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Floristic biodiversity and valorization of bioresources in Belezma National Park (wilaya of Batna)

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Dedication

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Resumé

Cette étude s'est concentrée sur l'évaluation de la biodiversité floristique du Parc National de Belezma, la caractérisation chimique de *Thymus algeriensis* Boiss. & Reut. et *Santolina rosmarinifolia* L. et l'étude des activités biologiques de leurs huiles essentielles et leurs extraits méthanoliques. Au total, 18 stations ont été échantillonnées sur une période de deux ans (2022-2023) en utilisant une méthode d'échantillonnage systématique, afin d'identifier les espèces végétales qui composent le cortège floristique du Parc. Les résultats d'étude floristique du Parc démontrent une diversité floristique, 288 espèces appartenant à 47 familles botaniques différentes et englobant 194 genres. Parmi les 288 espèces inventoriées, nous avons fait une redécouverte d'une espèce de grande importance au niveau de l'Algérie en général et du Parc en particulier: la redécouverte *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller. C'est une espèce rare et endémique Algéro-marocaine. En termes de représentation des espèces, les familles des Astéraceae, des Fabaceae et des Apiaceae sont les plus représentées, avec respectivement 20,27%, 10,83% et 8,04%. Les hémicryptophytes représentent 37,5% de la végétation de la région étudiée. En termes d'origine biogéographique, la majorité de ces espèces (51%) sont d'origine méditerranéenne.

Les huiles essentielles de *T. algeriensis* Boiss. & Reut. et *S. rosmarinifolia* L. obtenues par hydrodistillation ont été analysées par GC-MS. L'analyse chimique a montré que l'huile essentielle de *Thymus algeriensis* contient 89 composants chimiques dont (+)-2-bornanone (12,54 %), l'eucalyptol (8,65 %), le (1R)-2,6,6 triméthylidicyclo[3.1.1]hept-2-Eni (8,48 %), acétate d'alpha-terpinéyle (6,58 %) et linalol (6,23 %) sont les constituants principaux d'huile essentielle. L'huile essentielle de *S. rosmarinifolia* se compose de 96 composés, dont les principaux composants étant Bicyclo[3.1.1]heptane, 6,6-diméthyl-2-méthylène (18,39%), Eucalyptol (15,78%), 2(10)-Pinène (10,67%), (-)-Germacrène D (5,88%). Les huiles essentielles et les extraits méthanoliques testés ont montré une activité antibactérienne ; importante contre *Escherichia coli*, *Staphylococcus aureus* et faible contre *Pseudomonas aeruginosa*. Ces huiles et ces extraits ont une capacité antioxydante significative; mesurée par le test DPPH.

Mots clé : Parc National Belezma, inventaire floristique, redécouverte, *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller , *S. rosmarinifolia* L., *T. algeriensis* Boiss. & Reut., huile essentielle, activité biologique.

Abstract

This study focused on the evaluation of the floristic biodiversity of the Belezma National Park, the chemical characterization of *Thymus algeriensis* and *Santolina rosmarinifolia* and the study of the biological activities of their essential oils and their methanolic extract. In total, 18 stations were sampled over a two-year period (2022-2023) using a systematic sampling method, in order to identify the plant species that make up the floristic procession of the Park. The results of the floristic study of the Park demonstrate a floristic diversity, 288 species belonging to 47 different botanical families and encompassing 194 genus. Among the 288 species inventoried, we have made a rediscovery of a species of great importance at the level of Algeria in general and the Park in particular: the rediscovery of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller. It is a rare and endemic Algerian-Moroccan species. In terms of species representation, the Asteraceae, Fabaceae, and Apiaceae families are the most represented, with 20.27%, 10.83%, and 8.04%, respectively. Hemicryptophytes account for 37.5% of the study region's vegetation. In terms of biogeographic origin, the majority of these species (51 %) are of Mediterranean origin.

The essential oils of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L. obtained by hydrodistillation were analyzed by GC-MS. The chemical analysis showed that the essential oil of *Thymus algeriensis* contains 89 chemical components including (+)-2-bornanone (12.54%), eucalyptol (8.65%), (1R)-2,6,6 trimethyldicyclo[3.1.1]hept-2-Eni (8.48%), acetate alpha-terpineyl (6.58%) and linalool (6.23%) are the main constituents of essential oil. The essential oil of *S. rosmarinifolia* consists of 96 compounds, the main components of which are Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene (18.39%), Eucalyptol (15.78%), 2(10)- Pinene (10.67%), (-)-Germacrene D (5.88%). The essential oils and the methanolic extracts tested showed significant antibacterial activity against *Escherichia coli*, and *Staphylococcus aureus*, and weak antibacterial activity against *Pseudomonas aeruginosa*. These oils and these extracts have a significant antioxidant capacity; as measured by the DPPH test.

Key words: Belezma National Park, floristic inventory, rediscovery, *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller, *Santolina rosmarinifolia* L., *Thymus algeriensis* Boiss. & Reut., essential oil, biological activity

ملخص

ركزت هذه الدراسة على تقييم التنوع البيولوجي للنباتات في حديقة بلزمة الوطنية، وكذا القيام بالتحليل الكيميائي للزيوت العطرية لكل من *Santolina rosmarinifolia* L. و *Thymus algeriensis* Boiss. & Reut. استخدام الزيوت العطرية والمستخلصات الميثانولي لهذه الأخيرة لدراسة الأنشطة البيولوجية (مضادات البكتيريا وكذا مضادات الاكسدة).

تم اخذ العينات من 18 محطة مختلفة على مدار عامين (2022،2023) وذلك باستخدام طريقة اخذ العينات المنهجية، من اجل تحديد الأنواع النباتية المكونة للغطاء النباتي بالحظيرة الوطنية بلزمة.

أظهرت النتائج وجود تنوع في الغطاء النباتي بالحظيرة الوطنية بلزمة، 288 نوعا ينتمي الى 47 عائلة نباتية مختلفة و194 جنسا. من بين 288 نوعا تم جردها، قمنا بإعادة اكتشاف نوع ذو أهمية كبيرة على مستوى الجزائر بشكل عام والحديقة الوطنية بشكل خاص: إعادة اكتشاف *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller. هذا النوع من النباتات يعتبر نوعا جزائريا مغربيا نادر ومتوطن.

من حيث تمثيل الأنواع، فإن عائلات كل من النجميات (أستراسيا) وفاباسي، أبياسي تعد أفضل ثلاث عائلات ممثلة بالنسب 20.27 % و 10.83 % و 8.04 % على التوالي. من الناحية البيولوجية، تهيمن النباتات النصفية (37.5%) على الغطاء النباتي لمنطقة الدراسة. اما فيما يتعلق بالأصل الجغرافي الحيوي لهذه الأنواع، هناك عدد كبير من الأنواع تستوطن حوض البحر الأبيض المتوسط (51%).

أظهر التحليل الكيميائي ان الزيت العطري ل *T. algeriensis* Boiss. & Reut. يحتوي على 89 عنصرا كيميائيا، حيث يعتبر كل من le (1R)-2,6,6 (8,65 %), l'eucalyptol (12,54 %), (+)-2-bornanone (8,48 %), triméthylidicyclo[3.1.1]hept-2-Eni (6,58 %) et linalol (6,23%) العناصر الأساسية المكونة لهذا الزيت العطري. واما بالنسبة للزيت العطري *S. rosmarinifolia* L. فيتكون من 96 مركبا، مكوناته الأساسية هي Eucalyptol (18.39%), Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene (15.78%), 2(10)-Pinene (10.67%), (-)-Germacrene D (5.88%).

أظهرت الزيوت الأساسية والمستخلصات الميثانولي التي تم اختبارها نشاطا مضادا للبكتيريا هاما ضد الإشريكية القولونية (*Escherichia coli*)، المكورات العنقودية الذهبية (*Staphylococcus aureus*)، اما بالنسبة للزئافة الزنجارية (*Pseudomonas aeruginosa*) فقد أظهرت نشاطا مضادا للبكتيريا ضعيفا ضدها. تتميز هذه الزيوت وهذه المستخلصات بالقدرة الكبيرة كمضادات للأكسدة ؛ ويقاس باختبار DPPH.

الكلمات المفتاحية: الحظيرة الوطنية بلزمة، جرد الزهور، إعادة اكتشاف، *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller، *Thymus algeriensis* Boiss. & Reut.، *Santolina rosmarinifolia* L.، الزيت العطري، النشاط البيولوجي.

List of abbreviations

BNP : Belezma National Park.

ANOSIM: Statistical analysis of similarities

PARMANOVA: Multivariate Permutation Analysis of Variance

ACC: Canonical analysis of correspondences

C2: Hodna Mountains and Belezma

APG: Angiosperm Phylogeny Group

DGF: general directorate of forestry

M: maximum temperature

m: minimum temperature

P: precipitation

Med: Mediterranean

Ibero-Maur: Ibero-Mauretanian

Cosmop: Cosmopolitan

Eur: European

Atl: Atlantic

Euras: Eurasian

As: Asian

Irano-Tour: Irano-Touranian,

Paleo-Trop: Paleo-Tropical

Circumbor: Circumboreal

Paleotemp: Paleotemperate

End: Endemic

Alg: Algerian

N Afr: North African

Mor: Moroccan

Tun: Tunisian

Sah: Saharan

Sah-Sind: Sahara-Sindian

Sic: Sicilian

W: West, E: East

S: South

Oro: Montagnard.

AGE: gallic acid equivalents

QE : quercetin equivalents

DPPH: 2,2-diphenyl 1-1-picrylhydrazyl

HE = Essential oil.

IC₅₀ = Median inhibitory concentration.

CG/SM= Gas chromatography coupled to mass spectrophotometry

DW: dry weight

Figures list

Figure 1. Chemical structure of phenolic compounds.	22
Figure 2. Basic structure of Flavonoids.	23
Figure 3. Chemical structure of flavones and flavonols	24
Figure 4. Biosynthesis of phenolic compounds.	25
Figure 5. The general metabolic pathway for the biosynthesis of various terpene groups.	28
Figure 6. Location map of the Belezma National Park (Batna, North East of Algeria).	36
Figure 7. Soil map of the Belezma National Park.	37
Figure 8. Geological map of the Belezma National Park.	38
Figure 9. Hydrological map of the Belezma National Park.	39
Figure 10. Hydrothermal diagram (Batna Station 1991-2020).	47
Figure 11. Hydrothermal diagram (Point 1111 m 1991-2020).	47
Figure 12. Hydrothermal diagram (Point 1886 m 1991-2020).	48
Figure 13. Distribution map of the stations in Belezma National Park.	50
Figure 14. <i>Thymus algeriensis</i> Boiss. & Reut.	57
Figure 15. <i>Santolina rosmarinifolia</i> L.	55
Figure 16. Maceration.	58
Figure 17. Filtration.	56
Figure 18. Device of the rotary Rota-steam type (BÜCHI R- 120).	56
Figure 19. Protocol preparation of extracts.	59
Figure 20. Dry plant material.	60
Figure 21. Original hydrodistillation photo	58
Figure 22. Gas chromatography / mass spectroscopy apparatus.	60
Figure 23. Mechanism of reduction of the free radical DPPH by an antioxidant.	63
Figure 24. Original photo of a spectrophotometry device.	64
Figure 25. <i>Tulipa sylvestris</i> subsp. <i>primulina</i> (Baker) Maire & Weiller (photograph by N. Maalem).	69
Figure 26. Specimen of <i>Tulipa sylvestris</i> subsp. <i>primulina</i> (Baker) Maire & Weiller	70
Figure 27. Station 1: Fisdis sector (photograph by N. Maalem, 18 April 2022).	71
Figure 28. Station 2: Oued El Ma sector (photograph by N. Maalem, 16 May 2022).	72

Figure 29. Distribution map of <i>Tulipa sylvestris</i> subsp. <i>primulina</i> (Baker) Maire & Weiller in Algeria.....	72
Figure 30. Biological spectrum of species inventoried from Belezma National Park.....	73
Figure 31. ACC map of the distribution of botanical families in the studied stations.....	81
Figure 32. Contents in % of the major chemical components of the essential oils of aerial parts of <i>T. algeriensis</i> Boiss. & Reut.	86
Figure 33. The chemical classes of the essential oil of <i>T. algeriensis</i> Boiss. & Reut.	86
Figure 34. Contents in % of the major chemical components of the essential oils of <i>S. rosmarinifolia</i> L.	88
Figure 35. The chemical classes of the essential oil of <i>S. rosmarinifolia</i> L.	91

Tables list

Table 1. Classification of terpenes.....	29
Table 2. Characteristics identifying the weather station of Batna.	40
Table 3. Average monthly rainfall of the study region calculated over the periods 1991-2020). 41	
Table 4. Seasonal rainfall (mm) for the Batna station and the Belezma Mountains from 1991 to 2020.....	42
Table 5. Average monthly thermometric data in (°C) from the Batna station for the period from 1991 to 2020.	44
Table 6. Average monthly thermometric data in (°C) of the study region calculated by extrapolation over the period 1991-2020.....	44
Table 7. Maximum average amplitudes recorded at the stations studied during the period 1991-2020).	45
Table 8. Evaluation of the rainfall quotient Q2 over the period from 1991 to 2020.	49
Table 9. Sampling site data sheet.....	54
Table 10. List of bacterial strains tested	61
Table 11. floristic inventory in families, number and frequency of species recorded in the Belezma National Park.	65
Table 12. Distribution of the biological spectrum of flora identified in the study area.....	72
Table 13. Biogeographic distribution of the species inventoried in the BNP.....	75
Tableau 14. The total specific richness of the species found in BNP.....	77
Table 15. The totale values of the Sorensen similarity index for the species inventoried in the belezma National Park taken two by two.	78
Table 16. Result of Similarity Analysis (ANOSIM) of the species composition between sectors, bioclimatic floors, forest formations, orientations and zoning.	79
Table 17. PERMANOVA results for the different sectors, bioclimatic type, forest formations, orientations and zoning of Belezma National Park.....	80
Table 18. Quantity of essential oil of <i>T. algeriensis</i> Boiss. & Reut. And <i>S. rosmarinifolia</i> L. extracted by hydro distillation.	84
Table 19. Total phenol and flavonoid contents of the aerial parts of the methanolic extracts of <i>T. algeriensis</i> Boiss. & Reut. and <i>S. rosmarinifolia</i> L.	90

Table 20. Antibacterial activity of the aerial part of <i>T. algeriensis</i> Boiss. & Reut and <i>S. rosmarinifolia</i> L.	91
Table 21. Results of the antioxidant activity of the essential oils of <i>T. algeriensis</i> Boiss. & Reut, <i>S. rosmarinifolia</i> L. and that of BHT.	93

Table of contents

Resumé

Abstract

ملخص

Introduction..... 1

Chapter 1: General information on Biodiversity

1	Definition of biodiversity.....	6
2	Levels of biodiversity	6
3	Biodiversity and ecosystem functioning.....	7
4	Biodiversity in the world	7
5	Biodiversity in the Mediterranean basin	8
6	Biodiversity in North Africa	9
7	Floristic biodiversity in Algeria.....	10
8	Biodiversity conservation strategies in Algeria	11
8.1	In-situ conservation	11
8.1.1	Protected areas	11
8.1.2	National Parks.....	12
8.1.3	Nature reserves.....	13
8.1.4	Game Reserves.....	13
8.1.5	Biosphere Reserves	14
8.1.6	Ramsar Sites.....	14
8.2	Ex-situ conservation.....	15
9	Biodiversity of the Belezma National Park	15

Chapter 2 : Presentation of studied plant

1	Lamiaceae family.....	16
2	Genus <i>Thymus</i>	16
3	<i>Thymus algeriensis</i> Boiss. & Reut. specie.	17
3.1	Botanical description.....	17
3.2	Systematic position of <i>Thymus algeriensis</i> Boiss. & Reut.	17
3.3	Geographical distribution.....	18

3.4	Therapeutic properties and use.....	18
4	Astraceae Family	18
5	Genus Santolina	19
6	Santolina rosmarinifolia L. specie	20
6.1	Botanical characteristics.....	20
6.2	Classification in the systematics	20
6.3	Geographical distribution.....	21
6.4	Therapeutic properties and use.....	21

Chapter 3: Phytochemistry and biological activity

1	Secondary metabolism.....	22
1.1	Phenolic compounds	22
1.1.1	Flavonoids.....	23
1.1.1.1	The main classes of flavonoids	24
1.1.1.1.1	Flavones and Flavonols.....	24
1.1.2	Biosynthesis of phenoli compounds.....	25
2	What is an essential oil	26
2.1	Physical properties	26
2.2	Chemical properties.....	27
2.3	The role of essential oils.....	27
2.4	Toxicity of essential oils	27
2.5	Location of essential oils.....	27
2.6	Chemical composition of essential oils.....	28
2.7	Terpenes	28
2.8	Aromatic compounds	29
2.9	The Factors affecting the stability of essential oils	29
2.10	Essential oil extraction methods.....	30
2.10.1	Distillation.....	30
2.10.2	Hydrodistillation	30
2.10.3	Extraction by steam entrainment	31
2.10.4	Percolation (hydro-diffusion).....	31
2.10.5	Extraction by organic solvent	31
2.11	Essential oil analysis techniques	31

2.11.1	Analysis by gas chromatography (GC).....	31
2.11.2	Gas chromatography coupled with mass spectrometry (GC/SM)	32
3	Biological activities	32
3.1	Antioxidant activity.....	32
3.2	Antibacterial activity.....	33

Experimental part

Chapter 1: Materiel and Methods

First part: Study of the floristic biodiversity of the Belezma National Park

1	Presentation of the study area	35
1.1	History of the Belezma National Park.....	35
1.2	Geographical location	36
1.3	Pedology.....	37
1.4	Geology	38
1.5	Relief	38
1.6	Hydrology.....	39
1.7	Climate	40
1.7.1	Precipitation	41
1.7.1.1	Average monthly and annual rainfall	43
1.7.1.2	Seasonal precipitation regime.....	44
1.7.2	Temperatures.....	43
1.7.2.1	Average monthly and annual temperatures.....	45
1.7.2.2	Thermal amplitudes.....	47
1.8	Climate summary	46
1.8.1	Bagnouls and Gausson thermal diagram.....	48
1.8.2	Emberger's Climagram.....	50
2	Inventory	50
2.1	Choice of stations.....	Erreur ! Signet non défini.
2.2	Sampling method and species identification	50
3	Ecological indices	51
3.1	Total Specific richness (S)	51
3.2	SORENSEN similarity index.....	51

3.3	Disturbance index.....	52
4	Statistical analysis.....	52
4.1	Bi-varied statistical analysis.....	52
4.1.1	Similarity Analysis (ANOSIM)	52
4.1.2.	Permutational Multivariate Analysis of Variance" (PERMANOVA)	53
4.2.	Multivariate statistical Analysis	53
4.2.1.	Canonical correspondence analysis (CCA) of the floristic distribution in the Belezma National Park	53

Second part: phytochemistry and biological activities

1	Plant material	54
1.1	The Harvest of plants	54
1.2	Identification of the plant	54
1.3	Grinding the dry parts	55
1.4	Quantitative Characterization of Extracts	55
1.5	Maceration principle	55
1.6	Solid/liquid type extraction (Extraction by maceration in aqueous methanol): Preparation of extracts.....	55
2	Extraction of essential oils.....	58
2.1	Extraction by hydrodistillation.....	58
2.2	Calculation of the yeild.....	61
3 Chemical characterization of the HEs of <i>Thymus algeriensis</i> Boiss. & Reut and <i>Santolina rosmarinifolia</i> L.....	59
3.1	Determination of the chemical composition	59
3.2	Identification of chemical compounds	60
4	Determination of total phenols and flavonoids.....	60
5	Biological activities	61
5.1	Microbiological study	61
5.1.1	The strains tested.....	61
5.2	Evaluation of the antibacterial activity of <i>Thymus algeriensis</i> Boiss. & Reut and <i>Santolina rosmarinifolia</i> L.....	61
5.2.1	Principle	61
5.2.2	Preparation of culture medium.....	61

5.2.3	Preparation of dilutions of extract of the plants <i>Thymus algeriensis</i> Boiss. & Reut and <i>Santolina rosmarinifolia</i> L.....	62
5.2.4	Preparation of the discs.....	62
5.2.5	Reading the results.....	62
5.3	Antioxidant activity.....	63
5.3.1	Free radical test DPPH (2, 2-diphenyl 1-1-picrylhydrazyl).....	63

Chapter 2: Results and discussion

First part: Study of the floristic biodiversity in the Belezma National Park

1	Floristics inventory in the Belezma National Park.....	65
2.	Rediscovery of <i>Tulipa sylvestris</i> subsp. <i>primulina</i> (Baker) Maire & Weiller in the Belezma National Park.....	68
2.1	Taxonomy.....	68
2.2	Habitat description.....	70
2.3	Distribution.....	72
3.	Biological spectrum of the species inventoried in Belezma National Park.....	73
4.	Biogeographic distribution of the species inventoried in Belezma National Park.....	74
5.	Biodiversity Measurement.....	77
5.1	The diversity indices.....	77
5.1.1	The specific richness (S).....	77
5.1.2	Variation of the similarity according to the flora recorded in Belezma National Park.....	78
5.2	Multivariate Statistical Analysis.....	79
5.2.1	Statistical analysis of similarities (ANOSIM).....	79
5.2.2	Multivariate Analysis of Variance by Permutation "Permutational Multivariate Analysis of Variance" (PERMANOVA).....	80
5.3	Multivariate Statistical Analysis.....	81
5.3.1	Canonical correspondence analysis (CCA) of the floristic distribution in the Belezma National Park.....	81

Second Part: the phytochemistry and biological activities of *Thymus algeriensis* Boiss. & Reut. and *Santolina rosmarinifolia* L.

