



Ethnobotanical knowledge of *Achillea ligustica* All. in El Tarf region (Northeastern Algeria)

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Ethnobotany Research and Applications 29:73 (2024) - <http://dx.doi.org/10.32859/era.29.73.1-16>

Manuscript received: 10/11/2024 - Revised manuscript received: 18/12/2024 - Published: 18/12/2024

Research

Abstract

Background: The genus *Achillea* is one of the largest genera in the Asteraceae family. It is known for its medicinal and therapeutic virtues, which are highly interesting given the pharmacological activities of the phytochemical compounds present in its biological extracts. We therefore focused on Ligurian yarrow *Achillea ligustica* All., a little-known species in north-east Algeria, with the aim of gathering information on its therapeutic uses by the local population.

Methods: The study was carried out in 2023, using individual questionnaires for people living in two rural areas in El-Tarf region (Bougous and Bouhadjar), where the species had only been identified during a floristic survey. The surveys covered socio-demographic data, parts of the plant used, preparation, administration routes and diseases treated.

Results: We carried out a total of 253 surveys. The results indicate that women are much more familiar with and use this medicinal plant than men. The oldest people had the most ethnobotanical knowledge on its uses. The leaves were the most parts of the plant used, while infusion and poultice were the most common methods of preparation adopted. According to the respondents, this plant can be used to treat various types of ailments and diseases.

Conclusions: This preliminary and original ethnobotanical study has shown that *A. ligustica* is one of the valuable wild plants and plays a vital role. The information gathered constitutes an essential database for the discovery of new medicines and for validating ethnomedicinal knowledge.

Keywords: *Achillea ligustica* All., Algeria, Bougous, Bouhadjar, ethnobotanical surveys, population, phytotherapeutic uses.

Background

Throughout the world, plants have always been used as medicines. Herbal medicines are considered to be less toxic and milder than pharmaceutical drugs (Tahri *et al.* 2012). Today, the effectiveness of phytotherapy has been proven and its undeniable benefits for our health have allowed natural medicine to become part of our daily routine. Even the most developed countries have not been left behind (Pastor 2006).

Among the scientific fields interested in traditional herbal medicine, ethnobotany is considered to be a science that translates popular know-how into scientific knowledge. Moreover, the study of local knowledge of medicinal plants through ethnobotanical studies is becoming increasingly important in defining strategies for the conservation and sustainable use of plant resources (Jeruto *et al.* 2008, Da Silva *et al.* 2019).

Some studies carried out in the Mediterranean region have shown that many medicinal species have benefits for human health (Pieroni *et al.* 2006, Gonzalez-Tejero *et al.* 2008, Hadjichambis *et al.* 2008, Jdaidi & Hasnaoui 2016). In Algeria, the use of plants in phytotherapy is ancient and is currently enjoying renewed public interest (Lakhdari *et al.* 2016, Chohra & Ferchichi 2019, Baziz *et al.* 2020, Meddour *et al.* 2020, Adli *et al.* 2021, Belaidi *et al.* 2021, Mechaala *et al.* 2021), such is the case of the wilaya (province) of El Tarf (Boutabia *et al.* 2011, Lazli *et al.* 2019, Beldi *et al.* 2021), which has a fairly high lithological, structural and floristic diversity.

Among these many medicinal plants, we chose Ligurian yarrow *Achillea ligustica* All. (Asteraceae, Anthemideae). This aromatic plant is widespread in the Mediterranean region (Quezel & Santa 1962) and has been used in traditional medicine in several countries (Italy, Corsica (France), Sicily, etc.). Its range extends from Crete and the western Balkans to Italy, Sicily and as far north-west as Africa, where it grows in open forests and beside streams (Quezel & Santa 1962, Conforti *et al.* 2005, Bouteche *et al.* 2024). In these countries, it is used as a remedy to treat haemorrhages, stomach disorders, skin diseases and to relieve sprains and insect bites (Bader *et al.* 2022).

In Algeria, the species is found in the north-eastern region of the country, which is the most wooded and has a semi-humid climate, particularly in Mila, Jijel and El Tarf regions. In these areas, it is known by different names: in the coastal region of Jijel, the plant is known in the local dialect as "Belkisoum" or "Belkismoun", and in the far-eastern region such as El Tarf (on the Algerian-Tunisian border) as "Defret khadem or Defret khatem".

However, this species is still very poorly documented ethnobotanically. The few existing studies focus on the chemical composition of its essential oils and their biological activities (Boudjerda *et al.* 2008, Méradji & Tebbakh 2016, Bourouis *et al.* 2018, Habitouche & Maamar 2021, Boubertakh *et al.* 2024, Bouteche *et al.* 2024).

The aim of our work has been to gather as much information as possible on the virtues and therapeutic uses of *A. ligustica* All., from the populations of two regions of eastern Algeria, located on the Algerian-Tunisian border (Bougous and Bouhadjar) (Fig. 1), where it has been reported following flora inventories. These investigations will make it possible to promote the species and encourage its conservation in its natural habitat. In particular, ethnobotanical data on this medicinal plant is very fragmentary in North Africa, and particularly in Algeria, and any information concerning it will highlight ancestral knowledge that can be gathered and recorded for future generations.

Materials and Methods

Region and study sites

The province of El Tarf, located in north-east Algeria on the Algerian-Tunisian border, covers an area of 2,904 km² and is home to over 500,000 inhabitants. Renowned for its ecosystemic and landscape diversity, it is home to one of the most prestigious protected areas in the Mediterranean region, the El Kala National Park, classified as a Biosphere Reserve in 1990.

Two areas were chosen for this ethnobotanical study: Bougous and Bouhadjar (Fig. 1).

Bougous region: This is a mountainous area located to the south-east of the El Kala National Park, on the Algerian-Tunisian border. It covers an area of 22,000 ha and is composed of 21 Mechtas or douars scattered throughout the forest (Fig. 1). The total population is estimated at over 12,000, with the highest concentration in the main town, with over 1,000 inhabitants. The forests occupy the largest area, 16,140 ha, and the landscape is made up of a mosaic of ecosystems showing different plant groups: *Olea europaea* L. and *Pistacia lentiscus* L. formations, riparian, *Quercus suber* L. and *Quercus canariensis* Willd. forests. Agricultural activity is limited and mainly masked by extensive itinerant livestock farming (Lazli *et al.* 2019).

