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Corresponding Address
Current Perspectives on Medicinal and Aromatic Plants (CUPMAP),
Nazım Şekeroğlu
Gaziantep University, Faculty of Art and Sciences, 27310, Şehitkamil, Gaziantep - Türkiye
Phone: 0 342-317 19 22
Web: http://www.cupmap.org/
Contact: sekeroglunazim@gmail.com / editor@cupmap.org

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Web	http://www.cupmap.org/
Contact	Phone: 0 342-317 19 22/2960 E-mail:sekeroglunazim@gmail.com editor@cupmap.org

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Current Perspectives on Medicinal and Aromatic Plants (CUPMAP) is an open access, peer-reviewed and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary scientific studies in which results could easily be used in industrial production on MAPs. This international scientific journal publishes research papers related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on. CUPMAP publishes original research papers, applied studies, and review articles in MAPs science and technology. Special Issues devoted to important topics in the MAPs science and technology could also be published.

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Sincerely,
Prof. Dr. Nazım ŞEKEROĞLU
Editor-in-Chief
Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)
Contact: sekeroglunazim@gmail.com / editor@cupmap.org

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This international scientific journal publishes high-quality research articles related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on.

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RESEARCH ARTICLES

REVIEW ARTICLES

---------- Gaziantep University, Publishing House ----------
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Research article
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Anticholinesterase, Antidiabetic and Antioxidant Activities of Chloroform Extract of *Genista carinalis*

Hilmican CALISKAN[1], H. Hulya ORAK[2], Cansel CAKIR[3], Merve ARGON[1], Temine SABUDAK[1*], Mehmet OZTURK[3], Necmettin GULER[4]

[1] Department of Chemistry, Faculty of Science and Arts, Tekirdag Namik Kemal University, 59030, Tekirdag, Turkey, E-mail: tsabudak@nku.edu.tr , hlmcn.clskn@gmail.com , merweozer92@gmail.com ,
[2] Department of Food Technology., Vocational School of Technical Sciences, Tekirdag Namik Kemal University, 59030, Tekirdag, Turkey, E-mail: horak@nku.edu.tr
[3]Department of Chemistry, Faculty of Science, Mugla Sitki Kocman University, 48000, Mugla, Turkey, E-mail: mehmetozturk@mu.edu.tr , cansel.cakir@hotmail.com.tr
[4] Department of Biology, Faculty of Science, Trakya University, 22030, Edirne, Turkey, E-mail: nguler@trakya.edu.tr

*Corresponding author : tsabudak@nku.edu.tr

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Abstract

Alzheimer's disease and diabetes have become increasingly public health issues in recent years. On the other hand, synthetic drugs are expensive, could be inadequate to treat diseases, cause irritation, and have side effects. Therefore, increasingly more researchis being done on plant-derived formulas and, bioactive ingredients, which can be an alternative to synthetic drugs for treatments to solve basic health problems. In this study the antioxidant, anticholinesterase and antidiabetic activities chloroform extract of *Genista carinalis* Griseb. (Fabaceae) were determined. Based on the results, the extract was not observed inhibitory activities of α-glucosidase and α-amylase. It showed better activity for acetylcholinesterase activity than butylrylcholinesterase activity. The antioxidant potential of *G. carinalis* chloroform extract was determined with different assays. The TEAC value was determined to be 0.484 mmol TE/g, FRAP value was found to be 1023.20 µmol Fe^{2+}/g for *G. carinalis* chloroform extract. The EC_{50} value of DPPH assays of the extract was found to be 0.101 µg/ml.

Key Words: *Genista*, *G. carinalis*, Anticholinesterase inhibitory activity, Butyrylcholinesterase inhibitory activity, TEAC, α-Amylase inhibitory activity, α-Glucosidase inhibitory activity.

1. Introduction

Alzheimer's, one of the essential chronic diseases associated with loss of consciousness, is considered the most common type of dementia. Fifty million people are struggling with this disease and unfortunately, it is estimated to will increase to 82 million in 2030 and 152 million in 2050 (World Health Organization 2020). Acetylcholine is a neurotransmitter that inhibits first acetylcholinesterase (AChE) and then butyrylcholinesterase (BChE), which is thought to play a role in the pathology of Alzheimer's disease (Helbert et al. 1995). It is suggested that low acetylcholine level is a reasons for the progression of this disease (Talesa 2001). It is stated that AChE and

BChE inhibitors could treat Alzheimer's disease. (Orhan et al. 2004). *Diabetes mellitus* is an increasing health problem (Shaw et al. 2010). There is a need for effective anti-diabetic agents with few side effects for human health.

In this context, effective and safe plant-based inhibitors have been searching to replace synthetic inhibitors (Hasan et al. 2001). Antioxidants can prevent some inflammations that cause health problems by using mechanisms such as inhibiting reaction initiating radicals, breaking the chain reaction, and reducing localized oxygen concentrations (Dorman et al. 2003).

Genista is a genus of the Fabaceae (Legumes) family with approximately 100 species generally grown in the Mediterranean and Western Asia (Noccioli et al. 2011). It is stated that many *Genista* species show biological properties against various diseases (Rauter et al. 2009; Rainova et al. 1988; Bomtempo et al. 2013)

Expensive synthetic drugs, their inadequacy in curing diseases and their side effects, there is a tendency towards plant-derived formulas in the world (Jain et al. 2019) and confidence in traditional medicine is increasing (Craig 1999). In this study, enzyme inhibitory activities (AChE, BChE, α-amylase, α-glucosidase) and antioxidant activity of chloroform extract of *G. carinalis* were determined.

2. Material and Methods

2.1. Plant material and Extraction

Genista carinalis (2048.56 g) was collected from the Thrace region of Turkey (Location: 41°52'47.8"N 27°34'42.9"E and 41°52'29.5"N 27°34'36.4"E). The specimens (EDTU-16811) were identified by Asst. Prof. Guler at Trakya University, Faculty of Science, Department of Biology. First, the dried whole plants were cut into small pieces and extracted with methanol (Merck-1070184000) by maceration method. After the methanol evaporation under vacuum, a small amount of water was added to the crude extract. Later, crude extract was continues to extraction with n-hexane (Merck-1043742500) (46.85 g), chloroform (Merck-1070242500) (15.50 g), ethyl acetate (Merck-1007892500) (45.66 g) and n-butanol (Merck-1019902500) (434.51 g) according to the polarity order. Solvents were evaporated in the evaporator under vacuum to obtain crude extracts and the crude extracts were obtained. (Sabudak et al. 2021).

In this study, antioxidant, anticholinesterase and antidiabetic activities were aimed at the chloroform extract.

2.2. Anticholinesterase activity

The spectrophotometric method was used to determine of acetyl- and butyrylcholinesterase inhibitory activities (Ellman et al. 1961). Electric eel AChE and horse serum BChE were used as enzymes, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates. Cholinesterase activity was monitored using DTNB (5,5′-dithio-bis(2-nitrobenzoic)acid). Test extract and the galantamine were dissolved in ethanol (Ozturk et al. 2011).

Acetyl- and butyrylcholinesterase inhibitory activities of extract and galantamine were given as IC_{50}. To calculate IC_{50} value 50.0, 100.0, 200.0 and 400.0 µg/ml extract concentrations and 3.125, 6.25, 12.5 and 25.0 µg/ml galantamine concentrations were used. The IC_{50} values were calculated from the curve plotted against the % iğnhibition versus cencentration graph.

2.3. Antioxidant activity

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cations ($ABTS^{\bullet+}$) were used to evaluate the Trolox equivalent antioxidant capacity (TEAC) of G. carinalis chloroform extract. After $ABTS^{\bullet+}$ generation

in 2.45 mmol L-1aqueous sodium persulfate, reaction was carried out according Re et al. (1999) procedure. The results were estimated as mmol Trolox equivalents (TE) of per g extract.

The ferric-reducing antioxidant power (FRAP) of *G. carinalis* chloroform extracts were calculated as µmol Fe^{2+} equivalents per g of the extract by using Ferrous sulphate as standard and the absorbance values of the samples were measured at 593 nm (Benzie& Strain, 1996).

The DPPH (2,2-diphenyl-1picrylhydrazyl) capacity of *G. carinalis* chloroform was assayed by method against DPPH radical was monitored at 517 nm as described by Amarowicz et al. (2002). The radical scavenging capacity of extract for DPPH radical was given as EC_{50} values. EC_{50} values were calculated from the graph slope of absorbance versus extract concentration (ranging from 0.4 - 2.0 mg/ml concentration) and described as the µg/ml of extract needed to scavenge 50% of the $DPPH^{\bullet}$.

The CUPRAC assay was performed according to Apak et al. (2004). The method was based on the electron transfer to the media by the antioxidant sample. The extract was tested at four concentrations (25.0, 50.0, 100.0 and 200.0 µg/ml), while the positive antioxidant standarts at six concentrations (3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml). The results were given as $A_{0.50}$ (µg/ml), which corresponds to the concentration at 0.500 absorbance value. Absorbance was recorded at 450 nm.

2.4. α-Amylase and α-Glucosidase inhibitory activities

The α-amylase / α-glucosidase inhibitory activities of the chloroform extract of *G. carinalis* were investigated spectrophotometrically (Kim et al. 2010). The extract was tested in five concentrations (50.0, 100.0, 200.0, 400.0 and 800.0 µg/ml) to calculate the IC_{50}. Acarbose was used to compare both inhibitory activities.

3. Results and Discussion

The chloroform extract of *G. carinalis* was investigated for its bioactivities namely, antioxidant activity in four complimentary assays, anticholinesterase activity against Acetyl-butyryl-cholinesterase, and antidiabetic activity against α-amylase and α-glucosidase. Antioxidant activity, anticholinesterase activity and antidiabetic activity of chloroform extract of G. carinalis were studied herein for the first time.

Table 1 shows the α-glucosidase and α-amylase inhibitory activities. Based on the results, the extract inhibited α-glucosidase (IC_{50}: 117.9±14.5 µg/ml) better than that of acarbose (IC_{50}: 190.9±2.16 µg/ml) used as a positive standard. According to the α-amylase inhibitory activity test, the extract exhibited weak activity (IC_{50}: 418.9±14.5 µg/ml) and less than that of acarbose (IC_{50}: 85.6±1.56 µg/ml).

The results showed that the extract is slightly active against α-glucosidase but has less activity against α-amylase. According to the anticholinesterase activity the extract of *G. carinalis* exhibited weak activity (IC_{50}: 226.5±8.55 µg/ml and IC_{50}: 326.1±4.84 µg/ml) against AChE and BChE respectively (Table 2). The extract has a very weak capability to inhibit acetylcholinesterase and butyrylcholinesterase, the chief enzymes in Alzheimer's disease.

The antioxidant potential of *G. carinalis* chloroform extract was searched with different methods. The $ABTS^{\bullet+}$ scavenging activity of chloroform extract as shown in Table 3, explored that *G. carinalis* has 0.484 mmol TE/g TEAC activity. FRAP capacity (Ferric-reducing antioxidant power) of plant chloroform extract was found to be 1023.20 µmol Fe^{2+}/g.

Table 1. α-Glucosidase inhibition and α-Amylase inhibition activities of the chloroform extracts of *Genista carinalis*[a].

	Antidiabetic activity	
	α-Amylase Inhibitory Activity	**α-Glucosidase Inhibitory Activity**
	IC_{50} (µg/ml)	**IC_{50} (µg/ml)**
G. carinalis Extract	418.9±14.5	117.9±4.15
Acarbose[b]	85.6±1.56	190.9±2.16

[a] Values expressed herein are mean ± SEM of three parallel measurements p<0.05.
[b] Reference compounds.

Table 2. Anticholinesterase activity of the chloroform extracts of *Genista carinalis*[a].

	Anticholinesterase activity	
	Acetylcholinesterase inhibitory assay	**Butyrylcholinesterase inhibitory assay**
	IC_{50} (µg/ml)	**IC_{50} (µg/ml)**
G. carinalis Extract	226.5 ± 8.55	326.1 ± 4.84
Galantamine[b]	5.65±0.30	12.82±0.16

[a] Values expressed herein are mean ± SEM of three parallel measurements p<0.05.
[b] Reference compounds.

Based on EC_{50} value of DPPH assays, the EC_{50} value of *G. carinalis* chloroform extract was found as 0.101 mg/mL as shown in Table 3. The CUPRAC assay is a redox potential-based method, and according to results of the CUPRAC assay, the IC_{50} value of *G. carinalis* chloroform extract was found to be 99.81 µg/ml (Table 3). The EC_{50} value of DPPH of *G. carinalis* chloroform extract was found to be higher compared to previous studies, Meriane et al. (2014) found lower values in their study with the methanolic soluble fraction of *Genista saharae* (DPPH radical scavenging activity; IC_{50} = 8.27 µg /mL), and Boukaabache et al. (2013) obtained lower results (IC_{50} value of the extract against DPPH radical 61.64 µg/mL) in their study with ethyl acetate extract of *Genista quadriflora*. The difference could be explaining the difference between *Genista* species and extract types.

Table 3. Antioxidant activity of the chloroform extracts of *Genista carinalis*.

Antioxidant activity	
G. carinalis Chloroform Extract	
TEAC (mmol TE/g)	0.484 ± 0.11
FRAP (µmol Fe^{2+}/g)	1023.20 ± 3.45
EC_{50} value of DPPH capacity (µg/ml)	101.01 ± 0.02
IC_{50} value CUPRAC (µg/ml)	99.81 ± 3.24

4. Conclusion

Anticholinesterase, antioxidant and anti-diabetic activities among *Genista* species are available in the literatüre (Batista et al. 2015; Rauter et al. 2009). In this study, this plant was chosen because there is no biological activity study on *G. carinalis* in the literature. The antioxidant, anticholinesterase and antidiabetic activities of *G. carinalis* chloroform extract were studied in this report. Briefly, the extract exhibited weak anticholinesterase activity which is related with Alzheimer's disease. However, it inhibits α-glucosidase better than acarbose used as antidiabetic drug. Moreover, it has also low inhibitory activity against α-amylase which is also a chief enzyme for diabetic activity. What's more, the extract can be considered as powerful antioxidant activity. The TEAC

value was determined to be 0.484 mmol TE/g, ferric-reducing antioxidant power was found to be 1023.20 µmol Fe^{2+}/g for G. carinalis chloroform extract. The EC_{50} value of DPPH assays for extract was found to be 0.101 µg/ml.

This is the first report on the various bioactivities of *G. carinalis* chloroform extract. The results triggered us as a future study to isolate and elucidate the antioxidant compounds which may also have α-glucosidase inhibitory capacity.

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Author Contribution

TS, HHO and MO conceptualized the experimental procedures. NG identified the plant. HC, HHO, CC, MA, TS and MO conducted the experiments. HC, HHO, CC, MA and MO performed the data analysis. HC prepared the first draft of the manuscript. HC, HHO, CC, MA, TS and MO edited and revised the final draft of the manuscript. All authors approved the final version for submission

Conflicts of Interest

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The Contribution of Protected Areas to Human Health. A Case Study from Djurdjura Biosphere Reserve (Algeria), with New or Rarely Reported Medicinal Plants

Rachid MEDDOUR*, Ouahiba SAHAR

Department of Agronomy, Faculty of Biological Sciences and Agronomic Sciences, Mouloud Mammeri University of Tizi Ouzou, BP 17 RP, 15000, Tizi Ouzou, Algeria.
*Corresponding author : rachid_meddour@yahoo.fr

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Abstract

In the protected areas of the Mediterranean Basin, inventories on the ethnomedicinal uses of plants have been carried out both on its northern and southern shores. An outstanding wealth of ancestral knowledge on traditional medicine still exists in the mountainous area of the Djurdjura Biosphere Reserve. An ethnomedicinal survey was performed in the field with 64 informants from the villages of three municipalities, through a semi-structured questionnaire and direct interviews. It is especially illiterate women without activity, over 45 years old, who hold the best knowledge about this traditional medicinal practice. Overall, 121 plant species have been identified, with 42 plant species newly recorded. They belong to 108 genera and 56 families. The Lamiaceae are the most mentioned family with 13 species. The majority of these medicinal plants are growing in the wild (79.3%). They are used to treat a wide range of 83 diseases and symptoms. Digestive disorders are the disease group the most treated in the study region, with 63 species. Indigestion and diarrhea are the most commonly treated ailments by the local population, which mainly use the fresh leaves (48.51%) as infusion or decoction, the most common preparations. From the perspective of conservation and improvement of this ethnobotanical knowledge, the medicinal plants recorded, particularly the 12 endemic and/or rare species (e.g. *Origanum vulgare* L. subsp. *glandulosum* (Desf.) Ietswaart, which cures the highest number of diseases), deserve the greatest conservative attention for their patrimonial and therapeutic values.

Key Words: Ethnoflora, Ethnomedicinal Uses, Traditional Knowledge, Biodiversity, Protected Area, Djurdjura.

1. Introduction

Protected areas provide a variety of ecosystem services, including the direct use of plants, of which medicinal plants play a major role (Zank and Hanazaki 2011). The protected areas are particularly interesting from an ethnomedicinal point of view because they show not only the wide range of medicinal plants but also a huge knowledge on their uses by local populations (da Silva et al. 2019). In the protected areas of the Mediterranean Basin, inventories on the ethnomedicinal uses of plants have been carried out both on its northern shore (di Sanzo et al. 2013; Belda et al. 2013; Leto et al. 2013; Vitalini et al. 2015; Menale et al. 2016; Vinagre et al. 2019) than on its southern

shore (Bounar et al. 2013; Rhafouri et al. 2015; Rhattas et al. 2016; Boughrara and Belgacem 2016; Benaissa et al. 2018; Souilah et al. 2018; Chohra and Ferchichi 2019; Lazli et al. 2019; Hadjadj et al. 2019; Zatout et al. 2021). These inventories of medicinal plants and their uses in protected areas concerned some Algerian protected areas, such as those of El Kala, Taza, Belezma, Tlemcen and Djebel Aissa. On the other hand, to our knowledge, the Djurdjura Biosphere Reserve (DBR) has been the subject of only one published ethnobotanical study (Meddour et al. 2020). Without such ethnobotanical studies, information would not be recorded and, as the knowledge base evolves, it could eventually be lost. Moreover, the study of local knowledge on medicinal plants is becoming increasingly important in defining strategies for the conservation and sustainable use of plant resources (da Silva et al. 2019).

In this global framework, the major objective of this ethnobotanical study is to identify and document the indigenous knowledge on traditional uses of plant species used by the local populations for human health. The results will bring to limelight the plant species of high ethnobotanical value. This knowledge is also necessary to assist managers and decision makers in incorporating actual and potential valuable species into economic future planning, policy, and investment.

2. Material and Methods

2.1. Study area

The Djurdjura Biosphere Reserve (and National Park) is located 30 km south-east of Tizi Ouzou and 50 km from the Mediterranean Sea (Figure 1A), on the high slopes of the Djurdjura mountain range, between 800 and 2300 m a.s.l. It covers an area of 18550 ha, between latitudes 36°25'42'' and 36°32'02'' North and longitudes 3°57'23'' and 4°19'43'' East. This biosphere reserve, a Mediterranean mountainous site, very diversified in terms of flora and landscape (UICN 2015), was set up as a biosphere reserve in 1997. This study is carried out at the level of 14 villages surrounding or enclosed in the territory of the DBR. These villages depend on the municipalities of Iboudrarene (4 villages), Akbil (4), and Saharidj (6) (Figure 1B). The rural population of these three municipalities was estimated in 2008 at 22817 inhabitants in total, with a high human occupation density of 167 to 235 inhabitants per km^2 on northern slope and only 92 inhabitants per km^2 on southern slope (UICN 2015). In this wooded region of the biosphere reserve, with its mountainous and isolated relief (deep valley), traditional practices are preserved, in particular the use of herbal medicine by rural populations.

2.2. Data collection

To collect ethnomedicinal data, we carried out an inventory of current popular uses of medicinal plants as daily primary health care. We followed standard ethnobotanical data collection procedures (Bellakhdar 2008; Albuquerque et al. 2014). The choice of the sample was focused on the research of informants considered the most knowledgeable, with expertise regarding local medicinal plants. At the villages concerned by the survey, we applied the snowball technique (Martin 2004), to select the informants. The field survey was therefore performed with our key informants, who are native to the villages studied, via direct interviews in Kabyle (the Berber language of Northern Algeria) with a semi-structured questionnaire prepared in French. This implies an in-depth knowledge of the local culture.

During our survey in March-July 2019, we interviewed 64 informants from the villages. The code of ethics of the International Society of Ethnobiology (ISE 2006) was strictly followed. Prior informant consent (PIC) was obtained orally from all informants before beginning any of the interviews. They were

assured anonymity to participate in the survey and freely share their ethnobotanical knowledge (Vitalini et al. 2015). Direct face-to-face interviews alternated with botanical field trips, where the informant directly told us about the plant and its uses.

