of olive leaves (Oleaeuropaea L.). The extraction process was carried out according to the protocol established by Boubakeur (2017), with some minor modifications.

For the maceration extract, 50 grams of the plant material were soaked in 500 mL of distilled water and allowed to macerate at room temperature for three days. The infusion extract was prepared by adding 200 mL of boiling distilled water to 1 gram of the sample, which was then steeped at room temperature for 5 minutes before being filtered. This infusion was subsequently concentrated under reduced pressure. Lastly, the decoction extract was made by boiling 50 grams of powdered leaves in 500 mL of distilled water for 15 minutes. The mixture was then filtered through Whatman filter paper (3 mm) and concentrated under reduced pressure.

Determination of phenol contents

Total phenolic contentwas determined by the Folin–Ciocalteu method, following Singleton & Rossi (1965). To 500 μL of each extract were mixed with 2.5ml of Folin-Ciocalteu reagent and 2mL of 7.5% sodium carbonate solution (Na_2CO_3). The mixtures were then incubated in the dark for 30 min, absorbance was measured at 760 nm . Estimation of the phenolic content was carried out in triplicate and results were expressed as micrograms of Gallic acid per 100 mg of extract (µg AGE/ mg extract).

Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride method (Bahorun et al. (1996). 1 ml of a 2% solution of $AlCl_3$ was added to 1 ml of each extract. The mixtures were then incubated for 10 min, absorbance was measured at 430 nm . Estimation of the flavonoid content was carried out in triplicate and results were expressed as micrograms of Quercetin per milligram of extract (µg Q. Eq/mg extract).

Antioxydant activity

DPPH radical scavenging Assay

The DPPH free radical scavenging abilitymeasurements were carried out according to the method of Meriga et al. (2012). 4 ml of DPPH/methanol (60 μM) solution were added to 1 ml of various dilution of each extract. Mixtures were incubated for 30 min in the dark. the reduction in the DPPH radical was measured at 517 nm. The percentage of scavenging ability of the plant extract was calculated using this equation;

DPPH Scavenging activity (%)=(Ablank - Asample /Ablank) x 100

Where Ablank is the the absorbance of DPPH + methanol; Asample is the absorbance of DPPH mixed with the sample.

Statistical analysis

All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard error (SE). The data were analyzed using descriptive statistics, and IC_{50} values were determined using non-linear regression models in GraphPad Prism 9.0. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Ethnobotanical survey

Sociodemographic characteristics

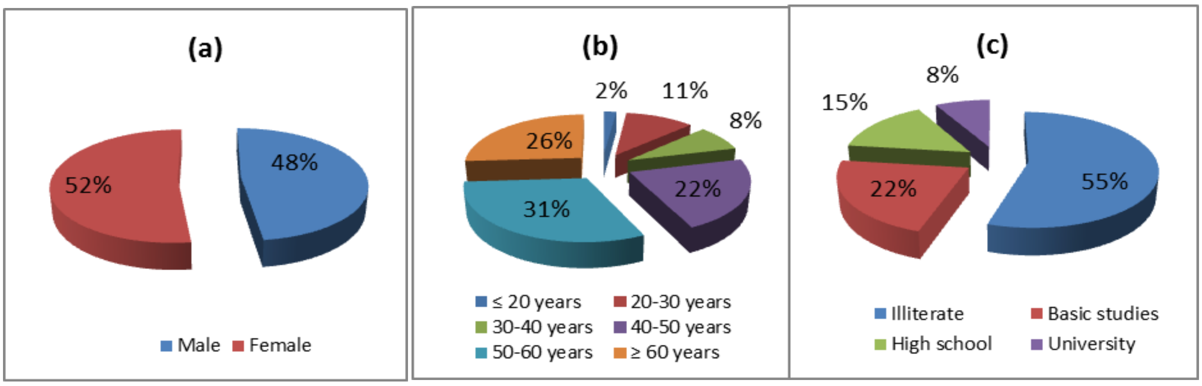

Figure 1. Demographic profile of the informants.(a) Sex,(b) Age and (c) Educational level

The ethnobotanical survey conducted in Souk-Ahras provides valuable insights into the demographic profile and traditional medicinal practices of the local population. With a total of 200 informants participating, the data reveals a balanced gender representation, with women constituting 52% and men 48% of the respondents (Figure 01). This distribution is particularly noteworthy as it highlights the significant role women play in the realm of herbal medicine.Many women reported acquiring their knowledge from maternal figures, traditional healers, and, more recently, through television programs. This transmission of knowledge underscores the pivotal role women play in preserving and disseminating traditional medicinal practices within their communities.