Bouhadjar region: it covers an area of around 45,000 km² and has a population of over 46,000, the majority of whom work either in agriculture or in education and health. The forest ecosystem covers an area of almost 27,900 hectares and includes various forest formations: cork oak *Quercus suber*, zeen oak *Quercus canariensis* Willd. (Fagaceae), Atlas cedar *Cedrus atlantica* Manetti (Pinaceae), etc. (Fig. 1).

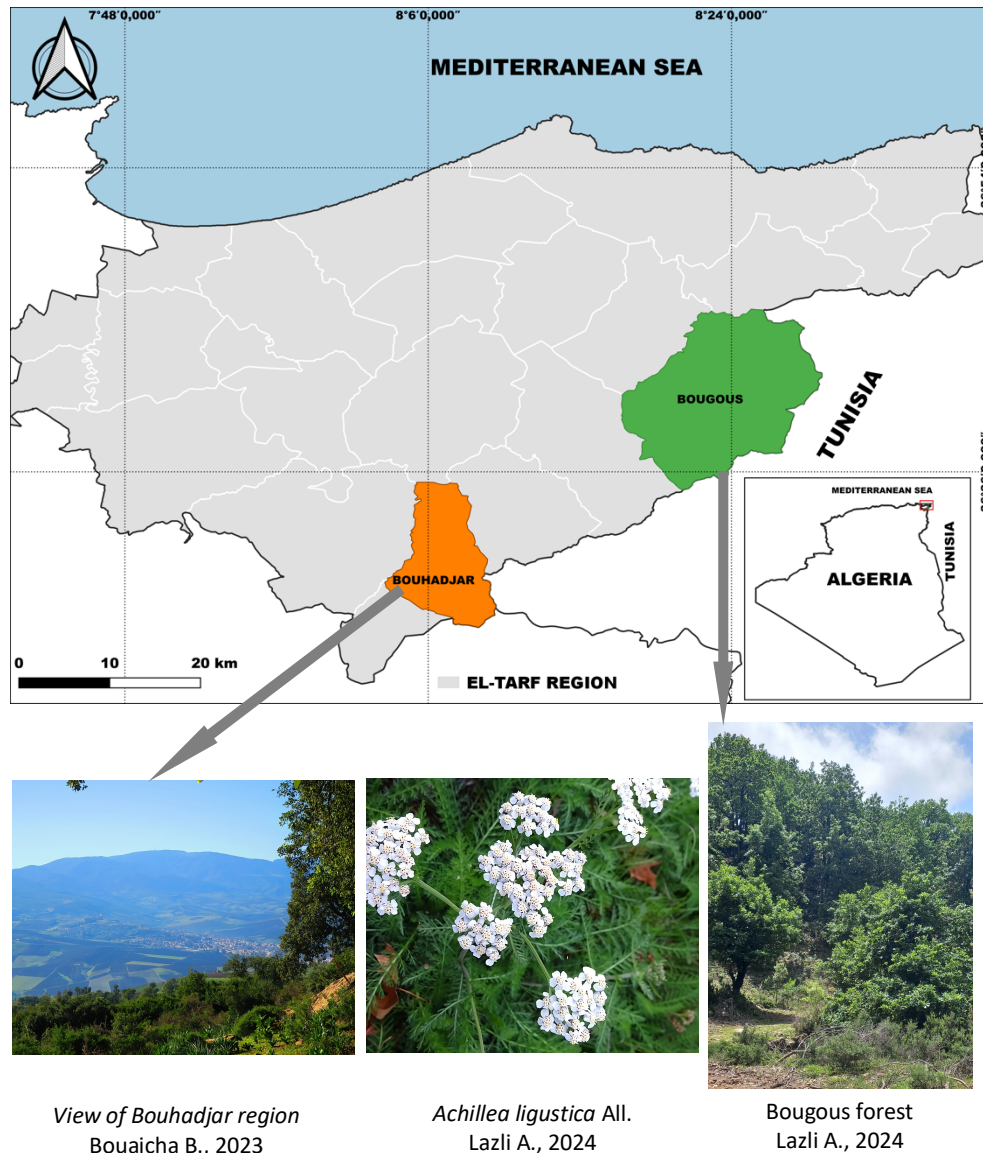


Figure 1. Geographical location of El Tarf region and the study sites (Bougous: green; Bouhadjar: orange)

Data collection

The ethnobotanical surveys were carried out between March and August 2023. Based on pre-established questionnaires, we interviewed people in the two zones under consideration (Fig. 2).

These questionnaires, which we filled in as we went along, related to the socio-demographic characteristics of the people interviewed (age, sex, level of education) and the ethnopharmacological characteristics of the plant studied, in particular the parts used, the diseases treated and the recommended methods of preparation. We also recorded any additional information on possible forms of toxicity or undesirable effects.

To make sure we were talking about the same plant, we showed photos of the plant to the people we interviewed, as well as the plant itself, freshly harvested (Fig. 3).