1	Plant material.....	84
1.1	Chemical characterization of the essential oils of <i>T. algeriensis</i> Boiss. & Reut. and <i>S. rosmarinifolia</i> L.	84
1.1.1	Yield of essential oils (R).....	84

1.2	Chemical composition of essential oils of <i>T. algeriensis</i> Boiss. & Reut.	85
1.3	Chemical composition of essential oils of <i>S. rosmarinifolia</i> L.	87
1.4	Determination of total phenols and flavonoids	90
2.	Biological activities	91
2.1.	Evaluation of the antibacterial activity.....	91
2.2.	Evaluation of the antioxidant activity	93
2.2.1	Free radical scavenging test DPPH (2,2-diphenyl-1-picrylhydrazyl)	93

Conclusion

References

Annexes

Introduction

Introduction

The Mediterranean basin is widely recognized for its exceptional biodiversity. It should be noted that the Mediterranean region, although it represents only 1.8% of the world's forest area, is home to a significantly higher number of forest species than the rest of Europe (Gauquelin et al., 2016).

The Mediterranean forests have experienced a series of modifications throughout history, the result of a multitude of factors, including fires, human activity (including their livestock and tools), and more recently, climate and global change. The dynamics of these forests can be considered a series of responses and feedbacks to anthropogenic activities and systems. It is crucial to acknowledge that overexploitation and global protection measures may potentially diminish the appeal and economic value of these terrestrial ecosystems (Peñuelas et al., 2017).

The terrestrial ecosystems of the Mediterranean are renowned for their high levels of biodiversity, particularly in terms of endemic species (FAO, 2013). Furthermore, the region is home to numerous protected areas and natural parks. Nevertheless, the region's abundant resources can occasionally give rise to conflicts between users and a potential depletion of resources (Houé, 1996).

Plants have been used for centuries for their medicinal properties, and many civilizations have developed knowledge of herbal medicine through empirical observation. The Ancients, in particular, were adept at identifying the healing and protective properties of medicinal plants, which have withstood the test of time (Carillon, 2009).

The demand for natural herbal substances is on the rise due to their diverse array of biological activities, which have been shown to have a positive impact on human health. These activities encompass a wide range of properties, including anticancer, antiviral, antibacterial, antimalarial, antifungal, antioxidant, and insecticidal properties. The scientific evaluation of traditional medicine can facilitate the development of effective herbal medicines.

Introduction

Algeria is acknowledged for its diverse biological resources, shaped by a variety of climates and geographies, which have given rise to a wide range of natural species. The country's varied flora is highly regarded for its plant genetic heritage, due to its spatial distribution and ecological importance (Snoussi et al., 2003). The Algerian flora is known for its great diversity, with 4449 (Dobignard & Chatelain, 2010-2013). In addition, it is home to a considerable number of rare species. According to the FAO (2012), there are 289 fairly rare species, 647 rare, 640 very rare and 35 extremely rare species.

The genus *Tulipa* L. and its subspecies are important on a local and worldwide scale since they contribute to the biodiversity of Enrique and the ecological value of the protected area. Chabert (1897), Marie (1958), and Quezel and Santa (1962) found the subspecies *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller to be common in Algeria in general and in Belezma National Park in particular, but these were ancient and limited studies. In our study, we rediscovered the presence of this species in Belezma National Park after 6 centuries, investigated the ecology of this rare plant, and attempted to estimate its dispersal throughout Algeria.

Medicinal plants are an essential part of the history of human medicine, used since ancient times to treat diseases. These plants distinguished by their natural diversity and their ability to produce chemical compounds with multiple health benefits. The use of medicinal plants is of paramount importance today due to their naturalness and their ability to improve health and well-being without harmful side effects. This research aims to explore the benefits of medicinal plants and their importance in the modern medical field (Belkacem, 2009).

However, due to the limited amount of research on its potential benefits, a significant portion of the potential of the flora remains unexplored (Boudoumi, 2014). The extraction of essential oils represents a potential use of this natural resource. These oils have been highly appreciated for their medicinal and flavoring properties since antiquity by various cultures, especially the Greeks, Egyptians, and Persians (Iserin, 2001).

Essential oils have a significant degree of variability, both in their composition and in their yield, which is a fundamental aspect given that their effects can differ considerably (Benini, 2007).

The *Thymus* and *Santolina* genus, which respectively belong to the Lamiaceae and Astraceae families, contain numerous medicinal plants that have been traditionally used for various purposes.

Introduction

It is crucial to acknowledge that the *Thymus* genus encompasses a multitude of botanical species that thrive in coastal and inland regions, including arid areas (Hammaz & Nafa, 2017). According to Quezel and Santa (1963), there are 12 species of *Thymus*, eight of which are endemic (Dob et al., 2006), and the genus *Santolina* includes more than 10 species distributed throughout the Mediterranean region, some of which are used as ornamental plants. (Ferrari et al., 2005, Kisiel et al., 2003; Palá et al., 2001).

Thymus algeriensis Boiss. & Reut. and *Santolina rosmarinifolia* L. are two medicinal plants frequently used in traditional Algerian medicine. These species have shown significant biological activity and have been the subject of numerous phytochemical studies, which have revealed a variety of bioactive secondary metabolites.

The objectives of this thesis are, first of all; identify the structural characteristics of plant communities in forest and pre-forest environments and to elucidate their composition. Furthermore, the thesis will examine the floristic dynamics of these communities in relation to local environmental. To achieve these objectives, a comprehensive floral inventory will be conducted, and the list of Belezma plants will be updated. This updated list will be analyzed in the context of promotion. Additionally, *Thymus algeriensis* Boiss. & Reut. and *Santolina rosmarinifolia* L. were selected for analysis. The objective was to examine the chemical composition of essential oils in aerobic parts and evaluate their antibacterial and antioxidant activities. Specimens were collected in Park during the flowering season.

A number of studies have been conducted at Belezma National Park, including Boukerker (2016), Smahli and Kalla (2017), and Benzina (2019). However, a comprehensive floristic inventory of the entire park is currently underway. The choice of Belezma National Park is primarily supported by its advantageous geographical location. It is considered a contact zone, a biogeographic crossroads between the Aurès Mountains to the east, the Bou Taleb Mountains to the west, constantinois's high plains to the north, and the Saharan Atlas Mountains to the south (Salhi, 2004). Belezma National Park boasts a diverse flora and an abundance of aromatic and medicinal plants. Its favorable geographical location and its varied climate provide optimal conditions for plant growth. Despite the park's diverse flora, few studies have been conducted on it. The park's floristic inventory has also not been updated.

Introduction

In the light of these postulates, we have asked ourselves a few questions, which we will try to answer at the end of this work. These questions first address vegetation and natural habitats: what is the organization of regional forest and pre-forest vegetation? Which plants groups make up these plant communities and what are their biological and phytogeographic affinities?

Regarding the *Thymus algeriensis* Boiss. & Reut. and *Santolina rosmarinifolia* L., what is the chemical composition of their essential oils? Moreover, what are their antibacterial and antioxidant properties?

In general, it is according to the above-mentioned hypotheses and questions, our work is divided into two parts. The first section is devoted to a bibliographic synthesis structured into three chapters. The first chapter provides general information about biodiversity; the second chapter describes the plants researched; and the third chapter discusses phytochemistry and biological activities, including antibacterial and antioxidant activity.

The experimental section is divided into two chapters. The first chapter is material and methods, and it is divided into two parts: the first on the park's floral diversity, and the second on phytochemistry and biological activities, specifically the hydrodistillation of essential oils of *Thymus algeriensis* Boiss. & Reut. and *Santolina rosmarinifolia* L., the analysis and identification of the chemical composition of essential oils using GC-MS, and the study of antibacterial and antioxidant properties. The second chapter presents and discusses the acquired results.

Bibliographic part

Chapter 1: General information on Biodiversity

1. Definition of biodiversity

The concept of biodiversity emerged in the late 1970s, referring to the variety of life and living beings inhabiting the biosphere (Dajoz, 2003; Ramade, 2009). In 1986, Wilson introduced the term "biodiversity" during the American National Forum on Biological Diversity, which was an immediate success and has been used worldwide since the Rio de Janeiro Conference on Environment and Development in 1992 (Du Bus De Warnaffe, 2002).

Biodiversity refers to the diversity of living organisms, encompassing the molecular scale to the biosphere scale. However, ecologists are more interested in populations, communities, and ecosystems (Krebs, 2001).

The OUN organized the Earth Summit, also known as the Rio de Janeiro Convention on Environment and Development, in 1992. According to his definition, biological diversity is the "variability of living organisms of all origins including, among others, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species and between species as well as that of ecosystems" (Lévêque & Mounolou, 2008).

For Frankel (1970), biodiversity refers to the variety and variability of all living organisms.

Biodiversity is a significant topic in ecology, encompassing the variety of living organisms at different functional levels. Nevertheless, as Barbault (1997) and Chevassus-au-Lewis (2005) posit, biodiversity is not merely the description of the diversity of organisms, however extensive it may be. However, in reality, this encompasses interactions within each functional level, between different functional scales, and with human societies.

2. Levels of biodiversity

There are three levels of biodiversity according to Robert-Pichette & Gillespie (2000):

- a. Ecological diversity or diversity of ecosystems.
- b. Specific diversity or interspecific diversity.
- c. Genetic diversity or intra-specific diversity.

3. Biodiversity and ecosystem functioning

The term "biodiversity" has historically been used to refer to the variety of species present within a given ecosystem (Gaston, 1996; Purvis & Hector, 2000; Magurran, 2004). This diversity is continuously changing due to natural factors, such as environmental stochasticity, and human disturbances (Pimm et al. 1995). The decline in biodiversity has given rise to numerous studies on its ecological importance, leading to the development of key concepts (Hooper et al., 2005; Naeem et al., 2009; Cardinale et al., 2012). Biodiversity promotes the stability and sustainability of ecosystems and communities, functioning as an environmental protection (Thébault & Loreau, 2005; Tilman et al., 2006).

This phenomenon, termed "species redundancy," permits the replacement of lost species by functionally equivalent species. Moreover, the diversity of organisms within a community is crucial for the fundamental processes of the ecosystem, including primary production rates, the decomposition of organic matter, and the recycling of nutrients (Hooper et al., 2005, 2012; Cardinale et al., 2017). Ecosystems with greater species diversity exhibit higher levels of process intensity than those with lower species diversity (Naeem et al., 1994).

The relationship between biodiversity and the functioning of ecosystems has been the subject of considerable attention at the interspecific level (Hooper et al., 2005). These studies have shown that changes in biodiversity can have multifaceted impacts on ecosystem processes (Naeem & Wright, 2003). In fact, species interact with each other in a complex way, modulating their individual effects (de Ruiter et al., 1995).

Thus, the relationships between biodiversity and ecosystem functioning, although sometimes criticized and questioned, can present a tendency to saturation where an increase in biodiversity translates into a corresponding increase in the ecosystem process considered (such as primary production) until reaching a plateau (Hooper et al., 2005).

4. Biodiversity in the world

According to Ramade (2008), biodiversity is unevenly distributed over the surface of the biosphere, encompassing both continental and oceanic ecosystems. As we travel the surface of the Earth, biodiversity generally decreases from the equator to the poles, with some exceptions in terrestrial and marine environments. A general principle is that warmer climates promote increased

variety in terrestrial environments. Equatorial forests constitute the most biologically diverse biome in the world, containing more than 70% of all the species of higher plants on the planet, with 180000 species located in the intertropical zone, representing only 40% of the earth's surface. Moreover, half of these species are found exclusively in dense tropical forests (Poncy & Labat, 1995 in Gimaret-Carpentier, 1999). Aquatic ecosystems, including lakes and rivers, represent only a fraction (0.01%) of the Earth's water. Nevertheless, these ecosystems are home to a significant level of biodiversity, with more than 100000 species identified, which represents about 6% of the global biodiversity of the planet (Dudgeon et al., 2006). The wide range of flora and fauna concentrated in small areas makes aquatic ecosystems hotspots of biodiversity (Strayer & Dudgeon, 2010). Thus, all levels of food webs, from primary producers to predators, present a great diversity (Nelson et al., 1994; Covich et al., 2004a; Clarke et al., 2008; Stomp et al., 2011). This diversity is crucial for the functioning of ecosystems and supports various ecosystem services (Covich et al., 2004a, 2004b, Rafard, 2023).

5. Biodiversity in the Mediterranean basin

The five Mediterranean climate ecoregions represent a relatively small area on a global scale, with only 1.6% of the terrestrial territory. Of this area, almost 80% is located around the Mediterranean (Médail & Quézel, 1997). It is worth noting that this region is home to a remarkable diversity of life forms (Cowling et al., 1996). It ranks third among the richest hot spots in the world in plant diversity (Mittermeier et al., 2004). The Mediterranean basin is home to more than 10% of the total plant species (phanerogams and pteridophytes) around the world. In Europe, Mediterranean plants represent 80% of endemic plants (Blondel & Aronson, 1999).

The specific richness is estimated between 25000 and 30000 species and subspecies (Médail et al., 2012), with a high richness in rare plants in families with a large number of species (Dominguez Lozano & Schwartz, 2005).

According to Sedjar (2015), Mediterranean forests are generally organized into successful vegetation levels based on altitude and thermal criteria. The author proposes the following terminology, which depends on the thermal criteria:

-The sub-Mediterranean floor: m between +7 and +3 C °, well represented in North Africa.

Chapter 1: General information on Biodiversity

-The Meso-Mediterranean floor: (also called eu-Mediterranean) is between zero and three, located between 400-500 m and 800-1000 m of altitude on the littoral and the sub-littoral of the North African countries and between 1200-1400 further south in the Tellian and Saharan Atlas.

-The supra-Mediterranean floor extends between 400-500 meters, 800, and 900 meters, with values up to 1400 and 1500 meters in North Africa. The temperature range is between 0 and -3 degrees Celsius.

-The Mediterranean mountain floor typically extends between 1600-1800 meters, 2300-2500 meters in North Africa. The values of m range from -3 to -7 degrees Celsius.

-The Oro-Mediterranean floor: is located beyond 2200-2500m on the high Mediterranean mountains where m is lower than a -7c °.

Although the history of Mediterranean forest is relatively well known, phytogeographers can contribute to our understanding of the potential spread of important tree species around the Mediterranean perimeter (Quézel, 1991).

Climate change is having a significant impact on the Mediterranean regions of Europe and North Africa. The vegetation cover of these areas will evolve more rapidly and more extensively in the long term than in other parts of the world (Hesselbjerg-Christiansen & Hewitson, 2007). However, this change will undoubtedly lead to a reduction in water availability during the growing season, especially at high altitudes (Vennetier & Ripert, 2010; Ozenda, 1997).

6. Biodiversity in North Africa

The flora of North Africa is relatively well documented, with about 125 endemic species (Maire, 1926). From a global point of view, Quézel conducted the first assessment in 1978, revealing the existence of 916 genera and 4034 species outside the Saharan regions, of which 1038 are endemic species (Medail & Quézel, 1997).

In 2000, Quézel was clear that "North Africa, which constitutes only a part of the Mediterranean world (about 15%)", does not currently have a precise assessment of the number of existing plant species." it is estimated between 5000 and 5300.

7. Floristic biodiversity in Algeria

In 1926, Maire differentiated the formations of the forest heritage in Algeria by classifying them into plant groups with coherent physiognomy. Algeria, like all Mediterranean countries, is troubled and confronted with the deterioration of its pastoral and forestry resources (Bestaoui, 2001).

The flora of Algeria has evolved relatively little since the separation of Africa and Europe. However, the situation in this country is probably less serious than that of other African countries. In 1999, these forests covered approximately 3.7 million hectares (Quezel and Santa, 1962-1963), (Babali, 2014). The region boasts a diverse range of floral species, with 4449 (Dobignard & Chatelain, 2010-2013) identified. A note sent to the press by the management on the International Day of Forests, celebrated every year on March 21, the DGF identified 289 species as "fairly rare," 647 as "rare," 640 as "very rare," and 35 as "extremely rare" (DGF, 2021).

The vulnerability of Algerian biodiversity is increasing due to the fragility of its ecosystems. Due to anthropogenic pressure, many endangered taxa are now on international lists such as CITES, CMS, and IUCN. All natural ecosystems have been affected by varying degrees of destruction. The ecosystems that have suffered the most damage include freshwater environments and marine coastal environments (Bakalem, 2012; Bouroumi, 2014), as well as forests and continental wetlands.

These environments exhibit a reduction in both area and biodiversity. The least productive terrestrial ecosystems are the steppe zones, and it is among the most exploited areas, resulting in a decline in biodiversity (MATE, 2014).

8. Biodiversity conservation strategies in Algeria

The conservation of national habitats and ecosystems in Algeria has been designated as the highest national priority in the national biodiversity strategy and action plan.

Many protected areas are ecosystems with fragile conditions and are affected by social, economic, and ecological forces, especially in the arid or semi-arid regions of the south. Nature reserves and national parks have structures and management plans that require reorientation or revision to reflect the objective of biodiversity conservation.

8.1. In-situ conservation

8.1.1. Protected areas

The term "protected areas" refers to IUCN management categories. These areas are primarily meant to preserve biological resources while also maintaining ecological balances and diverse habitats. The International Union for the Conservation of Nature (IUCN) has established six categories of protected areas, as specified by the Commission for National Parks and Protected Areas (CPNAP). The CPNAP sets the objectives and criteria for classifying these Protected Areas. According to IUCN 1994 and Adrian 1998 in Chalabi et al. (2002), protected areas include:

Category I: Nature reserve/wilderness area: Protected Area managed mainly for scientific purposes or the protection of wild resources.

Category II: National Park.

Category III: Natural Monument.

Category IV: Habitat or species management area.

Category V: Protected terrestrial or marine landscape.

Category VI: Protected area of managed natural resources.

Three additional protected areas may be established, specifically those designated as wetlands of global significance for water-birds (Ramsar sites), sites inscribed on the UNESCO World Heritage List, and biosphere reserves.

8.1.2. National Parks

National parks represent a vital element of national networks of protected areas in terms of in-situ conservation. Algeria's national parks collectively encompass over 50 million hectares, distributed across all ecological sectors within the country's biogeographic domains (Abdelguerfi, 2003).

- The creation of national parks in Algeria took place chronologically in six phases over about thirty years:

- 1972: Creation of the Tassili National Park, the first national park created since the recovery of the independence of Algeria.

- 1983: the creation of 04 national parks in the North of the country: El-Kala National Parks, de Chr a, from Djurdjura, and Th ni t-El-Had.

-1984: the creation of three other national parks, still in the north of the country: Belezma, Gouraya, and Taza National Parks.

-1987: creation of the Ahaggar National Park, the second Saharan park, and reorganization of the Tassili National Park.

-1993: creation of the Tlemcen National Park, still in the northern fringe of the country.

-2003: classification of the last national park in Algeria: Djebel A ssa National Park (wilaya of N ama), on 24600 hectares (executive decree n   03-148 of March 29, 2003 classifying the

Djebel A ssa National Park). This park is located on the Saharan Atlas. It is the first National Park established on the Saharan Atlas.

The cumulative area of Algeria's 11 national parks, including the Northern and Southern regions, is 53193837 hectares, or 22.33% of the country's total territory.

8.1.3. Nature reserves

Autonomous structures, such as nature reserves, are of paramount importance for the in-situ conservation of Algeria. By Algerian regulations, the objective of these reserves is to preserve, reconstitute, safeguard, conserve, and develop fauna and flora, as well as the soil, sub-soil, atmosphere, water, and any other natural environment of particular interest that must be protected. Furthermore, the encouragement of scientific research in nature reserves is encouraged (Belguerfi & Bellatreche, 2003).

Algeria has five nature reserves. Of these, only one was officially classified by executive decree in 2003: the Habibas Islands Marine Nature Reserve (executive decree n ° 03-147 of March 29, 2003).

According to the regulations in force, nature reserves must be administratively attached to the nearest national park. Thus:

- The Macta Nature Reserve must be attached to the Tlemcen National Park.
- The Babors Nature Reserve is attached to the Taza National Park.
- The Mergueb Nature Reserve must be attached to the Djurdjura National Park.
- The Beni-Salah nature reserve must be attached to the El-Kala National Park.
- The marine nature reserve of the Habibas Islands must be attached to the Tlemcen National Park.