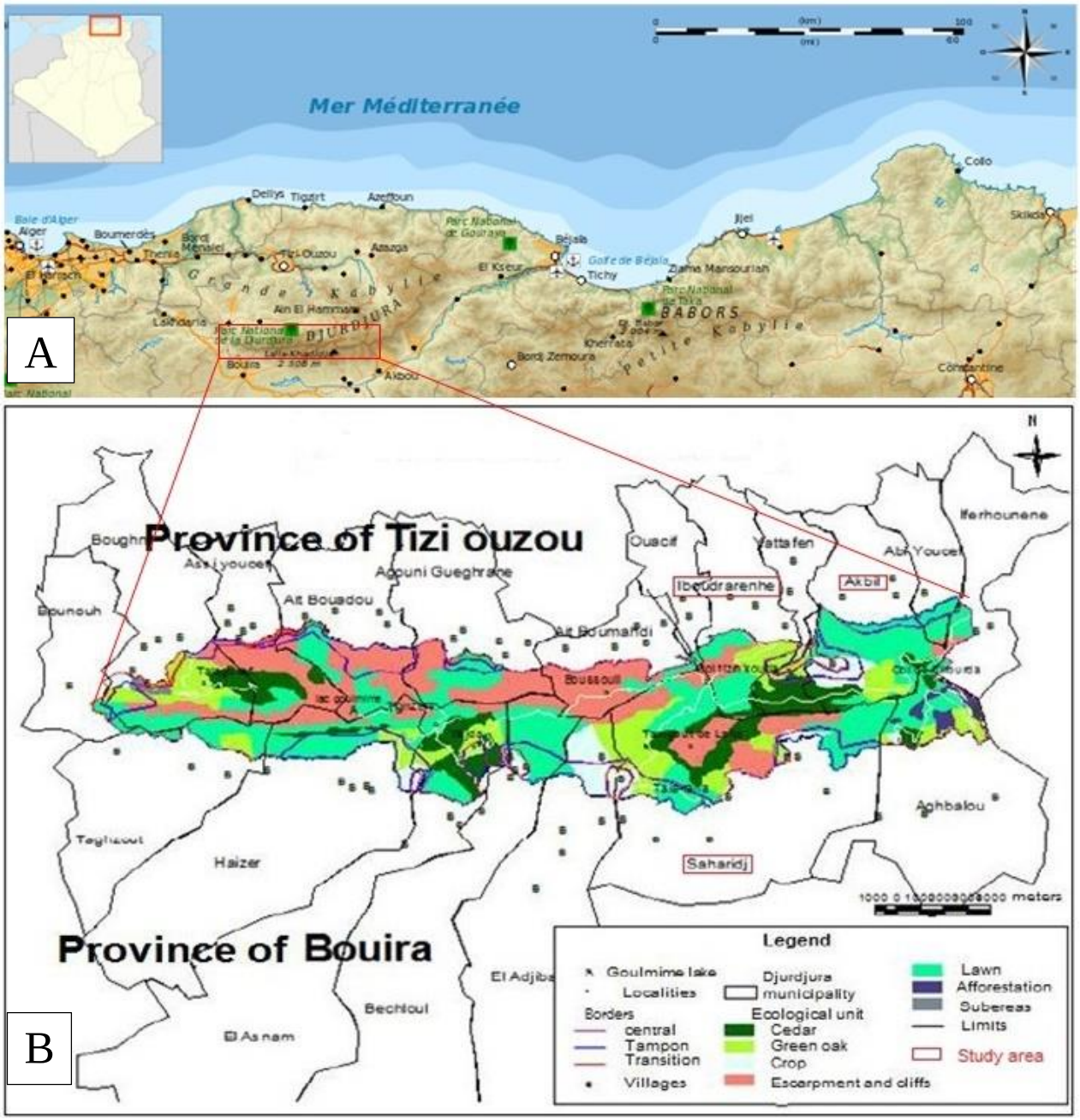

Figure 1. Location of the Djurdjura National Park in northern Algeria (A) (https://fr-ch.topographic-map.com/maps/4c2a/), and the study municipalities (B): Iboudrarene and Akbil are located on the northern slope of the DBR, and Saharidj on its southern

The systematic identification of plants, labelled with their vernacular names, was performed using the "Flora of Algeria" (Quézel and Santa 1962-1963). The nomenclature was updated according to the synonymic index of Dobignard and Chatelain (2010-2013). The specimens of plants collected were deposited at the Herbarium of the Faculty of Biological and Agronomic Sciences (Mouloud Mammeri University of Tizi Ouzou, Algeria).

2.3. Data analysis

The ethnobotanical data was entered into a table of raw data and processed using the Microsoft Excel® 2016 spreadsheet. We have standardized the information relating to the following aspects, frequency of use of medicinal plants, local medicinal uses and other uses of each plant, plant parts used, pharmaceutical preparation methods, administration modes (internal or external use), diseases and symptoms treated. To perform a simple statistical analysis of the collected data, we calculated the relative frequency of citation (RFC) at which each species of plant was used for its medicinal properties (Belda et al. 2013). This index, proposed by Tardío and Pardo-de-Santayana (2008), reflects the local therapeutic importance of each species and it results from the frequency of citation (FC), i.e. the number of informants who mentioned a given species, divided by the total number of informants (N); RFC = FC/N (0 < RFC < 1). The diseases and symptoms have been clustered into 10 major disease groups following the classification adopted in the Mediterranean region by Gonzalez-Tejero et al. (2008).

3. Results and Discussion

3.1. Informants' profile

We conducted our survey with 64 informants, whose characteristics are summarized in Table 1. Women have a numerical advantage over men (65.96% against 34.04%). Most of the surveyed population that has ethnobotanical knowledge is that of age groups over 45 years (45-60, 60-75, 75-90 years), a total of 65.96%. Age groups under 45 years (15-30, 30-45 years) are only represented by 34.04% (15-30 years old account for much less, i.e. 17.02%).

The holders of ethnomedicinal knowledge are thus people over 45 years of age and more often women, who are traditionally the legatees of ethnomedicinal information (Aquaron 2006). They are better informed about local medicinal practices compared to men, due to their social relationships, where they exchange more information related to family health care (Hoang et al. 2008; Sousa et al. 2012). These findings on the gender and age of the informants were likewise reported by other authors in Algeria (Boutabia et al. 2011), in Morocco (Mehdioui and Kahouadji 2007; Benkhnigue et al. 2011), and as well in Vietnam (Hoang et al. 2008). In addition, the younger generations compared to the elderly, know much less about the uses of plant species, as knowledge and experience are accumulated with age (Susanti and Zuhud 2019).

The people surveyed are housewives (27.66%), unemployed (10.64%), or retirees, with 8.51%. This shows that the majority (46.81% in all) of people surveyed are unwaged in this isolated mountainous region. Besides, farmer-herders are represented with 38.3%. Ethnobotanical information can be acquired from different sources. However, the main source of informants comes from family knowledge held by the elderly (65.45%). The other sources are represented by 12.73% for other persons (neighbors, friends), 16.36% for books (written sources), and 5.45% for the media and internet.

The majority of informants are illiterate, with a high percentage of 48.94%. It is obvious that there is a significant risk of loss due to illiteracy and especially to the non-transcription of local knowledge (Baydoun et al. 2017). The main source of ethnobotanical information for the holders of this ancestral knowledge is family knowledge transmitted by old people. This attests to the originality of ethnomedicinal knowledge, transmitted primarily orally within the family, between individuals, and from one generation to another in the Djurdjura Mountains, without resorting to the literature, as in other areas of Algeria

(Hamel et al. 2018). Mattalia et al. (2020) confirm likewise the vertical transmission of traditional medicinal knowledge from one generation to the next through family members (especially from mothers to daughters).

Table 1. Socio-demographic characteristics of the informants

Characteristics	Categories	Percentage of informants (%) (N = 64)
Gender	Men	34,04
	Women	65,96
Age group	15-30	17,02
	30-45	17,02
	45-60	25,53
	60-75	29,79
	75-90	10,64
Education level	Illiterate	48,94
	Primary	14,89
	Secondary	19,15
	High school	12,77
	University	4,26
Profession	Housewives	27,66
	Unemployed	10,64
	Retirees	8,51
	Students	4,26
	Farmers-herders	38,30
	Workers	10,64
Residence	Akbil	26,56
	Iboudraren	37,50
	Saharidj	35,93
Source of knowledge	Family members	65,45
	Other persons	12,73
	Books	16,36
	Media, Internet	5,45

3.2. Analysis of the medicinal flora recorded in the study region

In the DBR, informants from the villages reported 121 plant species that have medicinal uses for humans, belonging to 108 genera and 56 families. All these recorded medicinal plants are presented in Table 2, in alphabetical order of species and subspecies. The number of 121 plant species, although far from being complete, reflects a greater diversity of medicinal flora in Djurdjura Biosphere Reserve, compared to the first contribution (cf. Meddour et al. 2020), and with 42 plants newly recorded. On the other hand, it reveals also a bigger diversity compared to those recorded in similar ethnobotanical studies in other protected areas in Algeria. Indeed, the number of medicinal plants inventoried varies from 23 to 59, according to data from Bounar et al. (2013), Boughrara and Belgacem (2016), Benaissa et al. (2018), Chohra and Ferchichi (2019), Hadjadj et al.

(2019) and Lazli et al. (2019). However, Zatout et al. (2021) and Souilah et al. (2018) report 109 and 111 medicinal plant species in Tlemcen and El Kala National Parks, respectively.

Among the 56 families, the Lamiaceae are the most represented with 13 species (10.7% of all species recorded), followed by Asteraceae (9.1%), Apiaceae (7.4%), Rosaceae (6.6%), Poaceae, Amaryllidaceae, and Fabaceae (3.3% each), Fagaceae and Oleaceae (2.5% each). The remaining 47 families are represented by one or two species. The preponderance of families, such as Lamiaceae and Asteraceae, in medicinal flora is a well-established fact through the Mediterranean Region (Gonzalez-Tejero et al. 2008), particularly in Algeria (Hadjadj et al. 2019), in Morocco (Ennabili et al. 2000; Mehdioui and Kahouadji 2007), and in Spain (Belda et al. 2013). It has also been observed elsewhere in protected areas (e.g., Zank and Hanazaki 2011).

Allium (4 species), *Mentha* and *Prunus* (3 species each) are the most represented genera, followed by *Acer*, *Daphne*, *Malva* and *Quercus*, with two species each. The species most frequently cited by the informants with the highest RFC value (= 0.43) is *Origanum vulgare* subsp. *glandulosum* (Desf.) Ietswaart. It is followed by another *Lamiaceae*, *Marrubium vulgare* L., with a RFC value = 0.34. According to Rhattas et al. (2016), *Marrubium vulgare* L. is also one of the species most cited by informants in a protected area of Rif (Morocco). These aromatic plants are of very wide therapeutic use, given their efficacy, their status in the local pharmacopoeia, and are easily available in the DBR. Then, twenty-five species (20.7% of all recorded plants) are mentioned with a RFC value > 0.15, of which 12 have a RFC value > 0.21. However, a great number of species (70 or 58%) are cited with the lower RFC value (< 0.06).

On the other hand, the spontaneous plants growing in the wild participate with a high rate (79.3%) in the traditional pharmacopoeia of the DBR. Fully cultivated species are represented with only 20.7% (n = 25). Thus, local populations most often resort to wild flora, given the importance of spontaneous plant resources in this forested region. Belda et al. (2013) also found that medicinal plants are mostly collected from scrubland or forests. Globally, at least 60% of medicinal plants are gathered from the wild (Bonet and Vallès 2007). This demonstrates the strong connection of local populations with their immediate natural environment (Zank and Hanazaki 2011).

Most of the medicinal plants listed in the DBR are common species. However, the local population has recourse to some rare and/or endemic plants (10.8% of all plants), collected within this protected area, for medicinal and other uses. These rare and endemic plants species are *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Thymus numidicus* Poir., *Origanum vulgare* subsp. *glandulosum*, *Isatis djurdjurae* Coss. & Durieu, and rare non-endemic plants are *Artemisia absinthium* L., *Daphne laureola* L., *Lonicera etrusca* Santi, *Acer monspessulanum* L., *Acer obtusatum* Waldst. & Kit. ex Willd., *Ilex aquifolium* L., *Taxus baccata* L., and *Tussilago farfara* L. (montane plants). This part of the ethnoflora deserves the greatest conservatory attention for its heritage value. In particular, the Djurdjura pastel (*Isatis djurdjurae*), endemic to Algeria and Morocco, is used for skin diseases (boils and abscesses). Otherwise, the traditional exploitation of these species must be done in a reasonable way in order to preserve them and ensure their sustainability in this protected area. Local people should be informed on sustainable methods of harvesting plants to treat diseases today without compromising their availability for future use (Adaeze et al. 2018).

Table 2. Medicinal plants reported by the informants in the Djurdjura Biosphere Reserve with their ethnomedicinal uses.
For each species, we mention the scientific name, the family, the Kabyle vernacular names, its relative frequency of citation (RFC), whether spontaneous (S) or cultivated (C) type. Data such as therapeutic uses or treated diseases, plant parts used, mode of preparation, administration route, are likewise provided. * Newly recorded plant species compared to Meddour et al. (2020).

Plant species	Families	Vernacular names	Parts used	Preparation methods	Administration modes	Treated diseases/Therapeutic uses	Type	RFC
**Acer monspessulanum* L.	Sapindaceae	Adharchi	leaves	decoction	bath	hair loss	S	0,06
**Acer obtusatum* Waldst. & Kit. ex Willd.	Sapindaceae	Lqikev	leaves	decoction	bath	hair loss	S	0,04
Ajuga iva (L.) Schreb.	Lamiaceae	Chkentoura	leaves	infusion	oral ingestion	diabetes, stomach pain, circulatory disorders	S	0,15
			leaves	maceration	local application	scars		
Allium ampeloprasum L.	Amaryllidaceae	Tharnast	leaves, bulbs	cooking	oral ingestion	physical weakness, indigestion, dry cough	C	0,19
Allium cepa L.	Amaryllidaceae	Levsel	bulbs	raw	compresses	headache	C	0,09
			bulbs	raw	poultices	furuncles, abcesses		
			bulbs	juice	local application	warts		
Allium sativum L.	Amaryllidaceae	Thicherth	bulbs	raw	oral ingestion	cough, diabetes	C	0,04
			bulbs	raw	local application	bee stings		
Allium triquetrum L.	Amaryllidaceae	Vivras	aerial part	raw	oral ingestion	indigestion, general fatigue	S	0,06
**Aloysia citriodora* Paláu	Verbenaceae	Tizane, Zatage	leaves	infusion	oral ingestion	insomnia, headache, stomach pain	C	0,02
**Ampelodesmos mauritanicus* (Poir.) T. Durand & Schinz	Poaceae	Adless	leaves	decoction	gargle	oral conditions, canker sores	S	0,06
Apium graveolens L.	Apiaceae	Kravez	leaves	decoction	rinses	frostbites	C	0,02
Arbutus unedo L.	Ericaceae	Issisnou	leaves	decoction	oral ingestion	diarrhea	S	0,04
Artemisia absinthium L.	Asteraceae	Jaret meriem	leaves, flowers	infusion	oral ingestion	diabetes, diarrhea	S	0,23
			leaves	powder	oral ingestion	anorexia, nausea		
			leaves	maceration	oral ingestion	fever, stomach pain		
			leaves	juice, maceration	oral ingestion, local application	diarrhea, vomiting (babies)		
Arum italicum Mill.	Araceae	Aveqouq	leaves	decoction	oral ingestion	influenza, indigestion	S	0,09
**Arundo donax* L.	Poaceae	Aghanim	leaves	juice	oral ingestion	tonsillitis	S, C	0,06
			rhizomes	decoction	oral ingestion	tonsillitis		
**Asphodelus ramosus* L.	Asphodelaceae	Abarwaq	tubers	heating	instillation	otitis	S	0,02
Asplenium ceterach L.	Aspleniaceae	Thiwjrirhin	leaves	infusion	oral ingestion	kidney stones	S	0,21
Beta vulgaris L.	Amaranthaceae	Thividhest	leaves	raw	oral ingestion	anemia, indigestion	S, C	0,04
Blackstonia grandiflora (Viv.) Maire	Gentianaceae	Qlilu	aerial part	infusion	oral ingestion	anemia, diabetes, lack of appetite, nausea, indigestion	S	0,04
Borago officinalis L.	Boraginaceae	Ahledjedh, Chikh levqoul	leaves, flowers	juice	oral ingestion	indigestion	S	0,04
			flowers	infusion	oral ingestion	bronchitis, cold, joint pain		
Calicotome spinosa (L.) Link	Fabaceae	Uzzu	aerial part	lotion	local application	sores and injuries, hemorrhage	S	0,13
			seeds	powder	mask	headache		

Chamaeleon gummifer Cass.	Asteraceae	Addadh	flowers	crushed	oral ingestion	headache, cough	S	0,04
			flowers	crushed	bath	hemorrhoids		
**Castanea sativa* Mill.	Fagaceae	Abeludh urumi	fruits	raw	oral ingestion	diarrhea	S, C	0,02
Cedrus atlantica (Endl.) G.Manetti ex Carrière	Pinaceae	Avawel	leaves, bark	decoction	oral ingestion	bloating, cold	S	0,11
			bark, roots	tar (qedhran)	oral ingestion	cough, general fatigue		
**Celtis australis* L.	Cannabaceae	Ivikes	flowers	infusion	oral ingestion	hypertension, nervousness	S	0,06
**Centaurium erythraea* Rafn	Gentianaceae	Qlilu	flowers	infusion	oral ingestion	anemia, diabetes, lack of appetite, nausea, indigestion	S	0,09
**Ceratonia siliqua* L.	Fabaceae	Akharouv	leaves	infusion	oral ingestion	diarrhea, nausea, stomach pain, fever, insomnia	S	0,04
			leaves	juice	inhalation	nasal congestion		
			leaves	juice	oral ingestion	vomiting		
Citrus limon (L.) Osbeck	Rutaceae	Lkares	fruits	juice	oral ingestion	tonsillitis, cold, general fatigue	C	0,09
			leaves	infusion	oral ingestion	influenza, cold, anxiety		
**Coriandrum sativum* L.	Apiaceae	Leksvar	seeds	infusion	oral ingestion	bloating	S, C	0,06
			seeds	crushed	massage	joint pain, rheumatism		
Crataegus monogyna Jacq.	Rosaceae	Idhmim	fruits	infusion	oral ingestion	insomnia, hypertension	S	0,06
**Cupressus sempervirens* L.	Cupressaceae	Thaydha	resin	raw	local application	scars	C	0,09
			leaves	decoction	oral ingestion	influenza, cold		
			bark	powder	oral ingestion	stomach ulcer		
Cydonia oblonga Mill.	Rosaceae	Thakthounia	leaves	infusion	oral ingestion	colon pain	C	0,04
			fruits	cooking	oral ingestion	indigestion		
Cynara cardunculus L.	Asteraceae	Thaga	leaves	raw	oral ingestion	indigestion	S, C	0,06
Cynodon dactylon (L.) Pers.	Poaceae	Affar	leaves	infusion	oral ingestion	colon pain	S	0,04
			leaves	decoction	oral ingestion	kidney stones		
Daphne gnidium L.	Thymelaeaceae	Alezaz	leaves	decoction	oral ingestion	constipation	S	0,04
Daphne laureola L.	Thymelaeaceae	Telt drar	leaves	powder	oral ingestion	constipation	S	0,02
**Daucus carota* L.	Apiaceae	Thazdelt	seeds	crushed	poultices	burns, furuncles	S	0,04
**Daucus carota* subsp. *sativus* (Hoffm.) Arcang.	Apiaceae	Zrodia	roots	raw	oral ingestion	vomiting, heartburn, diarrhea	C	0,04
			roots	paste	mask	rough skin		
Dittrichia viscosa (L.) Greuter	Asteraceae	Amagraman	leaves	decoction	poultices	rheumatism, muscle aches	S	0,28
			leaves	juice	local application	scars, hemorrhages		
			aerial part	infusion	oral ingestion	colon pain		
			leaves	paste	local application	cracks in the feet		
Ecballium elaterium (L.) A.Rich.	Cucurbitaceae	Afequs lehmir	leaves	infusion	oral ingestion	hemorrhoids, jaundice	S	0,04
Erica arborea L.	Ericaceae	Akhlenj	flowers	decoction	oral ingestion	indigestion, nervousness	S	0,04
**Eriobotrya japonica* (Thunb.) Lindl.	Rosaceae	Thouvrest	leaves	decoction	oral ingestion	menstrual pains, indigestion	C	0,11
Eucalyptus globulus Labill.	Myrtaceae	Kalytous	leaves, flowers	infusion	inhalation	bronchial evacuation, cold, cough	C	0,04
			bark	decoction	mouthwash	dental pain		
Ferula communis L.	Apiaceae	Awli, Afougel	stem	splints	local application	fracture	S	0,06