The age demographic indicates that the most represented group is individuals aged 50 to 60 years, making up 31% of the participants. This suggests that the knowledge and practices surrounding herbal medicine are likely rooted in long-standing traditions, passed down through generations. The high percentage of illiteracy among informants, at 55%, aligns with findings from Si Yuan (2013), which suggest that those who are illiterate often possess a wealth of knowledge regarding traditional medicine.

Plant species usedin the treatment of diabetes

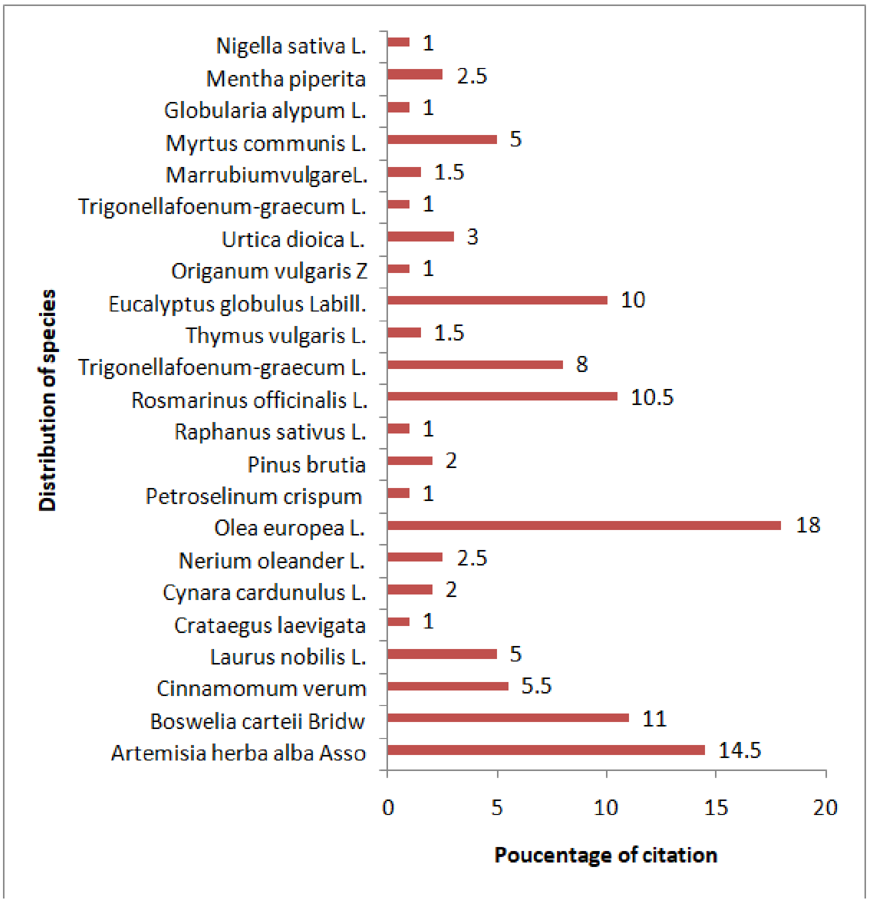

Figure 2. The frequency of citation of medicinal plants used for the treatment of diabetes in Souk Ahras region.

The most cited species in the present survey were Olea europaea L.(18%),Artemisia herba alba Asso(14.5%),Rosmarinus officinalis L.(10.5%) and Eucalyptus globulus Labill (10%) (Figure 2). These results seem consistent with those of other studies conducted in other areas of Algeriaand elsewhere (Aissa et al.,2019;Belmouhoub et al.,2022;Tahraoui et al .,2023) Importantly, Olea europaea L. reported as the most frequently used species to treat diabetes in different regions of Algeria.