8.1.4. Game Reserves

In Algeria, game reserves are sites where hunting is strictly prohibited, even if their name suggests otherwise. According to Algerian regulations, the purpose of hunting reserves is:

The protection and development of local game.

Develop the habitats of resident species.

Chapter 1: General information on Biodiversity

Provide a space for observation and experimentation on the biology, ecology, and ethology of existing species, and establish and maintain an inventory of the hunting heritage of the reserve.

-The current national network is composed of four hunting reserves, which are:

- The Djelfa hunting reserve (32400 ha) created in 1983.
- The Mascara hunting reserve (7000 ha), created in 1983.
- The Tlemcen hunting reserve (2000 ha), created in 1983.
- The Zeralda hunting reserve (1200 ha), created in 1984.

8.1.5. Biosphere Reserves

The MAB network, which is part of the UNESCO program on man and the biosphere, includes a network of representative ecological zones that integrate conservation, ecological research, monitoring, education, training, and conventional land use. In 1986, Algeria became a member of the MAB network by proposing the Tassili National Park as the inaugural Algerian biosphere reserve. Subsequently, three additional areas were integrated into the MAB network in Algeria: the El-Kala National Park Reserve in 1990, the Djurdjura National Park Reserve in 1997, and the Chr ea National Park Reserve in 2003. Currently, seven reserves of the UNESCO-MAB biosphere network are grouped, namely the National Parks of Chr ea, El Kala, Djurdjura, Tassili, Gouraya, Taza, and Belezma.

8.1.6. Ramsar Sites

Covering nearly three million hectares in Algeria, wetlands present diverse landscapes and species.

They are widely distributed in various natural regions, including lakes, ponds, lagoons, marshes, swamps, dam lakes, hill reservoirs, chotts, sebkhas, gueltas, and oases. Wetlands in Algeria are distributed across the country's various natural regions. The larger areas are renowned as winter habitats and gathering places for migratory waterfowl, which travel between Eurasia and Africa.

Algeria has 50 wetlands classified according to the Ramsar Convention of international importance, including Lake Tonga, Chott el Hodna, and Chott Chergui, among others. These

protected areas constitute only a fraction of the areas preserved in Algeria as part of its protected areas conservation program (Abdelguerfi 2003).

8.2. Ex-situ conservation

Ex-situ conservation is practiced in botanical and zoological gardens by the cultivation of potentially extinct species and the development of banks of genetic material, seeds, pollen, plants, tissue cultures, genes, and similar materials (Guerrant et al., 2004 b). This approach aims to preserve biodiversity and prevent the disappearance of valuable species. Nevertheless, this remains a secondary option for in-situ conservation or when there is a need to protect the genetic resources of species in case of emergency (Guerrant et al., 2004 a, Cohen et al., 1991; Maunder & Byers, 2005).

Indeed, protecting biodiversity is more than just preserving its existing makeup; it is also about ensuring its evolutionary potential. Conservation efforts take place in a variety of locations, including botanical gardens (Djelfa, Illizi, Laghouat, and Tlemcen), zoological gardens, gene banks (INRAA), and wild or agricultural variety conservatories (Crops and Selection) (MATE, 2014).

9. Biodiversity of the Belezma National Park

Belezma National Park is home to a wide range of flora and fauna that reflect the rich diversity of its ecosystems. The distribution of land in the park reveals distinct ecological units, which are entities of the natural environment that have a high degree of similarity in terms of physical characteristics (such as the nature of the substrate, the shape of the relief, and the altitude) and biological characteristics (such as vegetation cover and fauna). Ten ecological units have been identified in the BNP, according to their physical and biological characteristics, as outlined in BNP (2015). Nevertheless, a recent study on the classification of ecosystems within the national park revealed the existence of 45 different types of ecosystems. In addition, recent updates on the flora and fauna present in the BNP reveal 598 floral species, including 14 species specifically protected in Algeria. The fauna is composed of 745 species, including 574 invertebrates and 171 vertebrates, of which 68 are protected species (Benzina, 2020).

Chapter 2 : Presentation of studied plants

1. Lamiaceae family

The Lamiaceae family (Labiatae) is a family of plants widely distributed throughout the plant kingdom (Naghbi et al., 2005). Mainly includes herbaceous plants, shrubs, and some associated trees and lianas (Yuan et al., 2010). It is considered one of the largest families, comprising more than 200 genus and more than 7000 species. Plants of this family are found all over the world and are considered among the easiest plants to grow (Ramasubramania, 2012). This family is commonly used as a global source of spices and extracts due to its powerful antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Belkhiri & Baghiani, 2017).

It has been the subject of extensive chemical investigations aimed at isolating different types of compounds. A number of genus, including *Ajuga*, *Rhabdosia*, *Teucrium*, *Salvia*, *Scutellaria*, *Stachys*, *Leonurus*, *Ballota*, *Coleus*, *Thymus*, and *Phlomis*, have been subjected to extensive chemical investigation, resulting in the isolation of a multitude of secondary metabolites, particularly sterols, flavonoids, iridoids, sesquiterpenes, diterpenes, and triterpenes (Hoerni, 2001).

2. Genus Thymus

Thymus sp. (Thyme) is a small perennial herbaceous plant with therapeutic properties native to the Mediterranean basin, southern Italy, and Asia. (Messaoudi et al., 2019). This genus is widely known for its use as a spice and food preservative, as well as for its protective and healing properties in many food products (Hamdani et al., 2014). It has been found to have a range of biological activities, including antibacterial, antifungal, analgesic, carminative, antioxidant, spasmolytic, and antimutagenic effects (Messaoudi et al., 2019). According to Beloued (2009), this genus is commonly found in lawns and rockeries in the mountainous regions of Tell and generally blooms from April to June.

Thyme is a medicinal plant widely used in traditional Algerian medicine due to its various properties, including expectorant, antitussive, and antiseptic, stomach, antispasmodic, carminative, worming, and diuretic. It is also a popular condiment in cooking. Dried thyme is usually used for grilling, while essential oils extracted from the plant material are used as flavoring agents. Thyme is a well-known ingredient in perfumes and is frequently used in cosmetics. Thyme

essential oil is recognized for its antiseptic and healing properties, which makes it a preferred option in beauty products (Kabouche, 2005).

According to Tedone et al. (2001), several ecotypes of thyme vary in terms of morphological characteristics as well as their composition in essential oils (Corticchiato et al., 1998). Essential oils are known for their strong and penetrating smell, as well as for their sometimes-pronounced balsamic and spicy flavor. It has been observed that various species can present distinct intraspecific chemotypes and that the composition of essential oils can be influenced by factors such as the stage of plant development, the time of harvest, and the environmental conditions of the field (Senatore, 1996).

There are about 300 species of this genus in the world (Wafa & Zeyneb, 2017). Algeria is known for its rich variety of medicinal plants, which can be attributed to its vast area and the diversity of its bioclimates. The genus *Thymus* is home to many botanical species that can be found along the coastline and even in inland regions, including arid zones (Hammaz & Nafa, 2017). Quezel and Santa (1963) describe 12 species of *Thymus*, eight of which are endemic (Dob et al., 2006), including *Thymus algeriensis* Boiss. & Reut.

2.1. *Thymus algeriensis* Boiss. & Reut. specie

2.1.1. Botanical description

Thymus algeriensis Boiss. & Reut. is a fragrant herbaceous plant commonly used as an aromatic spice (El Hadjal et al., 2010). It reproduces by seeds and can reach 15 to 30 cm in height and 40 cm in width. This bushy, evergreen, wood-based subshrub has small, very aromatic gray-green leaves. It produces clusters of purple or pink flowers at the beginning of summer, with flowering between April and June (Zouari et al., 2011).

In North Africa and precisely in Algeria, this aromatic plant is commonly called "Zaater" (Khadraoui et al., 2016).

2.1.2. Systematic position of *Thymus algeriensis* Boiss. & Reut.

According to the classification of Quezel and Santa (1963), *Thymus algeriensis* Boiss. & Reut. is classified as a species belonging to :

Kingdom: Plantae

Branching: Spermaphytes

Under branch: Angiosperms

Class: Dicotyledons

Subclass: Metachlamydia

Order: Lamiales

Family: Lamiaceae

Genus: *Thymus*

Species: *Thymus algeriensis* Boiss. & Reut.

2.1.3 Geographical distribution

Thymus algeriensis Boiss. & Reut. is a species widely distributed in North Africa and endemic to Libya, Tunisia, Algeria, and Morocco (Sobeh et al., 2020). In Algeria, it is frequently found in the subsector of the Algerian and Oranese highlands (Khlefi & Medjani, 2018).

2.1.4. Therapeutic properties and use

Thymus algeriensis Boiss. & Reut. is a plant that is often used as a spice in culinary applications, whether fresh or dried. Furthermore, it is employed in traditional medicine for its medicinal properties, particularly in the treatment of respiratory and gastrointestinal disorders. Additionally, it is used for its antispasmodic effects (Howell et al., 2013; Ziani, 2019) and to relieve sciatica, kidney, and bladder pain. In certain instances, it has been employed to treat leprosy, paralysis, and nervous disorders (Madi, 2010).

3. Astraceae Family

The Asteraceae family, previously called Compositae (Composaceae, Compositae), is a vast family of the plant kingdom and widely distributed all over the world, especially in temperate regions. It includes the most important ovary plants. Although they are less common in tropical rainforests, they nevertheless play an important role in the ecosystem.

Asteraceae are composed of more than 1000 genus and about 20000 to 25000 species (Dupont & Guignard, 2007; Spichiger et al., 2004). The classification of the Asteraceae family varies among taxonomists. For example, Cronquist associates it with the superorder Asteridae and the order Asterales, while Dahlgren classifies it with the superorder Asteriflorae and the order Asterales (Spichiger et al., 2004). According to APG IV classification, established in 2016 with Angiosperm

Phylogeny Group, the Asteraceae family is classified into five subfamilies: Barnadesioidae, Mutisioidae, Carduoidae, Cichorioidae, and Asteroidea (Filleul,2019).

The Asteraceae family includes various plants, including herbs that can be erect or climbing, and sometimes shrubs or trees such as *Vernonia*. In the high tropical mountains, you can find *Senecio* and *Espeletia*, which are shrubs with a single stem and few leaves. In addition, some Asteraceae, such as *Senecio polyodon* and *Othonna clavifolia*, are also succulent. Plants in the Asteraceae family have a range of life cycles, including annual, biennial, and perennial. Their peculiarity is the flower head, a cluster of flowers located at the end of a branch or stem and surrounded by an involucre, a structure formed by floral bracts without peduncles. The involucre can take a cup-shaped or frill-shaped form (Barreda et al., 2010). According to Florin (2008), latex is frequently present in the stems. The inflorescence of these plants is in the form of flower heads, consisting of a common receptacle, usually flat, on which sterile green bracts (which can be scaly, hooked, or spiny) form an involucre. Then come small, fertile, non-green or straw-colored bracts, each with a flower, inserted from the base to the top in a spiral order. The family is commonly called "Composites" because of the compound flower it produces (Boullard, 1997; Dupont & Guignard, 2007).

4. Genus *Santolina*

The genus *Santolina* comprises more than 10 widely distributed species, native to the Mediterranean region (Ferrari et al., 2005; Kisiel et al., 2003). Among the most common species are *S. viridis* W. (found in the south of France and northern Spain), *S. pectinata* Lag. (found in the Iberian Peninsula), and *S. chamaecyparissus* (a common plant in the Mediterranean basin) (Liu et al., 2007). It is usually found between 20 and 60 cm tall and has many densely branched woody stems that form dense clumps. According to Giner Pons & Rios Canavate (2000), the Santoline is a subshrub known for its decorative and aromatic qualities. It includes ornamental plants (Gardner et al., 2005), traditionally used for medicinal purposes (Palá et al., 2001). In addition, their aromatic foliage is known to repel insects, which makes them popular as natural insecticides. The essential oils extracted from the genus *Santolina* are also used in the manufacture of perfumes (Gardner et al., 2005; Corana et al., 2009).

4.1. *Santolina rosmarinifolia* L. specie

4.1.1. Botanical characteristics

Santolina rosmarinifolia L. is a shrub that reaches 40 to 50 cm in height and has dark green foliage. Its fragrant flowers bloom in July–August (Ferrari et al., 2000; Kisiel et al., 2003) and are grouped in flower heads 8–15 mm in diameter. The flower heads are homogamous, discoid, and bear tubular and hermaphrodite flowers. The corolla has an evagination that covers the top of the ovary; the bracts of the involucre are oblong and accompanied by a scarious and lacerated appendix, arranged in a few rows (Dupont & Guignard, 2007, Ferrari et al., 2000, Kabissi, 1998).

The receptacle has a convex, sub-hemispherical, and paleaceous shape. The achenes are bald, tetragonal, and not winged (although the latter characteristic can be difficult to observe once dry). They are completely devoid of ribs and protruding libero-woody fascicles. The plant is a suffrutescent bushy subshrub with woody stems. It is branched and has a single sepal. The inflorescence is a dense corymbose. The leaves have a distinct aroma and are narrow linear in shape, with a length of 3 to 5 mm (Dupont & Guignard, 2007; Ferrari et al., 2000; Aniško, T. 2008).

4.1.2. Classification in the systematics

Kingdom: Plantae

Branching: Spermaphytes

Under branch: Angiosperms

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Gender: *Santolina*

Species: *Santolina rosmarinifolia* L.

4.1.3. Geographical distribution

Santolina rosmarinifolia L. is a species that has two subspecies. The first subspecies, '*rosmarinifolia*', found in all the regions where this species is distributed and presents a slightly tomentose appearance. The second subspecies, "*Canescens*", is found only in the southern regions of Spain and presents a densely tomentose appearance ranging from white to gray (Ferrari et al., 2000). However, it should be noted that in Algeria, this species does not have any subspecies.

4.1.4. Therapeutic properties and use

The plant species *S. rosmarinifolia* L. traditionally used for medicinal purposes in various regions (Ushakov et al., 1976). In Algeria, it has been used as a stimulant, antispasmodic, and anthelmintic. Likewise, in Portugal, the maceration of the dry flowers of the plant in water used as an antipyretic. In addition, the infusion of fresh or dried flowers prescribed as a liver protector, hypotensive, intestinal, anti-inflammatory, and aperitif (Novais et al., 2004).

1. Secondary metabolism

They are any complex organic molecule found in plants that are indirectly necessary for their survival and interaction with the environment (Beam, 2008). Autotrophic plants produce secondary metabolites from primary metabolites (Boudjouref, 2011), and they can be found in all regions of higher plants in low concentrations (Merghem, 2009).

Plants can produce numerous secondary metabolites, which are divided into three major classes based on their chemical structure (Crozier, 2006):

-Avoid phenolic and aromatic chemicals.

-Terpenoids.

-Alkaloids.

1.1. Phenolic compounds

The term "polyphenols" or "phenolic compounds" encompasses a vast array of over 8000 molecules, divided into approximately ten chemical classes. These molecules share a common feature: the presence of at least one aromatic ring with six carbons, which carries a variable number of hydroxyl (OH) functions (Hennebelle et al., 2004).

Polyphenols are the most prevalent antioxidants in nature and in our diets. They enable plants to defend themselves against oxidation phenomena, certain external aggressions, and rotting (Menat, 2006) (Figure1).

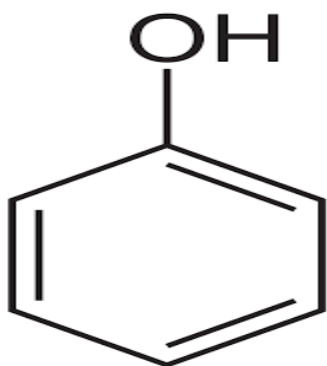


Figure 1. Chemical structure of phenolic compounds.

Phenolic compounds can be classified into numerous categories, distinguished by the intricacy of the basic skeleton, the extent of their modification, and the possible bonds of these basic molecules with others, including carbohydrates, lipids, and proteins (Herbert, 1989; Beta et al., 2005; Macheix et al., 2005).

1.1.1. Flavonoids

The term flavonoid (derived from the Latin word flavus, meaning "yellow") encompasses a vast array of naturally occurring compounds belonging to the polyphenol family. These diverse substances are present in both their free form (aglycone) and in the form of glycosides. In general, flavonoids are found in vascular plants (Erlund, 2004). These compounds can be localized in various organs, including roots, stems, wood, leaves, flowers, and fruits. Furthermore, flavonoids play a significant role in plant protection (Bruneton, 1993) (Figure2).

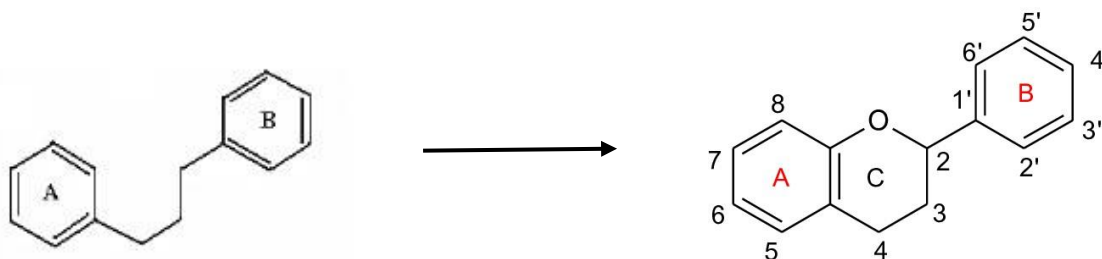


Figure 2. Basic structure of Flavonoids.

1.1.1.1. The main classes of flavonoids

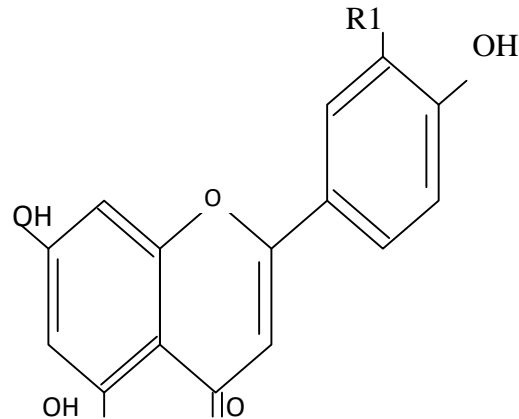
The majority of flavonoids have a similar chemical structure: two aromatic rings linked by three carbon atoms, which form an oxygenated heterocyclic compound.

Flavonoids are divided into six subcategories: flavonols, flavones, isoflavones, flavanols (catechins and proanthocyanidins), flavanones, and anthocyanidins (Benbrook, 2005).

1.1.1.1.1. Flavones and Flavonols

Flavones and flavonols are a class of flavonoids that possess a double bond at C2 and C3 (Figure 3). In general, yellow-hued flavones are distinguished by the presence of a carbonyl group (H₃CO-) in position 4 and a carbohydrate group, which is most often connected in position 7. The sole

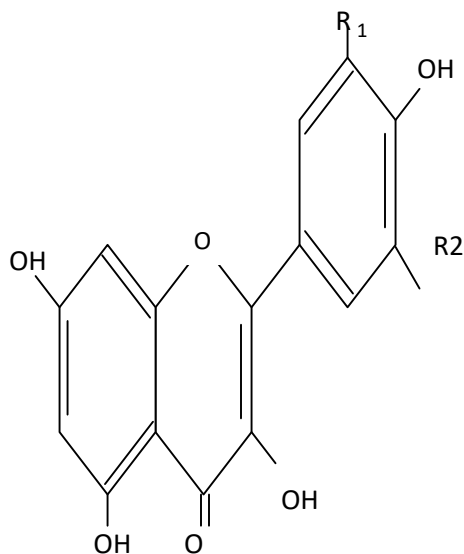
distinction between flavones and flavonols is the presence of a hydroxyl group at C3 in the latter, which distinguishes it from 3-hydroxyflavonols (Herrmann, 1976; Siewek et al., 1984).