Ficus carica L.	Moraceae	Thiqlets	fruits	raw	oral ingestion	general fatigue, constipation	S, C	0,15
Foeniculum vulgare (Mill.) Gaertn.	Apiaceae	Besvas	leaves, seeds	infusion	oral ingestion	bloating, lack of appetite, intestinal worms	S, C	0,06
			roots	decoction	oral ingestion	diarrhea, menstrual pains		
Fraxinus angustifolia Vahl	Oleaceae	Aslen	leaves	infusion	oral ingestion	fever	S, C	0,17
			bark	decoction	oral ingestion	stomach ulcer		
			seeds	powder	poultices	scars, joint pain		
**Fumaria officinalis* L.	Papaveraceae	Thijujar n yesghi	leaves	crushed	local application	rheumatism	S	0,04
Glebionis segetum Fourr.	Asteraceae	Wamlal	leaves	raw	oral ingestion	indigestion	S	0,04
			aerial part	juice	local application	rough skin, varicose veins		
Globularia alypum L.	Plantaginaceae	Thasselgha	leaves	infusion, decoction	oral ingestion	asthma	S	0,06
**Hyoscyamus albus* L.	Solanaceae	Bounarjouf	leaves	cooking	oral ingestion	infertility	S	0,02
			leaves	powder	mask	acne and pimples		
Hyoseris radiata L.	Asteraceae	Tughmas n temgharin	leaves	salad	oral ingestion	colon crisis, digestive disorders, bloating	S	0,16
**Ilex aquifolium* L.	Aquifoliaceae	Iskerchi	leaves	decoction	oral ingestion	?	S	0,04
Isatis djurdjurae Coss. & Durieu	Brassicaceae	Messlama	leaves	decoction	poultices	furuncles, abcesses	S	0,04
Juglans regia L.	Juglandaceae	Thajujets	roots (bark)	maceration	gargle, mouthwash	oral conditions, gum infection	C	0,06
			leaves	decoction	bath, rinçage	dull hair, bad foot odor		
Juniperus oxycedrus L.	Cupressaceae	Taqqa	leaves	decoction	oral ingestion	insomnia	S	0,02
Laurus nobilis L.	Lauraceae	Arihan, arend	leaves	infusion	oral ingestion	indigestion, insomnia	S, C	0,13
			leaves	decoction	oral ingestion	intestinal pain		
Lavandula stoechas L.	Lamiaceae	Amezir beghyul	flowers	infusion	oral ingestion	heartburn	S	0,11
			leaves	decoction	bath	scabies		
Lawsonia inermis L.	Lythraceae	L'Henni n yifer	leaves	powder	bath	eczema, hair loss	C	0,03
Lonicera etrusca Santi	Caprifoliaceae	Anaref	leaves, flowers	infusion, decoction	oral ingestion	angina, cough	S	0,06
Malva multiflora (Cav.) Soldano, Banfi & Galasso	Malvaceae	Mejjir	leaves	raw	poultices	boils, mumps	S	0,23
				infusion	inhalation	sinusitis		
**Malva sylvestris* L.	Malvaceae	Mejir	flowers	infusion	inhalation	bronchitis, cough	S	0,06
Marrubium vulgare L.	Lamiaceae	Marnuyeth	leaves	decoction	oral ingestion	diarrhea, intestinal worms, fever	S	0,34
			leaves, roots	crushed	poultices	weakness of children (thagdhit)		
			leaves, roots	decoction	instillation	otitis		
Melissa officinalis L.	Lamiaceae	Ifer tzizwith	leaves	infusion	oral ingestion	bloating, insomnia	S	0,19
			leaves	decoction	inhalation	influenza		
Mentha pulegium L.	Lamiaceae	Flegu	leaves	infusion	oral ingestion	cold, insomnia	S	0,28
			leaves	decoction	oral ingestion	indigestion, anemia, lack of appetite		
			aerial part	maceration	oral ingestion	general fatigue		
Mentha spicata L.	Lamiaceae	Naanaa	leaves, flowers	infusion	oral ingestion	insomnia, influenza, cough, menstrual evacuation, vomiting	C	0,13
Mentha suaveolens Ehrh.	Lamiaceae	Thimeja	aerial part	infusion	oral ingestion	fever, dizziness	S	0,23
			leaves	decoction	compresses	diarrhea		

			leaves	decoction	rinses	hair loss		
**Myrtus communis* L.	Myrtaceae	Chilmoun	leaves	infusion, decoction	oral ingestion	cold, bronchitis, hemorrhoids	S	0,04
Nerium oleander L.	Apocynaceae	Ilili	leaves	infusion	mouthwash	oral infection	S	0,11
			leaves	latex	local application	scabies, pimples, warts		
Nigella damascena L.	Ranunculaceae	Sanoudj	seeds	maceration	oral ingestion	lack of appetite	S	0,06
			seeds	infusion	gargle	dental pain		
Ocimum basilicum L.	Lamiaceae	Lahvek	leaves	infusion	oral ingestion	galactogenic	S, C	0,06
Olea europaea L. subsp. *europaea* var. *europaea*	Oleaceae	Azemour	fruits	oil	oral ingestion	food poisoning, sore throat	C	0,26
			fruits	oil	massage	lumbago, furuncles		
			leaves, fruits	decoction	oral ingestion	hypertension		
Olea europaea L. subsp. *europaea* var. *sylvestris* (Mill.) Lehr	Oleaceae	Ahechadh, Azeboudj	leaves	decoction	local application	heavy legs, varicose veins	S	0,13
			bark	decoction	oral ingestion	indigestion		
**Ophrys apifera* Huds.	Orchidaceae	Thiheythin thimeythin	bulbs	cooking, powder	oral ingestion	sexual impotence	S	0,06
**Opuntia ficus-indica* (L.) Mill.	Cactaceae	Akarmous	flowers	infusion	oral ingestion	diarrhea	C	0,06
			fruits	raw	oral ingestion	diarrhea, general fatigue		
			cladodes	raw	poultices	sciatica		
Origanum vulgare subsp. *glandulosum* (Desf.) Ietswaart	Lamiaceae	Zaatar	aerial part	infusion	oral ingestion	diabetes, general fatigue, fever, influenza, cold, cough, angina, indigestion, lack of appetite, stomach pain, nausea, vomiting	S	0,43
				crushed	poultices, friction, mask	joint pain		
				decoction	inhalation	insomnia, nervousness, headache, migraine, dizziness		
Papaver rhoeas L.	Papaveraceae	Wahrir, Djihbut	flowers	infusion	oral ingestion	cough, insomnia	S	0,09
Paronychia argentea Lam.	Caryophyllaceae	Latay n'lakhla	flowers	infusion	oral ingestion	intestinal problems	S	0,06
**Peganum harmala* L.	Nitrariaceae	Elharmel	aerial part	maceration	rinses	rheumatism	S	0,04
Petroselinum crispum (Mill.) Fuss	Apiaceae	Maadnous	aerial part	decoction	oral ingestion	cardiotonic, hypertension	S, C	0,11
			aerial part	raw	local application	dental pain		
			leaves	juice	instillation	otitis		
**Pimpinella anisum* L.	Apiaceae	Hebet hlawa	seeds	decoction	oral ingestion	cough, bronchitis	C	0,02
**Pinus halepensis* Mill.	Pinaceae	Azoumbi	seeds, bark	decoction	mouthwash	canker sores, oral infection	S	0,04
Pistacia lentiscus L.	Anacardiaceae	Thidhekth	leaves	infusion	oral ingestion	hypertension, bronchial evacuation	S	0,17
			leaves	decoction	oral ingestion	colon pain, stomach pain, diarrhea, dizziness		
			leaves	powder	oral ingestion	hemorrhoids		
Plantago lanceolata L.	Plantaginaceae	Thahchicht n'hmed	leaves	powder	local application	eczema, skin fungus	S	0,30
			leaves	raw	poultices	abcesses, foot nails		
			leaves	juice	local application	hemorrhages, scars		
**Prunus armeniaca* L.	Rosaceae	Elkhoukh	leaves	juice	poultices	?	C	0,04
**Prunus avium* (L.) L.	Rosaceae	Ardherim	fruits (peduncles)	infusion	oral ingestion	urine retention, diarrhea	S	0,06

Prunus cerasus L.	Rosaceae	Lesriz, Heb lemlouk	fruits (peduncles)	infusion	oral ingestion	urine retention, kidney stones, diarrhea, stomach pain	C	0,15
**Pteridium aquilinum* (L.) Kuhn	Dennstaedtiaceae	Ifelkou	rhizomes	decoction	oral ingestion	intestinal worms	S	0,02
			rhizomes	oil	massage	joint pain		
Punica granatum L.	Lythraceae	Reman	fruits	juice	oral ingestion	cold, fever, diarrhea, hemorrhoids, lack of appetite	C	0,23
			fruits	maceration	gargle	canker sores, oral infection		
			bark	powder	oral ingestion	indigestion, vomiting		
Quercus rotundifolia Lam.	Fagaceae	Abeludh	bark	decoction	oral ingestion	stomach ulcer	S	0,13
			fruits	infusion	bath	urinary tract infection		
**Quercus suber* L.	Fagaceae	Akarouch	leaves	decoction	oral ingestion	indigestion	S	0,04
			bark	decoction	bath	heavy legs		
Rhamnus alaternus L.	Rhamnaceae	Imliles	roots	decoction	friction, mask	jaundice	S	0,02
**Nasturtium officinale* R.Br.	Brassicaceae	Garninouch	aerial part	paste	mask	acne and pimples	S	0,04
			aerial part	raw	oral ingestion	heartburn		
Rosa canina L.	Rosaceae	Iaâfar	seeds	powder	oral ingestion	urine retention,	S	0,15
			fruits	raw	oral ingestion	diarrhea		
Rosmarinus officinalis L.	Lamiaceae	Amezir, Thiklilt	leaves	infusion	oral ingestion	menstrual pains	S, C	0,11
			leaves	powder	oral ingestion	menstrual pains		
Rubus ulmifolius Schott	Rosaceae	Inigel	leaves, roots	decoction	oral ingestion	diabetes	S	0,30
			leaves	infusion	oral ingestion	hypertension		
			leaves	maceration	oral ingestion	tonsillitis, tooth decay		
			fruits	juice	oral ingestion	goiter		
			leaves	raw	local application	wounds, burns, hemorrhages		
Rumex conglomeratus Murray	Polygonaceae	Tassemumt n yezgaren	leaves	raw	poultices	boils, sores, indigestion, diarrhea	S	0,13
**Ruscus aculeatus* L.	Asparagaceae	Icher n'yizem	leaves	infusion	oral ingestion	fever, inflammation urinaire	S	0,02
			roots	decoction	oral ingestion	improves blood circulation		
Ruta angustifolia Pers.	Rutaceae	Awermi	flowers	infusion	oral ingestion	anemia, anorexia, stomach pain	S	0,19
			roots	raw	poultices	burns, scars		
**Salix alba* L.	Salicaceae	Issemlel	bark	decoction	oral ingestion	colon pain, rheumatism, sciatica	S	0,06
**Salvia officinalis* L.	Lamiaceae	Marissem	leaves	infusion	oral ingestion	indigestion, diabetes	C	0,06
Sambucus nigra L.	Viburnaceae	Arwuri	leaves	raw	poultices	back pain	S	0,06
Scolymus hispanicus L.	Asteraceae	Thilitsen, Taghediwth	leaves (ribs)	sauce	oral ingestion	physical weakness, indigestion	S, C	0,26
Scrophularia canina L.	Scrophulariaceae	Harm larvi	aerial part	raw	poultices	back pain, joint pain, rheumatism	S	0,16
Silene vulgaris (Moench) Garcke	Caryophyllaceae	Thaghighachth	roots	crushed	compresses	infertility	S	0,09
			aerial part	raw	oral ingestion	general fatigue		
**Solanum tuberosum* L.	Solanaceae	Batata	tubers	raw	local application	migraine, headache	C	0,09
Sonchus oleraceus L.	Asteraceae	Thifaf	leaves	juice	local application	scars, burns	S	0,09
			leaves, stems	raw	oral ingestion	kidney stones		
Tamarix gallica L.	Tamaricaceae	Amemmay	leaves	decoction	oral ingestion	asthma, cough	S	0,03

**Taraxacum erythrospermum* Andrz. ex Besser	Asteraceae	Thughmes temgharth	leaves aerial part	raw juice	oral ingestion friction	indigestion jaundice	S	0,04
**Taxus baccata* L.	Taxaceae	Thifuzalet	leaves	decoction?	?	urinary disorders, rheumatism	S	0,02
Teucrium polium L.	Lamiaceae	Jaada	leaves leaves	decoction powder	oral ingestion oral ingestion	menstrual evacuation infertility	S	0,02
Thapsia garganica L.	Apiaceae	Adviv	roots leaves	decoction crushed	oral ingestion poultices	general fatigue, indigestion joint pain	S	0,13
Thymus numidicus Poir.	Lamiaceae	Thimezirth	aerial part aerial part	infusion rinses	oral ingestion rinses	bloating, cholesterol, cough wounds, rough skin	S	0,19
**Tragopogon porrifolius* L.	Asteraceae	Thawelment	leaves	infusion	oral ingestion	urine retention	S	0,11
**Trigonella foenum-graecum* L.	Fabaceae	Thifidhas	seeds seeds seeds	infusion powder powder	oral ingestion maceration mask	anemia, nausea, nervousness asthma hair loss	C	0,26
**Tussilago farfara* L.	Asteraceae	Ifer budhi	leaves leaves	decoction rinses	oral ingestion rinses	cough, indigestion rough skin	S	0,02
**Ulmus minor* Mill.	Ulmaceae	Oulmou	roots leaves	infusion raw	bath poultices	hair loss, scars joint pain	S	0,06
Umbilicus rupestris (Salisb.) Dandy	Crassulaceae	Thichoufthin	leaves	heating	poultices	furuncles, pimples	S	0,02
Urtica dioica L.	Urticaceae	Azegdouf	aerial part leaves, roots	infusion infusion	oral ingestion bath	anemia hair loss, pellicules, rheumatism	S	0,19
**Vicia faba* L.	Fabaceae	Ivawen	flowers fruits (pods)	raw raw	inhalation friction	food poisoning skin fungus (thifiri)	C	0,04
Vitis vinifera L.	Vitaceae	Thara thaezgeghth	leaves leaves	infusion juice	oral ingestion bath	anxiety, nervousness circulatory disorders	S, C	0,09
**Zea mays* L.	Poaceae	Akvel	seeds flowers (beard)	cooking infusion	oral ingestion oral ingestion	diarrhea urine retention, rough skin	C	0,06

3.3. Plants parts used, preparation and administration mode

The local population of the DBR uses plants mainly in their fresh form (68.46% of citations). This is linked to the direct relationship between local populations and nature and the daily harvest of fresh plants, which may eventually be also used for food. Conversely, the local population has little recourse to dried plants (12.61%). In addition, 18.91% of plants are used fresh or dried. Leaves are the most frequently used part in traditional medicine recipes, accounting for 48.51% of all citations by informants (Figure 3). This wide use of leaves has been largely reported in other similar ethnobotanical studies conducted in the Maghreb countries (Benkhnigue et al. 2011; Boutabia et al. 2011) or elsewhere (Leto et al. 2013). This can be explained by the availability and ease of collecting the leaves (Nasution et al. 2018), and as the leaves are the main organs of photosynthesis (Susanti and Zuhud 2019), which have chemicals that could be responsible for medicinal effects (Balick and Cox 1997). Otherwise, leaf collection, when moderate, does not damage or compromise the development of the plants (Brito et al. 2017). The underground vegetative organs (roots, bulbs, rhizomes, and tubers) come second with 12.87%. Aerial parts and fruits in third place (8.41% each), seeds (6.93%) and flowers (6.43%) follow them. The other organs (barks, stems) are mentioned less (5.94% and 2.47% respectively).

Infusion and decoction are the most common preparations among local populations of DBR, accounting for 26.53% of all citations each, followed by raw consumption with 13.78%. This reflects the ease of preparing the infusion and decoction with water. This is likewise the case in other studies (Estrada et al. 2007), where these preparations play a very important role in local traditional medicines, both for oral and topical routes (Vinagre et al. 2019). On the other hand, it is often enough for local populations of the DBR to consume wild plants uncooked (as salads), to cure the common diseases from which they suffer. Lastly, the other methods of preparation (juice, maceration powder, cooked, crushed and others) are cited with amounts of less than 10% each.

Oral ingestion is the predominant mode accounting for 58% of all citations. This high rate is related to the large number of internal diseases (digestive, circulatory and respiratory disorders) encountered during our study. The other modes of internal administration (inhalation, instillation, gargles and mouthwashes) are represented with 8.5% in all. In consent with Vinagre et al. (2019), internal administration, essentially the oral route, represents the most recommended mode. Local applications come in second, with 9.5% of citations, followed by other modes used externally, such as baths and rinses (9%), poultices (8.5%), masks, and friction (6.5%). They are mainly linked to skin ailments, but also musculoskeletal problems.

3.4. Traditional medicinal uses of recorded plant species

A wide range of 83 diseases and symptoms, reported by the local population of DBR during our investigation, were assembled into 10 disease groups, based on the body system (Table 3). The number of diseases in each group varies between 3 and 17. Three groups (circulatory, digestive, and skin disorders) include 10, 14 and 17 treated diseases, respectively. They are followed by mental-nervous, genitourinary group (8 each), respiratory group (7), skeletomuscular group (6), oral-dental and other diseases groups (5 each), and endocrine-metabolic-nutritional group (3).

Table 3. Major disease categories, diseases treated, and number of plant species used in the study area

Disease categories	Number of diseases treated	Number of species used per group	Diseases (number of species used per disease)
Digestive diseases	14	63	**indigestion (23), diarrhea (16)**, stomach pain (10), bloating (7), colon pain (7), lack of appetite (7), nausea (6), constipation (3), heartburn (3), intestinal worms (3), stomach ulcer (3), vomiting (3), food poisoning (1), hepatic-biliary insufficiency (1)
Skin diseases	17	35	**scars (11),** rough skin (8), hair loss (6), furuncles (4), abscesses (3), scabies (2), acne and pimples (2), wart (2), skin fungus (2), eczema (1), dandruff (1), burns (1), wound (1), bites (1), cracked feet (1), dull hair (1), bad foot odor (1)
Cardiovascular and circulatory diseases	10	27	anemia (7), hypertension (6), hemorrhages (4), hemorrhoid (4), jaundice (3), heavy legs (2), varicose veins (2), circulatory disorders (2), heart weakness (1), frostbite (1)
Respiratory diseases	7	23	**cough (11),** cold (9), influenza (5), bronchitis (3), angina (3), asthma (2), discharge of mucus (2)
Mental-nervous diseases	8	21	insomnia (9), headache (5), nervousness (4), sciatica (2), anxiety (2), dizziness (2), migraine (2), anorexia (2)
Other diseases	5	18	general fatigue (9), fever (8), otitis (4), tiredness (2), weakness of children (1)
Genitourinary and reproductive diseases	8	17	urine retention (7), kidney stones (4), infertility (3), menstrual pains (2), menstruation evacuation (2), genital impotence (1), galactogenic (1), urinary tract infection (1)
Skeletomusclar diseases	6	13	rheumatism (7), joint pain (5), muscle aches (2), lumbago (1), fracture (1), back pain (1)
Oral-dental diseases	5	12	oral infection (5), dental pain (5), tonsillitis (3), canker sores (2), gum infection (1)
Endocrine, metabolic and nutritional diseases	3	9	diabetes (6), cholesterol (1), goiter (1)

The disease group that is treated by the largest number of plants (63 in total) is those of digestive disorders (indigestion, diarrhea, stomach pain). This disease group is the most treated in several Mediterranean countries (Gonzalez-Tejero et al. 2008), thus confirming our observations and giving a broad character to this fact. The group of skin conditions takes second place, with 35 plants used to cure them; third place goes to the group of cardiovascular-circulatory disorders (27), fourth to respiratory problems (23). Nervous-mental group (21), other diseases (18), genitourinary group (17), skeletomuscular group (13), oral-dental group (12), and endocrine-metabolic-nutritional group (9) follow them. The predominance of digestive, skin and respiratory disorders treated by local populations is similarly observed in several Mediterranean countries (Scherrer et al. 2005; Gonzalez-Tejero et al. 2008; di Sanzo et

al. 2013; Menale et al. 2016). This is also the case in non-Mediterranean areas (Sousa et al. 2012; Safeer et al. 2017). Out of the 83 diseases identified in our survey, 31 of them are commonly treated with a large number of plants, at least four (Figure 2). Indigestion and diarrhoea are the most treated ailments by the local population, with 21 and 16 species respectively, followed by cough and scars, with 11 species each, and stomach pains, with 10 species. In addition, nine species can treat insomnia, cold or general fatigue. According to Belda et al. (2013), most of the reported species were likewise used to treat indigestion and diarrhea. In other studies, medicinal plants are mentioned mainly for the same therapeutic uses, indigestion (Sousa et al. 2012) or diarrhea (Qureshi 2012). Finally, the 41 remaining diseases are treated with one or two species.

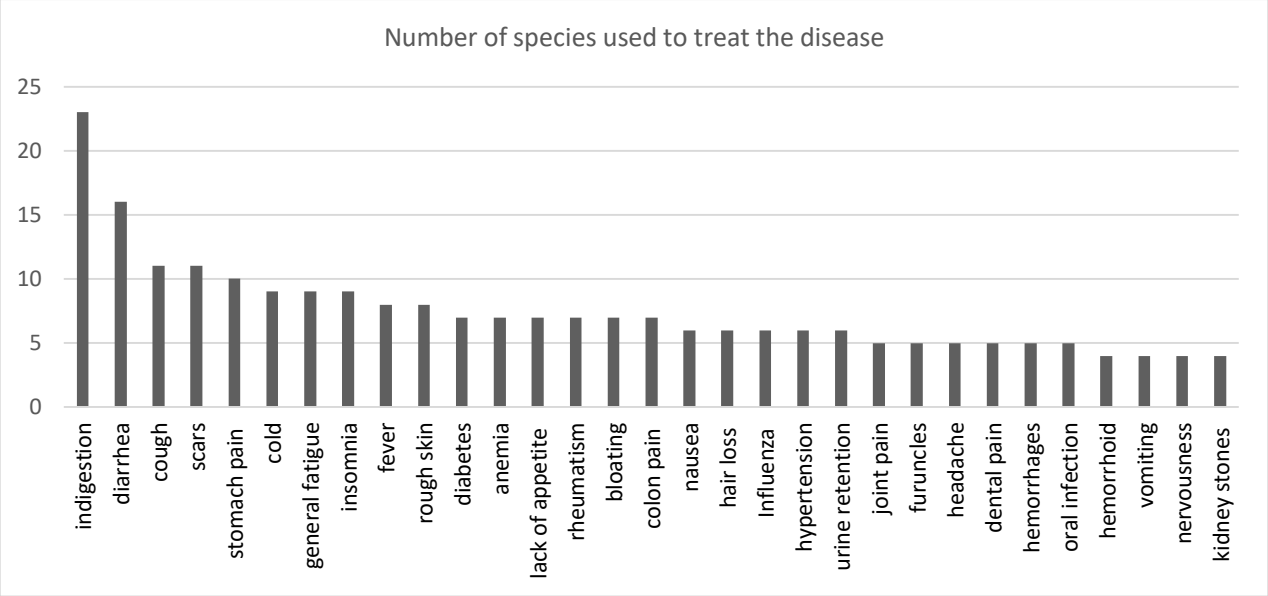

Figure 2. The most treated diseases and the number of species used in each case (≥ 4)

The DBR villagers use 22 medicinal plants to treat a minimum of five diseases per plant (Table 4). Oregano (*Origanum vulgare* L. subsp. *glandulosum* treats the maximum number of diseases (18), such as diabetes, insomnia, headache, digestive disorders (indigestion, stomach pain, nausea …) and respiratory problems (cough, cold, influenza, and angina). Then, *Rubus ulmifolius* Schott is used in eight therapeutic usages (diabetes, wounds, burns, hypertension, tonsillitis, haemorrhage, toothache, goitre). To treat seven diseases (angina, lumbago, back pain, hypertension, sore throat, food poisoning, furuncles), local people use the olive tree (*Olea europaea* L. subsp. *europaea* var. *europaea*); its oil is considered a panacea in local pharmacopoeia. These plants are followed by *Artemisia absinthium*, used to remedy seven diseases, such as anorexia, diabetes, fever, and digestive disorders (diarrhoea, stomach pain, nausea, vomiting), *Punica granatum* L. (diarrhea, indigestion, cold, haemorrhoids, canker sores…), and *Pistacia lentiscus* L. (colon pain, stomach pain, diarrhoea, hypertension, dizziness, etc.). The greater figures of therapeutic uses of these species can be explained by their abundance in the study area and by their reputation in the local pharmacopoeia. Lastly, the majority of the plants (82%) are used to treat one to four ailments each.