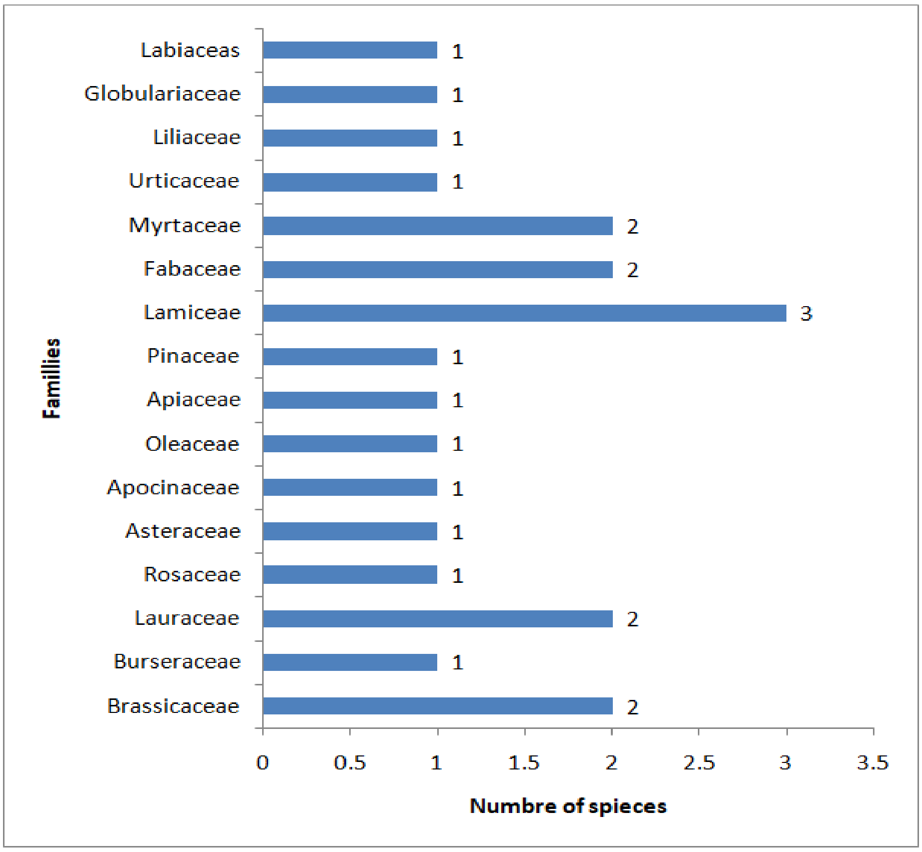

Figure 3. Distribution of species across different families of medicinal plants.

Overall, 23 medicinal plants belonging to 16 botanical families have been documented for the traditional treatment of diabetes in the region of soukahras(Algeria) (Figure.03).The Lamiaceae family is the most represented, with 3 species, followed by the Brassicaceae, Lauraceae,Fabaceae and Myrtaceae families(2 species). The remaining families are represented by a single species. These results are consistent with those reported by Bouzabata(2013), which indicated that the Lamiaceae family is one of the most species-rich families in traditional medicine in the Souk Ahras District.

Plant parts used

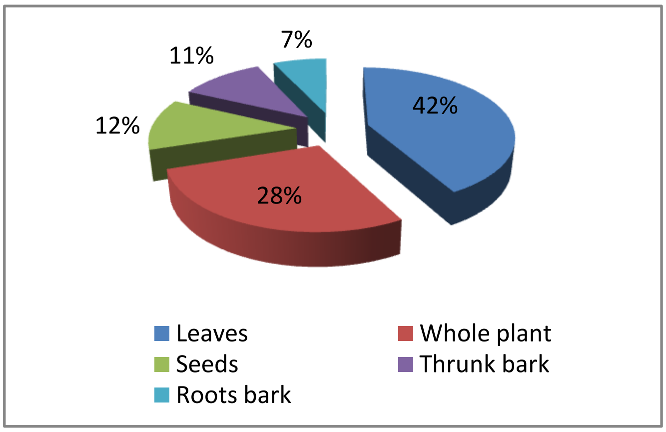

Figure 4. Distribution of plant part used.