Apigénine (R₁=H)

Lutéoline (R₁= OH)

Flavones



Quercétine (R₁= OH, R₂=H)

Kaempférol (R₁= R₂=H)

Myricétine (R₁= R₂= OH)

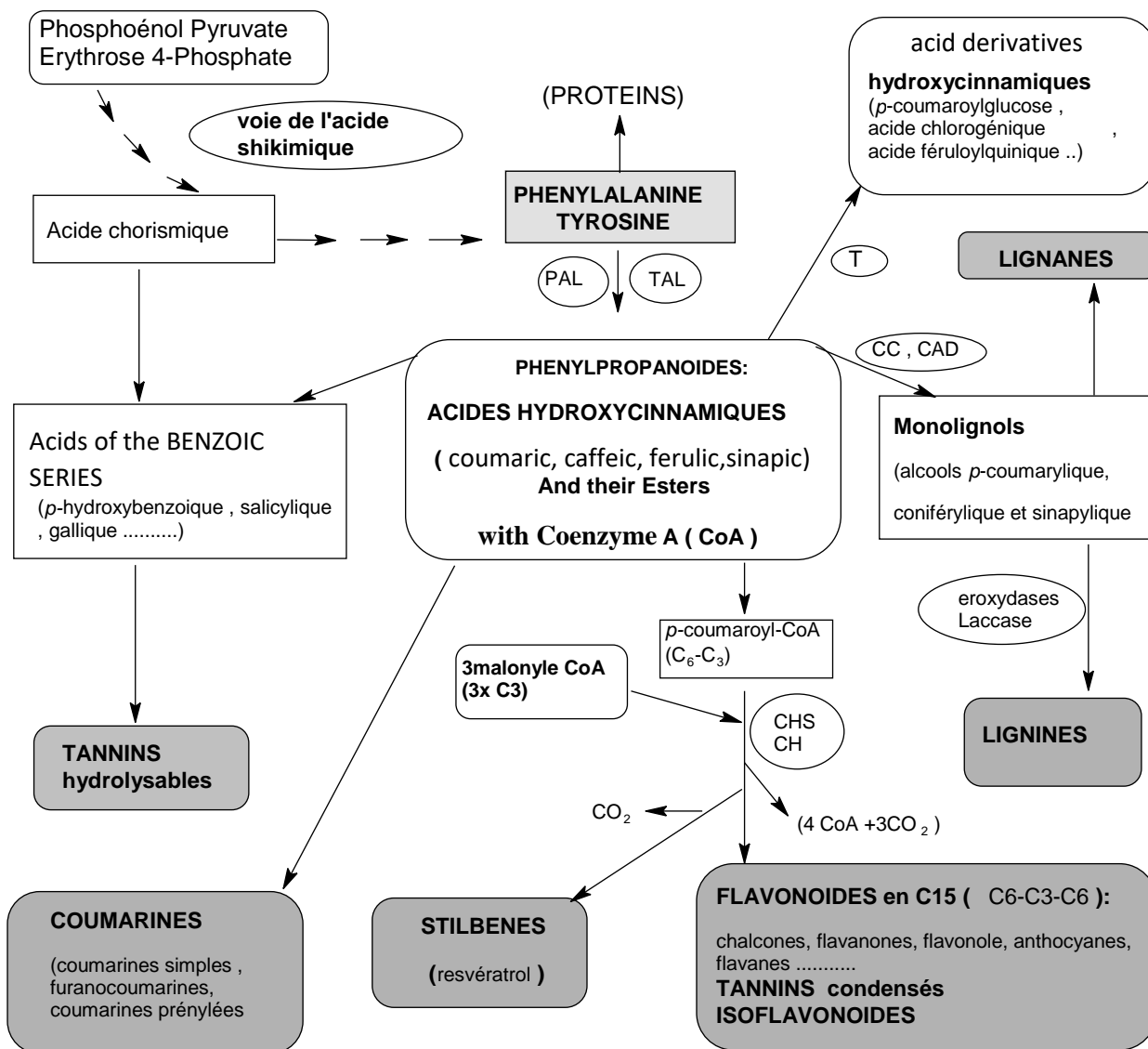
Flavonols

Figure 3. Chemical structure of flavones and flavonols (Bakker & timberlake, 1997; Andersen & Markham, 2006).

1.2. Biosynthesis of phenolic compounds

The main lines of the biosynthetic routes for the major phenolic compounds are now well understood. The two aromatic amino acids found in proteins (phenylalanine and tyrosine) are also responsible for the formation of the majority of phenolic molecules in plants. These

chemicals are produced through two major metabolic processes (Crépin ibingou, 2017) (Figure4).



The enzymes involved in biosynthesis L: phenylalanine ammonia lyase; TAL: tyrosine ammonia lyase; CCR: cinnamate CoA reductase; CAD: cinnamyl alcohol dehydrogenase; CHS: chalcone synthase; CHI: chalcone flavanone isomerase; TR: transferases

Figure 4. Biosynthesis of phenolic compounds.

2. What is an essential oil

Essential oil contains a variety of molecules to be analyzed by a chemist, a pleasant aroma to be incorporated by a perfumer, and the essence of a plant. Essential oils are aromatic and volatile compounds extracted from specific trees and plants, which produce ingredients that give each oil its distinct fragrance. The term "volatile" refers to essential oils that evaporate swiftly. It is consequently critical to store them correctly to maintain the integrity of their active components (Moro-Buronzo, 2008).

"Essential oils are compounds made up of various molecules, each with their unique properties" (Franchomme & Penoel, 1990).

These oils are known for their volatile and odorous characteristics, secreted and excreted by aromatic plants (Duraffourd & Lapraz, 2002)."

Essential oils are highly concentrated liquids naturally present in many plants, herbs, flowers, fruits, woods and spices (Cusson, 2007).

Essential oils exist almost only in higher plants. They are synthesized in the cytoplasm of secretory cells and usually accumulate in specialized glandular cells located on the surface of the cells and concealed by a cuticle. They can be preserved in different organs, encompassing flowers, leaves, bark, wood, roots, rhizomes, fruits or seeds (Brunetton, 1987).

2.1. Physical properties

At room temperature, essential oils are in a liquid and volatile state, which distinguishes them from fixed oils. Except for the blue-green *Matricaria* essential oil, their colors range from colorless to pale brown. With a range of 0.850 to 0.950, essential oils typically have a lower density than water, except oils from sassafras, clove, and cinnamon. Wintergreen essential oil is the densest, measuring 1.160 to 1.195 at 20°C (Franchomme et al., 1990).

The high refractive index of essential oils causes them to deflect polarized light. These oils dissolve in fats and non-polar solvents, and their solubility is generally high in alcohols, but very low in water, which makes them fat-soluble (Brunetton, 2008).

2.2. Chemical properties

The essential oils undergo oxidation in the presence of light, which leads to their resinification by absorbing oxygen. As a result of this process, their smell also changes. In addition, these oils can absorb chlorine, bromine, and iodine, which leads to the release of heat. In addition, they can combine with water to form hydrates. The lipophilic nature of essential oils allows their effective penetration through the physiological membranes, in particular the cutaneous membranes.

2.3. The role of essential oils

Plants use them to defend themselves against viruses, and they are often confused with plant hormones (Willem, 2009), but others see them as intermediaries between parasites and microbes (Guendouzen & Haddouche, 2006).

Plants can survive due to essential oils. According to Festy (2011), they possess an "effective protection system that includes antibiotics, anti-solar and other properties. They also play several roles, such as attracting pollinators, protecting plants from sunburn, predators, and diseases, and helping to heal wounds and various attacks. »

Essential oils contribute to the ecological balance of plant-plant and plant-animal interactions and could even serve as communication channels for selective biological messages by transfer (Rober et al., 1993).

Research suggests that monoterpenes and sesquiterpenes can have a significant impact on the relationship between plants and their environment (Holley, 1999).

2.4. Toxicity of essential oils

Essential oils can have serious side effects due to their potency. For example, essential oils rich in thymol or carvacrole can irritate, while essential oils rich in cinnamaldehyde can trigger allergic reactions (Smith et al., 2000). In addition, essential oils containing furocoumarin can cause phototoxic effects (Naganuma et al., 1985).

2.5. Location of essential oils

Essential oils are not commonly found in plants. Of the 1500000 plant species recorded, only 10% can synthesize an essence. As a result, these plants are qualified as "aromatic" (Bruneton, 1999; Degrise et al., 2008).

Essential oils are usually present in various parts of aromatic plants, such as flowers, leaves, fruits, stems, rhizomes, roots, seeds, and wood (Teixeira et al., 2013).

2.6. Chemical composition of essential oils

Essential oils are complex mixtures of various chemical compounds that dissolve in homogeneous solutions. The main components of the oils are divided into two distinct groups of odorous compounds, depending on the metabolic pathway: terpene compounds (mono- and sesquiterpenes) and aromatic compounds derived from phenylpropane.

2.7. Terpenes

In the mid XIXe century, the study of turpentine led to the creation of the term "terpenes" to describe hydrocarbons with the empirical formula $C_{10}H_{16}$. Terpenes constitute a family of compounds that are widespread in the plant kingdom (Wichtl & Anton, 2003). They are natural hydrocarbons that have a cyclic or linear structure. Their most notable structural feature is the presence of an isoprene unit comprising five carbon atoms (C_5H_8) within their framework (Hernandez-Ochoa, 2005; Fillatre, 2011)(Figure 5).

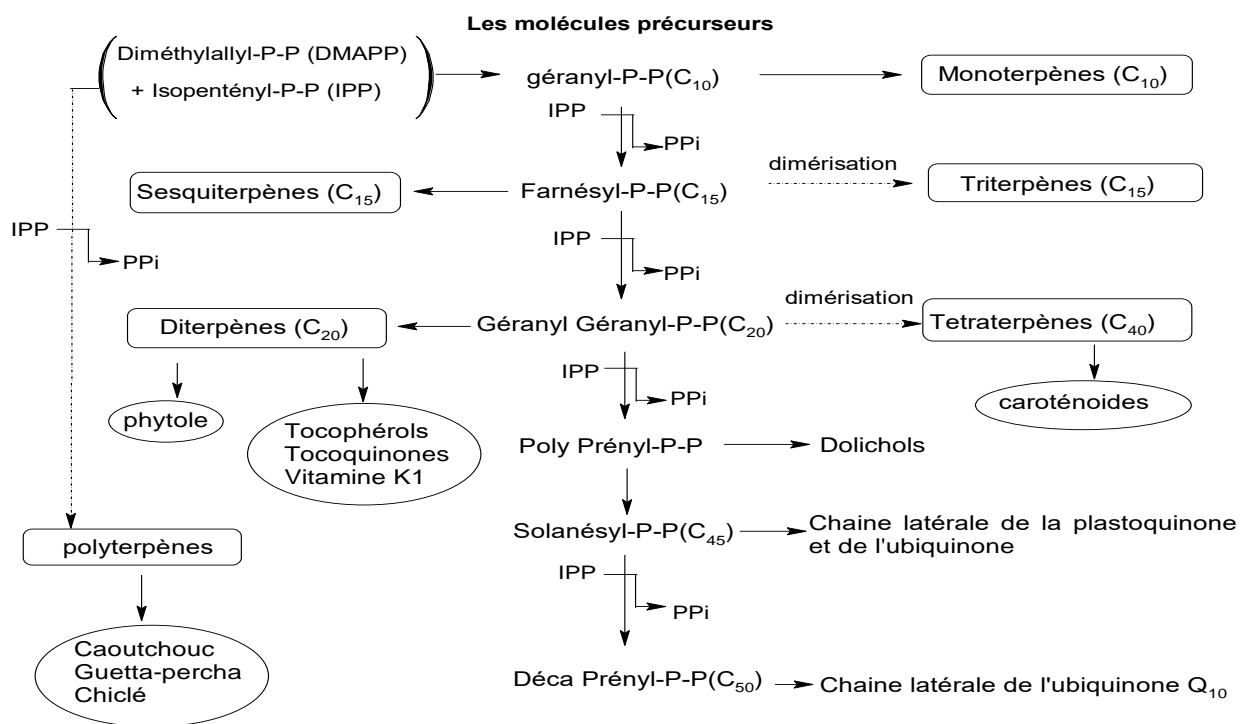


Figure 5. The general metabolic pathway for the biosynthesis of various terpene groups.

We thus distinguish according to the number of carbon constituting the molecules of this group (table 1):

Table 1. Classification of terpenes.

Classe	Formule brute	Nombre d'isoprène
Hémiterpènes	C ₅ H ₈	1
Monoterpènes	C ₁₀ H ₁₆	2
Sesquiterpènes	C ₁₅ H ₂₄	3
Diterpènes	C ₂₀ H ₃₂	4
Triterpènes	C ₃₀ H ₄₈	6
Polyterpènes	(C ₅ H ₈) _n	45-30000

2.8.
Aromatic
compounds
According
to Bakkali et

al. (2008), aromatic compounds originating from phenylpropane (C₆-C₃) are less widespread than terpenes. Aromatic compounds include:

Aldehydes: Cinnamaldehyde

Alcohol: cinnamic alcohol

Phenol: chavicol, eugenol

Methoxy derivatives: anethole, estragole

The chemical composition, quantity, and quality of essential oils are influenced by various factors.

Some of them include intrinsic factors such as genetic constitution, location, maturity level, and physiological stage of the plant.

Extrinsic factors such as soil, environmental conditions, crop protection, crop type, and harvesting method also play a role, while technological factors such as the extraction method affect the result more.

2.8. The Factors affecting the stability of essential oils

Essential oils exhibit significant instability regarding various extrinsic and intrinsic factors. The measurement of chemical indices (for example, the peroxide value and the acid number), the determination of physical quantities (such as the refractive index, the rotary power, the miscibility with ethanol, and the density), and the carrying out of chromatographic analyses can easily reveal the possible degradation of essential oils (Turek & Stintzing, 2013).

Some of the main degradation reactions include, among others, oxidation, isomerization, polymerization, racemization, thermal rearrangement, and volatilization (Turek & Stintzing, 2013). In addition to the loss or structural modification, degradation can also lead to changes in the organoleptic properties of essential oils, as well as the creation of toxic secondary compounds. The conservation of essential oils (HE) requires special precautions. It is therefore recommended to use small tinted or opaque glass bottles to reduce exposure to air, protect from light, oxygen, moisture, and contaminants, and avoid any potential incompatibility with certain plastic containers. There are precise standards governing the packaging and storage of essential oils (Afnor standard NF T 75-001, 1996), as well as the labeling of containers containing them (standard NF 75-002, 1996).

2.9. Essential oil extraction methods

2.9.1. Distillation

The oldest and most widely used method of extracting essential oils consists of entraining aromatic substances using water vapor (Franchomme et al., 1990; Bruneton, 1999). There are three distinct processes that use this principle: hydro-distillation, steam distillation, and hydro-diffusion (Piochon, 2008).

2.9.2. Hydrodistillation

The process consists of immersing the raw material in a water bath and bringing it to a boil. It is generally carried out at atmospheric pressure. The distillation can be carried out with or without the cohobing of the aromatic waters acquired during the decantation. This process has disadvantages, primarily related to the impact of boiling water or water vapor. Some plant parts, particularly flowers, are too delicate to support steam distillation or hydrodistillation (HD) procedures (Farhat, 2010).

The direct contact between the constituents of the essential oil (HE) and the water leads to chemical reactions that modify the final composition of the extract (Raaman, 2006; Walton et al., 1999). The distillation time and the other operating conditions have a significant impact on the yield and composition of the essential oil.

2.9.3. Extraction by steam entrainment

It is one of the permitted methods of producing HE (European Pharmacopeia, 2007). During this extraction procedure, the plant material is exposed to a stream of steam without first being macerated. The vapors, which are rich in volatile compounds, are then condensed and poured into the gasoline where they segregate into an aqueous phase (HA) and an organic phase (HE).

The absence of direct contact between the water and the vegetable materials, then between the water and the aromatic molecules, avoids a possible hydrolysis or degradation that could negatively affect the quality of the oil (Raaman, 2006).

2.9.4. Percolation (hydro-diffusion)

The Swiss company Schmidt (1981) has developed this method, which consists of sending water vapor at very low pressure (0.02-0.15 bar) through the plant mass from top to bottom instead of the conventional bottom-up approach. Some plants, such as conifers and cinnamon, benefit from this faster method because it reduces the risk of deterioration in the quality of essential oils. The substances obtained contain non-volatile components, which give rise to percolating essences rather than essential oils.

2.9.5. Extraction by organic solvent

The solvents most commonly used today are hexane, cyclohexane, and ethanol, with dichloromethane and acetone being less frequently used. An authorized solvent must be stable to heat, light, or oxygen. It must also have a low boiling point to accelerate its elimination and avoid chemical reactions with the extract. The extraction is carried out using a Soxhlet device. These solvents have an extraction power greater than that of water, which gives extracts containing not only volatile compounds but also numerous non-volatile substances, in particular waxes, pigments, fatty acids, and others (Hubert, 1992).

2.10. Essential oil analysis techniques

2.10.1. Analysis by gas chromatography (GC)

Gas chromatography (GC) is the most suitable method for analyzing essential oils. It separates the compounds in the gaseous state according to their partition coefficient, which refers to their distribution between a stationary liquid phase impregnated on an inert solid support (chromatography column) and a gaseous mobile phase (the carrier gas) (Browning, 1971).

The mixture of components penetrates through the head of the column and vaporizes at the injector. Different components are eluted through the column at a speed determined by the linear velocity of the carrier gas, as well as by their solubility in the stationary phase and their volatility (Nguyen et al., 1993).

The separated substances exit the column as gaseous compounds and pass sequentially via a detector, which indicates the proportion of each substance on an integrating recorder. The recorder generates a succession of peaks, each with a specific retention time and area. The length of the column determines separation efficiency. "Capillary" columns, with a small diameter of around ~1 millimeter and lengths of up to 100 meters, achieve the highest level of separation.

Chromatography Gas allows qualitative and quantitative analyses. However, the main disadvantage of this method lies in the need to spray the products, which could lead to structural changes.

2.10.2. Gas chromatography coupled with mass spectrometry (GC/SM)

Gas chromatography/mass, spectrometry (GC/MS) coupling is the current standard technique in the analysis of essential oils. The concept of adding a second analytical dimension to chromatography through the integration of another physical method of investigation after chromatographic separation was realized in 1960 when gas chromatography and mass spectrometry were combined. This coupling method is abbreviated as GC/MS (De Maack and Sablier, 1994).

The method works by transporting isolated substances from gas chromatography into the mass spectrometer via the carrier gas (mobile phase). In the mass spectrometer, these compounds will break into ions of varying masses, which will be sorted based on mass. A computer comparison of an unknown peak's spectrum with one or more reference libraries can be used to determine whether the similarity of the unknown and reference spectra is adequate and whether the retention indices are the same under operational conditions (Desjobert et al., 1997; Bruneton, 1999).

3. Biological activities

3.1. Antioxidant activity

Antioxidants are compounds that have the ability to protect the body from the harmful effects of oxidative stress (Beirão & Bernardo-Gil, 2006). There are three categories of antioxidants: enzymatic antioxidants, repair enzymes, and non-enzymatic antioxidants. Non-enzymatic

antioxidants include natural substances such as essential oils. Antioxidants can have primary or preventive (indirect) activity, the latter has the ability to delay oxidation because of its reduction of oxygen mechanisms. (Madhavi et al., 1996). Direct-acting antioxidants can donate electrons to oxygen radicals, trapping them and thus preventing the destruction of biological structures. It also act as reducing agents, transferring electrons to ROS and removing those (Kohen & Nyska, 2002). According to several studies, more effective than synthetic antioxidants (Hussain et al, 2009).

The antioxidant effects of essential oils and plant extracts are mainly due to the hydroxyl groups present in their chemical structure (Hussain et al., 2009).

It can concluded that biological activities directly depend on the chemical composition of essential oils. It concluded that the chemical composition of essential oils is directly responsible for biological activities.

3.2. Antibacterial activity

An antibacterial or bactericidal substance has the ability to eradicate bacteria, depending on specific structural and metabolic properties that distinguish them from other microorganisms, in particular lower fungi and protozoa (Loiseau-Marollean, 1968).

The antibacterial properties of aromatic and medicinal plants have been recognized since ancient times. These properties come from the essential oil fraction present in plants (El-Kalamouni, 2010). Due to the variations in the quantities and profiles of the components of the essential oils, their antibacterial activity cannot attributed to a single mechanism. It is rather likely that they act on several cellular sites of action (Carson et al., 2002).

The chemical composition of essential oils is complex, leading to a mode of action difficult to understand from a molecular point of view. Each constituent of essential oils is likely to have its own mechanism of action. The mode of action of essential oils is mainly based on the nature and characteristics of their active components, in particular their hydrophobic property, which allows them to penetrate into the double phospholipid layer of the bacterial cell membrane. This can trigger a modification of the conformation of the membrane (Cox et al., 2000; Carson et al., 2002).

Experimental part

Chapter 1: Materiel and Methods

First part: Study of the floristic biodiversity of the Belezma National Park

1. Presentation of the study area

1.1. History of the Belezma National Park

The Belezma National Park (BNP), created by Presidential Decree No. 84/326 of November 3, 1984, in parallel with the creation of the Gouraya and Taza National Parks (Atlas of National Parks), enjoys legal and financial independence as a public administrative institution, operating under the supervision of the Ministry of Agriculture, Rural Development, and Forestry Administration (Boukerker, 2016). The classification was motivated by the vast expanses of Atlas cedars in an area strongly influenced by the Saharan and Mediterranean climates, the invaluable archaeological and historical heritage present, and a mosaic of wetlands in the northeastern part (Moulai, 2020). UNESCO in June 2015 classified the park as a biosphere reserve. It is one of the richest parks in plant diversity in Algeria after that of El Kala (B.N.E.F., 1986).

The chronology of the history of BNP, covering the period from 1953 to 2010, is summarized below:

1953: P. Boudy recommended a development for 50 years for the Belezma massif.

1971: Idea of developing part of the Belezma national forest into a National Park (idea emanating from the forest services).

1973: The Cedar Forest is landscaped by the F.A.O. "Algeria Project 15" for the exploitation of wood. The latter has never taken place for the problem of natural and/or artificial regeneration of cedar.

1977: The Cedar wooded area was the subject of a development study to erect it as a National Park (BNEDER).