Figure 2. Photos of people interviewed during surveys

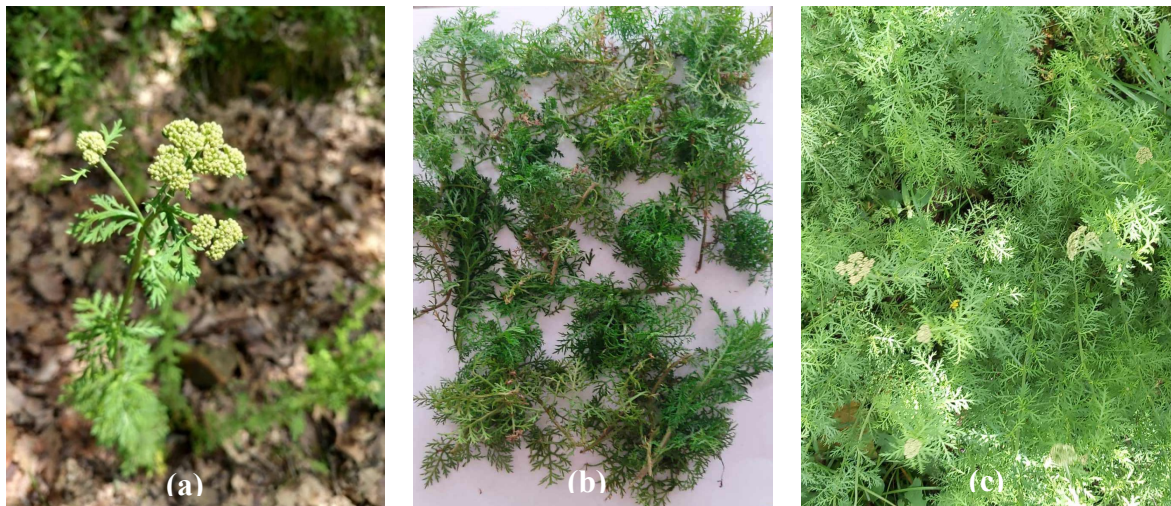


Figure 3. Photos (a, c) and fresh plant (b) of *Achillea ligustica* All. shown to respondents during the investigations. Gherib I. 2023

Legal aspects

The interviewees were informed that the surveys were being conducted as part of a scientific research project. Discussions were open, free and unconditional.

Data analysis

Once the fieldwork had been completed, the questionnaires were analysed. The data collected was sorted and entered a matrix from which we produced various graphs and tables using Excel software (Microsoft Office 2010 version). A factorial correspondence analysis was performed using ADE-4 Software to test the relationship between observations and variables (Lebart Piron & Morineau 1997).

As quantitative ethnobotanical data, we calculated the fidelity level (FL). Developed by Friedman *et al* (1986), this is an index used to measure the importance of specific uses of a plant within a particular category. The values obtained for this index range from 0% to 100%. It is calculated according to the following formula:

$$FL = (N_i/N) * 100$$

Where N_i is the number of informants who cited the specific use; and N is the total number of informants.

Results and Discussion

Socio-demographic characteristics of respondents

The survey enabled us to interview 253 people, distributed between the two considered regions. Through these interviews, we were able to meet people of different ages and education levels. Their knowledge allowed us to gather several therapeutic and traditional uses of *A. ligustica*.

The discussions initiated with the respondents were friendly, simple and were conducted in the local dialect to put the older and illiterate people at ease. Some of those interviewed were unfamiliar with studied plant: 40 in Bougous region, i.e. 26%, and 36 in Bouhadjar, i.e. 36% (Fig. 4). This unfamiliarity with the plant could be attributed to the places in which it grows. In fact, the areas where it has been found are far from habitations and at an altitude of around 800 m in humid forests, under trees, near small streams or along roadsides. In Italy, Bader *et al.* (2007) indicated that Ligurian yarrow grows on arid slopes between 0 and 800 m.

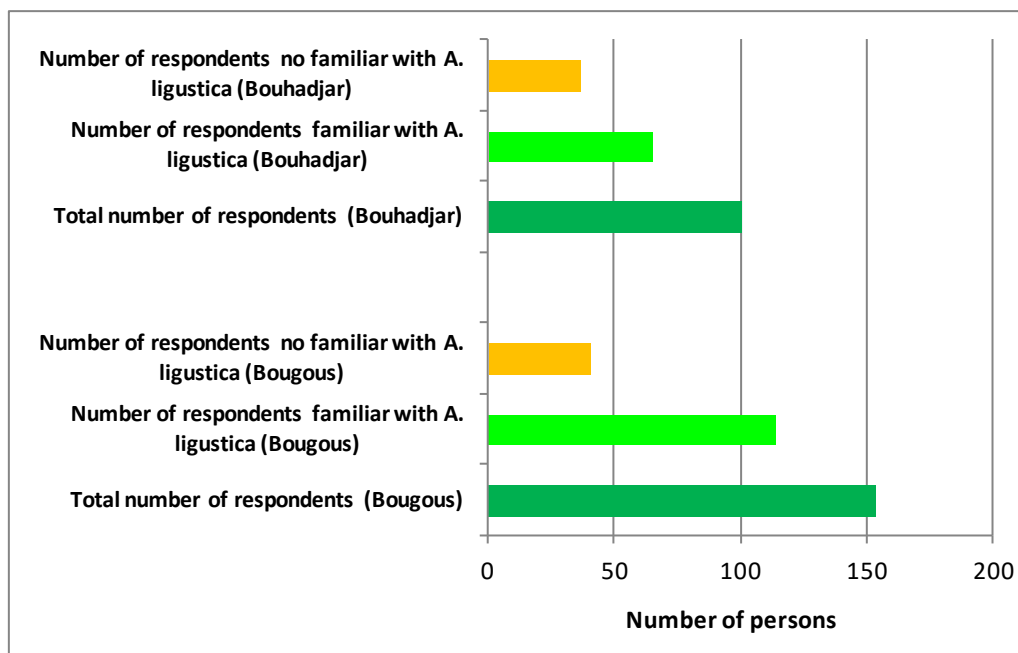


Figure 4. Statistics for interviewees in the two study regions

Profile of respondents

Of the 253 people interviewed during this study, 113 were able to provide information on the use of *A. ligustica* in the Bougous region and 64 in Bouhadjar. This plant does not seem to be very well known in these two localities, particularly in the second, which is a little less rural than the first.

According to gender

The results indicate that in both areas, women know and use this medicinal plant much more than men, almost 70% in Bougous and 61% in Bouhadjar (Tables 1, 2). These results agree with those of various previous studies, which have shown that women are the most frequent users of medicinal plants. In fact, women are the holders of traditional herbal knowledge, having learned it from their mothers and grandmothers. They know how to use useful plants and prepare plant-based recipes for the ailments of every member of the family, especially children. This type of finding has been reported in various studies, including those by Voeks (2007), Boughrara *et al* (2016), Miara *et al* (2018), Ben Salah *et al* (2019), Lazli *et al* (2019), Maamar

Sameut *et al* (2020), Beldi *et al* (2021), Bouafia *et al* (2021) and Karous *et al* (2021). On the other hand, men are generally responsible for collecting medicinal plants, especially in areas considered dangerous or faraway.