The analysis of the plant parts utilized in this study reveals that leaves (42%) and the whole plant (28%) were the most commonly used, as illustrated in Figure 04. In contrast, the use of seeds (12%), trunk bark (11%), and root bark (7%) was less frequent. The quantity and quality of phytochemical compounds can vary significantly depending on the part of the plant used. Numerous ethnobotanical studies have emphasized the frequent use of leaves in herbal remedies (Adnan et al., 2014; Sher et al., 2015). The preference for aerial parts may be attributed to their easy accessibility. Some researchers advocate for the use of leaves due to their rich content of secondary metabolites, suggesting that this practice could promote the sustainable management of medicinal plants (Lumbu,2005; Ramde-Tiendrebeogo,2019).This sustainable approach not only helps preserve plant populations but also ensures that these vital resources remain available for future generations.

Method of preparation and administration

Table 1. Methods of preparation of medicinal plants.

Methods of preparation	**Percentage**	**Number of citation**
Maceration	20%	40
Decoction	26.5%	53
Infusion	39%	78
Powder	14.5%	29

The methods of preparation of the plants used included several modes, mainly infusions (39%) ,decoctions (26.5 %), and maceration (20 %) (Table 01). Patients can use the same plant in the form of an infusion, decoction, or maceration, likely because the active ingredients in these plants are heat-stable. However, if the active constituents of raw medicinal plants (or their parts) are heat-sensitive, using decoction as the extraction method can lead to their decomposition under high temperatures, resulting in a loss of their characteristic properties. Therefore, further studies are necessary to identify the optimal preparation method for effectiveness in treating diabetes (Bouzabata, 2013; Elyebdri et al., 2017; Azzi et al., 2012; Bouzid et al., 2016; Benarba et al., 2015).

Total polyphenols and flavonoids estimation

Table 2. Total polyphenols and total flavonoid content.

Method extracts	**Total polyphenols (µgGAE/mg extracts)**	**Total flavonoids (µgQE/mg extracts)**
Infusion	161.45±0.03	86.77± 0.021
Décoction	144.26±0.04	94.2±0.032
Macération	120.48±0.08	47.44±0.02

The polyphenol content in the extracts ranged from 120.48±0.08µg to 161.45±0.03 µg GAE per mg. In contrast, the flavonoid content varied from 47.44±0.02 µg to 94.2±0.032 µg QE per mg (Table .02). Notably, the infusion extract demonstrated a significant total polyphenol content of 161.45±0.03 µg GAE per mg. Meanwhile, the decoction extract exhibited the highest flavonoid content, reaching 94.2±0.032 µg QE per mg.Several factors contribute to the variability in polyphenol and flavonoid content, including the extraction method, as noted by Lee et al. (2003). The efficiency of extraction can be influenced by the temperature, time, and solvent used, which can alter the solubility and stability of these compounds.

Antioxidant activity

Table 3. IC50 DPPH activity

Method extracts	**IC_{50} (µg/ml)**
Infusion	98.8 ±0.14
Décoction	153.2±0.45
Macération	148.9±0.17

The antioxidant capacity was assessed by measuring the inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The samples extracted using the infusion method exhibited the most potent antioxidant activity, with an IC_{50} value of 98.8 ± 0.14 µg/mL (Table 03). This enhanced activity can be attributed to the infusion method being a gentle extraction technique that helps preserve thermosensitive compounds, which play a crucial role in boosting antioxidant activity.

CONCLUSION

In conclusion, the ethnobotanical study of antidiabetic medicinal plants has provided significant insights into traditional practices for managing diabetes in the study area, highlighting Olea europaea L. as the most commonly utilized species. However, to validate the antidiabetic properties of these plants and to explore their phytochemical profiles more comprehensively, further scientific research is essential. Such investigations will not only enhance our understanding of these traditional remedies but also contribute to the development of effective and natural therapeutic options for diabetes management.

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