1984: Creation of the Belezma National Park by Decree N ° 84-326 of November 03, 1984.

1985: Development of part of the Belezma National Forest by the BNEF into a National Park (26,250 ha of the 61,000 ha).

1986: The Belezma National Park becomes operational.

1987: First implementation of program authorizations.

1998: Elaboration of the first management plan (1998-2002).

2006: Elaboration of the second management plan (2006-2010).

2002-2008: Appearance of cedar dieback; INRF missions; dendrochronology study established by the Arizon Institute.

2010: Elaboration of the third management plan (2010-2014).

1.2. Geographical location

It is located in the eastern part of northern Algeria, about 7 kilometers northwest of Batna, a hundred kilometers north of Biskra, and south of Constantine (Boukerker, 2016). The plain of El-Madher borders it to the east, the Oued Barika to the west, and the plains of Merouana and Ain Djasser to the north. (B.N.E.F., 1986). The mountain massifs in concern are elongated and face south-west or northeast (Boukerker, 2016). It covers an area of 26250 ha. Its privileged location has made it a contact area, a biogeographic crossroads between the Aurès Mountains to the east, the Bou Taleb Mountains to the west, the high plains of Constantinois to the north, and the Saharan Atlas Mountains to the south (Salhi, 2004). The BNP is divided into three sectors: Oued Chaaba, Fesdis, and Oued El-Maa. In addition, there are four forest houses in the park: the forest houses of Oued El-Maa, Oued Hamla, Oued Chaaba, and Timezouegh (Figure 6).

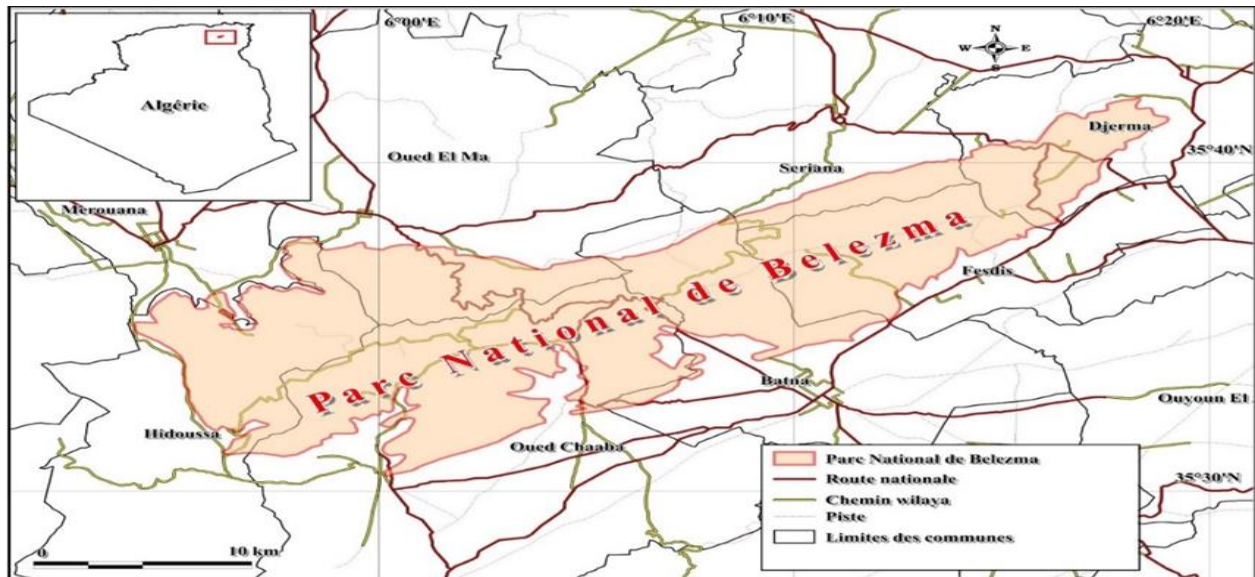


Figure 6. Location map of the Belezma National Park (Batna, North East of Algeria) (Maalem N,2023).

1.3. Pedology

According to Schoenberger (1970) and Abdessmed (1981), the BNP soils are relatively young, thick, and have a low degree of erosion.

They are currently forming rendzine or are close to brown limestone or Terra Rosa (soft limestone, marl).

The cedar forests of Bordjem and Theniet-el-Gontas contain brown soils with a minimum of limestone. The sandstone underlies them at altitudes ranging from 1400 to 1800 m on the northern slopes, while the surface decalcified rendzines are present on the Djebel Tichaou and the Tuggurt at altitudes of 1,800 m, where the slope exceeds 75% in some areas. It should be mentioned that although no pedological research has been carried out in Belezma National Park, we have encountered this type of soil (Figure 7).

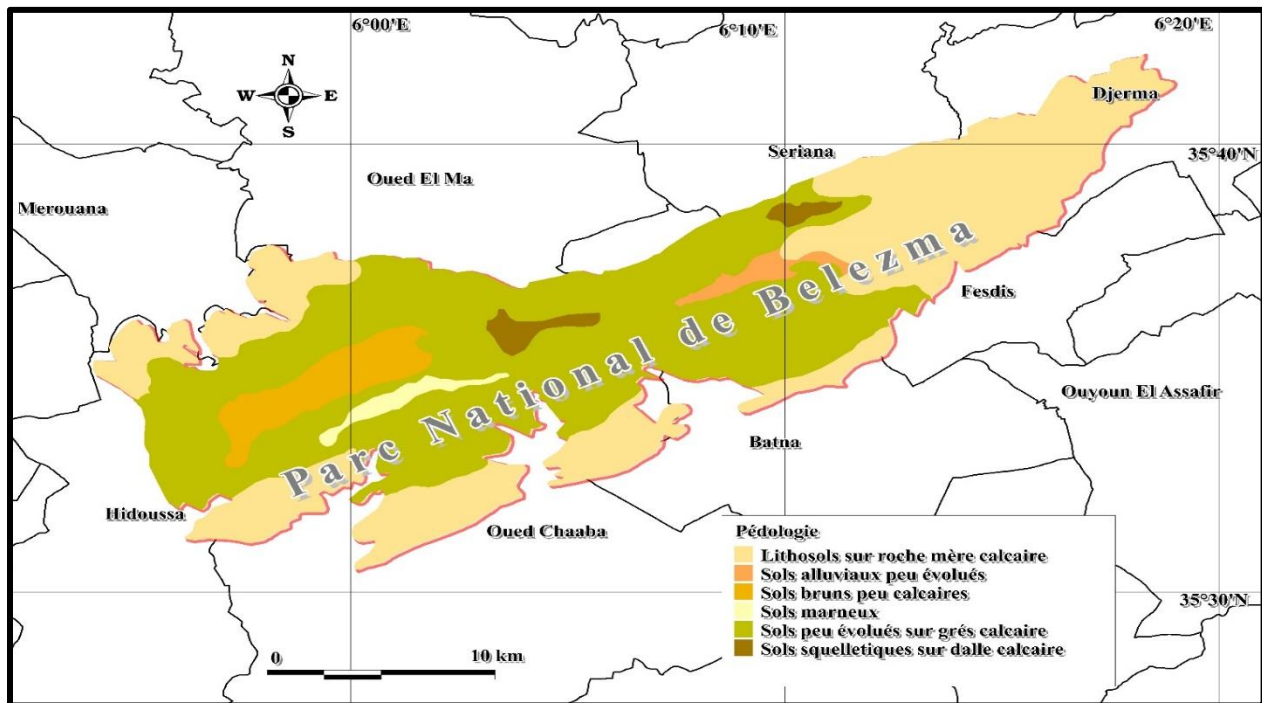


Figure 7. Soil map of the Belezma National Park (Maalem N, 2023).

1.4. Geology

The only known evidence on the geology of the BNP is Abdessamad's (1981) paper, which is based on Laffite's (1939).

The principal geological structures of the region can be characterized as follows:

The Boumerzoug region consists of marls in the bottom part and sandstones in the upper section. The summit of Djebel Touggour, which reaches an elevation of 2010 m, consists of marls in the lower half, dolomitic sandstones in the middle, and sandstones in the upper portion.

The Bordjem and Chellaâlaâ area is distinguished by the presence of sandstone in the bottom half, dolomitic limestones in the middle, and sandstone in the upper half (Figure 8).

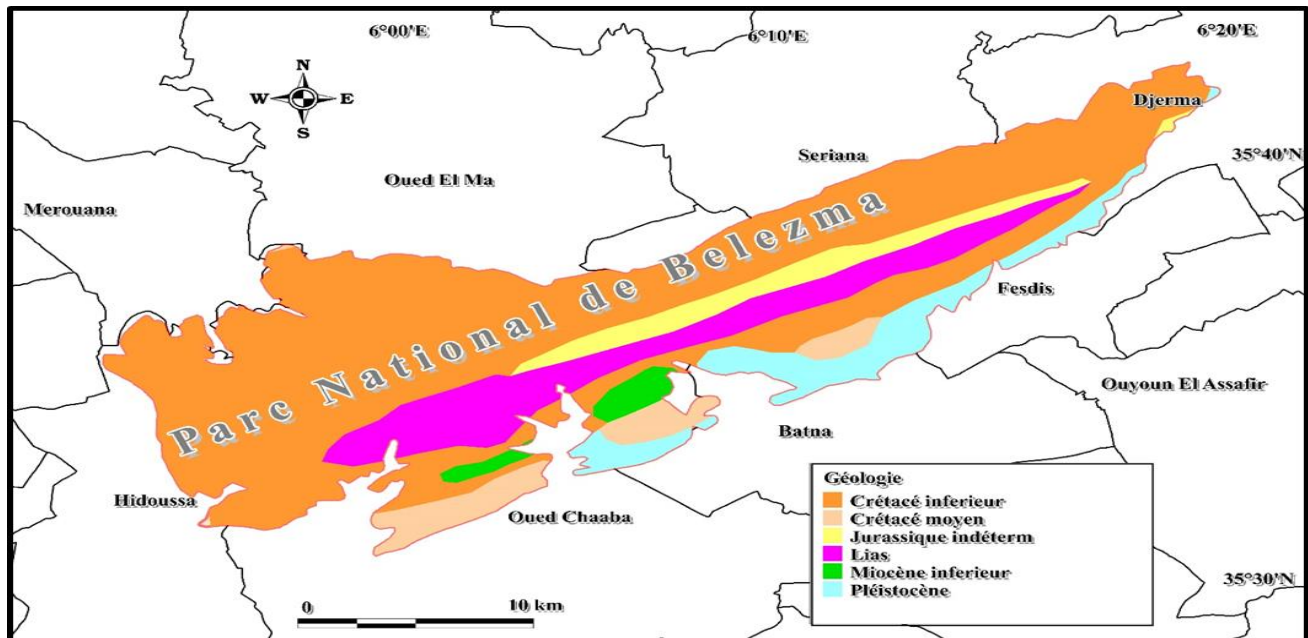


Figure 8. Geological map of the Belezma National Park (Maalem N, 2023).

1.5. Relief

Belezma National Park is characterized by a steep mountain range with slopes of up to 50% in some places, in particular the Bourdjem, Chelalaa, and Tuggurt Mountains (B.N.E.F., 1986). This mountain range marks the beginning of the Aurès mountain range and extends through high and medium-altitude mountainous regions, including Djebel Maâguel at 1500 m, Djebel Kasserou at 1641 m, Djebel Touggour at 2090 m, Djebel Tichaou at 2136 m, and Djebel Rafâa at 2178 m (PGPNB, 2019).

1.6. Hydrology

The topography is responsible for the hydrographic system of the region, which creates two distinct networks based on the two ridgelines of the Belezma Mountains.

The northern hydrographic network includes Oued Ketami, Oued H'rakta, Oued Enadjerime, Oued El Ma, and Oued Châabet Islan.

The southern hydrographic system is composed of Oueds and ravines, including Oued Bouilef, Oued Nafla, Oued Châaba, Oued Hamla, and Oued Skène.

The southern hydrographic system is composed of Oueds and ravines, including Oued Bouilef, Oued Nafla, Oued Châaba, Oued Hamla, and Oued Skène. Only two permanent rivers, the Oued Châaba and the Oued Bouilief, have low flows in the area (B.N.E.F., 1986).

Most of the Oued within the national park export their water beyond the limits of the protected area, mainly comprising temporary streams that observe seasonal (winter) and torrential cycles. Runoff from the southern slopes frequently leads to flooding along the northwestern ring road of Batna (Figure 9).

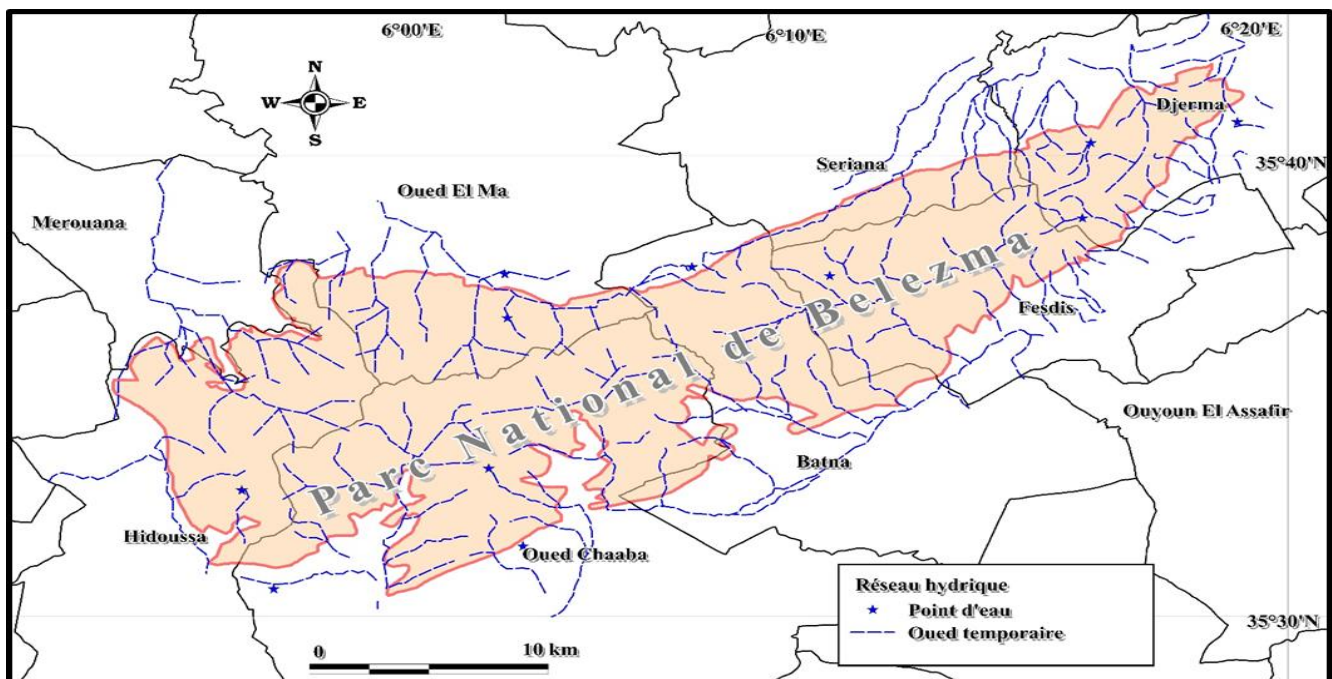


Figure 9. Hydrological map of the Belezma National Park (Maalem N, 2023).

1.7. Climate

The climate represents the average state of the atmosphere and its changes in a particular location. This definition includes two concepts: the notion of an average climate, which concerns the collection of circumstances that characterize the average atmosphere in a given place or region, and the notion of climatic variability, which relates to the statistical distribution of climatic characteristics around their average measurement (Guyot, 1997).

It plays a crucial role in the development, individualization, and distribution of living beings. Certain climatic factors, such as rainfall, water availability, and temperature, are essential for the life of plants (Ramade, 1984).

In order to better understand the different climatic factors and to carry out a bioclimatic study, we used climatic data from the Ain Skhouna Batna meteorological station as a reference because no meteorological station was present in the surveyed area. The data concerned a period of 29 years, from 1991 to 2020. Climatic corrections have been made according to the rainfall gradient of Seltzer (1946). In his study on the climate of Algeria, he suggests that a decrease of 0.7°C occurs for every 100 m of altitude difference for maximum temperatures (M) and that a decrease of 0.4°C occurs for every 100 m of altitude difference for minimum temperatures (m). When taking into account the thermal and rainfall parameters, correction coefficients were calculated to provide more accurate data for the evaluated points (table2).

Table 2. Characteristics identifying the weather station of Batna.

characterization of the study area	Geographic Coordinates		Altitude(m)	Available data	Periods	Data sources
	Lat.	Long				
Belezma National Park	35.55°N	6.18°E	1052 m	T-P	1991-2020	Weather station Ain skhouna Batna

P = Précipitation (mm), T = Température ($^{\circ}\text{C}$)

1.7.1. Precipitation

Le Houérou et al. (1977) noted that rainfall is a critical factor in determining the dryness of a global climate. The source of rainfall in Algeria is mainly orographic. The levels of precipitation depend on the altitude, the orientation of the mountain range, and the exposure (Seltzer, 1946). As a result, the north- and north-west-facing slopes receive more rainfall than the other exposures. The amount of rain increases from bottom to top, following an altitudinal rainfall gradient (Seltzer, 1946; Kadik, 1987).

1.7.1.1 Average monthly and annual rainfall

A number of authors, including Seltzer (1946), Lucido (1972), Le Houérou (1974), Chaumont & Paquin (1979) in Le Houérou (1989), have observed a linear correlation between precipitation levels and altitude. As altitude increases, rainfall tends to do so as well. For instance, Seltzer (1946) posits that there is an increase of approximately 50 mm for every 100 m of altitude difference.

Table. 3 shows the rainfall correction for the study area. This correction is based on the calculation of precipitation correction coefficients (Cp) 00 that is calculated using the following formula:

Cp: (precipitation correction coefficient)

$$Cp = (A + 352.5) / 352.5.$$

Where A is the rainfall increase in mm.

$$A = d \times 50 / 100.$$

d: difference in altitude between the two stations.

352.5: being the sum of the average monthly rainfall precipitation at the reference station (Ain Skhouna 923m)

Table 3. Average monthly rainfall of the study region calculated over the periods 1991-2020).

Months	Jan	Feb.	Mar	Apr.	May	June	July	Aoug	Sep	Oct.	Nov.	Dec.	Total
Station Batna 1052m	37.7	22.5	34.5	37.4	44.7	20.7	6.5	21.5	33.2	28.1	27.9	37.7	352.5
Point 1 Alt :1111 m	40.71	24.3	37.26	40.39	48.27	22.35	7.02	23.22	35.85	30.34	30.13	40.71	380.55

Chapter 1: Materiel and Methods

Point 2 Alt :1866 m	81.05	48.37	74.17	80.41	96.10	44.50	13.97	46.22	71.38	60.41	59.98	81.05	757.61
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P: monthly rainfall averages

The examination of Table 3 reveals differences in the distribution of monthly and annual rainfall between the Ain Skhouna-Batna station and the Belezma National Park. Specifically, the Ain Skhouna resort experiences an annual average of only 352.5 mm of rainfall, while the examined region receives an average of between 380.55 mm and 757.61 mm.

In July, the Batna station observed the minimum rainfall of 6.5 mm, while the months January, April, May, and December experienced the highest level of rainfall with 37.7 mm, 44.7 mm, 37.7 mm, and 37.4 mm, respectively.

May was the wettest month in the Belezma Mountains, with a maximum rainfall of 40.23 mm on the southern slope and 96.10 mm on the northern slope. The lowest levels were recorded in June and July, the two summer months, and in February, the winter month, with 24.3 mm (Southern slope) and 48.37 mm (Northern Slope). July was the least rainy month, with a minimum of 7.02 mm on the southern slope and 13.97mm on the northern slope. On the other hand, the resort experiences varying levels of precipitation (23.22mm/46.22 mm) during the month of August, generally marked by tumultuous showers.

1.7.1.2 Seasonal precipitation regime

It has been discovered that vegetation is mainly affected by the distribution of rainfall throughout the year rather than by its overall quantity (Menasri, 2014). To provide a more accurate representation, it is necessary to study seasonal rainfall patterns. Halimi (1980) defines the four average seasonal rainfall totals, ordered in descending order, to form the seasonal indicator (table4).

Table 4. Seasonal rainfall (mm) for the Batna station and the Belezma Mountains from 1991 to 2020.

Season Station	Winter (H)	Spring (Sp)	Summer (E)	Automn (A)	Annual Precipitation (P mm)	Type of seasonal regime
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Chapter 1: Materiel and Methods

Batna Station	97.9	116.6	48.7	89.2	352.4	SpWAS
Point 1111 m	105.72	125.92	52.59	96.32	380.55	SpWAS
Point 1866 m	210.47	250.68	104.69	191.77	757.61	SpWAS

Examination of the table indicates that the seasonal pattern is SpWAS, with the greatest amount of precipitation occurring during the winter and spring months. During the winter season, rainfall varies from 97.9 mm to 210.47 mm. The summer period experiences low rainfall, mainly in the form of stormy rains.