Table 1. Sociodemographic characteristics of informants using *A. ligustica* All. in Bougous region

Characteristics	Subgroup	Number	Percentage
Sex	Women	79	69,91%
	Men	34	30,08%
Age	<20	7	6,19%
	20-40	45	39,82%
	40-60	55	48,67%
	>60	6	5,30%
Educational level	Analphabetic	54	47,78%
	Primary	22	19,46%
	College	17	15,04%
	Secondary	15	13,27%
	University	5	4,42%

Table 2. Sociodemographic characteristics of informants using *A. ligustica* All. in Bouhadjar region

Characteristics	Subgroup	Number	Percentage
Sex	Women	39	60,93%
	Men	25	39,06%
Age	<20	0	0
	20-40	31	48,44%
	40-60	29	45,31%
	>60	4	6,25%
Educational level	Analphabetic	16	25,00%
	Primary	9	14,06%
	College	7	10,94%
	Secondary	13	20,31%
	University	19	29,68%

According to age

Investigations carried out showed that people aged between 40-60 years in Bougous and those aged 20-40 years in Bouhadjar, are the most concerned in the exploitation of the plant (Tables 1, 2; Fig. 5). In fact, their properties and uses are generally acquired following many years of experience accumulated and transmitted from one generation to the next (Benlamdini *et al.* 2014, Beldi *et al.* 2021). Generally, older people are those who have the most ethnobotanical knowledge and are the most likely to provide reliable information on the use of medicinal plants (González-Tejero *et al.* 2008, Bouasla & Bouasla 2017, Miara *et al.* 2018, Lazli *et al.* 2019, Senouci *et al.* 2019, Bouafia *et al.* 2021).

The youngest respondents under 20 years old, have a mixed opinion of medicinal plants uses and have an exploitation rate ranging from 0% to 6.14%, respectively between Bouhadjar and Bougous (Tables 1, 2; Fig. 5). For this age group, the practice of phytotherapy remains unimportant because modern medicine seems safer and more up to date. This is why, nowadays, the transmission of this ancestral knowledge is in danger because it is not always guaranteed.

According to educational level

In Bougous, most people surveyed on the therapeutic use of Ligurian yarrow were illiterate, representing a percentage of 47.78%. People with primary, secondary and high school levels have a percentage of information varying respectively between 19.46%, 15% and 13.27% (Fig. 6). However, those with a university level use medicinal plants very little (4.42%). Thus, the low level of education does not constitute a constraint for the knowledge of plants, their uses and the transmission of ethnobotanical information, from one generation to the next orally, without any recourse to literature.

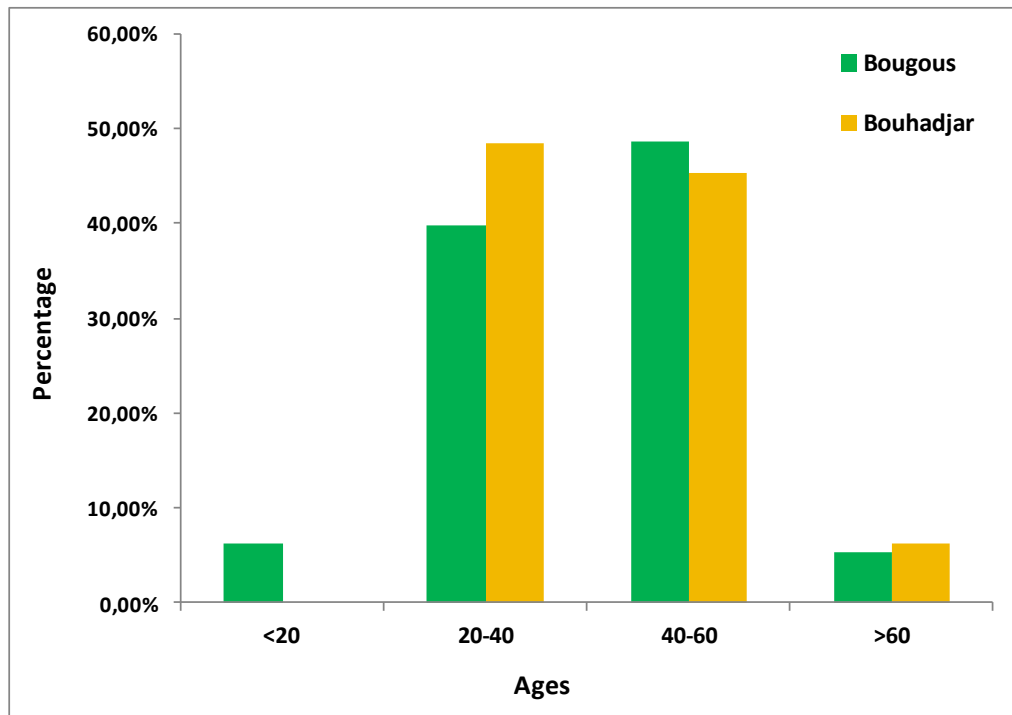


Figure 5. Distribution of respondents by age

In Bouhadjar, respondents with higher education, particularly university, gave the highest percentage of responses for the plant studied, 29.68%, compared to other user categories and compared to the more riverside population of Bougous (Fig. 6). Next in descending order were illiterate people, followed by those of high school, primary school and secondary school, with 25%, 20.31%, 14.06% and 10.94% respectively (Fig. 6).