Table 4. Classification of the most used plants treating more than five diseases

Medicinal plants treated	Relative Frequency of citation (RFC)	Number of diseases
Origanum vulgare subsp. *glandulosum* (Desf.) Ietswaart	0,43	18
Rubus ulmifolius Schott	0,3	8
Olea europaea L. subsp. *europaea* var. *europaea*	0,26	7
Artemisia absinthium L.	0,23	7
Punica granatum L.	0,23	7
Pistacia lentiscus L.	0,17	7
Plantago lanceolata L.	0,3	6
Mentha pulegium L.	0,28	6
Thymus numidicus Poir.	0,19	6
Urtica dioica L.	0,19	6
Petroselinum crispum (Mill.) Fuss	0,11	6
Ceratonia siliqua L.	0,04	6
Marrubium vulgare L.	0,34	5
Dittrichia viscosa (L.) Greuter	0,28	5
Trigonella foenum-graecum L.	0,26	5
Asplenium ceterach L.	0,21	5
Ruta angustifolia Pers.	0,19	5
Fraxinus angustifolia Vahl	0,17	5
Mentha spicata L.	0,13	5
Centaurium erythraea Rafn	0,09	5
Citrus limon (L.) Osbeck	0,09	5
Blackstonia grandiflora (Viv.) Maire	0,04	5

3.5. New or rarely reported medicinal plants and their uses

To evaluate the degree of originality of the ethnoflora reported in this study, we have compared our list of medicinal plants with: i) those reported in other regions of Kabylia (Ait Youssef 2006; Meddour and Meddour-Sahar 2015), ii) a large corpus of recently published articles (about 50) on Algerian ethnobotany (e.g. Gonzalez-Tejero et al. 2008; Boutabia et al. 2011; Bounar et al. 2013; Benarba et al. 2015; Boughrara and Belgacem 2016; Ouelbani et al. 2016; Benaissa et al. 2018; Souilah et al. 2018; Hamel et al. 2018; Hadjadj et al. 2019; Chohra and Ferchichi 2019; Lazli et al. 2019; Baziz et al. 2020, Zatout et al. 2021, etc.), and iii) the information available for Algeria in the Prelude database (2022).

From this comparison, it appears that eight species did not appear in this recent bibliography on medicinal plant uses in Algeria. Those are *Acer monspessulanum* and *Acer obtusatum* (both fights hair loss). *Blackstonia grandiflora* (Viv.) Maire (anaemia, diabetes, lack of appetite, nausea, indigestion), *Celtis australis* L. (hypertension, nervousness), *Daphne laureola* L. (constipation), *Isatis djurdjurae* (furuncles, abscesses), *Ophrys apifera* Huds. (sexual impotence) and *Tragopogon porrifolius* L. (diuretic). These plants are cited for the first time in Algeria as

medicinal, and their traditional therapeutic uses are therefore to be considered new and previously undocumented for the Algerian pharmacopoeia. Some other plants are rarely reported in Algerian ethnobotanical studies. They are confined in forest ecosystems of mountainous humid area. We will review them underneath.

Ilex aquifolium, used as a diuretic by local population of Djurdjura, is likewise diuretic and treats rheumatism in the Northeastern part of Algeria (Hamel et al. 2018). *Lonicera etrusca*, used to treat respiratory problems (angina, cough) in Djurdjura, is known as suitable for skin problems in Belezma National Park (East of Algeria) (Chohra and Ferchichi 2019). *Ruscus aculeatus* L., which improves blood circulation, and treats fever and urinary inflammation in the DBR, is mentioned for the treatment of several diseases (cardiovascular diseases, digestive disorders, spasms, sinusitis, and psoriasis) in the regions of Constantine and Mila (Ouelbani et al. 2016). *Taxus baccata* is used to manage urinary disorders and rheumatisms according to local population of Djurdjura. Elsewhere in Algeria, it is known to treat reproductive problems in Mitidja, Algiers region (Gonzalez-Tejero et al. 2008), anaemia and nervousness in Eastern Algeria (Ouelbani et al. 2016). *Tussilago farfara*, which has several local uses (cough, indigestion, rough skin) in Djurdjura, is similarly used to treat cough in Northwest of Algeria (Benarba et al. 2015). Finally, *Umbilicus rupestris* (Salisb.) Dandy, whose leaves treat skin disorders (furuncles, pimples) in Djurdjura, is used precisely to treat skin diseases (inflammation, rough skin (as softener), wound healing) in Aures mountains at eastern Algeria (Baziz et al. 2020), and also in Italy (Gonzalez-Tejero et al. 2008). In the northern Mediterranean rim, the leaves of this plant are used against inflammation of the skin, wounds, burns, and as an ophthalmic disinfectant (Benhouda et al. 2014). There is therefore a high consensus on the therapeutic indications of this plant in the Mediterranean basin.

4. Conclusion and outlooks

This ethnobotanical survey in the Djurdjura Biosphere Reserve, among rural populations isolated in a mountainous area, permitted the faithful transcription of noteworthy medicinal knowledge. The ethnobotanical data acquired are evidencing a diversity of medicinal flora and traditional therapeutic uses of plants and a vital role of the protected area in preserving the human health of local populations. The long-lasting interest of local populations in the therapeutic virtues of plants was clearly reflected in the important number of medicinal plant species (more than a hundred) used to manage primary human health care. Indeed, the local populations have succeeded in preserving the ancestral therapeutic practice of plants until today, by a use of plants deeply rooted in the local tradition that can make a significant contribution to sustainable development.

Finally, if management and decision-making are conducted through a participatory approach, local populations can play a crucial role in the *in situ* conservation of plant resources, by incorporating their traditional ecological knowledge (TEK) into a strategy for the sustainable management of this protected area, of national and international importance.

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Author Contribution

RM and SO settled the research and data collection protocol, and wrote the manuscript. RM did the fieldwork. SO performed the data analysis and processing.

Conflicts of Interest

The authors reported no conflict of interest.

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Impacts of Essential Oil and Extracts Obtained from Coriander Cultivars (*Coriandrum sativum* L.) on Important Some Pathogenic Bacteria

Esma ERSİN[1*], Belgin COŞGE ŞENKAL[2*]

[1] Department of Field Crops, Faculty of Agriculture, University Yozgat Bozok, 66900, Yozgat, Türkiye
[2] Department of Field Crops, Faculty of Agriculture, University Yozgat Bozok, 66900, Yozgat, Türkiye

*Corresponding author : belgin.senkal@yobu.edu.tr

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Abstract

In this study, the antibacterial activity of the essential oil obtained from the seeds of two coriander (*Coriandrum sativum* L.) cultivars (Gürbüz and Arslan) and extracts obtained from the seeds, leaves and stems with methanol and ethanol against seven Gram-negative (*Escherichia coli* ATCC®25922, *Pseudomonas aeruginosa* ATCC®9027, *Salmonella typhimurium* ATCC®14028, *Serratia marcescens* ATCC®13880, *Proteus vulgaris* ATCC®6380, *Enterobacter cloacae* ATCC®13047, and *Klebsiella pneumoniae* ATCC®4352), and one Gram-positive bacteria (*Staphylococcus aureus* ATCC®6538) were evaluated. Summer sowing was carried out for the supply of herbal materials and the plants were grown in Yozgat / TURKEY climatic conditions. Linalool (average 74%) has been noted as the main component in essential oils, and γ-terpinene, geraniol and camphor have been identified as other important components. It has been determined that essential oils exhibit varying levels of activity against *S. aureus, E. coli, S. tyhimurium, S. marcescens, P. vulgaris*, and *E. cloacae* bacteria in this study. Especially, the essential oil obtained from Arslan cultivar exhibited strong activity against *P.aeruginosa* and *S.aureus* bacteria.

Key Words: Clevenger, Fruit, GC/MS, Kirby-Bauer Method, Linalool, Methanol

1. Introduction

Coriander (*Coriandrum sativum* L.) is an economically important spice and essential oil plant from the Apiaceae (Umbelliferae) family. It is an annual herbaceous structure. Light or dark green leaves are segmented and usually 3-lobed. The flowers are white or light pink in the form of umbrellas at the ends of the branches and bloom in June-July. The fruits are light yellow-brown, spherical schizocarp with a diameter of 2-4 mm. 1000 fruit weight varies between 5-18 g (Diederichsen and Hammer, 2003; Yeung and Bowra, 2011). The leaves, stem, flowers and fruits of the plant contain high levels of essential oil. The amount and chemical composition of the essential oil differ according to the organs of the plant (Mandal and Mandal, 2015).

The predominant component in the essential oil obtained from coriander fruits is linalool. In some essential oils, the linalool content can reach up to 80%. The essential oil contains "α-pinene, ɣ-terpinene, camphor, geranial acetate, geraniol, borneol, citronellol, thymol,

β-caryophyllene and caryophyllene oxide", although at lower concentrations than this component (Zekovića et al., 2011; Vasconcelos Dos Santos et al., 2019; Satyal and Setzer, 2020; Al-Khayri et al. 2023).

Coriander has essential oil that exhibits broad biological activity (antifungal, antibacterial, antioxidant, antibacterial, antidiabetic, antidepressant etc.) (Gkogka et al., 2013; Al-Khayri et al. 2023). Studies have shown that coriander essential oil has determined the inhibitory efficacy against *Bacillus subtilis, Candida albicans, Enterococcus faecalis, Enterococcus faecium, Klebsiella pneumoniae, Listeria innocua, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella infantis*, and *Salmonella kentucky*. (Serban et al., 2011; Niamah and Alali 2016; Ozkinali et al., 2017; Rizk et al., 2022; Al-Khayri et al., 2023). In this study, it was aimed to determine to what extent essential oils and extracts of coriander cultivars grown in cultural conditions inhibit the growth of important pathogenic bacteria.

2. Material and Methods

2.1. Plant Material

Two coriander cultivars (cv. Arslan-large fruited and cv. Gürbüz- small fruited) registered by Ankara University/TURKEY were used as plant material.

2.2. Production area

Production of plant material to be used in analysis was carried out in Yozgat Bozok University, Research and Application Area (Yozgat/TURKIYE; Locality: 39° 45' 08" N, 34° 48' 11" E, Altitude:1267 m). The precipitation, temperature and humidity values of the vegetation period of the production area, and soil properties are given in Figure 1 and Table 1, respectively.

Table 1. The soil analysis results of the production area (Yakupoglu, 2018)

Clay	Silt	Sand	pH	Salt	$CaCO_3$	Organic matter	Total N
(g kg^{-1})				(%)			
476	138	386	7.09[1]	0.178[2]	7.15[3]	2.49[4]	0.15[5]
P	K	Ca	Mg	Fe	Cu	Zn	Mn
(µg g^{-1})							
78[6]	728[6]	7060[6]	5604[7]	8.08[6]	2.84[5]	0.62[8]	4.07[8]

1 Neutral, 2 Slightly salty, 3 Medium calcareous, 4 Medium, 5 Enough, 6 Much, 7 Excessive, 8 Little

The semi-arid continental climate of the Central Anatolia Region is dominant in the Yozgat Province. Summers are hot and dry, as it is closed to sea influence; winters are cold and rainy. Temperature differences between day and night, and summer and winter are high. The total precipitation, the average temperature and the average relative humidity were 177 mm, 18.74 C, and 45.24%, respectively in the production area during the vegetation (Figure 1). The production area with medium level of organic matter has a heavy structured soil (Table 1).

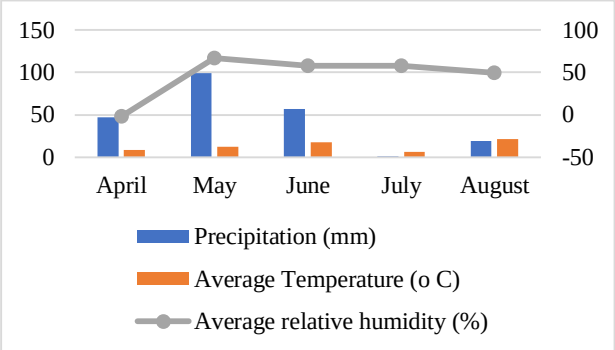

Figure 1. Climate data recorded during vegetation

2.3. Production practices

The seeds of the coriander cultivars were 30 cm between rows and 3 m in row length, 1 g of seeds were planted in rows. Sowing was made on April 21, 2017, with 30 rows of each variety. Weed control has been done when necessary. Any irrigation and fertilization have not been done. Plants were harvested in two different periods.

Period 1: Fresh plant harvesting: All above ground parts of plants were collected when the plants were in full flowering stage. (5 July 2017, 12:00)

Period 2: Dried plant harvesting: During the seed ripening period, all plant parts were harvested (12 August 2017, 11:00). The harvested fresh plants (Period 1) were dried in the shade. Plants in Period 1 were divided into three parts as flower, stem and leaf; Plants in Period 2 were divided into two parts as seed and stem (Figure 2).

Figure 2. Development stages of coriander and its fruits

2.4. Preparation of extracts

The dried plant parts (flower, green stem, green leaf, seed and dry stem) were ground separately with a laboratory type blender. The dry matter of 1 g from the flowers, 2 g from the green stem and leaf, and 4 g from the dry stem was used. Two different solvents (methanol and ethanol, 1/10 w / v) were used in the study. The samples mixed with the solvent were first incubated in the oven (Elekto-mag M 5040p) at 40 ° C for 24 hours. Then, the solvents were removed from the extracts filtered through Whatman No 1 filter paper with the help of a rotary evaporator, and then it was left in the oven for another 24 hours to ensure complete drying. The dried extracts were dissolved in 2 ml of methanol and stored at +4 ° C until analyzed (Zakaria et al., 2019).

2.5. Essential oil extraction

100 g of the ground seeds of the coriander varieties were weighed and after they were placed in 2 L balloon flasks, 750 mL of water was added to them. It was subjected to water distillation in Clevenger device for 3 hours. Essential oil values (%, v/w) were calculated as volume on dry matter. The essential oils obtained as a result of distillation were kept at +4 C in dark glass bottles until analyzed (Baj et al., 2015).

2.6. Determination of the chemical composition of essential oil

Chemical component determination of essential oils ob tained as a result of the research was made by Shimadzu, QP2010 ULTRA Gas Chromatography-Mass Spectrometer (GC / MS) at Yozgat Bozok University, Science and Technology Application and Research Center. Separation of the components was made on an Rxi-5ms capillary column with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 µm. The split rate was set to 10, the flow rate was set to 1.10 mL/min. In the analysis, a temperature program that ends at 70°C for 1 minute, with an increase of 20°C/min to 180°C, 1 minute at 180°C, and 280°C with an increase of 10°C/min was applied. Helium was used as the carrier gas, and the injector temperature was kept at 250°C. Substances exiting the column were screened in the mass range of

50-550 (m/z) (70eV). The transfer temperature was set to 250°C and the ion source temperature to 200°C. Identification of components was done using FFNSC 1.2 library search software and literature data (Babushak et al., 2011).

2.7. Antibacterial activity test

In the study, three different medium was prepared, as below:
1:Tryptic Soy Agar (TSA, a general purpose growth medium for a wide variety of microorganisms),
2:Tryptic Soy Broth (TSB, a high nutrient-containing bacteria and fungus growth medium,) and
3:Muller-Hinton Agar (MHA, having international validity in antimicrobial susceptibility tests). The contents of the medium are given in Table 2. While preparing the medium, 40 g TSA, 30 g TSB and 38 g MHA were weighed on a precision scale and mixed with 1L distilled water, then autoclaved at 121 °C for 15 minutes to sterilize the media. Medium were stored at + 4C until analysis.

Disk diffusion technique (Kirby-Bauer method) was used to evaluate the antimicrobial activity (Prescott et al., 1990). In the tests, 7 Gram-negative (*Escherichia coli* ATCC®25922, *Pseudomonas aeruginosa* ATCC®9027, *Salmonella typhimurium* ATCC®14028, *Serratia marcescens* ATCC®13880, *Proteus vulgaris* ATCC®6380, *Enterobacter cloacae,* ATCC®13047, and *Klebsiella pneumoniae* ATCC®4352), and 1 Gram-positive bacteria (*Staphylococcus aureus* ATCC®6538) were used. These pathogenic organisms were selected for the study considering their clinical and pharmaceutical importance (Table 3). Bacteria were transferred to test tubes containing 2 ml TSB and incubated in an oven at 37 ° C for 3 hours, and at the end of 3 hours, each microorganism was spread on TSA containing medium and inoculated.

Table 2. TSA, TSB and MHA contents

Tryptic Soy Agar (TSA)	
Typical Formula	**gL^{-1}**
Pancreatic Digest of Casein	15.0
Piapiac Digest of Soybean Meal	5.0
Sodium chloride	5.0
Agar (gelatin)	15.0
pH =7.3 ± 0.2	
Tryptic Soy Broth (TSB)	
Typical Formula	**gL^{-1}**
Pancreatic Digest of Casein	17.0
Soybean Meal Piapiac Digest	3.0
Sodium chloride	5.0
Dipotassium Phosphate	2.5
Glucose	2.5
pH =7.3 ± 0.2	
Mueller-Hınton Agar (MHA)	
Typical Formula	**gL^{-1}**
Beef, dried infusion	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
pH=7.3 ± 0.1	

It was left in the oven at 37 ° C for another 2 days. At the end of the two days, 4-5 loops bacteria were taken from pure cultures, transferred to medium containing 20 ml TSB and incubated at 37 ° C until the morning. 0.5Mc Farland (McF) unit bacteria were put into tubes containing 2 ml TSB with the help of a densitometer. Then, 100 µl of the obtained suspension was taken and spread in Petri dishes containing MHA and the medium were prepared for antibacterial test. 5 separate antibiotic discs "Erythromycin (15µg), Ampicillin (10 µg), Carpenicillin (100µg), Tetracycline (30µg), Chloramphenicol (30µg)" were used for controls. All stages were carried out in a laminar flow sterile cabin. Filter papers to be used in the study were cut in 0.22 µm shape and autoclaved for 15 minutes at 121 ° C and sterilized. With the help of a densitometer, 0.5Mc Farland (McF) unit was set from the bacteria, 100 µl was drawn with a pipette, transferred to the medium containing MHA and spread on the media with an iron loop and waited for 30 minutes. Sterile discs were dipped in extracts (5 mL) and essential oils (2 mL), and the excess water was removed on sterile blotting paper and placed in petri dishes. The trial was set

up in 2 replications. Petri dishes were kept in the oven at 37 ° C for 24 hours, and then, the diameters of the inhibition zones around the discs were measured in mm with the help of a digital caliper. (Figure 3).

Table 3. The effects of bacteria used in the research on human health (WHO, 2001; Hogg 2005)

Pathogen	Diseases
Escherichia coli	Urinary tract infection, Chronic renal failure, Stomachache, Diarrhea, Vomiting, Dysentery, Meningitis, Liver abscess
Pseudomonas aeruginosa	Eye disease, External and middle ear infection, Burn and wound infections, Meningitis, Bronchitis, Cramp, Nausea, Epidemic diarrhea, Death in infants
Salmonella typhimurium	Fever, Nausea, Headache, Diarrhea, Tuberculosis, Meningitis
Serratia marcescens	Urinary tract, upper respiratory tract and wound infections, Meningitis
Proteus vulgaris	Urinary tract and wound infections, Meningitis Diarrhea, Sepsis (organ failure)
Enterobacter cloacae	Urinary tract infection, Fever, Shortness of breath, Cough
Klebsiella pneumoniae	Sudden fever, Wound infections, Shortness of breath, Bronchitis, Heart disease
Staphylococcus aureus	Meningitis, Vomiting, Fatigue, Inflamed wounds, Sweating, Skin and Organ infections

All tests and analyzes performed in the study were carried out with 3 repetitions, and the mean of the values was given with the standard deviation (mean ± SD). Antibacterial activity results were evaluated according to the criteria reported by Davis and Stout (1971) (Table 4).

Table 4. The criteria for evaluating antibacterial activity

Inhibition zone diameter (mm)	Evaluation
<5	Weak
5-10	Moderate
10-19	Strong
>20	Very strong

3. Results and Discussion

3.1. Extract yield

Flowers, leaves and stems were harvested during the flowering period, and seeds and stem were harvested at full maturity. The values obtained from the extracts prepared by using ethanol (E) and methanol (M) of these plant samples are given in Table 5.

Table 5. Extract amount and yield of plant samples

Fresh Plant Organs				
Cv.	Plant Organ	Solvent[1]	Extract Amount (g)	Extract Yield (%)
Arslan	Flower	E	0.027±0.0022	2.54±0.20
		M	0.080±0.0046	7.63±0.18
	Leaf	E	0.037±0.0035	1.77±0.15
		M	0.083±0.0075	5.67±0.38
	Stem	E	0.017±0.0012	0.80±0.06
		M	0.045±0.0009	2.23±0.07
Gürbüz	Flower	E	0.021±0.0004	2.02±0.07
		M	0.077±0.0030	7.37±0.31
	Leaf	E	0.021±0.0010	1.05±0.05
		M	0.088±0.0092	4.31±0.46
	Stem	E	0.018±0.0014	0.86±0.07
		M	0.053±0.0040	2.60±0.23
Dry Plant Organs				
Cv.	Plant Organ	Solvent	Extract Amount (g)	Extract Yield (%)
Arslan	Seed	E	0.029±0.0029	1.42±0.13
		M	0.038±0.0031	1.87±0.19
	Stem	E	0.026±0.0052	0.32±0.26
		M	0.017±0.0049	0.82±0.25
Gürbüz	Seed	E	0.061±0.0264	2.99±1,29
		M	0.042±0.0046	2.03±0.25
	Stem	E	0.038±0.0030	0.19±0.14
		M	0.011±0.0022	0.52±0.97

The extract yields of the fresh plant parts obtained from the flowering period were higher than the seed and stem obtained from the full maturity period. In general, it was observed that the yields of extracts prepared using methanol were higher than those prepared with ethanol. Palmieri et al.

(2020) reported that the yields of extracts prepared using different traditional and non-traditional methods in coriander fruits varied between 0.57-2.36% (w / w). Jangra et al. (2018), who prepared extracts from coriander leaves using acetone, ethanol and water, obtained extract yields (g 100 g-1) of 2.22-2.45, 2.59-3.35, and 1.99-2.4, respectively, and they had emphasized that higher yields were obtained from extracts prepared with ethanol. It is stated that in general, extraction yield is lower in seeds and higher in stems and leaves (Palmieri et al. 2020). According to the literature findings, we can say that the yields of herbal extracts vary according to the organ of the plant, the solvent and method used.