1.7.2. Temperatures

Temperature is a crucial limiting factor because it regulates all metabolic phenomena and consequently regulates the distribution of species and living communities within the biosphere (Ramade,1984).

Plant physiology informs us of the importance of temperature and water in the processes that maintain plant life (Heller et al., 1993; Lüttge et al., 1992; Soltner, 1992).

1.7.2.1 Average monthly and annual temperatures

The thermometric data of the study region for the periods 1991–2020 are presented in the tables before and after extrapolation. These extrapolated values were determined from an altitudinal gradient of 100 meters, calculated according to the maximum (M) of -0.7°C and the minimum (m) of -0.4°C (Seltzer, 1946) (table5,6).

Chapter 1: Materiel and Methods

Table 5. Average monthly thermometric data in (°C) from the Batna station for the period from 1991 to 2020.

Month	Jan	Feb	Mar	Apr	May	June	July	Aoug	Sep	Oct	Nov	Dec
Max	11.5	13.1	16.6	20.6	26.2	30.9	36.0	35.3	29.4	24.1	17.2	13.0
Min	0.5	0.6	3.2	6.2	9.9	14.4	17.6	17.3	14.4	9.9	4.8	1.6
M+m / 2	6	6.85	9.9	13.4	18.05	22.65	26.8	26.3	21.9	17	11	7.3

M: averages of maximum temperatures; m: averages of minimum temperatures; $m + M / 2$: monthly averages.

Table 6. Average monthly thermometric data in (°C) of the study region calculated by extrapolation over the period 1991-2020.

Station	Station 1 : 1111 m			Station 2 : 1866 m		
	Month	T max	Tmin	M+m/2	Tmax	Tmin
Jan	8.74	-0.12	4.31	5.81	-2.76	1.52
Feb	10.34	-0.02	5.16	7.41	-2.66	2.37
Mars	13.84	2.58	8.21	10.91	-0.05	5.43
Apr	17.84	5.58	11.71	14.91	2.95	8.92
May	23.44	9.28	16.36	20.51	6.65	13.60
June	28.14	13.78	20.96	25.21	11.15	18.17
July	33.24	16.98	25.11	30.31	14.35	22.32
Aug	32.54	16.68	24.61	29.61	14.05	21.82
Sept	26.64	13.78	20.21	23.71	11.14	17.42

Chapter 1: Materiel and Methods

Oct	21.34	9.28	15.31	18.41	6.65	12.52
Nov	14.44	4.18	9.31	11.51	1.55	6.52
Dec	10.24	0.98	5.61	7.31	-1.65	2.82
Totale	240.78	92.96	166.87	205.62	61.37	170.19

M: averages of maximum temperatures; m: averages of minimum temperatures.

Tables 6 and 7 illustrate the average annual temperature of Batna and the study area, which varies from year to year. The temperatures in the search area vary from 1.52 °C to 26.63 °C. January experienced the lowest minimum temperatures in all stations, ranging from -2.76°C to 0.62°C. On the other hand, the hottest month for all stations is July, with temperatures ranging from 33.24°C to 36.22°C.

1.7.2.2 Thermal amplitudes

The difference between the highest and lowest temperatures in a year, also known as the annual temperature amplitude, can indicate the level of continentality of a climate (Seltzer, 1946). To calculate the thermal amplitudes, the method of Debrach (1953) is used with respect to this amplitude (Table 7).

We can distinguish four types of climates:

- $M-m < 15^{\circ}\text{C}$: Island climate
- $15^{\circ}\text{C} < M-m < 25^{\circ}\text{C}$: Coastal climate
- $25^{\circ}\text{C} < M-m < 35^{\circ}\text{C}$: Semi-continental climat
- $M-m > 35^{\circ}\text{C}$: Continental climate

Table 7. Maximum average amplitudes recorded at the stations studied during the period 1991-2020).

Station	Ain- skhuna Batna Weather Station 1991-2020	BNP point 1 (1111 m) 1991-2020	BNP point 2 (1886m) 1991-2020
M	36	33.24	30.31
M	0.5	-0.12	-2.76
M-m	35.5	33.36	33.07

M: maximum average amplitude, m: minimum average amplitude

The table 7 reveals that the Batna station experiences a continental climate, while the study area experiences a semi-continental climate for northern slope and continental climate for southern slope.

1.8. Climate summary

1.8.1 Bagnouls and Gaussen thermal diagram

The Gaussen and Bagnouls ombrothermal diagram is a meteorological graph that illustrates the monthly fluctuations of one or more climate-related variables, thus approximating the duration of the dry and wet seasons (Bagnouls & Gaussen, 1957).

On a graph, the X-axis represents the months, while the Y-axis represents the precipitation (P) and the temperature (T), with $P = 2T$ (Frontier et al., 2004). A month classified as "dry" if the precipitation is less than twice the average temperature, and "humid" if it exceeds this value.

The graphs illustrating the hydrothermal data (Figure 10, 11, 12) display the typical precipitation and temperature patterns in the study area. From here, we can conclude the duration of the summer drought.

From these diagrams, we can make the following observations: A 5 month dry spell occurs at the Batna station, from June to October. The 1111 m station is experiencing a drought from Mid-May to early September, lasting 5 months. For the station located at 1866 m, the period of drought extends over 3 months, from Mid-June to August.

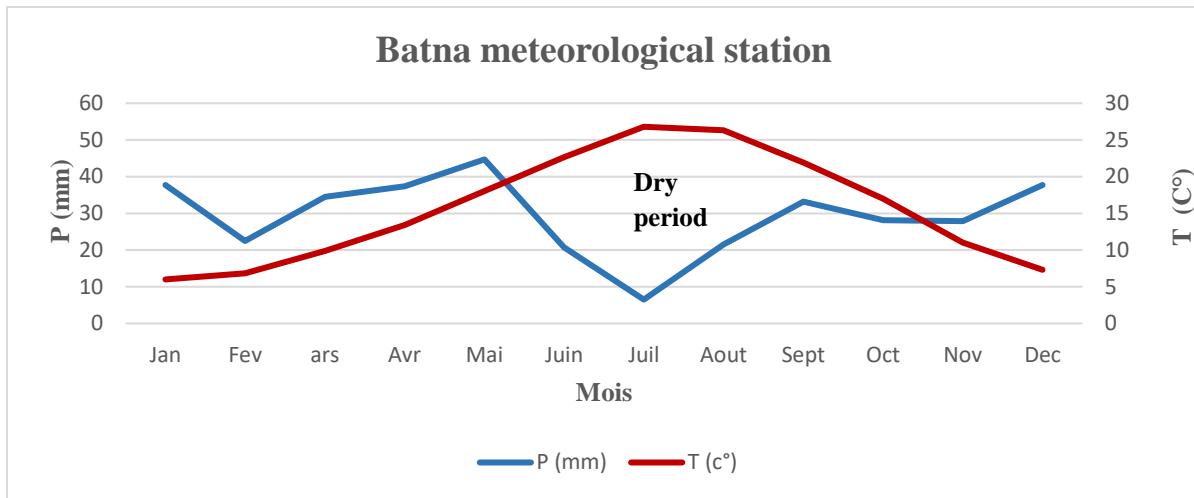


Figure 10. Pluviothermic diagram (Batna Station 1991-2020).

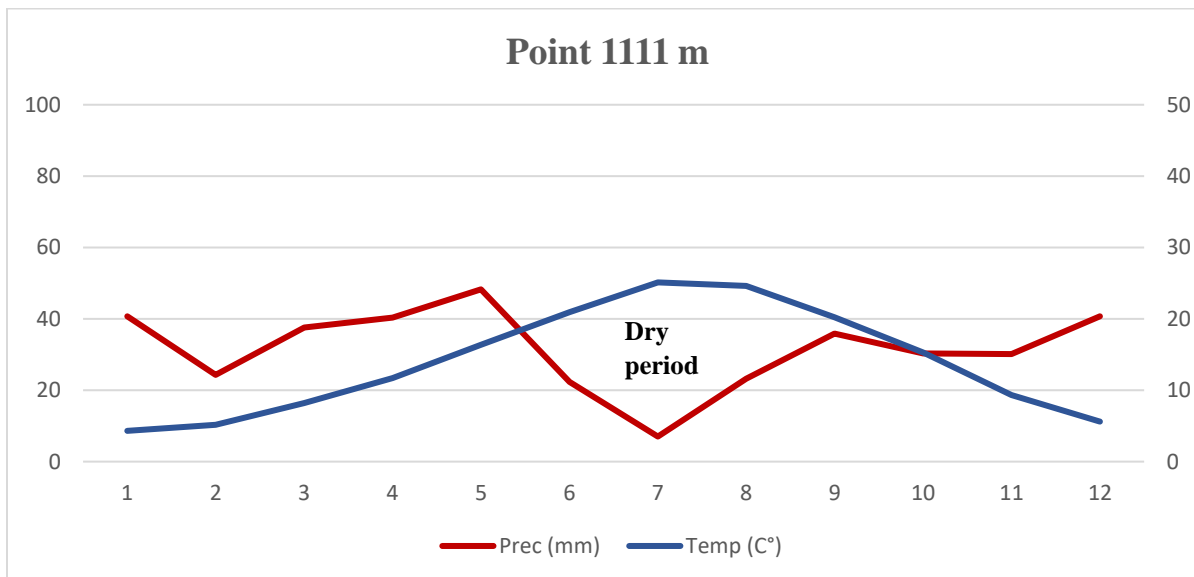


Figure 11. Pluviothermic diagram (Point 1111 m 1991-2020) (Maalem N, 2023).

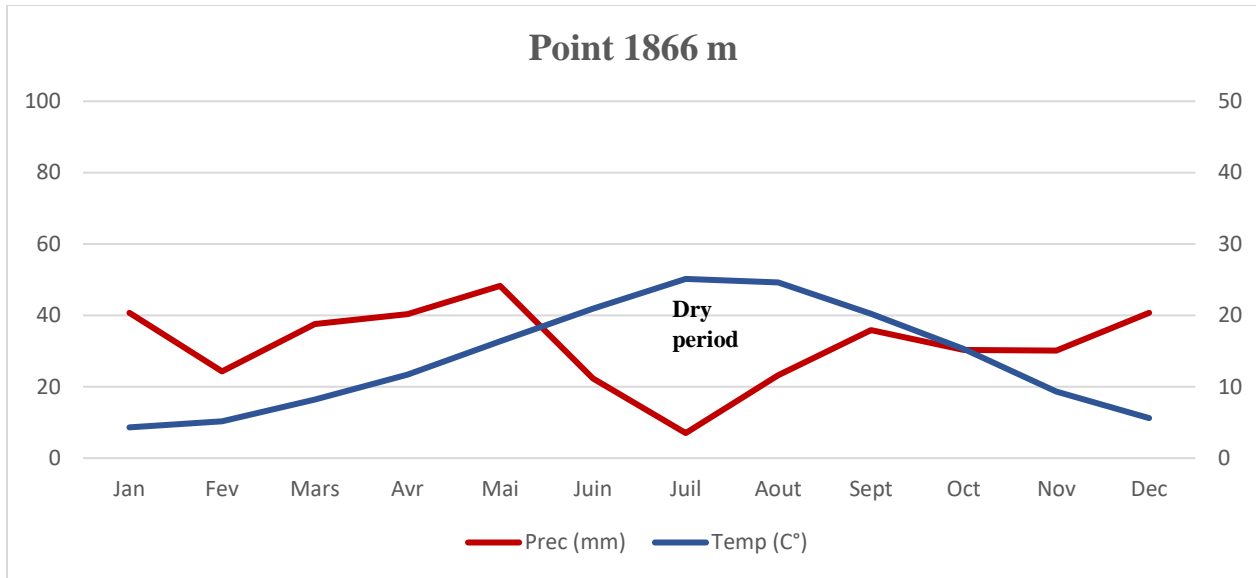


Figure 12. Pluviothermic diagram (Point 1886 m 1991-2020) (Maalem N, 2023).

1.8.2 Emberger's Climagram

The Emberger bioclimatic index is one of the bioclimatic indices commonly used in North Africa and other Mediterranean regions (Emberger, 1931; Gaussen, 1954; Daget, 1977; Quezel, 1979). Its objective is to establish and categorize the distinct varieties of the Mediterranean climate, ranging from extremely arid regions to high-altitude mountainous regions (Dajoz, 1985–2003).

This classification involves two crucial factors. First, it takes into account the drought, which is represented by the rainfall-thermal quotient Q_2 on the ordinates. Secondly, we consider on the abscissa the average minimum temperature of the coldest month.

It is calculated using the modified Emberger formula (Stewart, 1969).

$$Q_2 = 3.43 \times (P/M - m)$$

P: annual rainfall in mm.

M: maximum average temperature of the hottest month in °C.

m: minimum average temperature of the coldest month in °C.

To establish the bioclimatic floor of the study area, which includes Belezma National Park and the Ain Skhouna Batna station, we estimated the rain quotient at different altitudes (Table 8). We have determined the precipitation gradients (M, m, and P) explained in sections I-1-7-1 and I-1-7-2 to guide our analysis.

Chapter 1: Materiel and Methods

Table 8. Evaluation of the rainfall quotient Q2 over the period from 1991 to 2020.

Station	P (mm)	T min (c°)	Q2	Climatic floor
Ain Skhouna Batna	352.5	0.5	34.05	Semi-arid in cool winter
Point 1111 m	317.16	-0.12	39.68	Semi-arid in cold winter
Point 1886m	757.61	-2.76	78.58	Sub-humid a cold winter

The results of table 8 shows that Belezma National Park occupies the semi-arid cold-winter bioclimatic floor on the southern slope, and on the northern slope, it occupies a sub-humid cold-winter bioclimat.

2. Inventory

2.1. Choice of stations

We selected 18 stations based on the information provided by Belezma National Park staff, their significant expertise and understanding of all the park's routes, and our field inspections in the three sectors (6 stations per sector). These stations are distinguished by their variable exposures, slopes, and altitude, as well as by their different types of trees, all with the aim of making known as many species as possible. It should be noted that these stations are both secure and easily accessible, with sufficient surface area to study the various influencing factors and provide a complete representation of the station (Figure13).

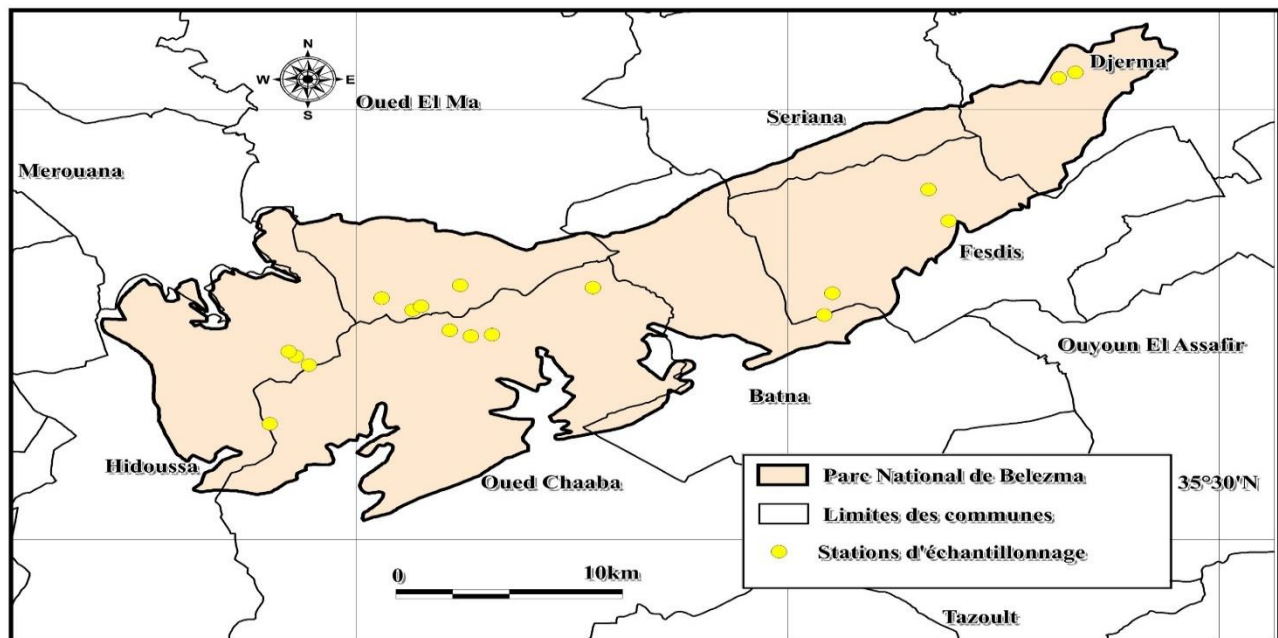


Figure 13. Distribution map of the stations in Belezma National Park (Maalem N, 2023).

2.2. Sampling method and species identification

The large area of the BNP and the variation in abiotic conditions from one sector to another prompted us to use systematic sampling techniques, where The sampling technique employed in this study is subjective, as it is the simplest and most straightforward method of selecting homogeneous and representative areas (Gounot, 1969).

The minimum air should be determined when the species studied no longer show signs of increasing their population (Gounot, 1969; Gland et al., 2003).

Chapter 1: Materiel and Methods

The specimens are collected and kept in herbariums. The identification is then carried out in the laboratory for all the species that have not been identified during the fieldwork, using the New Flora of Algeria by Quezel & Santa (1962-1963) as a reference. The identified plant species revised from the Synonymic Index of the Flora of North Africa by Dobignard and Châtelain (2010–2013), and the POWO 2023 website.

Concerning *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller, whom we rediscovered in the park, its identity was confirmed by Gordo Belkacem (a Professor at Oran University), we used POWO website and African database to check her current name. We studied the ecology of this rare plant and tried to estimate its dispersion throughout Algeria.

For the analysis of the floristic composition, we have compiled and studied a list of species inventoried in 18 stations. At each station, we evaluated the number of families, genus, and species. In our comprehensive analysis, we have included the biological type and the types of phytogeographic distribution for all species.

3. Ecological indices

3.1. Total Specific richness (S)

The specific richness S defined as the total or average number of species per unit area (Grall & Coic, 2006).

S = represents the number of species in the study area

3.2.SORENSEN similarity index

The similarity index evaluates the similarity between two surveys by dividing the common species that appear in both surveys by those specific to each survey. This measure highlights the degree of affinity between the different stations according to their populations (Magurran, 1988, 2004; Bougurnna, 2011).

There are different similarity indices, but the SORENSEN index is the most suitable for evaluating the correlation between two sites. The formula for determining:

$$S = (2 C/A+ B) \times 100$$

S: Similarity index.

A: Total number of taxa in the middle 1.

B: Total number of taxa in the middle 2.

C: Total number of taxa common between the two environments.

This index varies from 0 to 100%. A value close to 0 indicates that the two sites have no common species and are dissimilar. On the other hand, a value close to 100% indicates that the species of the two localities are identical, i.e., that the similarity is complete. The higher the percentage of similarity, the more similar the compared media are, whereas a value close to 0 means that the media are significantly different.

3.3. Disturbance index

The disturbance index evaluates the state of degradation of individual groups (Habrard et al., 1995), and it is formulated by the relationship between Loisel & Gamila (1993).

$$IP = [(N \text{ Chamephytes} + N \text{ Therophytes}) / N \text{ total species}] * 100$$

IP: disturbance index

N: number of species

4. Statistical analysis

4.1. Bi-varied statistical analysis

4.1.1. Similarity Analysis (ANOSIM)

Ecological studies that take into account several variables, such as the abundance or biomass of different species in various samples, can generate data matrices that are difficult to analyze using conventional statistical approaches. To solve the many statistical problems involved, Field et al. (1982) have described a robust nonparametric multivariate strategy. Clarke (1993) developed and clarified this analytical method, which has since evolved (Clarke et al., 2014a). The essence of the method is to utilize grouping (Clarke et al., 2016) and ordination to reflect patterns between samples established by appropriate measures of similarity (Clarke et al., 2006), following adequate data pretreatment (Clarke et al., 2014b). These models are then examined using a variety of hypothesis tests and associated studies (for example, Clarke et al., 2008), with a focus on categorized similarities. To assist the discussion, similarities between samples considered

dissimilar in all that follows; nevertheless, the methods are also relevant to other types of resemblance, such as similarities or distances.

The ANOSIM statistic compares the mean of ranking dissimilarities between groups and within groupings. An R-value close to "1.0" indicates dissimilarity between groups, whereas an R-value close to "0" indicates a consistent distribution of high and low rankings within and between groups. R values less than "0" indicate that dissimilarities are larger within groups than between them (Pauls et al., 2013).

Xlstat software was used to conduct the analyses. Differences were judged significant below the threshold of $\alpha = 0.05$.

4.1.2. Permutational Multivariate Analysis of Variance" (PERMANOVA)

The PERMANOVA is a multivariate nonparametric test used to compare groups and test the null hypothesis that the groups' centroids and dispersion, as specified by the measurement space, are equal for all groups. The PERMANOVA performs significance tests by comparing the value of F obtained from the estimation of the real data to that obtained from random permutations of the items between the groups. In cases of observed significance for a component or an interaction of factors, the pairwise function adonis created, functioning as a post-hoc (Oksanen et al., 2019).