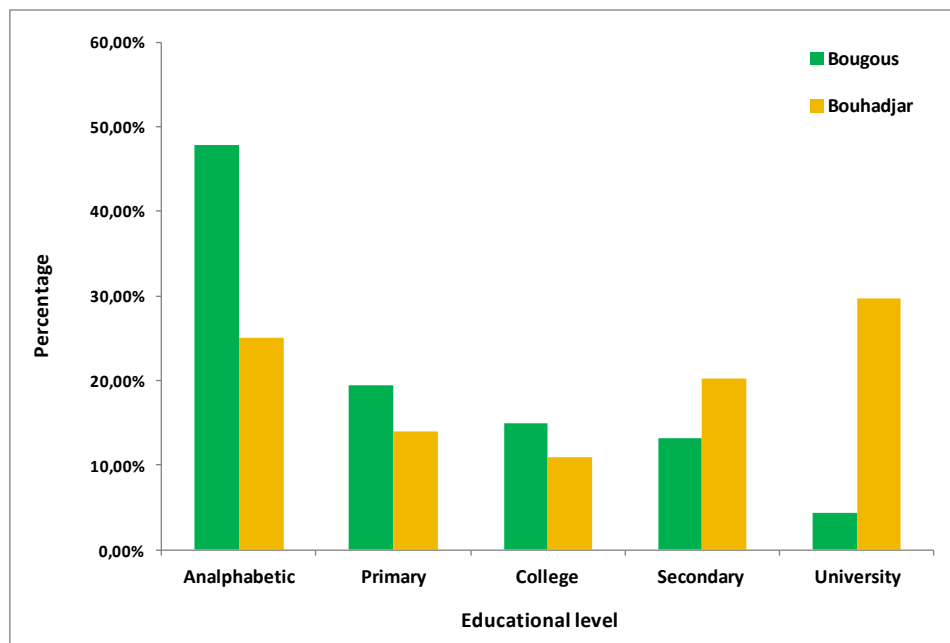


Figure 6. Distribution of respondents according to their educational level

In Belezma region (Batna, eastern Algeria), Chohra & Ferchichi (2019) found that academics predominated in the use of medicinal plants. This was also the case for the studies conducted by Yaici (2020) in the Tell Sétifien and Guechi (2022) in the massif of Maadid (M'Sila - Eastern Algeria). According to Boughrara & Legseir (2016), the development of herbalism culture, the ease of information exchange and awareness of the benefits of medicinal plants has led this category of the population to gradually adopt herbal medicine.

In addition, since the Covid-19 pandemic, a large proportion of the world's population, particularly intellectuals and therefore academics and people with a secondary education level, have turned to certain categories of medicine with preventive and curative properties, such as herbal medicine, aromatherapy, etc. Various scientific studies have also highlighted the importance of using some medicinal plants in phytotherapy and those that could have clinical value due to their richness in certain active ingredients to treat Covid-19 (Gangal *et al.* 2020, Hamdani & Houari 2020, Helalia *et al.* 2020, Vroh 2020). This trend has also been facilitated by the current lifestyle of many people based on access to the web and in particular social networks.

Ethnopharmacological data about the plant

Parts used

Depending on the part of the medicinal plant (leaves, flowers, roots, bark, fruit, seeds, rhizome), various active ingredients (secondary metabolites) are present. These are essential components of many of our medicines and healthcare products (Pelt 2004).

Ethnobotanical surveys carried out among local populations in the two areas studied indicate that the leaves and aerial parts are the most widely used parts of the plant (Fig. 7).

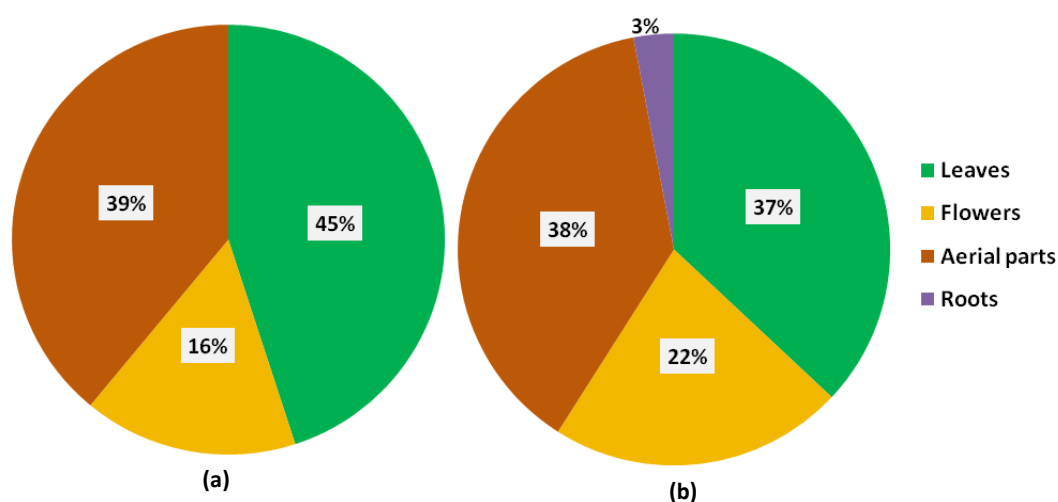


Figure 7. Percentage of Medicinal plant parts used. (a): Bougous region; (b): Bouhadjar region).

In Bougous, leaves dominate with almost 45% of uses, followed by aerial parts (39.06%) and lastly flowers (16%). On the other hand, in Bouhadjar, aerial parts account for the highest percentage of use (38%), followed by leaves, flowers and then roots (Fig. 7).

Various studies of folk medicine in Sicily and Sardinia have reported the use of *A. ligustica* leaves to treat certain diseases (Viegi *et al.* 1992, Bruni *et al.* 1997, Bader *et al.* 2007, Mohammadhosseini, 2017).

This frequent use of leaves and aerial parts can be explained by the ease and speed with which these plant parts can be harvested (Benarba *et al.* 2015, Chermat & Gharzouli 2015, Kadri *et al.* 2018, Miara *et al.* 2018, Lazli *et al.* 2019, Senouci *et al.* 2019, Guechi 2022), but also and above all by the fact that these organs are the seat of photosynthesis and sometimes the storage site for the secondary metabolites responsible for the plant's pharmacological properties (Salhi & Fadli 2006, Mangambu *et al.* 2014, Raterta *et al.* 2014, Francis Xavier *et al.* 2015; Lazli *et al.* 2019, Senouci *et al.* 2019). This is in agreement with other studies, since leaves are the powerhouse of photochemical reactions and therefore rich in active ingredients, and they are easy to collect. Moreover, leaves use poses no danger to plant regeneration and ensures the conservation of the flora richness (Poffenberger *et al.* 1992). In fact, there is a clear relationship between the part of the plant used and the effects of this use on its existence (Cunningham 1996).

Types of use

The majority of people interviewed in the two study areas preferred to use *A. ligustica* in its fresh form, 66.88% for Bougous and 62.90% for Bouhadjar (Fig. 8). However, others prefer the use of the plant in the form of herbal teas after drying in a dry

place, at room temperature and protected from light, which allows the preservation of the majority of the plants' active ingredients (Chraïbi *et al.* 2018).