3.2. The chemical compositions and content of essential oil

Mean values belong to the ratio and chemical composition of light yellow-colored essential oils obtained from coriander varieties are presented in Table 6. The fruit essential oil ratio of cv. Arslan (large-fruited) and cv. Gürbüz (small-fruited) were recorded as 0.31% (v/w) and 0.42% (v/w), respectively. Beyzi et al. (2017) worked with the same cultivars, essential oil contents of cultivars detected 0.30% in cv. Arslan and 0.33% in cv. Gürbüz. Generally, the essential oil content of small-fruited varieties (0.8-1.8%, v/w) is higher than the varieties with large-fruited (0.1-0.35%, v/w) (Burdock and Carabin, 2009). Similar results were obtained in our study, but the essential oil content of the small-fruited cultivar was found to be lower than the values stated in the literature. In a study conducted in Bangladesh, fruit essential oil was found to be 0.42% (Bhuiyan et al., 2009). In other studies, the essential oil content of coriander fruits was reported as 0.39% by Ravi et al (2006), 0.35% by Msaada et al. (2007), and 0.23% by Mansori et al. (2018). Although coriander fruits contain 0.2-1.5% essential oil, in some genotypes this rate is up to 2.6% (Ebrahimi et al., 2010). However, it is also possible to come across genotypes containing essential oil higher than 2.6%. For example, the essential oil yields obtained by hydro distillation method from fruits belonging to coriander genotypes originating from different countries have taken values in the range of 0.1-5.2 % (Orav et al., 2011). It is observed that there are differences in the essential oil content of coriander fruits grown in different geographies.

Table 6. Chemical composition and content of fruit essential oils of coriander cultivars (%)

Compound	RT[1]	cv. Arslan	cv. Gürbüz	EPS[2]
α-pinene	3.760	1.03	1.67	3-7
Camphene	3.909	0.11	0.15	
Sabinene	4.093	0.13	0.13	
β-Pinene	4.152	0.23	0.31	
β-Myrcene	4.182	0.21	0.26	
Ortho-Cymene	4.537	0.73	0.67	
Limonene	4.578	1.43	1.45	1.5-5
p-cymene				0.5-4
γ-Terpinene	**4.831**	**8.11**	**6.94**	1.5-8
Trans-sabinenehydrate	4.930	0.01	0.02	
Linalool	**5.203**	**73.52**	**74.38**	65-78
Citronellal	5.615	-	0.02	
Camphor	**5.667**	**3.67**	**4.22**	3-6
1-Borneol	5.836	0.14	0.22	
Terpinen-4-ol	5.911	0.19	0.18	
α-Terpineol	6.013	0.21	0.25	0.1-1.5
Myrtenol	6.074	0.27	0.29	
Geraniol	**6.444**	**6.11**	**8.45**	0.5-3
Cis-myrtanol	6.615	-	0.01	
Caryophyllene	8.109	0.21	0.31	
Bicyclogermacrene	8.833	-	0.06	
Geranyl acetate	-	-	-	0.5-4
TOTAL		**96.31**	**99.99**	
Essential oil content (%)		**0.31 ±0.025**	**0.42 ±0.03**	**min. 0.3**

[1]RT: Retention Time; [2]EPS: European Pharmacopoeia Standards (Gebarowska et al., 2019).

In the cv. Arslan, 17 components representing 96.31% of the oil and in the cv. Gürbüz, 20 components representing 99.99% of the oil were detected. It was observed that the main component is linalool in both essential oils. Also, γ-terpinene, geraniol and camphor were determined as other important components in essential oils (Table 5). Considering the literature data and our study results, linalool was determined as the component

with the highest value in the essential oil obtained from coriander fruits. Overall, it was reported that the linalool content in the essential oils obtained from coriander fruits ranged from 37.3% to 87.5% (de Figueiredo et al.2004; Msaada et al., 2007; Bhuiyan et al., 2009; Ebrahimi et al., 2010). γ-terpinene, α-pinene, p-cymene, camphor, and geranyl acetate were determined as the other characteristic components of the essential oil (Orav et al., 2011; Zekovića et al., 2011; Vasconcelos Dos Santos et al. 2019; Satyal and Setzer, 2020; Al-Khayri et al. 2023). Micića et al. (2019) stated that they detected α-pinene (7.31%), geranyl acetate (5.76%), γ-terpinene (5.59%), camphor (4.245), p-cymene (3.83%), and limonene (1.60%) in addition to linalool (64.04%) in the essential oil of coriander fruits. When we compare the data obtained from our study with EP standards; α-pinene and limonene values are low, geraniol and γ- terpinene values are very high, and essential oil content, linalool and camphor values are among the limit values. On the other hand, geranyl acetate and p-cymene were not detected in the essential oil samples we studied (Table 6).

Although some of the findings we obtained from our study are compatible with the literature data, there are differences. In this context, cultural conditions are an important factor (Mandal and Mandal, 2015). Within the scope of the study, coriander varieties were grown in arid conditions and no additional irrigation was done. The literature data indicate that the essential oil content and composition may differ depending on the climatic conditions of the region, the growing conditions of the plant, cultural practices (sowing time, harvest time etc.) and the genotype of the plant (Gebarowska et al., 2019; Delibaltova 2020).

3.3. Antibacterial activity

Inhibition zone diameters (mm), which are the indicators of the activity of the research materials against one Gram-positive and seven Gram-negative bacteria, are given in Table 7. The antibacterial activity of essential oils obtained from Arslan and Gürbüz cultivars was similar. While none of the antibiotic discs in the study showed any effect against *S. aureus*, the only Gram-positive bacteria included in the study, essential oils were effective. When evaluated the antibacterial activity according to the criteria reported by Davis and Stout (1971), EO (essential oil) Arslan was strong and EO-Gürbüz was medium. The CB-100 antibiotic used as a control showed the highest activity against *E. coli* bacteria. A moderate activity recorded in essential oils was similar to that of the C-30 and TE-30 antibiotics. The essential oil of both varieties showed no activity against *P. aeruginosa* bacteria. In most studies examining the effect of essential oils against pathogens, it is revealed that the effect of essential oils against Gram-positive bacteria is more than Gram-negative bacteria. The cell wall of Gram-negative bacteria is surrounded by lipopolysaccharides, which prevents the antibacterial effect. The outer membrane surface of Gram-negative bacteria such as *P. aeruginosa* is rich in hydrophilic and lipopolysaccharide molecules. Therefore, they show a very different internal resistance to essential oils (Dorman and Deans, 2000; Esen et al., 2007; Turker and Usta, 2008).

The highest activity against *S. tyhimurium* bacteria was observed with antibiotic discs. However, the activity exhibited by essential oils was close to E-15. M-10 and E-15 control antibiotics were ineffective against *S. marcescens* bacteria. C-30 and CB-100 antibiotics exhibited the highest activity against this bacterium. It was noted that the activity (moderate) exhibited by essential oils was close to the TE-30 control disc. TE-30, C-30 and CB-100 control discs, respectively, exhibited the highest activity against *P. vulgaris* bacteria.

Table 7. Antibacterial activity of essential oils and extracts from coriander varieties against tested bacteria

Mean inhibition zone diameters (mm±SD)				
Material	**1[1]**	**2**	**3**	**4**
EO-A	11.34±0.29(S)	9.99±0.34 (M)	-	8.36±0.23 (M)
EO-G	10.23±0.72 (M)	9.56±0.55 (M)	-	7.30±0.06 (M)
Fruit (Ethanol-G)	-	7.08±0.94 (M)	-	-
C (30 µg)	-	10.73±0.00 (M)	23.92±2.03 (VS)	22.80±2.26 (VS)
TE-(30 µg)	-	8.64±1.02 (M)	22.14±0.43 (VS)	20.09±1.02 (VS)
AM-(10 µg)	-	-	13.46±0.00 (S)	17.13±4.18 (S)
CB-(100 µg)	-	22.16±0.00 (VS)	21.38±0.02 (VS)	10.44±1.68 (M)
E-(15 µg)	-	-	8.97±0.05 (M)	9.29±0.20 (M)
	5	**6**	**7**	**8**
EO-A	9.17±0.21 (M)	9.24±0.36 (M)	11.43±0.07 (S)	-
EO-G	10.79±0.22 (M)	9.48±0.68 (M)	10.52±0.00 (M)	-
Fruit (Ethanol-G)	-	-	-	-
C (30 µg)	23.41±0.40 (VS)	22.25±0.69 (VS)	24.67±0.71 (VS)	29.65±1.37 (VS)
TE-(30 µg)	11.58±0.00 (S)	22.26±1.19 (VS)	16.53±0.00 (S)	22.52±4.09 (VS)
AM-(10 µg)	-	-	21.32±0.04 (VS)	-
CB-(100 µg)	25.51±1.42 (S)	19.64±0.57 (S)	31.93±0.21 (VS)	-
E-(15 µg)	-	-	-	18.43±0.06 (S)

[1]1: *S. aureus*, 2: *E. coli*, 3: *P. aeruginosa*, 4: *S. tyhimurium*, 5: *S. marcescens*, 6: *P. vulgaris*, 7: *E.cloacae*, 8: *K. pneumoniae*
EO: Essential oil, A: cv. Arslan, G: cv. Gürbüz, C: Chloramphenicol, TE:Tetracycline, AM:Ampicillin, CB:Carbenicillin , E:Erythromycin, M: Moderate, S: Strong, VS: Very Strong

AM-10 and E-15 antibiotics were ineffective against the bacterium in question. The inhibition zone diameter recorded for these bacteria, in the essential oils was lower than that of in the controls, with an average of 9.36 mm. CB-100 showed the highest activity against *E. cloacae* bacteria with an inhibition zone diameter of 31.93. C-30 (24.67 mm, VS), AM-10 (21.32 mm, VS) and TE-30 (16.53 mm, VS) followed respectively. E-15 had no activity against this bacterium. EO-Arslan and EO-Gürbüz showed strong and moderate activity, respectively, against this bacterium. Essential oils and AM-10 and CB-100 antibiotics were ineffective against *K. pneumoniae* bacteria. C-30 exhibited the highest activity, followed by TE-30 and E-15, respectively (Table 7).

It is known that essential oils obtained from different organs of coriander (such as fruits and leaves) inhibit microorganisms in a wide spectrum (Bhuiyan et al., 2009; Begnami et al., 2010; Mandal and Mandal, 2015; Beyzi et al., 2017; Al-Khayri et al., 2023). Coriander essential oil has been observed to be effective against both Gram-positive and Gram-negative bacteria (*E. coli, Salmonella* spp., *S. aureus*, *S. typhimurium*, *K. pneumoniae*, *P. aeruginosa, E. cloacae*) (Delaquis et al., 2002; Asgarpanah and Kazemivash, 2012; Dima et al., 2015; Mandal and Mandal, 2015; Rezaei et al., 2016). In our study, essential oils were ineffective against *K. pneumoniae* and *P. aeruginosa* bacteria.

In the study we carried out, only the extract prepared with ethanol from the fruits of the cv. Gürbüz showed moderate activity against *E. coli* bacteria. However, this activity was lower than both essential oils and control antibiotics (Table 7). None of the other extracts exhibited antibacterial activity against tested microorganisms. In the studies conducted also, it has been stated that the coriander aqueous extract had no antimicrobial activity (Sahib et al., 2013).

It has been reported that the antimicrobial activity of coriander essential oil is due to active ingredients such as linalool, α-pinene, β-pinene, p-cymene and ɣ-terpinene (Dorman and Deans, 2000; Koutsoudaki et al., 2005; Xianfei et al., 2007). In addition, it was determined that the antimicrobial activity of coriander essential oil is not only related to the linalool amount of the essential oil, but also the interaction of other components in the essential oil is important on the antimicrobial activity (Begnami et al., 2010).

Although the amount of linalool (63% and 66%) in coriander fruit essential oils obtained by two different methods is similar, it was determined that the essential oil with higher ɣ-terpinene, α-pinene and p-cymene contents exhibited a stronger activity against *S. aureus* bacteria (Sourmaghi et al., 2015). In our study, since the essential oil composition of the cultivars was similar, their activity against the tested bacteria was also found similar (Table 5 and Table 7).

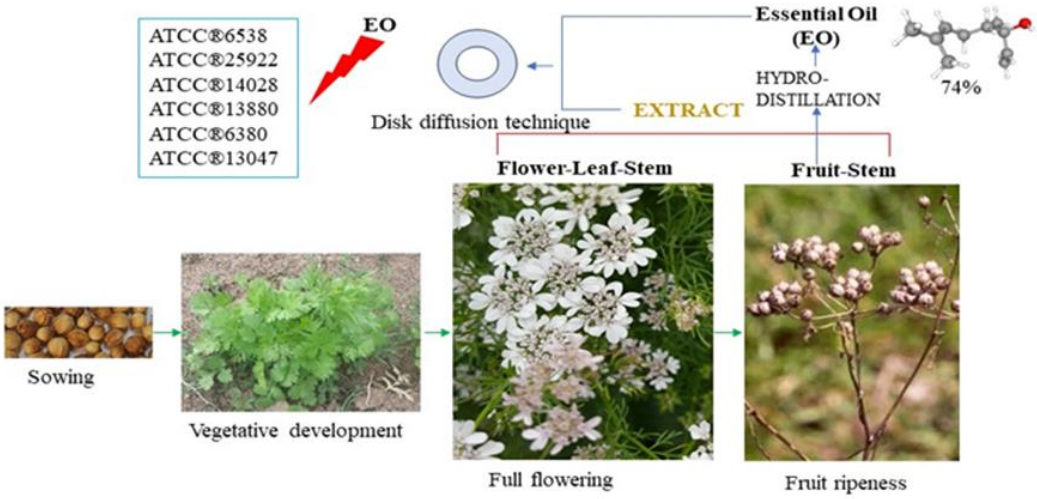

Figure 3. Procedures in this research

4. Conclusion

In this study, the antimicrobial activities of essential oils obtained from the seeds of two coriander varieties grown under semi-arid culture conditions and extracts obtained from different parts against pathogenic bacteria that threaten human health were evaluated (Figure 3). All but one of the extracts (Fruit /Ethanol-Gürbüz) showed no activity against test microorganisms. It has been determined that essential oils exhibit varying levels of activity against *S. aureus, E. coli*, *S. tyhimurium, S. marcescens, P. vulgaris*, and *E. cloacae* bacteria. In line with the findings, these essential oils may inhibit the growth of different bacteria. Therefore, more research with different bacteria is needed to better explain the healthcare use of these essential oil samples.

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Author Contribution

This article was produced from a graduate test.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Enzyme inhibitory and antioxidant activities and HPLC quantification of chlorogenic acid in *Helichrysum stoechas* (L.) Moench and *H. stoechas* subsp. *barrelieri* (Ten) Nyman

Nurten ABACI KAPLAN[1], Hasya Nazlı GÖK[1], Mustafa ASLAN[1,2], Ilkay Erdogan ORHAN [1,3*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Türkiye
[2]Department of Pharmacognosy, Faculty of Pharmacy, Cyprus International University, Lefkoşa, KKTC
[3]Principal Member, Turkish Academy of Sciences (TÜBA), Vedat Dalokay Cad., No. 122, 06670 Ankara, Türkiye
*Correspondence: iorhan@gazi.edu.tr

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Abstract

The inhibitory effects of ethanol (80%) and aqueous extracts of *Helichrysum stoechas* (L.) Moench and *H. stoechas* subsp. *barrelieri* (Ten) Nyman on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), two sister enzymes associated with the pathogenesis of Alzheimer's disease (AD), as well as on elastase and collagenase, linked to inflammation and skin aging, were investigated. Simultaneously, the antioxidant activity of the extracts was assessed through DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and metal-chelating activity assays since oxidative damage plays a critical role in AD pathophysiology and skin aging. Total phenol and flavonoid contents in the extracts were spectrophotometrically determined. The highest AChE inhibitory activity (44.60 ± 4.4% at 2000 µg/mL) was found in the ethanol extract of *H. stoechas* subsp. *barrelieri* collected from Hatay, and the uppermost BChE inhibitory activity at same concentration was found in the aqueous extract of *H. stoechas* subsp. *barrelieri* collected from Izmir (80.24 ± 2.63%, IC_{50}: 38.52 ± 1.41 µg/mL). Both of them inhibited AChE and BChE in a concentration-dependent manner. Nevertheless, none of the extracts from the two plants inhibited elastase and collagenase. Although both ethanolic and aqueous extracts had significant antioxidant activity in DPPH radical scavenging and FRAP assays, they demonstrated inadequate antioxidant activity in the metal-chelating assay. Chlorogenic acid was quantified in the extracts using HPLC. The mentioned two extracts with strong cholinesterase (ChE) inhibition also had the highest chlorogenic acid content. The ethanol extract of *H. stoechas* (Hatay sample) and the aqueous extract of *H. stoechas* (Izmir sample) seem to contain promising ChE inhibitors, which deserve further investigation.

Key words: *Helichrysum stoechas*, enzyme inhibition, Alzheimer's disease, antioxidant activity, chlorogenic acid.

1. Introduction

Helichrysum stoechas (L.) Moench, a member of Asteraceae family, is a perennial medicinal plant that grows naturally in the Western and Eastern regions of Türkiye. Aside from Türkiye, it grows naturally in Italy, the Balkans, Cyprus, and Lebanon, where it is cultivated in many parts of the world due to its medicinal importance. This plant is called by many different names such as "altın çiçeği, guddeme çiçeği, kudama, ölmez çiçek, altın otu" in diverse parts of Anatolia. *Helichrysum stoechas* subsp. *barrelieri* (Ten) is a sub-taxon of *H. stoechas* that grows in the southern

Marmara, Aegean, and Mediterranean regions of Türkiye as well as Albania, Cyprus, Egypt, Greece, and Italy (Aksoy et al., 2011; Aslan, 1994; Eroğlu, 2018). The ethnopharmacological records of *H. stoechas* indicate that the plant's capitulum is used for its diuretic, emmenagogue, anticoagulant, kidney stone-lowering, appetite-raising, and anthelmintic properties along with its utilization in the treatment of dermatological illnesses such as dermatitis, burn-wound treatment, and body crack healing (Gras et al., 2017; Memariani et al., 2018; Tsioutsiou et al., 2022).

Alzheimer's disease (AD) is an incurable and progressive neurological disease that deprives people of their memory and cognitive abilities, primarily affecting the elderly population. The specific cause of the illness is unknown, while there is no proven therapy yet, to seize the disease. According to the cholinergic theory, levels of two neurotransmitters, *i.e.* acetylcholine and butyrylcholine, have been low in the brains of AD patients, which break down by two sister enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), respectively. Inhibition of cholinesterases (ChE) is critical for the treatment and thus, ChE inhibitors are currently used to treat AD symptoms. Relevantly, current FDA-approved pharmacotherapy for AD includes ChE inhibitors, *e.g.* rivastigmine, galantamine, tacrine, and donepezil, along with *N*-methyl D-aspartate (NMDA) receptor antagonists (*e.g.* memantine) (Terry & Buccafusco, 2003). In a study, which inspired us to carry out the present work, inhibition by the extract prepared from *H. stoechas* capitula on AChE (IC_{50} value of 260.7 µg/mL) shown by *in vitro* and *in silico* molecular docking methods was determined, where chlorogenic acid, cynarin, and arzanol were reported to be responsible for the inhibitory activity of the extract (Silva et al., 2017). On the other hand, skin aging ranks as one of the most widespread dermatologic concerns; it is a multifaceted and unavoidable process of human existence. Elastin and collagen, two key proteins that make up connective tissue, are responsible for the skin's resilience and suppleness. With the skin's natural aging process, their breakdown by elastase and collagenase, which free oxygen radicals would activate, promotes wrinkle development. Many researchers working on skin aging have turned their attention to plant extracts after discovering that cosmetic products containing synthetic agents, including such sodium lauryl sulphate and triethanolamine as active ingredients cause side effects such as allergic or irritant contact dermatitis, phototoxicity, and photoallergic responses. Natural skin care goods are acknowledged to be also effortlessly received through into the epidermal layer and to be typically hypoallergenic. Although so called """natural beauty products" "" appear to be remarkably popular amongst consumers throughout the globe, yet, the overwhelming of them lack scientific evidence to back up their putative aesthetic effects (Mukherjee et al., 2011).

It should be mentioned that free radicals and reactive oxygen species (ROS) have been identified as playing a crucial part in both skin and brain aging, *i.e.* AD. The capitula of *H. stocheas* were previously revealed to possess high antioxidant activity (Haddouchi et al., 2014). Furthermore, a single research examining antiaging action of *H. petiolare* has described its ability to prevent skin aging, which should be explored further (Sagbo & Otang-Mbeng, 2020). Based on the aforementioned little data, we were inspired to do research on the skin and brain aging potential of *H. stoechas*. For this purpose, *H. stoechas* and *H. stoechas* subsp. *barrelieri* were subjected to enzyme inhibition assays towards AChE, BChE, collagenase, and elastase, as well as antioxidant assays using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric-reducing antioxidant power (FRAP), and metal-chelating activity as well as HPLC analysis, which will constitute the first study of its kind on this species and sub-taxon.

2. Materials and methods

2.1. Plant materials

The cultivated samples of *H. stoechas* obtained from a farm in Izmir (Türkiye) and naturally growing samples *H. stoechas* subsp. *barrelieri* collected from two provinces of Türkiye (*e.g.* Izmir and Hatay) were used as the plant materials in this study. Firstly, the capitula of these species were manually separated and utilized for extraction and bioassays performed herein. The species were identified by one of us (M.A.) and their herbarium species are preserved at Faculty of Pharmacy, Gazi University (Ankara, Türkiye).

2.2. Preparation of the extracts

The separated capitula of the plant samples were dried in circulating air. The dried plant materials were homogeneously pulverized using a mechanical grinder and each sample was weighed accurately (4.5 g) on a digital scale (Shimadzu, Japan). Each plant material was extracted with ethanol (EtOH, 80%, 150 mL) and hot water (150 mL), sequentially, through maceration for 24 hours with periodic handshaking. The EtOH phases were evaporated under reduced pressure by a rotary evaporator (Büchi, Switzerland) to obtain the crude extracts. A lyophilizer (Telstar Lyo Quest, Japan) was used to freeze-dry the aqueous extracts. A total of six extracts was obtained from three plant samples. The crude extracts were kept in the refrigerator at +4°C until bioassays were performed. Table 1 displays the yields of the extracts obtained from each plant.