4.2. Multivariate statistical Analysis

4.2.1. Canonical correspondence analysis (CCA) of the floristic distribution in the Belezma National Park

Canonical correspondence analysis (CCA) is a multivariate method for determining the links between biological assemblages of species and their environments. The method aims to extract synthetic environmental gradients from ecological data sets. Gradients provide the foundation for clearly explaining and exhibiting taxavia's variable habitat preferences (niches) in an ordination diagram (Anderson, 1993).

The objective of the analysis of canonical correspondences (ACC) in our study is to determine the impact of altitude and bioclimate on the floristic composition of the 18 stations of Belezma National Park. The data on the flora coded in presence-absence and linked to a matrix of explanatory variables, in particular environmental variables such as altitude and bioclimate (Ter Braak, 1995).

This analysis performed using the xlstat computer language, version 3.0.1 (Team, 2014).

Second part: phytochemistry and biological activities

1. Plant material

1.1. The Harvest of plants

Santoline (*Santolina rosmarinifolia* L.) is common in Fisdis, while the *Thymus* (*Thymus algeriensis* Boiss. & Reut) is found in all three sectors of Belezma National Park. Because this is the most common species, we sampled the aerial parts (leaves and flowers) of these two common plants during their blossoming phase in the morning. Santoline samples taken during the end of April 2022 at 10 a.m. in Tagherbit, Fisdis sector, while *Thymus* samples obtained during the first week of May at 11 a.m. in Theniet Guntess, Mont Bourdjem, Oued El Maa sector. The table below summarizes data from the sample site (Table 9).

Table 9. Sampling site data sheet.

Stations	Sectors	Geographical coordinates	expositions	Altitude	Type of population
Station 5	Fisdis	35°37'25''N 6°13'43'' E	Est	1021 m	<i>Juniperus oxycedrus</i> <i>Juniperus phoenicea</i> <i>Quercus ilex</i> <i>Olea oeuropa</i>
Station 17	Oued Imaa	35°34'16''N 5°58'36'' E	Nord	1607 m	<i>Cedrus atlantica</i> <i>Quercus ilex</i> <i>Juniperus oxycedrus</i>

1.2. Identification of the plant

The identification of our species carried out in the laboratory of Biodiversity and Biotechnological Techniques for the Valorization of Plant Resources (BTB-VRV), Faculty of Nature and Life Sciences at the Mohamed Boudiaf University of M'sila, using the new flora of Algeria from Quezel and Santa 1962–1963 (Figure14, 15).



Figure 14. *Thymus algeriensis* Boiss. & Reut.

(Maalem N,2023)



Figure 15. *Santolina rosmarinifolia* L.

(Maalem N,2023)

1.3. Grinding the dry parts

Selected plants are crushed in a mortar, which results in obtaining a vegetable powder.

1.4. Quantitative Characterization of Extracts

The method of extracting phenolic compounds from the leaves and flowers of these plants involves the use of an extraction technique called aqueous methanol maceration.

1.5. Maceration principle

Maceration is a method of extracting soluble compounds from a solid by immersing it in a cold liquid or by soaking it in the liquid to obtain its aroma or flavor, preserve it, or decompose it. Alcoholic solvents can increase the permeability of cell walls, thus facilitating the extraction of a greater number of polar, medium, and weak molecules.

1.6. Solid/liquid type extraction (Extraction by maceration in aqueous methanol):

Preparation of extracts

After grinding the dried components, macerate 50 g of powdered aerial plant parts in a solution containing 80% methanol and 20% distilled water for 28 to 48 hours. Repeat this operation three times at room temperature. The extract is then filtered on filter paper and evaporated to dryness

Chapter 1: Materiel and Methods

under reduced pressure ($T = 40^{\circ}\text{C}$) using a rotary evaporator (BÜCHI R-120). The dry residue is taken up with a few milliliters of methanol and stored at $+4^{\circ}\text{C}$. The result is the crude hydromethanolic extract of the plant (aerial parts) (EBr.pln.) (Figure 16,17,18,19).

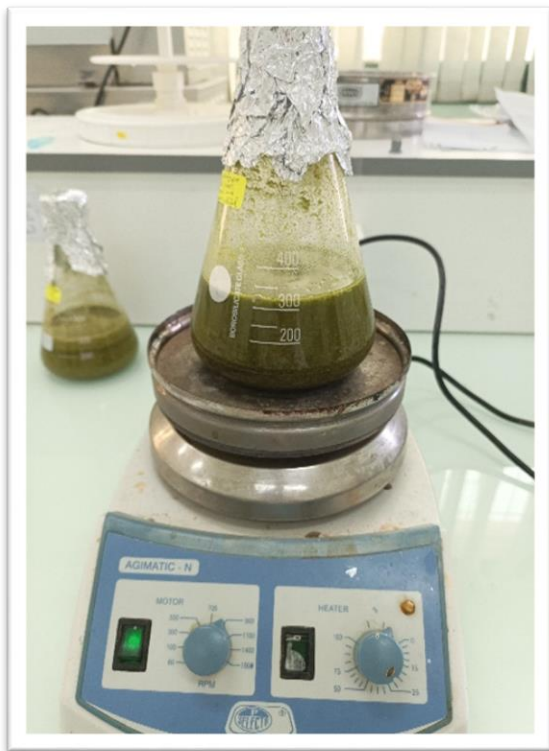


Figure 16. Maceration.

(Maalem N., 2023)

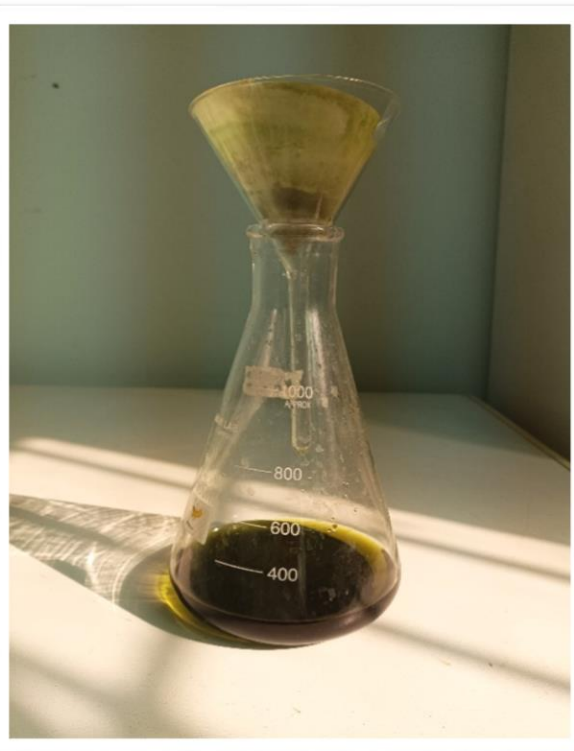


Figure 17. Filtration.

(Maalem N., 2023)



Figure 18. Device of the rotary Rota-steam type (BÜCHI R- 120) (Maalem N., 2023).

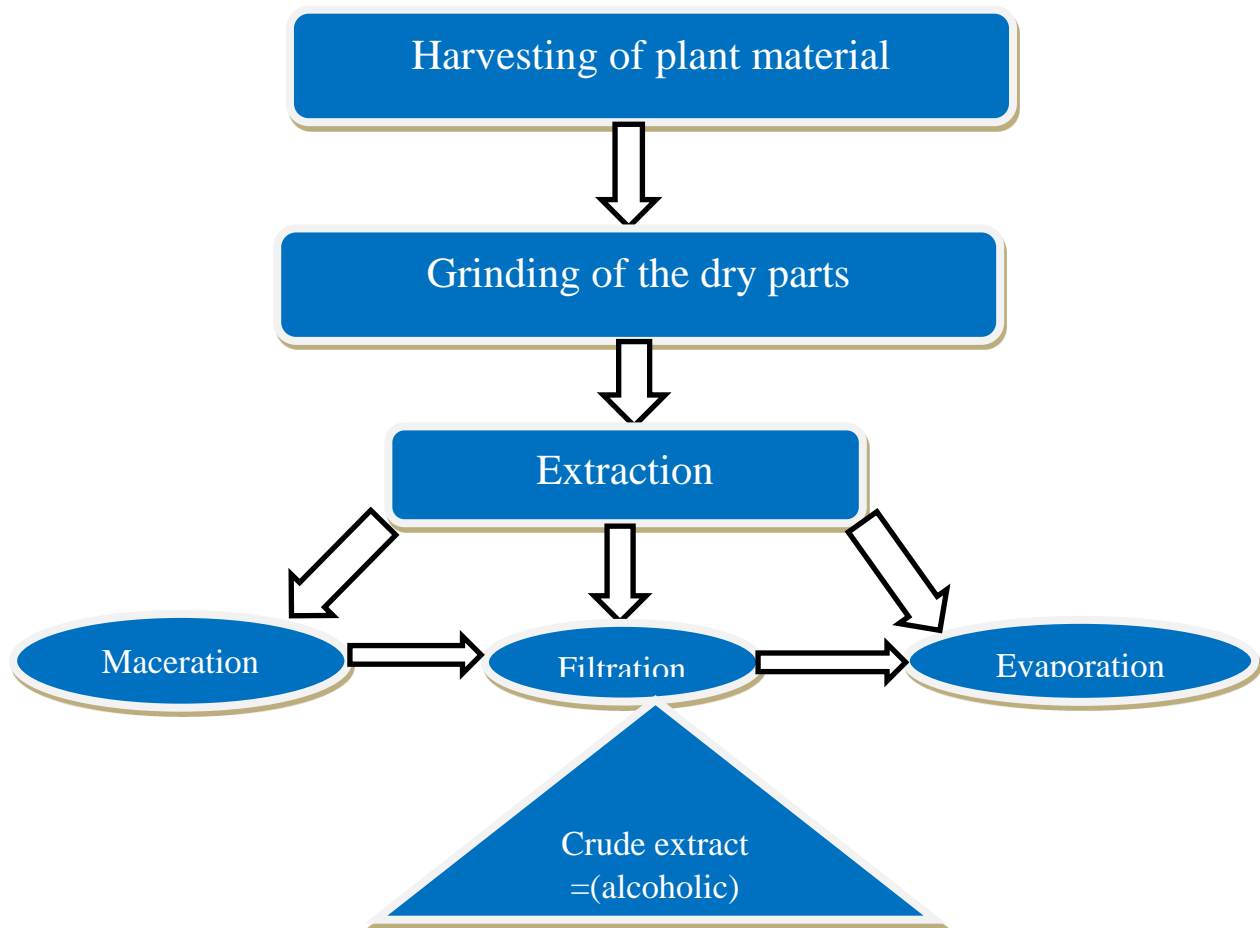


Figure 19. Protocol preparation of extracts.

2. Extraction of essential oils

2.1. Extraction by hydrodistillation

The leaves and flowers were separated from the twigs and dried in a place protected from the sun for 15 days. The two plants were then placed in paper bags and taken to the laboratory in order to extract the essential oils using a Clevenger water distillation apparatus (before using the apparatus, it must be cleaned with acetone and then distilled water in order to avoid contamination). This process requires introducing 100 g of the plant material of each species into two 2-liter balloons containing 1000 ml of distilled water for one cycle. The whole thing is brought to a boil for 3 hours using a flask heater. The essential oils obtained were dried with anhydrous sodium sulfate (Na_2SO_4) and stored in opaque glass bottles at 4 °C, protected from light (Gardeli et al., 2008) (Figure 20,21).



Figure 20. Dry plant material.
(Maalem N., 2023)



Figure 21. Original hydrodistillation photo of clevenger type (Maalem N., 2023)

2.2 Calculation of the Yield

According to the AFNOR standard (1986), the essential oil yield refers to the percentage ratio of the mass of essential oil obtained after its extraction relative to the mass of plant material used.

The formula for calculating the essential oil yield is as follows:

$$\text{RHE} = \text{M}'/\text{M} \times 100$$

RHE: Essential oil yield in %.

M': mass of essential oil in grams.

M: mass of the plant in grams.

3. Chemical characterization of the HEs of *Thymus algeriensis* Boiss. & Reut and *Santolina rosmarinifolia* L.

The chemical composition of the essential oils of the species studied analyzed and identified by gas chromatography coupled to mass spectrometry (GC-IMS), a chromatographic technique. This method allowed the separation, quantification, and identification of compounds by referencing spectral databases. The approach used was objective and impartial, leading to accurate results.

3.1. Determination of the chemical composition

The essential oils were analyzed at the Scientific and Technical Research Center for Physico-Chemical Analysis (CRAPC) in Elhadjeb-Biskra using a Nexis GC-2030 SHIMADZU chromatograph connected to a GCMS-QP2020 NX SHIMADZU mass spectrometer (Figure 22). The compounds were separated using an HP-5MS UI capillary column measuring 30 m x 0.25 mm and a film thickness of 0.25 μm . The temperatures of the ion source and of the interface were maintained at 280 °C, respectively. Helium (He) was used as carrier gas, with a flow rate of 0.5 ml/min. To be precise, 0.2 μl of the essential oil sample were injected in fractional mode according to a division ratio of 1:80.

The oven has been programmed to heat from 60°C (for 8 minutes) to 250°C with a gradual increase of 2 °C per minute. The mass spectra were obtained by electron impact ionization with an energy of 70 eV, scanning a mass range ranging from 30 to 550 units.



Figure 22. Gas chromatography / mass spectroscopy apparatus (Maalem N., 2023).

3.2. Identification of chemical compounds

The identification of the compounds carried out by comparison of their mass spectra with those obtained from the Wiley 7n.L. and NIST11.L (National Institute of Standards and Technology) data libraries (Massada, 1976; Jennings and Shibamoto, 1980; Swigar and Silverstein, 1981; Davies, 1990; Adams, 1995, Hamdi et al., 2015). The use of compound retention times has also been implemented to ensure accurate identification.

3.3. Determination of total phenols and flavonoids

The crude extracts' phenol and flavonoid contents were determined using pyrocatechol and quercetin equivalents, and they were calculated according to the following equations (Ertaş et al., 2014):

$$\text{Absorbance} = 0.0035 \text{ pyrocatechol } (\mu\text{g}) + 0.045$$

$$\text{Absorbance} = 0.205 \text{ quercetin } (\mu\text{g}) - 0.1528$$

4. Biological activities

4.1. Microbiological study

4.1.1. The strains tested

For the study, we evaluated the sensitivity of reference bacterial strains from the microbiology laboratory of the University of Mohamed Boudiaf, M'sila. We carried out tests on three bacterial strains, including one gram-negative and two gram-positive (Table 10). All the strains tested were recognized for their pathogenic and destructive qualities of food and were frequently associated with a whole series of bacterial diseases, in particular infections and cases of food poisoning.

Table 10. List of bacterial strains tested

Strain name and Reference code	Gram
<i>Escherichia coli</i> ATCC 8739	Gram negative
<i>Pseudomonas aeruginosa</i> ATCC 27853	Gram negative
<i>Staphylococcus aureus</i> ATCC 25923	Gram positif

4.2. Evaluation of the antibacterial activity of *Thymus algeriensis* Boiss. & Reut and

Santolina rosmarinifolia L.

4.2.1. Principle

The process works by impregnating a paper disc with extract at various concentrations. The disc placed directly on an agar plate that has been uniformly seeded with the germ that will be tested. The distance at which the germs' development inhibited relative to the disk is proportional to their sensitivity to the extract. The limit of the inhibition zone, as determined by the eye, corresponds to the start of bacteria growth.

4.2.2. Preparation of culture medium

The recommended culture medium for this study is Muller-Hinton. To completely dissolve the agar, it must be boiled in a water bath. Then pour the middle into the Petri dishes and let it cool.

4.2.3. Preparation of dilutions of extract of the plants *Thymus algeriensis* Boiss. & Reut and *Santolina rosmarinifolia* L.

The extracts of *Thymus algeriensis* Boiss. & Reut were dissolved in dimethyl sulfoxide (DMSO) to obtain four concentrations: C1 = 2, C2 = 4, C3 = 8, and C4 = 16. The same process repeated for *Santolina rosmarinifolia* L.

4.2.4. Preparation of the discs

This method involves cutting filter paper into circular discs about 6 mm in diameter to obtain an easily measured inhibitory zone (Benlahrache & Boukerzaza, 2022).

4.2.5. Reading the results

The readings are acquired by measuring the diameter of the inhibition zone surrounding each disk using a millimeter ruler. The results expressed by the diameter of the inhibition zone, which can be represented by signs based on the strains' sensitivity (Ponce, 2003).

- * Not sensitive (-) or resistant: diameter < 8mm
- * Sensitive (+): diameter between 9 and 14 mm
- * Very sensitive (++) : diameter between 15 and 19 mm
- * extremely sensitive (+++) : diameter > 20 mm.

4.3. Antioxidant activity

Several experiments are carried out to determine the antioxidant efficacy of the extracts. Generally, these tests are based on the color alteration or discoloration of a reagent within the reaction medium. Our study used the DPPH test to assess antioxidant activity.

4.3.1. Free radical test DPPH (2, 2-diphenyl 1-1-picrylhydrazyl)

The study evaluated the antioxidant activity of the extracts by means of in vitro tests using 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable, violet-colored free radical that can be measured photometrically at 517 nm. Its stability is attributed to the unpaired electron on a nitrogen bridge atom. The antioxidants, which act as hydrogen donors (AH), reduce the compound to a non-radical form of DPPH-H (2, 2-diphenyl-1-picrylhydrazine) (Figure 23). This reduction causes the compound to turn yellow, resulting in a decrease in absorbance (Brand-Williams et al., 1995; Maataoui et al., 2006). The absorbance can be determined with a Jenway 7305 UV-Visible spectrophotometer (Brand-Williams et al., 1995).

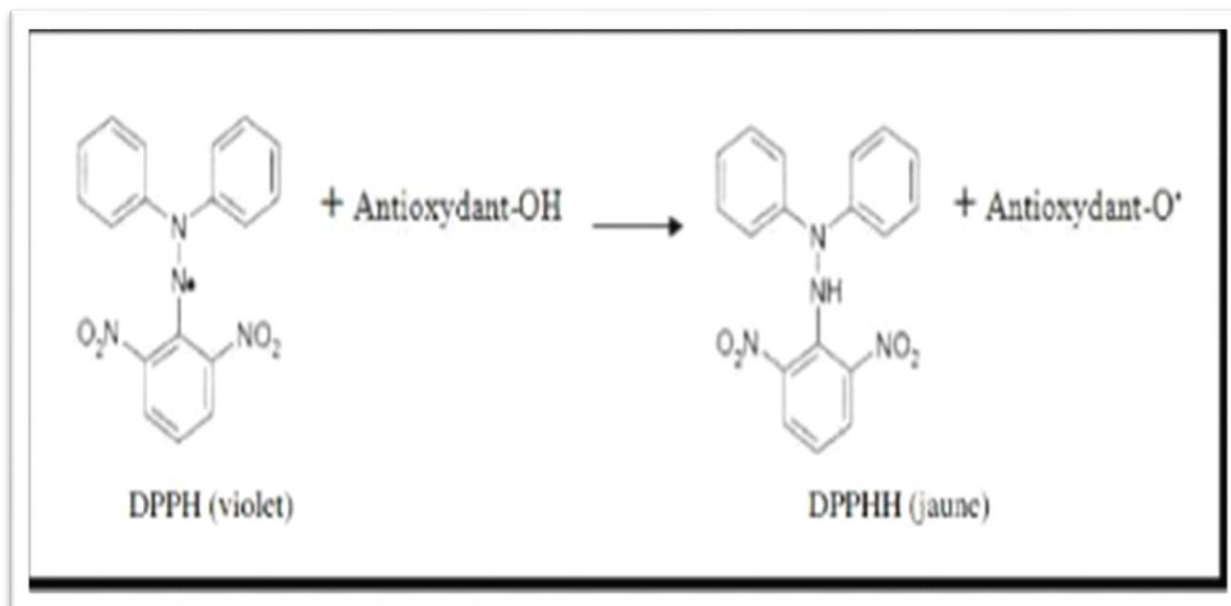


Figure 23. Mechanism of reduction of the free radical DPPH by an antioxidant.

4.3.2. Practical implementation

The DPPH is dissolved in ethanol, yielding a solution containing 4 mg of DPPH per 100 ml of ethanol. The solution is then mixed with different amounts of extracts of *Thymus algeriensis* Boiss. & Reut. and *Sontolina rosmarinifolia* L. (10, 25, 50, 100 µg/ml) (Figure 24).

The mixture stirred vigorously and then incubated in the dark at room temperature for 30 minutes.

Reducing the DPPH leads to a color change from purple to yellow in the solution. The readings are obtained by spectrophotometric analysis at a wavelength of 517 nm.

$$\% \text{ inhibition} = (\text{Abs control} - \text{Abs test}) \times 100 / \text{Abs control}$$

Such as:

Abs control: Absorbance of the negative control at the wavelength 517 nm.

Abs test: Absorbance of the sample at the wavelength 517 nm.