Various studies have mentioned the use of the plant studied, whole or in some of its parts, in fresh or dried form (Viegi *et al.* 1992, Bruni *et al.* 1997, Bader *et al.* 2007, Mohammadhosseini 2017).

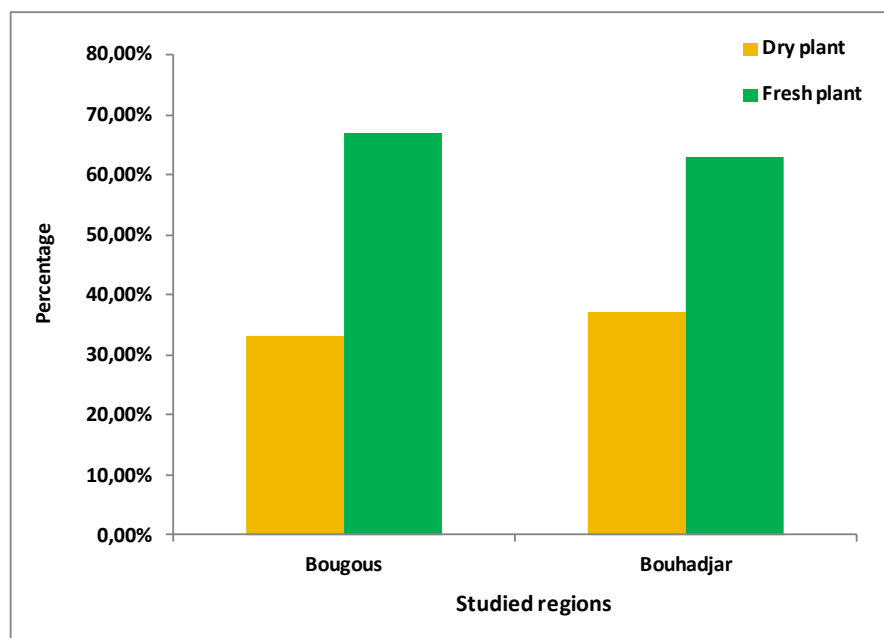


Figure 8. Ways of uses of the studied plant in the two regions under consideration

Mode of preparation

Information on how medicinal plants are used, and their therapeutic properties can vary from one person to another. Analysis of the ethnobotanical surveys obtained shows that in Bougous, infusion is the most recommended method of preparation, with 44%, followed by decoction, 41%. The percentages of the other remaining methods varied between 1% and 6% (Fig. 8). In Bouhadjar, cataplasm, infusion and decoction were the most common methods of administration, with 26%, 25% and 21% respectively. The other methods were cited with percentages varying between 2% and 9% (Fig. 9).

Several investigations have highlighted the use of Ligurian yarrow according to the preparation modes mentioned in the present study (Simonpoli 1993, Bruni *et al.* 1997, Conforti *et al.* 2005, Filippi *et al.* 2006, Muselli *et al.* 2009, Mohammadhosseini 2017). The best use of a plant would be the one that preserves all its properties while allowing the extraction and assimilation of the active ingredients (Dextreit 1984).

Various studies conducted on medicinal plants in Algeria and other Mediterranean countries agree with this work results (Benitez *et al.* 2010, Fakchich & Elachouri 2014, Jdaïdi & Hasnaoui 2016, Ouelbani *et al.* 2016, Sargin *et al.* 2013, Sarri *et al.* 2014, Miara *et al.* 2018, Lazli *et al.* 2019). The predominance, especially, of infusion and decoction of medicinal plants has been mentioned in numerous studies, including those of Meddour *et al.* (2020), Mechaala *et al.* (2021) and Guechi (2022). Indeed, it has been reported that these two modes allow the collection of most active ingredients and attenuate or cancel the toxic effect of certain preparations or mixtures of plants (Salhi *et al.* 2010).

Azzouz (2007) indicated that infusion is suitable for delicate plant parts such as leaves, flowers and flowering tops. On the other hand, Decoction is recommended for hard and compact parts (wood, bark, stems, and roots) which require a certain boiling time to release their active ingredients. This may explain why people in Bougous and Bouhadjar regions are more inclined to use leaves and flowers in infusions and the aerial parts and roots in decoctions.

In both regions, the plant studied was mainly administered orally: 85% of cases in Bougous and 46% in Bouhadjar (Fig. 9). Other methods of use, particularly for dermatological and rheumatic diseases, accounted for 51% of cases, including cataplasms, ointments and massage for the populations of both regions (Fig. 9). The use of *A. ligustica* as cataplasm has been mentioned in the literature by Bruni *et al.* (1997), Conforti *et al.* (2005) and Filippi *et al.* (2006).

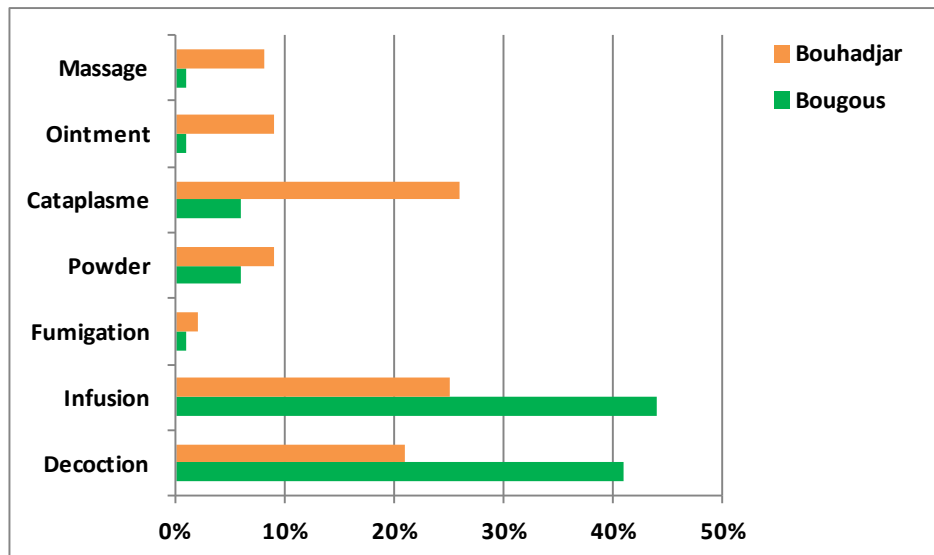


Figure 9. Percentage of *Achillea ligustica* administration modes in the two study regions

Treated diseases

Analysis of the results obtained concerning the relationship between the medicinal species studied and the types of illnesses treated showed that the species was used to treat gastrointestinal illnesses in the Bougous region (86%) and dermatological illnesses in the Bouhadjar region (49%) (Fig. 10). Other diseases were also mentioned during the interviews, in particular diseases of cardiovascular system and respiratory diseases (Fig. 10).