Table 1. Yields of the extracts (w/w)

Extracts	Yields (%, w/w)
EtOH extract (80%) cultivated in Izmir (CE)	10.89
Aqueous extract cultivated in Izmir (CW)	14.91
EtOH extract (80%) grown naturally in Izmir (NIE)	9.65
Aqueous extract grown naturally in Izmir (NIW)	13.55
EtOH extract (80%) grown naturally in Hatay (NHE)	7.88
Aqueous extract grown naturally in Hatay (NHW)	14.29

2.3. Determination of total phenol and flavonoid content in the extracts

Total phenolic contents of EtOH (80%) and aqueous extracts were determined using the Folin-Ciocalteau colorimetric technique with some slight modifications (Singleton & Rossi, 1965). To construct the calibration curve, gallic acid (GA) solutions (used as standard) at various concentrations (1, 0.5, 0.25, 0.125, 0.125, 0.0625, and 0.03125 mg/mL) were prepared to calculate total phenol content. The aqueous and EtOH (80%) extracts were dissolved in EtOH (96%). Both samples and varying concentrations of GA were treated with 30 µL of Folin-Ciocalteau reagent (diluted twice, Sigma, USA) and 150 µL of sodium carbonate (3.5%). After half an hour at 40°C incubation, absorbance at 760 nm was measured with an ELISA microplate reader (Molecular Devices, Spectramax i3 microplate reader, USA). The experiment was performed in triplicate. GA equivalents (mg/g extract) were used to express the results. With some slight changes, total flavonoid content of the extracts was determined in the extracts using aluminum chloride colorimetric technique (Woisky & Salatino, 1998). Numerous concentrations of quercetin (1, 0.5, 0.25, 0.125, 0.125, 0.125, 0.0625, and 0.03125 mg/mL) employed as standard were prepared to create the calibration curve. In a 96-well microplate, each sample and quercetin solutions at the given concentrations were mingled with 75 µL of EtOH (96%), 5 µL of $AlCl_3$ (10%), 5 µL of 1 M sodium acetate, and 100 µL of distilled water, respectively. The samples and standard solutions were then incubated for

30 minutes in the dark at room temperature. Absorbance of each sample was measured at 415 nm using an ELISA microplate reader (Molecular Devices, Spectramax i3 Microplate Reader, USA). The experiment was conducted on three parallel assays. The results were represented in milligrams of quercetin equivalent (mg/g extract). Table 2 demonstrates total phenol and flavonoid contents of the extracts.

Table 2. Total phenolic and flavonoid content of the extracts

Extracts	Total phenol content[a] ± S.D[b]	Total flavonoid content[c] ± S.D[b]
CE	118.77 ± 4.88	91.87 ± 0.81
CW	71.50 ± 9.27	47.21 ± 1.92
NIE	113.60 ± 5.37	79.86 ± 1.44
NIW	94.62 ± 1.95	48.09 ± 3.74
NHE	141.55 ± 2.93	75.45 ± 2.05
NHW	108.76 ± 1.46	65.20 ± 2.02

[a]Data expressed in mg equivalent of gallic acid to 1 g of extract, [b]Standard deviation (n: 3),[c]Data expressed in mg equivalent of quercetin to 1 g of extract.

2.4. Antioxidant activity assays

2.4.1. DPPH radical scavenging activity assay

The bleaching characteristic of a violet-colored methanol solution of DPPH (Sigma, USA) was used to determine the antioxidant capacity of the extracts prepared in 80% ethanol and water. Stable DPPH radical scavenging activity was tested utilizing (Hatano et al., 1988) method with mild modifications (Barros et al., 2007). Samples/references (10 µL for each) dissolved in EtOH (96%) were transferred to 96-well plates. After then, using a calibrated multichannel pipette (Eppendorf Research, Germany), 90 µL of DPPH solution (1.5×10^{-4} M) prepared in EtOH was poured *per* each well. Table 3 illustrates DPPH radical scavenging capabilities of the extracts.

Table 3. Antioxidant activities of the extracts

Extracts	Metal-chelating capacity (Capacity % ± S.D.[a])	Ferric-reducing antioxidant power[b] (FRAP) (Absorbance at 700 nm ± S.D.)	DPPH radical scavenging activity (Inhibition % ± S.D.)
CE	NA[c]	0.98 ± 0.01	83.58 ± 1.43
CW	6.58 ± 0.74	0.72 ± 0.02	64.97 ± 1.81
NIE	4.96 ± 0.74	1.23 ± 0.04	83.58 ± 1.72
NIW	22.7 ± 2.35	0.89 ± 0.01	70.68 ± 1.45
NHE	NA*	1.49 ± 0.0	87.39 ± 0.17
NHW	6.29 ± 0.82	0.83 ± 0.09	69.98 ± 0.6
References	94.75 ± 0.13[d]	1.34 ± 0.02[e]	85.51 ± 0.17[f]

[a]Standard deviation (n: 3), [b]Higher absorbance indicates higher antioxidant activity in FRAP, [c]No activity, [d]EDTA (2 mg/mL), [e]Quercetin (1 mg/mL)

2.4.2. FRAP assay

Ferric-reducing potential of the EtOH (80%) and aqueous extracts as well as quercetin (reference) was investigated using Oyaizu's method (Oyaizu, 1986) with minor modifications. The test is based on the diminishing power of the transformation of ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}), which leads to the creation of a blue complex (Fe^{2+}/TPTZ) that enhances absorbance at 700 nm. In summary, 10 µL of samples and reference in EtOH (96%) were transferred to

a 96-well microplate and pre-incubated at 50°C for 20 minutes with 25 µL phosphate buffer (pH: 6.6) and 25 µL [$K_3Fe(CN)_6$] (1%, w/v, Sigma, USA). FRAP values of the extracts are tabulated in Table 3.

2.4.3. Metal-chelating assay

For evaluating metal-chelating activity, Carter's modified approach was utilized with ethylenediaminetetetraacetic acid (EDTA, Sigma, USA) provided as reference (Carter, 1971). In sum, 20 µL of each extract and reference were incubated for 10 minutes at ambient temperatures with EtOH (96%), 2 mM $FeCl_2$ (Sigma, USA), and 5 mM ferrozine (Sigma, USA). Through the use of an ELISA microplate reader (Molecular Devices, Spectramax i3 microplate reader, USA), absorbance of the formed ferrozine-Fe^{2+} complex was measured at 562 nm. Metal-chelating activity of the extracts is presented in Table 3.

2.4.4. Data analysis for antioxidant activity assays

The subsequent DPPH radical scavenging and metal-chelating results of the extracts and references were determined and expressed in percent inhibition (I%) in accordance to the formula below:

$$\%I = \frac{A_{blank}\ A_{sample}}{A_{blank}}\ x\ 100$$

A_{blank} indicates absorbance of the control reaction (all reagents except the test sample) and A_{sample} represents absorbance of the extracts/references. The bioassays were carried out three times, and the results were expressed as means with standard deviations (S.D.).

2.5. Enzyme inhibition assays

2.5.1. ChE Inhibition assays

Inhibitory capacity of the extracts against AChE and BChE was assessed using a slightly altered version of Ellman's procedure (Ellman et al., 1961). Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma) and equine serum BChE (EC 3.1.1.8, Sigma) were employed as enzyme sources, whilst also acetylthiocholine iodide and butrylthiocholine chloride were employed as the reaction substrates (Sigma, St. Louis, MO, USA). The activity of ChEs was determined using 5,5'-dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA). Sodium phosphate buffer (0.1 mM, pH 8.0, 140 µL) was added to the 96-well microplate using a multichannel automated pipette (Eppendorf Research, Germany), followed by 20 µL of the samples/EtOH (negative control) at dilutions ranging from 25-200 µg/mL. After that, 0.2 M AChE/BChE solution (20 µL) was incorporated using a multichannel automated pipette (Gilson Pipetman, France). Having followed that, it was incubated for a total of ten minutes at room temperature. As substrates, 0.2 M acetylthiocholine iodide/butyrylthiocholine chloride (10 µL) were supplied to the 96-well microplate to initiate the reaction. Thiocholine is produced by hydrolyzing thiol esters employing AChE or BChE. The yellow byproduct of thiocholine-DTNB reaction is 2-nitro-5-thiobenzoate (TNB). The rate of production and color intensity of the product created as a result of the reaction were analyzed with an ELISA microplate reader (Molecular Devices, Spectramax i3x microplate reader, USA) at 412 nm. Galantamine hydrobromide (Sigma, USA) was utilized as a reference in both investigations. Table 4 displays AChE and BChE inhibition by the extracts.

2.5.2. Elastase inhibition assay

The spectrophotometric approach of Kraunsoe et al. (as modified by Lee et al.) was used to investigate elastase inhibition (Kraunsoe et al., 1996; Lee et al., 2009). The enzyme source was porcine pancreatic elastase (Type IV, Sigma, EC 3.4.21.36), while the substrate was *N*-Suc-(Ala)3-*p*-nitroanilide (Sigma, USA). The reaction is based on determining the quantity of nitroaniline emitted from the substrate at 410 nm. The substrate (1.015 mM) was

prepared in 0.1 M Tris-Cl (pH 8.0) buffer and blended with 10 µL for each extract dissolved in DMSO in a 96-well microplate. After pre-incubating the microplate for 5 minutes at 25°C, 15 µL 0.5 units/mL of the enzyme solution was added. After adding the enzyme, the microplate was incubated at 25°C for 30 minutes, and the quantity of *p*-nitroaniline released from the substrate was measured using an ELISA microplate reader at 410 nm. The reference and control were oleanolic acid (Sigma, USA) and DMSO, respectively. Elastase inhibition percentage of the extracts was estimated using the formula below. The assay was performed in triplicate, and the data are shown as the mean standard deviation of % inhibitions from experimental trials.

Inhibition% = 100 - [(A_1 / A_2) × 100]

In this formula, A_1 represents the absorbance of the sample solutions at 410 nm, and A_2 represents the average absorbance of the control solutions at 410 nm. Table 5 displays the elastase inhibition findings of the extracts tested.

2.5.3. Collagenase inhibition assay

The slightly modified spectrophotometric approach established by Wart and Steinbrink was used to evaluate collagenase inhibition (Barrantes & Guinea, 2003; Van Wart & Steinbrink, 1981). The enzyme source was *Clostridium histolyticum* (Sigma, EC 3.4.23.3), and the substrate was *N*-(3-[2-furyl]acryloyl)-Leu-Gly-Pro Ala (FALGPA) (Sigma, USA). The enzyme was dissolved in a tricine buffer with a pH of 7.5. FALGPA was dissolved in the same buffer at a concentration of 2 mM. Each well in a 96-well microplate was filled with 25 µL of buffer, 25 µL of DMSO or extract, and 25 µL of the enzyme. After 15 minutes of pre-incubation, FALGPA (50 µL) was added. Absorbance was measured at 340 nm in an ELISA microplate reader. The reference was (-)-epigallocatechin gallate (Sigma, USA), while the control was DMSO. Percentage collagenase inhibition of the extracts was estimated using the formula below. Each assay was performed in triplicate and the data is displayed as the mean standard deviation of % inhibitions.

Inhibition % = 100 - [(A_1 / A_2) × 100]

In the formula given above, A_1 represents the absorbance of the sample solutions at 340 nm and A_2 represents the average absorbance of the control solutions at 340 nm. Table 5 displays the collagenase inhibition findings of the samples tested.

Table 4. Cholinesterase inhibitory activities of the extracts and their IC_{50} values

Extracts	**(Inhibition % ± S.D.[a]) at 200 µg/mL[b]**	
	AChE	**BChE**
CE	7.36 ± 4.87	16.08 ± 4.98
CW	31.33 ± 4.79	12.36 ± 0.65
NIE	13.18 ± 6.07	25.67 ± 1.76
NIW	23.12 ± 5.86	80.24 ± 2.63 (IC_{50}: 38.52 ± 1.41 µg/mL)
NHE	44.60 ± 4.41	67.24 ± 3.74 (IC_{50} =157.3 ± 6.93 µg/mL)
NHW	30.14 ± 3.01	32.39 ± 2.17
Reference[c]	97.57 ± 2.59 (IC_{50}= 0.67 ± 0.02 µg/mL)	89.56 ± 0.8 (IC_{50} = 92.34 ± 5.05 µg/mL)

[a]Standard deviation (n: 4), [b]Final concentration, [c]Galantamine hydrobromide (200 µg/mL)

Table 5. Elastase and collagenase inhibitory activities of the extracts

Sample	Collagenase Inhibition (Inhibition % ± S.D.[a]) 133 µg/mL[b]	Elastase Inhibition (Inhibition % ± S.D.[a]) 133 µg/mL
CE	NA*	6.47 ± 4.4
CW	NA*	2.44 ± 2.1
NIE	NA*	1.79 ± 3.49
NIW	NA*	2.4 ± 1.5
NHE	NA*	8.3 ± 3.11
NHW	28.8 ± 1.48	NA*
Reference	80.83 ± 11.53[c]	99.65 ± 0.08[d]

[a]Standard deviation (n: 4), [b] Final concentration,*No activity, [c] Epigallocatechin gallate (1 mM), [d]Oleanolic acid (1 mM)

2.6. RP-HPLC analysis of chlorogenic acid in crude extracts

Chlorogenic acid is utilized to standardize *Helichrysum* crude extracts according to Turkish Pharmacopoeia as an approved standard. Chlorogenic acid analysis was carried out in crude extracts prepared with 80% EtOH and water using the technique defined for RP-HPLC (Gök et al., 2022). Each extract solution was prepared with 25% (v/v) acetonitrile at a concentration of 1 mg/mL and transferred to vials through membrane filters. Chlorogenic acid (Sigma, USA) was prepared at various concentrations using 25% aqueous acetonitrile, including 100, 50, 20, 5, and 1 ppm. An HP Agilent 1260 series LC System and an ACE_5 C18 (5 m, 150 mm x 4.6 mm) column with a diode array detector (DAD) was employed for the analysis. Temperature inside the column was adjusted to 25°C. Solvent A (acetonitrile:H_2O:formic acid/80:20:0.1) and solvent B (H_2O:formic acid/100:0.5) were used as the mobile phase. A gradient method has been used to separate the peaks appropriately,. The flow chart was adjusted as follows; from 0-minute 5% A, to 10 minutes 15% A, to 17 minutes 15% A, to 22 minutes 20% A, to 32 minutes 30% A, to 50 minutes 100% A, to 53 minutes 100% A. Duration of the analysis was set at 53 minutes. Injection volume was 20 µL, and the flow rate was 0.8 mL/min. The calibration equation and correlation coefficient for chlorogenic acid were determined as y = 124.09x - 9.988 and r2 = 0.9999, respectively. Table 6 and Figure 1 demonstrate chlorogenic acid quantities in each extract.

Table 6. Amount (%, w/w) of chlorogenic acid in the extracts

Extracts	Amount (%, w/w)
CE	1.14 ± 0.000
CW	0.15 ± 0.000
NIE	0.81 ± 0.000
NIW	1.36 ± 0.000
NHE	1.95 ± 0.000
NHW	1.22 ± 0.000

3. Results and discussion

3.1. Enzyme inhibition findings

AChE and BChE inhibitory activities of aqueous and EtOH extracts prepared from *H. stoechas* subsp. *barrelieri* (Izmir and Hatay samples) and *H. stoechas* (Izmir sample) are shown in Table 4. The outcomes revealed that AChE inhibitory activity was low in the extracts, whereas the highest activity was found in EtOH extract of the plant collected from Hatay (NHE) with moderate inhibitory activity (44.60 ± 4.41 %). The inhibitory activity was nearly half that of galantamine, the reference (97.57 ± 2.59 %). It was determined that the most effective extract against BChE was the aqueous extract of the plant collected from Izmir (NIW, IC_{50}: 38.52±1.41 µg/mL), and the extract demonstrated more activity than galantamine (IC_{50}: 92.34 ± 5.05 µg/mL). In addition, NHE was also found to exhibit a high inhibition (IC_{50}: 157.3 ± 6.93 µg/mL) against BChE.

Collagenase and elastase inhibitory activity results of the extracts indicated that only water extract of the plant collected from Hatay (NHW) extract inhibited collagenase moderately. The other extracts possessed low or no inhibition against both of the enzymes (Table 5).

3.2. Antioxidant activity results

Antioxidant activity of the extracts was analyzed *via* determination of metal-chelating capacity, FRAP, and DPPH radical scavenging activity methods (Table 3). The extracts did not exert remarkable activity in metal-chelating capacity and FRAP assays. Only the NIW extract had a moderate level of metal-chelating capacity (22.7 ± 2.35 %). On the other hand, all of the extracts demonstrated high DPPH radical scavenging activity from 64.97 ± 1.81 % to 87.39 ± 0.17 %. Especially, the EtOH extracts exerted high activity nearly as reference quercetin (85.51 ± 0.17 %).

3.3. Total phenol and flavonoid content in the extracts

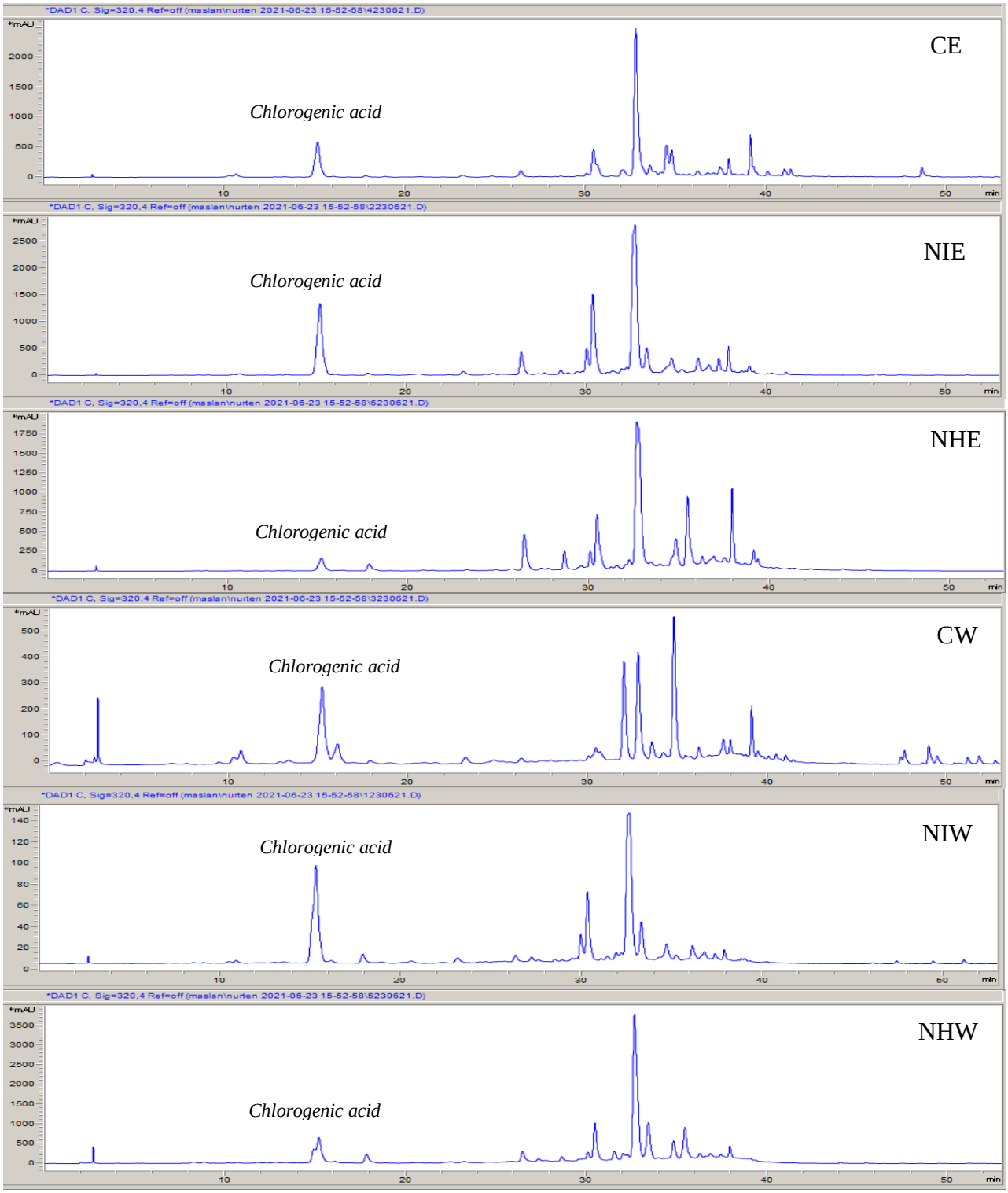
Total phenol and flavonoid contents of the extracts were analyzed spectrophotometrically and expressed in mg equivalent of gallic acid and quercetin, respectively (Table 2). The results demonstrated that the total phenolic and flavonoid contents in the EtOH extracts were found to be higher than those of the aqueous extracts. The highest total phenolic content was found in the NHE extract (141.55 ± 2.93 mg/g gallic acid equivalent, GAE), while the highest total flavonoid content was found in the EtOH extract of the cultivated plant in Izmir (CE) extract (91.87 ± 0.81 mg/g quercetin equivalent, QE). The results were found to be consistent with FRAP and DPPH activity results. The extract with the highest phenolic content was NHE which displayed the highest FRAP (1.49 ± 0.0) and DPPH (87.39 ± 0.17 %) activity. Also, the extracts demonstrated AChE, elastase, and prominent BChE inhibitory activity.

3.4. Quantification of chlorogenic acid in the extract using RP-HPLC

Chlorogenic acid as one of the major compounds in *H. stoechas* flowers (capitula) was determined in the extracts by using HPLC-DAD (Table 6). As aforementioned, the compound is also used for the standardization of *H. stoechas* subsp. *barrelieri* and *H. plicatum* subsp. *plicatum* flower monographs in Turkish Pharmacopoeia (Pharmacopoeia, 2019). The equation of the chlorogenic acid calibration curve was y = 124.09x – 9.988 (r^2: 0.9999) and the test range was 1-200 ppm. The results indicated that the highest chlorogenic acid content was also found in NHE extract (1.95 ± 0.000 %, Figure 1). It might also be related to the highest AChE, BChE, and elastase inhibitory activity of the extract.