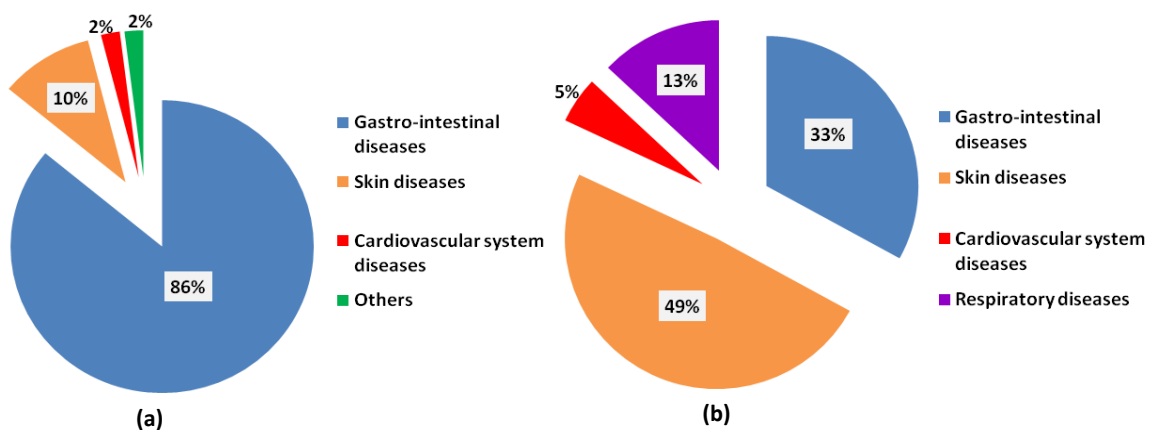


Figure 10. Main therapeutic indications (a: Bougous region ; b: Bouhadjar region)

Analysis of sociodemographic characteristics and different uses of respondents by Factorial Correspondence Analysis (FCA)

The graphical representations (Fig. 11a and 12a) carried out by Factorial Correspondence Analysis (FCA) shows different correspondence spaces between sociodemographic variables and different use-reports between the two study areas.

Figures 6a and 7a highlight the variables in order of importance according to region. For example: university level, which shows a higher percentage of knowledge in Bouhadjar (29.68%) compared with Bougous (4.42%) (Fig. 11a); or the example of the high percentage of use of Ligurian yarrow for gastrointestinal diseases in Bougous (86%) compared with Bouhadjar (33%) (Fig. 12a).

Therefore, the Factorial Correspondence Analysis revealed the different levels of interest in this plant among the two rural populations.

The correlation circles drawn up on the basis of the factorial correspondence analysis of the two study regions show an opposite situation on axis 2 on the same factorial plane (Fig. 11b and 12b). This situation can be explained by the following facts: in the two study stations, we observe the same uses, but with a predominance of use of certain variables for each region. Note that the two study sites are mountainous regions located in a rural wilaya.

Our results show that *A. ligustica* is reported to treat 5 categories of diseases. Among these, the treatments of gastro-intestinal diseases and skin diseases were associated with very high level of fidelity, with values of 82.3% (Bougous region) and 46.9% (Bouhadjar region). Respiratory and Cardiovascular system diseases come next category (Table 3). These findings highlight the perceived effectiveness of this medicinal plant in treating these diseases according to the informants in this study.

The same findings have also been highlighted by several studies conducted all around the Mediterranean basin. In Algeria, particularly Jijel and Mila, the plant is used by local population for stopping hemorrhage and for stomach pains (Bouteche et al. 2024). In Corsica (France), it is used against gastro-intestinal disorders, to relieve sprains and insect bites and against haemorrhages (Conforti et al. 2005, Filippi et al. 2006, Muselli et al. 2009, Mohammadhosseini 2017). In Sardinia (Italy), the species is used against gastric and stomach pains, skin diseases and rheumatic diseases (Bruni et al. 1997, Bader et al. 2007, Muselli et al. 2009, Mohammadhosseini 2017). In Sicily (Italy), *A. ligustica* is used to treat stomachache, to relieve gastralgia and neuralgia and also used as an anthelmintic agent (Bruni et al. 1997, Viegi et al. 2003, Bader et al. 2007).

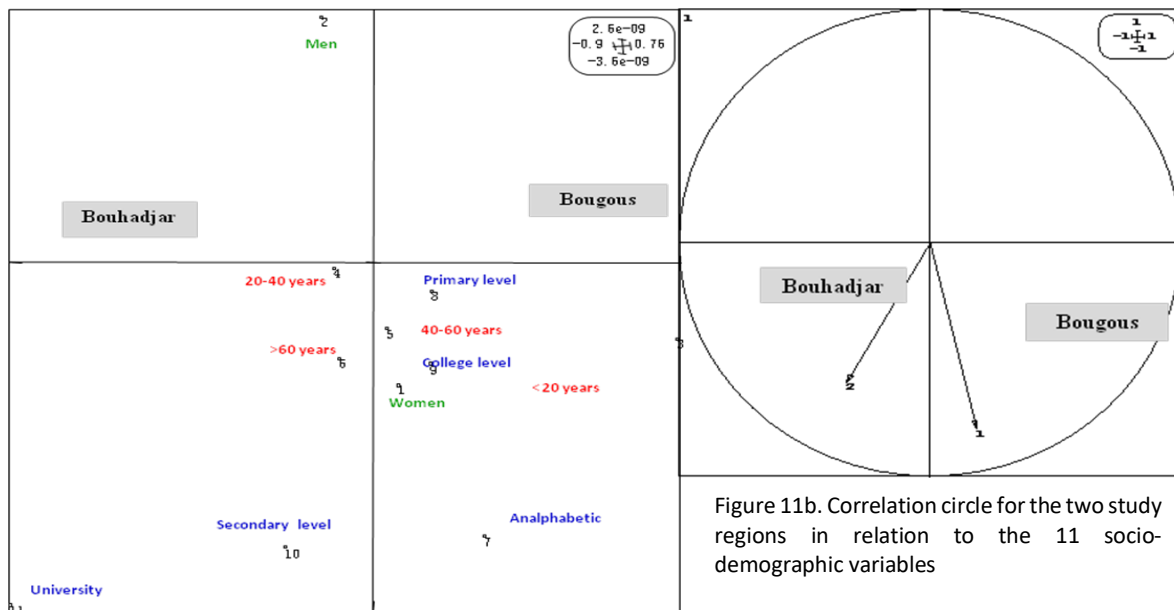


Figure 11b. Correlation circle for the two study regions in relation to the 11 socio-demographic variables