In a study, AChE inhibitory activity of the flower and stem/leaf water extracts of *H. stoechas* was analyzed, and their chemical profiles were detected by using liquid chromatography-mass spectrometry (LC-MS/MS) and HPLC-DAD (Silva et al., 2017). The extracts inhibited AChE with IC_{50} values of 260.7 and 654.8 µg/mL, respectively. The phytochemical analysis revealed presence of chlorogenic acid, myricetin-3-*O*-glucoside, cynarin, dicaffeoylquinic acid, dicaffeoylquinic acid, and arzanol in both of the extracts, whereas malonyl-dicaffeoylquinic acid was found only in the stem/leaf water extracts. Molecular docking studies pointed out that arzanol, chlorogenic acid, and cynarin was compatible with the anti-AChE active site channel and prohibited all access to the catalytic triad. In a similar study, enzyme inhibitory, antioxidant activities, total phenolic and flavonoid contents, and phytochemical analysis using UHPLC/MS of five extracts of *H. stoechas* subsp. *barrelieri* obtained by different extraction techniques were analyzed (Zengin et al., 2020).

Figure 1. HPLC-DAD chromatograms of the extracts



The results demonstrated that the extracts were able to inhibit AChE in a range from 3.56 ± 0.04 to 4.23 ± 0.07 as galantamine equivalent (GAE), BChE from 5.42 ± 0.01 to 6.05 ± 0.03 (GAE), α-glucosidase from 1.59 ± 0.01 to 1.66 ± 0.01 acarbose equivalent (ACE), α-amylase from 0.46 ± 0.01 to 0.63 ± 0.02 ACE and tyrosinase from 174.50 ± 2.71 to 183.32 ± 0.78 kojic acid equivalent (KAE). Besides, the extracts exhibited antioxidant

activity to some extent *via* DPPH in a range from 90.05 ± 0.92 to 219.92 ± 3.21 mg trolox equivalent (TE)/g, ABTS radical scavenging activities from 138.68 ± 1.20 to 313.12 ± 8.42 mg TE/g, FRAP from 285.14 ± 5.25 to 662.87 ± 20.41 mg TE/g, cupric-reducing antioxidant capacity (CUPRAC) from 335.97 ± 9.89 to 927.39 ± 11.19 mg TE/g, metal-chelating capacity from 2.91 ± 0.43 to 17.11 ± 0.69 mg EDTA equivalent/g, and phosphomolybdenum assay from 1.65 ± 0.07 to 2.27 ± 0.20 mmol TE/g. In ultraperformance liquid chromatography-mass spectrometric (UHPLC/MS) characterization, a total of 107 compounds was identified, 40 of which were found as hydroxycinnamic acid derivatives, while 50 of them were flavonoids. Quercetin, 5-O-caffeoyl-quinic acid, and *p*-hydroxybenzoic acid were reported as the major molecules in highest amount.

Except *H. stoechas*, various *Helichrysum* species also demonstrated ChE inhibitory and antioxidant activities in previous studies. For instance; *H. pallasii* inhibited AChE (IC_{50}: 1.49 mg/mL), BChE (IC_{50}: 1.98 mg/mL), α-amylase (IC_{50}: 2.09 mg/mL), α-glucosidase (IC_{50}: 0.51 mg/mL), tyrosinase (IC_{50}: 0.68 mg/mL), and pancreatic lipase (IC_{50}: 42.5 µg/mL) (Nejmi et al., 2023). Furthermore, antioxidant activity of *H. pallasii* was shown by FRAP (2205 µmol Fe^{2+}/gE), oxygen radical scavenging (ORAC) capacities (2540 µmol Trolox Eq./gE), DPPH (IC_{50}: 0.58 mg/mL), CUPRAC (IC_{50}: 0.37 mg/mL), phosphomolybdenum (IC_{50}: 1.34 mg/mL), and metal-chelation (IC_{50}: 1.42 mg/mL) methods. In another study, *H. plicatum* inhibited AChE by 41.15 ± 1.68 % at 500 µg/mL concentration. At 200 and 100 µg/mL, it is shown similar results, which was indicative of dose-independent activity (Jovanović et al., 2020). In another study, methanol extract from *H. plicatum* DC. subsp. *plicatum* inhibited carbonic anhydrase I (hCAI), hCAII, AChE (IC_{50}: 115.50 mg/mL), BChE (IC_{50}: 117.46 mg/mL), and α-glycosidase (IC_{50}: 81.53 mg/mL) (Aydin, 2020). The study also described isolation of apigenin, β-sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside, helichrysins A and B, isosalipurposide, and nonacosanoic acid. All of the compounds inhibited AChE and BChE, ranging between IC_{50} values of 1.69–2.90 and 1.09–3.89 µM, respectively. AChE and BChE inhibitory activity of some other species, *e.g. H. chionophilum* and *H. plicatum*, was demonstrated in another report (Acet et al., 2020). In particular, EtOH and ethyl acetate extracts of the flower and stem of *H. chionophilum* and *H. plicatum* inhibited AChE and BChE at moderate levels, where their BChE inhibitory activity was found higher than their AChE inhibition, which is consistent with our data. In the study conducted by Taşkın et al. (Taşkın et al., 2020), anti-ChE, anti-inflammatory, antioxidant, antimicrobial, and anti-urease activities of methanol extracts of *H. plicatum* subsp. *plicatum* by Soxhlet extraction and conventional maceration techniques were investigated. Furthermore, phytochemical analysis of the methanol extracts obtained by maceration and Soxhlet using HPLC-DAD and LC-MS/MS presented 42.10±0.79 % and 58.51±0.85 % of AChE inhibitory activity at 500 µg/mL concentration, which later led to identification of chlorogenic acid, dicaffeoylquinic acid, luteolin, luteolin-7-*O*-glucoside, naringenin-O-hexoside, and isoquercitrin. ChE inhibitory activity of chlorogenic acid described as one of the major molecules in genus *Helichrysum* was demonstrated in former studies. Chlorogenic acid inhibited AChE and BChE with IC_{50} values of 8.01 ± 0.01 and 6.30 ± 0.02 µg/mL, respectively (Oboh et al., 2013), whereas, in another study, it was able to inhibit AChE only IC_{50} value of 98.17 µg/mL (Oboh et al., 2013).

Our literature survey indicated no any previous study relevant to the effect of *H. stoechas* on elastase and collagenase. However, elastase and collagenase inhibitory activity of leaf EtOH extract of *H. petiolare* was weak (Sagbo & Otang-Mbeng, 2020). The

essential oil of aerial parts of *H. italicum* subsp. *italicum* inhibited collagenase and elastase activities with IC_{50} values of 36.99 ± 1.52 and 135.43 ± 6.32 µg/mL, respectively (Fraternale et al., 2019).

4. Conclusion

Our present study revealed water and EtOH (80%) extracts prepared from *H. stoechas* subsp. *barrelieri* capitula growing naturally in two localities (Hatay and Izmir), and water and EtOH (80%) extracts prepared from the capitula of cultivated *H. stoechas* sample inhibited AChE weak to moderate levels. Aqueous extract of the plant collected from Izmir (NIW) and EtOH extract of the plant collected from Hatay (NHE) extracts inhibited BChE strongly with 38.52 ± 1.41 and 157.3 ± 6.93 µg/mL IC_{50} values. The activity of NIW was more than galantamine, the reference drug. The extracts exhibited potent DPPH radical scavenging and FRAP activities; but the metal-chelating capacity was weak except NIW extract (22.7 ± 2.35 %). In addition, amount of chlorogenic acid in the extracts was determined and the highest chlorogenic acid was found in NHE and then in NIW. The findings suggested that chlorogenic acid may be one of the substances responsible for the tested activities.

The current study discloses the first outcomes on AChE, BChE, elastase, and collagenase inhibitory activities and antioxidant data of the capitulum extracts from *H. stoeachas* and *H. stoechas* subsp. *barrelieri* from Türkiye as well as their chlorogenic acid quantification.

Conflict of interest

The authors declare no conflict of interest.

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Echinacea Genus: An Endless Natural Therapeutic Resource? An Overview

Aurel VASIU[1], Carmen Dana ŞANDRU[1,2], Emeline CHANOVE[1], Diana Ioana OLAH[1], Emoke PALL[1,2], Gheorghiţă DUCA[2], Marina SPÎNU[1,2]*

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Division of Infectious Diseases, University of Agricultural Sciences and Veterinary Medicine, 400372, Cluj-Napoca, Romania, marina.spinu@gmail.com
[2]Institute of Research and Development for Montanology, 557085, Cristian-Sibiu, Romania

*Corresponding author :icdmcristian@gmail.com

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Abstract

Echinacea spp., Asteraceae family represented one of the important genera of cultivated biologically active plants, well-known in traditional medicine. Its multiple effects were investigated along time, within the research for alternative therapies to synthetic drugs. Due to those effects the *Echinacea* genus represented a valuable resource for medicine in general and for veterinary medicine especially. In the latter, the fight to increase resistance against diseases counter-balances the antimicrobial therapies; further, the potential adjuvant role of *Echinacea* products provide perspectives of an enhanced innate rather than adaptive immunity along with antibacterial effects.

Still, the involvement of plant extracts in therapy and prevention of diseases in general, even by enhancing immunity or increasing the post-vaccination responses needs careful species and age-based tailoring to avoid unwanted or noxious side effects. Could this genus represent more than an alternative? Are its effects more valuable in medicine than thought before? This mini-review is exploring those possibilities.

Key Words: *Echinacea* spp., general biological effects, innate immunity, specific immunity

1. Botanical Description

Compared to other species with medicinal uses, the history of *Echinacea* is relatively short. *Echinacea* includes several plants of the Asteraceae family: Echinacea purpurea (L.) Moench, *Echinacea angustifolia* DC. and *Echinacea pallida* (Nutt.) Nutt. In Methodus Plantas Horti Botanici et Agri Marburgensis, Conrad Moench (1794) accepted that *Echinacea* (*E. purpurea* (L.) Moench) was equivalent to *Rudbeckia purpurea* L. from the family Asteraceae.

The currently accepted taxonomy for *Echinacea* species is based on morphological and anatomical studies by McGregor (McGregor , 1968, American Herbal Pharmacopoeia and Therapeutic Compendium, 2007). According to these data, the genus comprises nine to ten (WFO, 2023) species and two varieties. The ten accepted species are: *Echinacea angustifolia* DC. (Narrow-leaf coneflower), *Echinacea atrorubens* (Nutt.) Nutt. (Topeka purple coneflower), *Echinacea laevigata* (C.L. Boynton & Beadle) S.F.Blake (Smooth

coneflower, smooth purple coneflower), *Echinacea pallida* (Nutt.) Nutt. (Pale purple coneflower), *Echinacea paradoxa* Britton (Yellow coneflower, Bush's purple coneflower), *Echinacea purpurea* (L.) Moench (Purple coneflower, eastern purple coneflower), *Echinacea sanguinea* Nutt. (Sanguine purple coneflower), *Echinacea simulata* McGregor (Wavyleaf purple coneflower) and *Echinacea tennesseensis* (Beadle) Small (Tennessee coneflower)(The International Plant Names Index and World Checklist of Vascular Plants 2023). According to Bauer and Wagner (1990), Rudbeckia serotina Sweet (Table 1) represented just a synonym for *Echinacea purpureea* (L.) Moench.

The *Echinacea* spp. distribution is defined by the natural or introduced status of the plant, broader geographical areas being lately covered by the introduced *Echinacea* (Fig.1).

Corresponding to the Royal Botanic Kew Gardens "Plants of the World Online", native Echinacea spp. are found in the North American continent, while introduced species are spread from Western Europe to South Eastern Asia (Fig.1, https://powo.science. kew.org/).

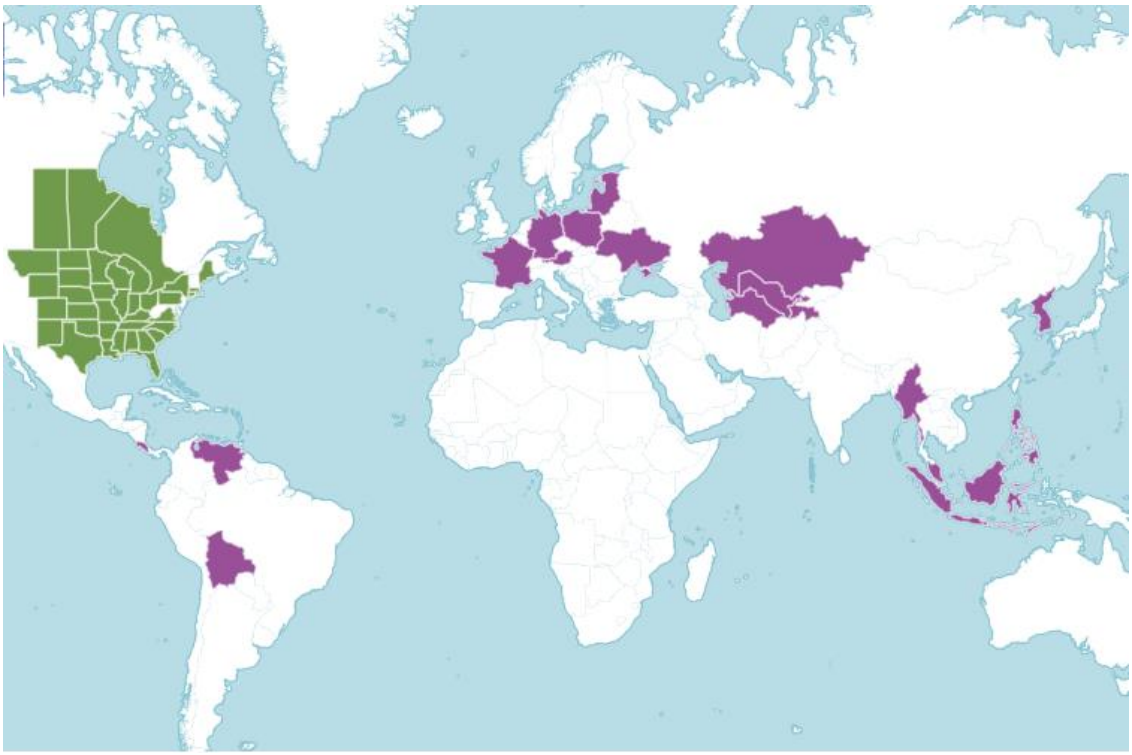


Figure 1. The distribution map of *Echinacea* spp. native or introduced species (http://www.ipni.org , https://powo.science.kew.org/). The green color indicated the native species distribution, while introduced plants are represented by purple color.

Echinacea spp. are perennial herbaceous plants, growing from either tap- or fibrous (E. purpurea) roots in relatively dry climate. The stems are erect up to 1.4 m, with no branches in most species and the rough, hairy leaves are arranged alternately. The leaves decrease in size towards the top of the plant, showing a linear, lanceolate, ovate or elliptic shape, with entire or dentate/serrate margins, showing species specific differences (Belaeva and Butenkova, 2018, WFO 2023). As in all Compositae, the flowers of *Echinacea* spp. are inflorescences, with the outer pink or sometimes yellow florets pointing downward and the middle florets are positioned in a cone-shaped head (cone-flower- WFO 2023).

Table 1. Taxonomy of the genus ECHINACEA, after MCGregor (1968)(Bauer R., Wagner H., 1990)

Echinacea angustifolia DC. var.*angustifolia*
Synonyms: *Brauneria angustifolia, Echinaceea pallida* var. *angustifolia* (DC) Cronq
Echinacea angustifolia DC. Var. *strigosa,* McGregor
Echinacea alrorubens Nutt
Synonyms: *Rudbeckia alrorubens* Nutt.
Echinacea levigata (Boynton & Beadle) Blake
Synonyms: *Brauneria levigata* Boynton & Beadle*, Echinaceea purpureea* (L.) Moench var.*levigata Cronq*
Echinacea pallida (Nutt.) Nutt.
Synonyms: *Echinaceea angustifolia* Hooker*, Rudbeckia pallida* Nutt.*,Brauneria pallida* Britton, *Echinaceea pallida* (Nutt.) Nutt. *F. albida* Steyerm.
Echinacea paradoxa (Norton) Britton var. *paradoxa*
Synonyms: *Brauneria paradoxa* Norton, *Echinaceea atrorubens* Nutt. var*. paradoxa* (Norton) Cronq.
Echinacea paradoxa (Norton) Britton var.*neglecta* McGregor
Echinacea purpureea (L.) Moench
Synonyms: *Rudbeckia purpureea* L., *Rudbeckia hispida* Hoffmgg., *Rudbeckia serotina* Sweet*, Echinaceea purpureea* (L.) Moench var*., arkansana* Steyerm., *Echinaceea purpureea* (L.) Moench f. *ligettii* Steyerm., *Echinaceea speciosa* Paxton, *Echinaceea intermedia* Lindley, Brauneria *purpureea* (L.) Britton*.*
Echinacea simulata **McGregor**
Synonyms: *Echinaceea speciosa* McGregor.
Echinacea sanguinea **Nutt**
Echinacea serotina **(Nutt.)** DC.
Echinacea tennesseensis (Beadle) Small**,** Synonyms: *Brauneria tennesseensis* Beadle, *Echinaceea angustifolia* DC. var*. tennesseensis* (Beadle) Blake

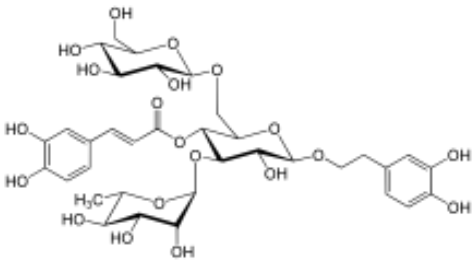
2. **Chemical Composition**

Echinacea spp. has a complex composition. The chemical constituents have been more or less defined over the years, and chemical investigations have even identified different species of Echinacea that are difficult to differentiate botanically. The various plant preparations belonging to the genus Echinacea must be classified according to the plant species (*Echinacea purpurea*, *E. pallida* or *E. angustifolia*), the processed portion of the plant (root, aerial part or whole plant) and the method of processing. Significant pharmacological effects have been described both *in vivo* and *in vitro* for the extract from the aerial parts of E. purpurea and for alcoholic extracts from the roots of *E. pallida, E. angustifolia* and *E. purpurea*. This activity is mainly directed towards the non-specific

cellular immune system. The active components of these plants are polysaccharides, glycoproteins, caffeic acid derivatives (cychoric acid) and alkamides (Bauer et al., 1999, Nyalambisa et al., 2016).

When chemists and pharmacologists started showing interest in Echinacea, numerous constituents were isolated such as polysaccharides, echinacosides, cyclochoric acid, ketoalkenes and alkyl amides (El-Gengaihi et al., 1998). These extracts have shown immune stimulating capacity and were mainly used in prophylaxis and therapy of colds, influenza and septic disorders, however the identity of the active principles was not well known (Hostettmann, 2003).

The main biologically active constituents are: echinacoside (*E. pallida*), verbascoside (*E. angustifolia, E. pallida*)(Fig.2), quinic acid derivatives (cynarin)(*E. angustifolia*, *E. tennesseensis*), chicoric acid (*E. purpureea*)(Fig.3), flavonoids; essential oils, polyacetylenes, alkylamides, alkaloids, polysaccharides and other constituents (resins, acids: oleic, linoleic, cerotic, palmitic, etc.).



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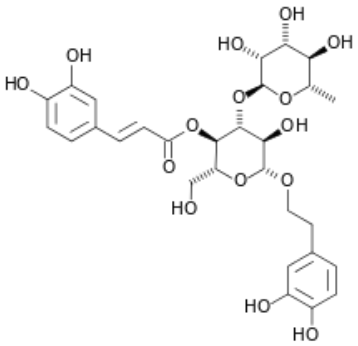

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Figure 2. Echinacoside(a) (*E. pallida*) and verbascoside (b) (*E. angustifolia, E. pallida*) – caffeic acid glycosides from the phenylpropanoid class

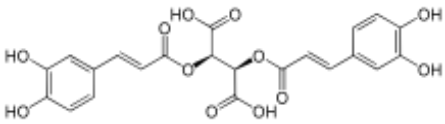

Figure 3. Chicoric acid (*E. purpureea*) a hydroxycinna-mic acid, an organic compound of the phenyl-propanoid class

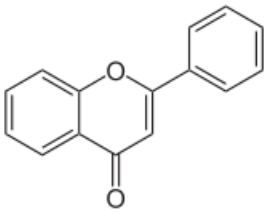

Figure 4. The flavonoid backbone

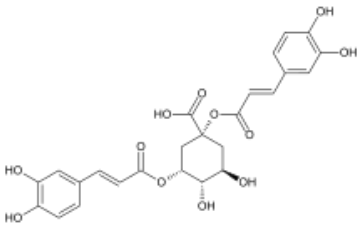

Figure 5. Cynarine)(*E. angustifolia*) hydroxy-cinnamic acid derivative

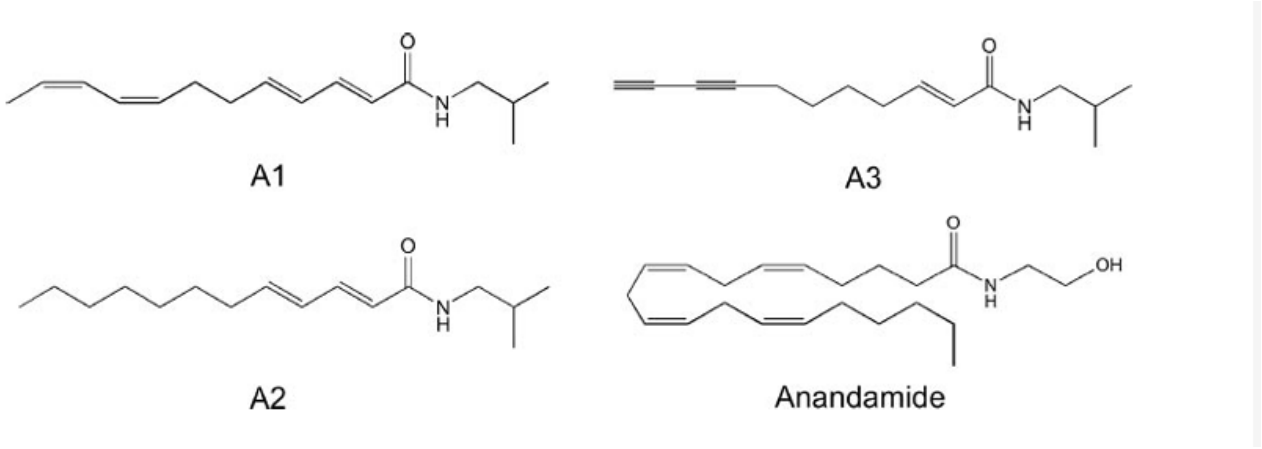

Figure 6. Alkylamides (*E. angustifolia, E. tennesseensis*) hydroxy-cinnamic acid derivative (Raduner et al., 2006)

Chemical evaluation by HPLC showed a differentiated distribution of active compounds in the roots of *E. angustifolia, E. purpurea* and *E. pallida*. Cyclic acid and verbascosides predominated in *E. purpurea* extracts while cynarin and dodeca-2E,4E,8Z,10Z/E-tetraenoic acid isobutylamide represented a major component of *E. angustifolia* extract. Echinacoside and 6-O-caffeoyl-echinacoside were dominant in extracts from *E. pallida* roots. Characteristic alkamides were also examined by tandem electrospray mass spectrometry (MS/MS), finding their characteristic fragmentation. All root and leaf extracts of the three plants showed antioxidant properties in the free radical assay and in the lipid peroxidation assay (Sloley et al., 2001; Kahlos et al., 1989). The flowers of Echinacea spp. contained monoterpenes and monoterpenoids, sesquiterpenes nd sesquiterpenoids, as well as other hydrocarbons, the most prevalent being Sesquiterpenic hydrocarbons identifiable by HS-SPME-GC/MS (Kaya et al., 2018).