Figure 11a. FCA. Axes (1,2). Dispersal model of the ethnobotanical survey for 11 socio-demographic variables of the two study areas for the species *Achillea ligustica*

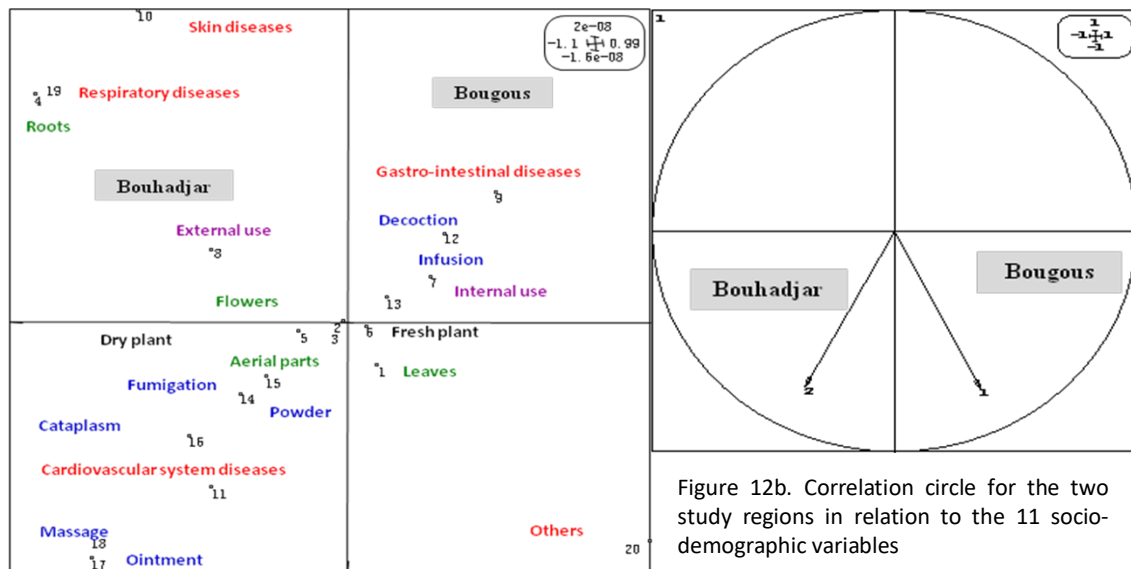


Figure 12b. Correlation circle for the two study regions in relation to the 11 socio-demographic variables

Figure 12a. AFC. Axes (1,2). Dispersal model of the ethnobotanical survey of 20 variables of the two study areas for the species *Achillea ligustica*.

Table 3. Percentage of Fidelity level of diseases treated by *A. ligustica* in the two study regions

Diseases	Region of Bougous		Region of Bouhadjar	
	Number of citations	Fidelity Level (FL)	Number of citations	Fidelity Level (FL)
Gastro-intestinal diseases	93	82.3%	23	35.9%
Skin diseases	15	13.3%	30	46.9
Cardiovascular system diseases	3	2.7%	3	4.7%
Respiratory diseases	-	-	8	12.5%
Others	2	2%	-	-

Toxicity

None of the respondents mentioned any health warnings about toxicity. In neighbouring countries, particularly Corsica, the plant is added to cakes and fritters on Good Friday (Simonpoli 1993, Filippi *et al.* 2006).

In north-western Italy, Ligurian yarrow is mixed with *Urtica*, *Beta vulgaris*, garlic and eggs to make a highly energetic soup recommended for men and children during arduous work (Cornara *et al.* 2014, Mohammadhosseini 2017).

Conclusion

This first ethnobotanical study conducted in the northeastern Algeria, and notably in two regions of El Tarf department (Bougous and Bouhadjar), at the Algerian-Tunisian borders, revealed a very rich local knowledge in term of traditional herbal medicine relating to *Achillea ligustica* All. very poored documented species in North Africa.

The results obtained provide an appreciable database that could be exploited for the development and promotion of a plant that could have an impact on the socio-economic life of local populations, while preserving it.

Furthermore, these results can serve as foundational data for future investigations in phytochemical and pharmacological studies.

We recommend expanding future ethnobotany research to cover broader regions and compare findings with regional and international studies.

Declarations

Ethics approval and consent to participate: All respondents were informed of the study purpose and provided prior informed consent.

Consent for publication: All people shown in images gave their consent to have the images published.

Availability of data and materials: Data used in this article are available for any requests.

Competing interests: Authors declare no conflict of interest

Funding: Authors have not received any funding during this research, as part of the preparation of a Doctorate thesis at Chadli Bendjedid University (El Tarf- Algeria).

Authors' contributions:

AL designed the questionnaire. IG conducted the personal interviews. AL conducted the most part of statistical analysis. IG, FG and AL supervised the study. IG and AL wrote the original draft of the manuscript. All the authors analysed the data and revised the manuscript. All the authors approved the final draft of manuscript after revision.

Acknowledgements

The authors are grateful to Bougous and Bouhadjar local populations for accepting to share their knowledge and practices. They also thank Dr Louhi-Haou Sihem for her help in a part of statistical analysis, Pr Bouchecker Abdennour for his assistance with the GIS, as well as all peoples whose participate to this investigation.

This work was carried out as part of Ms. Imene GHERIB's doctoral thesis. We also thank the Directorate-General for Scientific Research and Technological Development (DGRSDT) and the Ministry of Higher Education and Scientific Research.

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