3. Biological Effects

The plant is native to North America and has served in the traditional medicine of native Indians. There are records of this plant being used medicinally by the Cheyenne, Dakota, Fox, Kiowa, Crow, Delaware, Comanchee and other North American tribes. It is likely that the *Echinacea* used by them belonged to the species *E. angustifolia, E. purpureea* or *E. pallida.* The earliest archaeological evidence of the use of the plant for therapeutic purposes dates from the 18th century. The first *Echinacea* preparation, known as Meyers Blood Purifier, was marketed around 1880 for rheumatism, neuralgia and snakebites. In the early 20th century, Echinacea was used as the most popular herbal preparation in the US. Commercial cultivation began in Germany around 1939 and since 1950, Vogel has cultivated the plant in Switzerland (Bauer et al., 1988).

For a long time, *Echinacea* species were regarded as esoteric medicinal plants, their use being restricted to a few areas in Germany and the United States. Due to ignorance of its mechanism(s) of action, no relations were sought between its wound healing activity or anti-snake venom effect and modern pharmacology. Until 1930, *Echinacea* extracts were used in experiments of varying scientific accuracy in the treatment of abscesses, puerperal sepsis, septicemia, uremia, malaria, septic shock, typhus, tuberculosis, tetanus or other bacterial toxemias (Bauer and Wagner, 1990).

After 1940, clinical trials became more circumscribed, based on intravenous

administration of *Echinacea* EchinacinR extract or intramuscular administration of Myo-EchinacinR, more rarely oral, drops or external ointments. The main clinical indications of these extracts were shown in Table 2.

Table 2. Clinical results of EchinacinR extracts applied externally or injected (Bauer and Wagner, 1990)

External use
Burns, chemical burns, frostbite, radiation ulcers
Post-operative soft tissue injuries
Atherosclerotic soft tissue and bone wounds, not prone to suppuration, phlegmon, fistulization
Decubital ulcers in elderly, cachectic patients
Ulcus cruris
Eczema (including industrial eczema)
EchinacinR , ampoules
Internal medicine and paediatrics**:** Septic conditions, rheumatoid arthritis, antibiotic resistance. Whooping cough, flu, catarrhal infections. Chronic upper respiratory tract infections.
Gynaecology**:** endodermatitis, parametritis, post-infectious abortion treatment, pelviperitonitis, chronic adnexitis.
Surgery**:** chronic osteomyelitis.
Urology**:** non-specific prostatitis, non-specific urethritis, epididymitis.
Dermatology**:** Psoriasis arthropathica, psoriasis vulgaris, erythroderma of various origins, pemphigus vulgaris, endogenous eczema, atonic skin ulcers (ulcus cruris, irradiation ulcers, decubital ulcers).

Extensive studies carried out in the last decades highlighted that the plant's active substances have beneficial effects on the non-specific immune system. Establishing, without doubt, the botanical identity of the plant and identifying the effects of isolated components, which was possible both in vivo and *in vitro* experiments, allowed a more precise definition of immune modulating activity (Schranner and Losch, 1986, Schranner et al., 1989). Phylloxanthobilins (PBs) which result from degrading of clorophylle while the plant is aging contained in *Echinacea purpurea* extracts could be ensuring part of the plant's biological activity (Karg et al., 2019).

Traditionally, *Echinacea* has been prescribed externally for various conditions such as wounds, burns, skin or lymph node swellings, insect bites. The roots have been used to combat dental and neck pain. Internally, it has given results in headaches, digestive cramps, coughs, colds, measles, gonorrhea or various intoxications (including snakebite). The roots are the most widely used part of the

plant. The fresh plant has been used as a paste or macerate, less often as an infusion.

4. Biological Activities other than on the Immune System

4.1. Local Tissular Activity: Originally, *Echinacea* extracts were used for their healing properties. Using EchinaceaR, its anti-hyaluronidase action was observed, probably associated with other effects, due to indirect intervention on the hyaluronic acid-hyaluronidase system, expressed by changes in fibroblasts (Koch, cited by Bauer R., Wagner H., 1990). EchinacinR appears to induce synthesis of mesenchymal mucopolysaccharides, suggesting also the pituitary-adrenal axis as a target of influence (Koch cited by Bauer and Wagner, 1990).

Echinacea extract, used in equine feed for 42 days, acts as a haematinic agent, improving blood quality, decreasing haemoglobin concentration and erythrocyte count and thus oxygen transport, thereby increasing physiological parameters of exercise and sports performance (O'Neill et al., 2002).

4.2. The Anti-inflammatory Activity: Factor A, a compound isolated from aqueous extracts of Echinacea purpureea, shows cortisol-like activity and a mixture of polysaccharides from *E. angustifolia* show effects in the rat model of edema disease. An alkylamide fraction has also been found to be responsible for anti-inflammatory effects (Bauer, 2002). Anti-inflammatory effects have also been observed for the isolated polysaccharide fraction from *E. angustifolia* (Tubaro et al., 1988).

Echinacea crude extract (Echinacea B) inhibited auricular edema induced in mice by application of 0.015 ml of a 0.25% croton oil emulsion in water, during its peak (6 h) or decline (18 h), by topical application, the effect being dose-dependent. Moreover, this anti-edema effect has been shown to be stronger than that of benzidamine, a non-steroidal topical anti-inflammatory drug. Intravenous administration one hour prior to inoculation of a 1% carrageenan extract amounting in 0.05 ml, in the posterior plantar perineum of the rat, inhibited edema in the histamine phase as well as the inflammatory process (Tragni et al., 1985, 1988; Tubaro et al., 1988).

Components such as phyllobilins (PBs)(phylloleucobilin, dioxobilin-type phylloleucobilin, phylloxanthobilin, etc.) were also found in E. purpurea. Pharmacological activities such as anti-inflammatory and anti-oxidative have been described for PBs of Echinacea purpurea , including those present in infusions of the plant (Gorfer et al., 2023).

4.3. Antiviral, Antibacterial, Antifungal, Oncolytic and Insecticidal Activities: Echinacosides isolated from *E. angustifolia* strains have anti-staphylococcal activity, at the lower limit of antibiotic activity. Polyacetylenic compounds from the roots of *E. angustifolia* and E. *purpureea* have been shown to inhibit the growth of bacteria (*E.coli, Pseudomonas aeruginosa*) and fungi. High dilutions (1:1000) of an E. angustifolia extract completely inhibited the growth of Epidermophyton interdigitale. Antiparasitic effects of these extracts were also mentioned, thus *in vitro*, an alcoholic extract of E. angustifolia weakly inhibited the growth of Trichomonas vaginalis.

EchinacinR, the already mentioned extract, was shown to be effective against encephalomyocarditis and vesicular stomatitis virus in cell cultures. Extracts of *E. purpureea* reduced the number of plaques in cell cultures treated with influenza virus, herpesvirus and vesicular stomatitis virus respectively (Wacker and Hilbig, 1978). This activity has been described as "interferon-like", but without inducing interferon synthesis.

Peroral treatment for four weeks with *Echinacea* extract in combination with Eupatorium perfoliatum and Thuja

occidentalis (*Echinacea* complex) in patients undergoing surgery for tumour diseases, did not induce the activation of leukocyte or lymphocyte populations or amplification of the secretion of IL-1-α, IL-1-β, IL-2, IL-6, TNF-α, or IFN-γ in supernatants of stimulated whole blood cultures compared to the untreated group of patients (Elsässer-Beile et al, 1996; Bodinet and Freudenstein, 1999; Block and Mead, 2003).

Phytochemical preparations obtained from Echinacea spp., by stimulating non-specific immune effectors (micro- and macrophages), act in the first line of defense against virally infected/transformed cells. Increased levels of these cells in the bone marrow following treatment in rats indicate that at least one of the mechanisms of action of the active compounds is the stimulation of the appearance of new cells in situ (Sun et al., 1999, 2005, Burlou-Nagy et al., 2022).

PBs of *E. purpurea* were proven to bind to actin and inhibit cancer cell migration (Vollmar and Moser, 2023). Further, phylloxanthobilin, induces cell cycle blockage and apoptosis in cancer cells, an increase in anti-proliferative activity being induced by esterification inactive phylloleucobilin and depending on the chain lengths of the alkyl esters (Karg et al., 2020)

4.4.Toxicity: Acute toxicity tests by Lorke (1983) in mice using two of the *Echinacea* polysaccharides resulted in death following intraperitoneal inoculation of over 5000 mg/kg-1, but the nature of the histological lesions (pronounced alveolar and interstitial edema with localized leuko-diapedesis) suggested acute circulatory collapse (decompensated shock) caused by the syrupy nature of the extract and not the plant polysaccharides themselves.

Neutral polysaccharides from *E. purpureea* have been tested for possible genotoxicity in human lymphocyte cultures (Schimmer and Leimeister, 1989). No sister chromatid exchange or chromosomal aberrations occurred in either long or short term experiments.

Experiments carried out recently (Balciunaite et al., 2020) identified by LC-MS/MS glycoproteins were homologous with the lysine motif (LysM) domain containing lectins, common to several Asteraceae plants. Tested in vivo, these induced toxic effects expressed by statistically significant kidney glomerular vacuolization and tubular necrosis.

5. Immunological Investigations

A review of research conducted over the last 40 years (January 1966-July 1999) to confirm the immune stimulating effects of *Echinacea* extracts or purified Echinacea components revealed a lack of agreement between these results obtained by different working groups, with arguments pro and against immunomodulatory activity but not for the safety of using these extracts (Giles et al, 2000).

According to some authors (Văgîi et al., 1996), the immune modulating influence of Echinacea extracts is global, with usefulness in immune deficiencies, particularly in combination with extracts from Epilobii herba, *Usnea barbata*, Urticae folium, Alchemillae herba, Hyperici herba, Crataegi folium cum flores.

By correlating the results obtained with the composition of the different parts of the plant or with that of the different Echinacea species, it could be stated that the immune stimulating effects of alcoholic or aqueous extracts depended very much on the combined action of their constituents. Most chemical analyses have been carried out on Echinacea angustifolia, particularly the older ones, while immune activity has been tested mainly on Echinacea purpurea (Schumacher and Friedberg, 1991).

5.1. Influence on the Complement System: Due to the extremely important physiological role of the complement system, its modulation (inhibition or stimulation) is a target of interest in the development of various drugs. Some plant polysaccharides are known to have complement-modulating activities. However, the classically used hemolysis assay does not allow differentiation between complement inhibitors or activators due to low hemolysis.

Comparative evaluation of the inhibitory and activating effects, respectively, using heparin (inhibitor) and an arabino-galacatan from Echinacea purpurea extract demonstrated that the latter was a complement activator, both in the classical and alternative pathways, by altering the complement incubation periods. Removal of the side chains of the tested arabinogalactan significantly reduced complement stimulating activity, proving that the three-dimensional structure was an essential component of the activating potential. The involvement of arabinogalactan in complement activation could represent one of the attributes of the immune stimulating efficacy traits of Echinacea purpurea extract (Alban et al., 2002).

5.2. Influence on the Phagocytic System: The positive effects of Echinacea extracts on phagocytosis have been followed on isolated rat liver (Bauer, 1996). In order to determine any differences between extracts from plants belonging to different species, the carbon particle inclusion test was performed and extracts of E. purpurea, E. angustifolia and E. pallida were tested. Phagocytosis was amplified in the presence of alcohol extracts by 20-30% at doses of 10-2 to 10-4.

The E. purpurea extract was found to be the most active, and the chloroform-extracted fractions of the ethanolic extract were more effective than the hydrophilic ones (Fig. 7). The results of this *in vitro* assay correlated with those obtained in vivo by oral administration of the alcoholic extract.

Of the isolated compounds, chicoric acid induced a significant increase in phagocytosis, confirmed by the carbon particle inclusion assay.

The polysaccharide fraction favored phagocytosis after intravenous injection (EchinacinR), an effect supported by assessing the percentage of lymphocytes and granulocytes labelled with tritiated thymidine in Cohen's experiment (1980) (Table 3).

In vitro investigations using a polysaccharide-rich mixture from the aerial parts of E. purpurea demonstrated its predilect influence on the mononuclear cell subsystem. The same mixture stimulates macrophages to release interleukin-1, but does not induce T lymphocyte proliferation (Lowenthal and MacDonald, 1986; Lowenthal et al., 1999, 2000, Barrett, 2003). Macrophage stimulation and induction of cytokine secretion are due not only to the polysaccharide fraction but also to the glycoprotein fraction (Bauer, 2002).

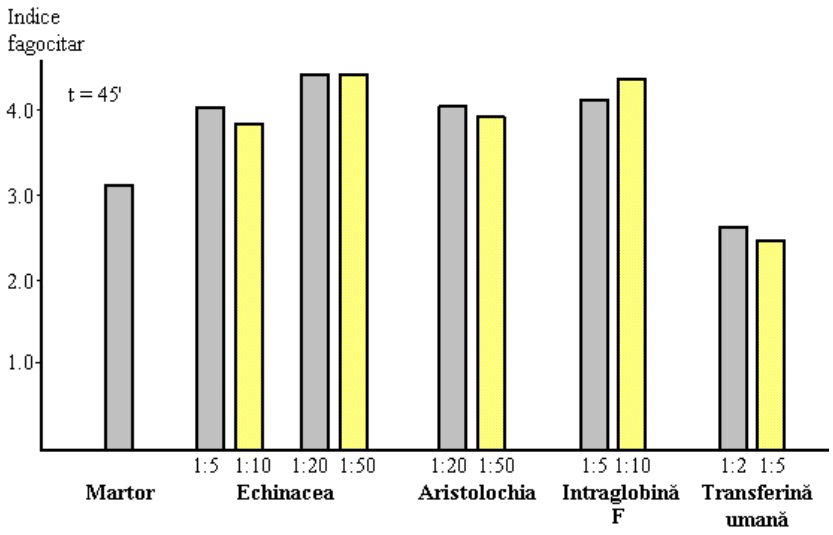

Figure 7. Influence of *Echinacea* extract versus other principles on phagocytic activity in humans (after Bauer R. et al., 1999)

Legend: Echinacea preparations used: 1:5 - Myo Echinacin 5%, 1:20-1:50 - Echinacea angustifolia extract 5:1/30%; Aristolochia - Tardolyt Madaus; Intraglobin F, Ch-B 4111107 Biotest, Human Transferrin - Ch-B 1672 Behringwerke

Echinacea purpurea extracts, stabilised, are well-tolerated, allergic reactions, particularly skin reactions, are reversible and can occur particularly in people with contact hypersensitivity to plants of the Compositae family. Pharmacological data suggested that cold-pressed Echinacea preparations stimulate the non-specific immune system and increase resistance to respiratory infections by stimulating oxidative shunt and modulating monokine secretion (Kligler, 2003, Bauer, 2002; Bauer et al., 1988). These claims are supported by stimulation of natural killer cell activity (Barrett, 2003).

A series of studies in mice using polysaccharides purified from Echinacea cell cultures have shown an immune stimulating effect in cell cultures from mice or in animals injected intraperitoneally. These effects include amplification of phagocytic activity, chemotaxis and respiratory shunting of neutrophils and macrophages respectively. Peritoneal macrophages from treated animals produced increased amounts of tumour necrosis factor (TNF), interleukins (IL-1, IL-6 and IL-10) and were more active in destroying WEHI 164 lineage of tumour cells as well as cells infected with Leishmania enriettii or Candida albicans. Similar effects were observed after administration of Echinacea extracts even in mice suppressed with cyclophosphamide or cyclosporine. These studies highlight the immune stimulating activity of Echinacea polysaccharides in both healthy and immunosuppressed animals (Percival, 2000).

A comparative study carried out to investigate the immune-biological effects of some components of Echinacea extract (cychoric acid, polysaccharides and alkylamides) demonstrated the maximum efficacy of alkylamides at a dose of 12 µg/kg body weight/day in significantly increasing phagocytic activity as well as the phagocytic index of alveolar macrophages. Simultaneously, alveolar macrophages obtained from this group produced significantly more TNF-α and nitric oxide after *in vitro* stimulation with LPS than any other component or the control. None of the concentrations of the investigated components induced TNF-α, IFN-γ and IL-2 release by splenocytes. The immunomodulatory effects of alkylamides appear to be more pronounced on lung

immunocompetent cells than on splenic cells (Goel et al., 2002).

Western blot analysis of the *in vivo* effects of *Echinacea purpurea* (L.) Moench extract showed that it induced, along with changes in lipopolysaccharide (LPS) response, IFN-γ-induced cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS) expression in peritoneal macrophages. Thus, treatment with 100 mg kg(-1) of Echinacea extract reduced only COX-2 expression, demonstrating that the anti-inflammatory effect of the extract may be due to this mechanism (Raso et al., 2002).

Table 3. Percentages of labelled lymphocytes and granulocytes in the total population (after Bauer and Wagner, 1990)

Time for thymidine inoculatin (h)	Lymphocytes	Granulocytes
24	11	0
48	15	6
72 (Echinacin application)	7	34
78	40	89
97	7	59

5.3. Influence on Specific Humoral-Mediated Immunity: Numerous immunomodulatory effects have been attributed to Echinacea angustifolia extracts, however little is known about the stimulation of antigen-specific immunity. Studies in rats shed some light on these issues, indicating increased synthesis of immunoglobulins by enhancing the primary and secondary IgG immune response to antigen (Rehman et al., 1999). Contrary to these results, administration of Echinacea preparations of different origin (standard or officinal products) to male and female rats decreased the concentration of antigen-specific immunoglobulins (standard products) or had no effect (officinal products) in females, but was neutral in efficacy in males (South and Exon, 2001).

5.4. Influence On Specific Cell-Mediated Immunity: Although Echinacea extract acted immune stimulating, not all cell categories were encouraged. Thus, B lymphocytes were not activated nor did they produce antibodies in increased concentrations against a thymus-dependent antigen, sheep red blood cells (Barett, 2003). In some papers, a weak stimulation of T lymphocytes was reported, but those cells did not synthesize increased amounts of IL-2, IFN-β2, or IFN-γ. Delayed hypersensitivity, a T lymphocyte-mediated reaction, was not affected by treatment with Echinacea extract. These data strongly indicate the action of purified polysaccharides from E. purpurea on the non-specific side of the immune response, i.e. phagocytes, rather than on the specific side of immunity (Percival, 2000). Other works (Wagner et al., 1984, 1985; Wagner, 1991; Wagner and Jurcic, 2002) suggest that Echinacea purpurea extract in mixture with extracts from other plants (*Glycyrrhiza glabra*), in tablet form (Revitonil), exerted a remarkable stimulating effect both on phagocytic activity, measured by the carbon particle engulfment test and chemiluminescence, and on T lymphocytes (30-50%) in the CD69 bioassay at a concentration of 100 microg-1/ml.

6. Is There More?

Several reseachers mention that their results open gates for further investigations on the biological effects of Echinacea genus, given the sometimes substantial composition differences, which were found among the species. Similarly, some of the biological effects of these plants supported by traditional medicine uses still remain unexplained. Therefore, several

areas/directions to be tackled by further research and several unanswered questions could be identified.

There is an obvious need for identifying new compounds, whose biological effects remained insufficiently unveiled, i.e., further research on phyllobilins and all the compounds included in this group, where investigations are at their beginnings.

Similarly, not all the effects connected with single components have been revealed, therefore new research techniques, mainly *in vivo* studies with respect to the three R (replacement, reduction, refinement) could be applied to increase the database on the biological efficacy result of these plants.

One of the difficulties in preparing plant products for medicinal use consist of standardization, therefore the use of single purified components seems to be a solution to the problem. But will the overall effects be those expected under these circumstances? Will the patients' reactions be the same? Broadening the variety of subjects, will the "species" factor become a more influential one? Other questions yet to be answered in the future.

Finally, all the available data suggest that the "pharmacy of nature" withholds numerous secrets and to avoid the Pandora box effect only depends on those involved in its research.

7. Conclusion

Due to multiple biological effects, the *Echinacea* genus represents a valuable resource for medicine in general and especially for veterinary medicine, where the fight of increasing resistance against diseases counter-balances the antimicrobial therapies; further, the potential adjuvant role of *Echinacea* products provide perspectives of an enhanced innate rather than adaptive immunity along with antibacterial effects.

Still, the involvement of plant extracts in therapy and prevention of diseases in general, even by enhancing immunity or increasing the post-vaccination responses needs careful species and age-based tailoring to avoid unwanted or noxious side effects.

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Author Contribution

All the authors gave the same effort and contribution to this review and they approved the final version for the submission.

Conflicts of Interest

There is no conflict of interest for any of the authors of this article.

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