4.3.3. Calculation of inhibitory concentrations at 50% "IC₅₀"

According to Torres et al. (2006), the IC₅₀ (inhibitory concentration of 50%), also called the EC₅₀ (effective concentration of 50), is the concentration of the test sample necessary to decrease the DPPH radical by 50%. The IC₅₀s are calculated graphically in the form of inhibition percentages based on various concentrations of the test extract.



Figure 24. Original photo of a spectrophotometry device (Maalem N.,2023) .

Chapter 2: Results and discussion**First part: Study of the floristic biodiversity in the Belezma National Park****1. Floristics inventory in the Belezma National Park**

The present study conducted in Belezma National Park; wilaya of Batna (Algeria).The floristic inventory has identified 288 species and 194 genus belonging to 47 families (Table 11).

Table 11. Floristic inventory in families, frequency of species recorded in BNP.

Families	Species frequency (%)
Asteraceae	20.13%
Fabaceae	10.76%
Apiaceae	7.98%
Brassicaceae	6.25%
Poaceae	5.55%
Lamiaceae	5.20%
Caryophyllaceae	4.86%
Plantaginaceae	4.86%
Cistaceae	3.12%
Geraniaceae	2.08%
Caprifoliaceae	1.73%
Crassulaceae	1.73%
Papaveraceae	1.73%
Rosaceae	1.73%
Ranunculaceae	1.73%
Convolvulaceae	1.39%
Rubiaceae	1.38%
Asparagaceae	1.38%

Chapter 2: Results and discussion

Cupressaceae	1.04%
Oleaceae	1.04%
Iridaceae	1.04%
Primulaceae	1.04%
Amaranthaceae	0.69%
Resedaceae	0.69%
Polygalaceae	0.69%
Malvaceae	0.69%
Boraginaceae	0.69%
Asphodelaceae	0.69%
Liliaceae	0.69%
Plumbaginaceae	0.69%
Euphorbiaceae	0.69%
Pinaceae	0.69%
Heliotropiaceae	0.34%
Gentianaceae	0.34%
Ephedraceae	0.34%
Dipsaceae	0.34%
Cyperaceae	0.34%
Anacardiaceae	0.34%
Aspleniaceae	0.34%
Alliaceae	0.34%
Berberidaceae	0.34%
Linaceae	0.34%
Nitrariaceae	0.34%
Oxalidaceae	0.34%
Polygonaceae	0.34%

Sapindaceae	0.34%
Fagaceae	0.34%
Total	100%

In terms of species representation, the families Asteraceae, Fabaceae, and Apiaceae are the three best represented families. The Asteraceae family representing 20.13% of the total number of species. The Fabaceae family representing 10.76%. The family Apiaceae (7.98%). Conversely, the families Brassicaceae, Poaceae, Lamiaceae, Caryophyllaceae, and Plantaginaceae have fewer than 19 species, while the remaining 39 families represented by between one and nine species.

Of the 288 species identified, we have rediscovered the presence of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller. in Belezma National Park. We focus on its ecology, and in addition, we try to delimit its distribution in Algeria.

2. Rediscovery of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller in the Belezma National Park.

2.1. Taxonomy

Tulips are perennials that grow to be 20–25 cm tall and have a round, hairy, tunneled bulb with a diameter of 5 cm. Flat, linear-lanceolate leaves, 3-6, 10-15 cm long, on upright stems (Figure 25,26). Flowers are solitary (each on a single stem), fragrant, and vary in color: pinkish white or sulfur yellow with purplish, washed-out dorsal surfaces of tepals (parts of the perianth). The tepals have ciliated interior surfaces that turn yellow at the base. We concur with Chabert (1897), who observed that the stigma is tiny and the stamens are half as long as the tepals. Quezel & Santa (1962) describe the fruit as a capsule containing fattened, subtriangular, and narrowly margined seeds. To corroborate our identity, we consulted the specimens of this tulip deposited in POWO (2023). Similarly, we consulted the photos of this species in the database of plant biodiversity in southwest Morocco: <https://www.gbif.org/fr/species/5299357>.



Figure 25. *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller (photograph by Maalem N., 2023).



Figure 26. Specimen of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller (Maalem N., 2023).

2.2. Habitat description

In the spring of 2022, we observed this tulip species at two stations in Belezma National Park. The first is located on the southern slope of Mount Kessrou at an altitude of 1117 m in the Fisdis sector (35°35'45.0"N, 06°11'01.0"E) (Figure 27), where we observed only one individual. There, matorral (shrubland) with trees dominates, characterized by some *Quercus ilex* subsp. *ballota* (Desf.) Samp. and *Pinus halepensis* Mill. The numerous shrubs and bushes include *Helianthemum hirtum* (L.) Mill., *Juniperus phoenicea* L., *Juniperus oxycedrus* L., *Phillyrea angustifolia* L., and *Pistacia lentiscus* L. The herbaceous layer contains chiefly *Arenaria serpyllifolia* L., *Hippocrepis multisiliquosa* L., *Lotus ornithopodioides* L., and *Plantago lagopus* L. In 2009, this area suffered a fire that destroyed 3/4 of the plant cover, according to the park's technical managers. This contributed to the modification of the vegetation and landscape, where *Macrochloa tenacissima* (L.) Kunth. spread to the detriment of many forest species.



Figure 27. Station 1: Fisdis sector (photograph by Maalem N., 18 April 2022).

The second station, situated at an altitude of 1792 m, is located in the scree on the northern slope of Mount Bourdjem in the Oued El Ma sector (35°35'20.0"N, 06°01'18.0"E) (Figure 28), where we found 20 individuals. It has favorable environmental conditions, including humidity, elevation, northern exposure, which is reflected in the nature of the plant cover, marked by the presence of an endemic Algerian-Moroccan tree, the Atlas cedar, *Cedrus atlantica* (Endl.) Manetti ex Carrière. The shrub layer includes *Crataegus monogyna* Jacq., *Juniperus oxycedrus* L., and *Quercus ilex* subsp. *ballota*. The herb layer includes *Anacyclus clavatus* (Desf.) Pers, *Aphanes arvensis* L., *Bromus hordeaceus* L., *Cynoglossum creticum* Mill, *Erodium cicutarium* (L.) L'Hér., *Fumaria capreolata* L., *Polygala monspeliaca* L., *Sonchus oleraceus* L., *Torilis arvensis* (Huds.) Link, *Trifolium campestre* Schreb., and *Veronica agrestis* L.



Figure 28. Station 2: Oued El Ma sector (photograph by Maalem N., 16 May 2022).

2.3. Distribution

In order to better understand the distribution of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller in Algeria, we referred to the works of Chabert (1897), Maire (1958), Quezel & Santa (1962), and our own observations. This allowed us to draw up a map on which we have indicated these reports (Figure 29). The distribution of the species is limited to northern Algeria, where the water conditions are favorable. From a biogeographical point of view, this taxon has a disjunct distribution, since it has been recorded in 2 distinct biogeographical sectors. The first area of distribution is more northerly and corresponds to C2 (Hodna Mountains and Belezma) according to the biogeographic division of Quezel & Santa (1963) modified by Meddour (2010). The second one concerns the Jebel Aïssa (Western Saharan Atlas), which constitutes its southern limit.

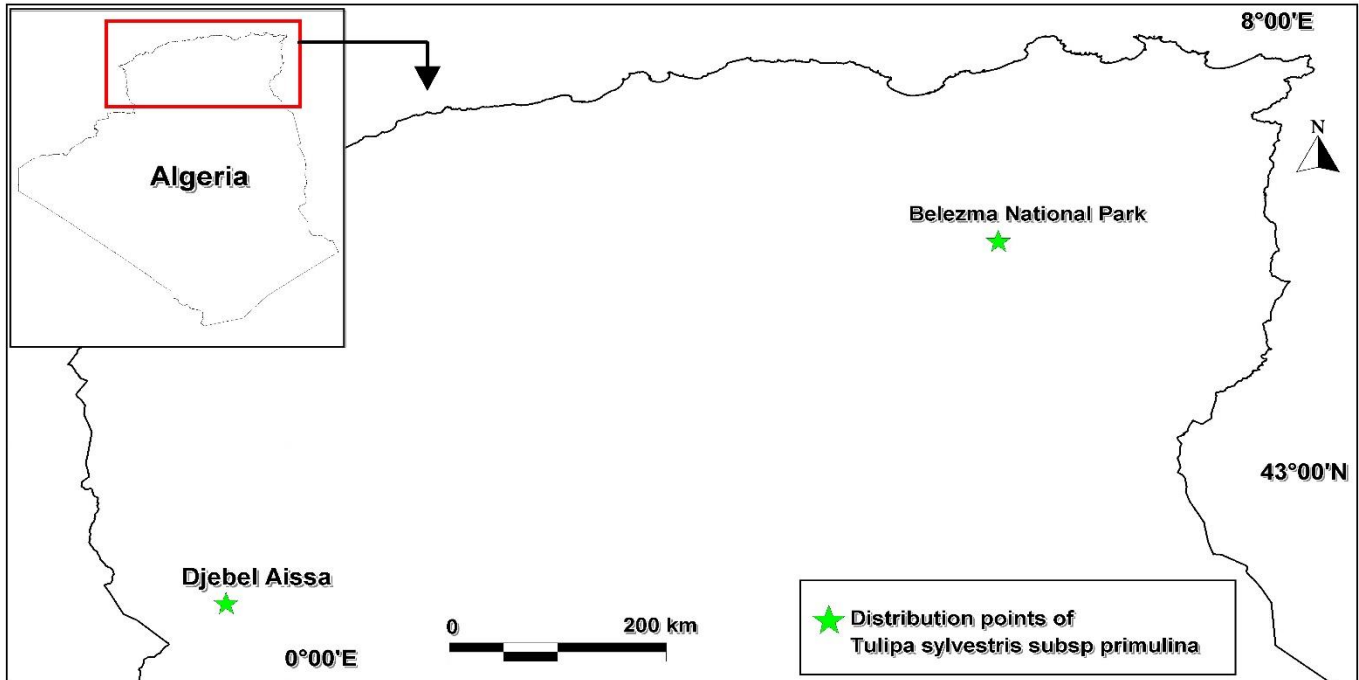


Figure 29. Distribution map of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller in North Algeria (Quezel & Santa, 1963) (Modified).

3. Biological spectrum of the species inventoried in Belezma National Park

In terms of biological spectrum, the results indicate that hemicryptophytes and therophytes have the highest representation, with 37.5%, 37.15%, respectively. In contrast to geophytes, which represented only 6.25% (Table 12).

Table 12. Distribution of the biological spectrum of flora identified in the study area.

Biological form	Percentage
Hemicryptophytes	37.5%
Therophytes	37.15%
Chamephytes	11.80%
Phanerophytes	7.29%
Geophyte	6.25%

The biological spectrum analysis suggests that hemicryptophytes and therophytes have the highest representation, with (37.5%) and (37.15%) respectively. Chamephytes accounted for 11.80%, phanerophytes for 7.29%, and geophytes for 6.25% (Figure 30)

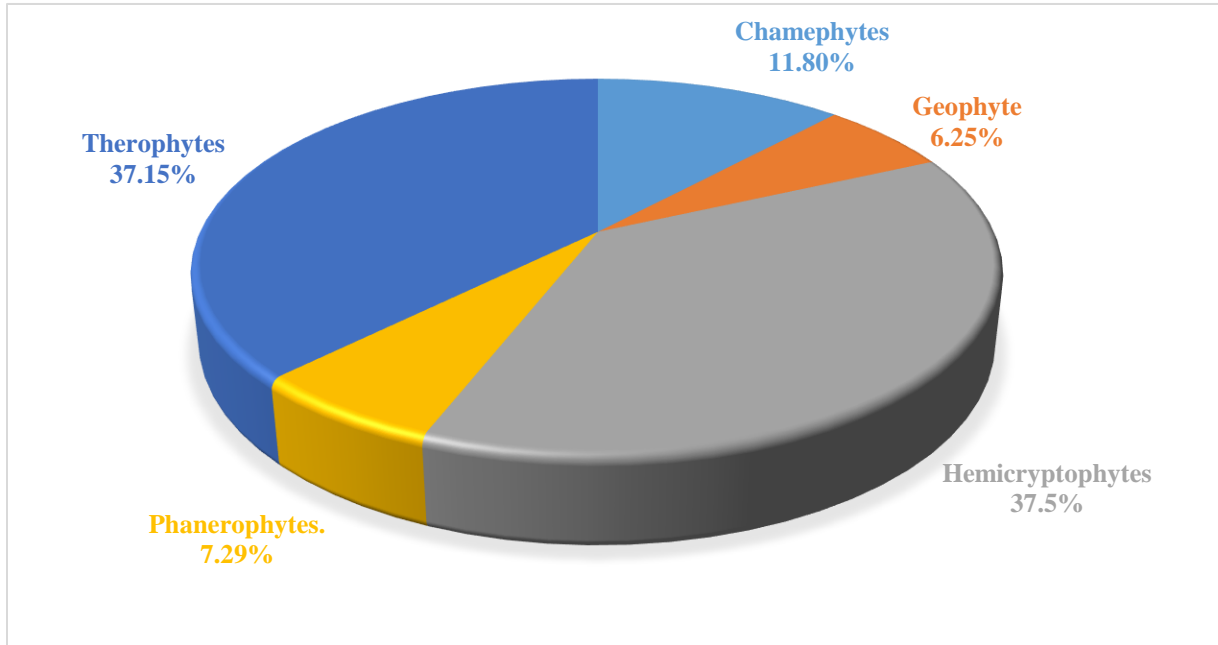


Figure 30. Biological spectrum of species inventoried from Belezma National Park.

The proportion of hemicryptophytes in this study differs from that reported in other studies. In concrete terms, the proportion is lower than that reported by Beghami (2013) for the Chélia flora (46%) and by Bouchibane et al. (2021) for the Kabylia mountains of Babors (43.8%), but higher than that reported by Touati et al. (2021) in the wilaya of Souk Ahras (31.93%). The dominance of hemicryptophytes attributed to various factors, including rainfall, cold temperatures (especially in winter), soils rich in organic matter, and high altitudes (Floret et al., 1990).

The therophytes occupy an important place, representing almost 37.15% of the flora. This percentage is higher than that reported by Bouchibane et al. (2021) for the Kabylia of the Babors (22.8%), which demonstrates the richness of the flora of the region compared to the Babors. Moreover, this percentage is similar to the percentage reported by Rebbas (2014) for the Gouraya National Park (37.3%), which suggests a consistency in the flora of the region and that it is subject to the influence of the Mediterranean climate. Nevertheless, it is lower than the percentage reported by Beghami (2013) for the flora of the Chelia massif (41%).

The BNP shelters a diversified vegetation, in particular forest formations composed mainly of cedars (*Cedrus Atlantica* (Endl.) Manetti ex Carrière), holly (*Ilex aquifolium*), oak groves (*Quercus ilex*), junipers (*Juniperus oxycedrus* and *Juniperus phoenicea*), pine forests (*Pinus halepensis* Mill.), and pistachio trees (*Pistacia lentiscus* L.). This impressive range of flora highlights the ecological richness of the park and underlines its importance as a conservation area. In addition, there are mosaic matorrals composed of *Cistus albidus* L., *Cistus salviifolius* L., *Globularia alypum* L., *Rosmarinus Officinalis* L., various lawns based on Poaceae, Fabaceae, Brassicaceae, and Apiaceae, and maquis based on Fagaceae and Cupressaceae (APN, 2006).

The study of the various forms of plant adaptation can enhance our comprehension of their ecological circumstances.

Therophity commonly considered an adaptive strategy in adverse circumstances. Champhytes, in comparison with other biological types, are better adapted to aridity. They are able to cope with drought by reducing the surface area of their leaves and developing their root system (Floret et al., 1990). It should be noted that the presence of therophytes could be used to calculate the disturbance index of a medium. The disturbance index value is 49.30%. A comparison of our results with those of other studies reveals that the disturbance index in our study area is lower than that observed in the Tlemcen National Park (Bensissa, 2020), which represents 74%, and in Mellal (2022) in Theniet El Had. The percentage of disturbance in Theniet El Had area is 55.43%, as indicated by station 1. However, station 2 in Theniet El Had region, which is situated at an altitude of 1700 m, exhibits a similar disturbance index (47.5%). The disturbances caused by humans and their livestock are numerous and correspond to two increasingly severe situations, ranging from matorralisation to steppisation (Berbero et al., 1990 in Benkhattou et al., 2015).

4. Biogeographic distribution of the species inventoried in Belezma National Park

The vegetation of BNP is composed of a wide range of elements of various origins. Mediterranean species are the majority with 51%, followed by the Nordic elements with 20.82%, cosmopolitan species, and wide distribution with 17.68%. The Saharan species represents 0.34% of the flora of the park.

For endemic species, the BNP has 29 species, which represents 10.06% of the total flora identified (Table 13).

Table 13. Biogeographic distribution of the species inventoried in the BNP.

Biogeographic Types	Percentage
Mediterranean	51%
Cosmopolitan and wide distribution	17.68%
Nordic	20.82%
Endemic	10.06%
Saharan	0.34%

Our findings, in conjunction with those of other studies conducted in Algeria, suggest that the Mediterranean distribution represents the predominant biogeographic spectrum. This is evidenced by the studies conducted by Sarri et al. (2014) in El-Kala, the study of Bounar et al. (2013) in Taza National Park, and the 2018 study of green spaces in Boussaâda. The high proportion of Mediterranean species in the Mediterranean climate can be attributed to their natural inclination towards the Mediterranean climate. Therefore, the Mediterranean strain tends to prevail, as observed by Quezel (2002) and Gharzouli (2007).

The importance of cosmopolitan species and wide distribution in the BNP can be attributed to the sensitivity of the park to anthropogenic pressures, in particular to agricultural activity.

The research of Vêla and Benhouhou from 2007 indicates that the BNP is a habitat to 27.7% of the endemic species discovered in the biogeographic sectors of the Tell Constantinois (C1), the Constantinois Highlands (H2), and the Constantinois Saharan Atlas (AS3). The same authors have indicated that the BNP has 53 endemic species, which represents almost 13% of the endemic flora of northern Algeria, with 407 species. The BNP's endemism is comparable to that of Gouraya National Park, which has 27 species (Rebbas 2014), and Ouled Yagoub in Chélia, which has 28 species (Beghami, 2013). The significance of Algerian-Moroccan endemics, in conjunction with North African endemics, can be elucidated by the Aurès region's resemblance to the Moroccan Atlas territory in terms of the dominant plant formation. Indeed, the Aures region's and Moroccan Atlas are distinguished by sparse low vegetation and forest formations dominated by *cedrus atlantica* (Endl.) Manetti ex Carrière and *Pinus halpensis* Mill., both of which are absent among the Tunisian territory. In addition, it should be mentioned that the Saharan distribution is represented by only one species.

5. Biodiversity Measurement

5.1. The diversity indices

5.1.1. The specific richness (S)

The total specific richness analysis indicates that sector 2 (Oued chaaba) is the most diversified across all sectors. The formation with the greatest species diversity is the Atlas Cedar Formation (F1), while the North orientation (O2) exhibits the greatest species richness. The most diverse zone is the buffer zone (Z3), while the most diverse bioclimatic stage is the Sub-humid (Bioclimat 2) (Table14).

Table 14.The total specific richness of the species found in BNP.

	Bioclimate	Sectors	Zoning	Orientation	F.formation
Total specific richness	B1- B2	S1-S2-S3	Z1-Z2-Z3	O1-O2-O3-O4-O5	F1-F2-F3-F4-F5
	97-191 Species	90-101 Species	58-118 Species	29-138 Species	23-167 Species

The sub-humid bioclimate (B2) is distinguished by its great specific richness, followed by the semi-arid bioclimate (B1). The three sectors, Oued Chaaba, Fisdis, and Oued el Maa, seem to have almost a notable differences in the vegetative composition of the sectors.

The buffer zone (Z3) is the richest in species, followed by the peripheral zone and the primitive zone.

It should be noted that the northern orientation (O2) has the most species, followed by the southeast orientation.

The first Formation (formation of *Cedrus atlantica* (Endl.) Carrière) has a relatively high number of species compared to other formations. Moreover, the fifth formation (*Pinus halpensis* Mill.) is the second richest, followed by the other formations.

The stations belonging to the sub-humid bioclimate and to the buffer zone, oriented towards the north and characterized by cedar formations in the sectors of Oued chaaba and Oued el Ma, appear to be the most diversified. This observation can be attributed to the more favorable ecological conditions at these stations.

5.1.2. Variation of the similarity according to the flora recorded in Belezma National Park

The presence or absence of species at different sectors, bioclimate, orientation, forest formations, zoning and stations, and, as well as sectors, were considered in order to compare the detected

species based on the aforementioned factors. Each factor was then compared two by two (Table 15) using the Sorensen similarity index.

Table 15. The total values of the Sorensen similarity index for the species inventoried in the Belezma National Park according to Bioclimate taken two by two.

	Bioclimate	Sectors	Zoning	Orientation	F. Formation	Stations
Similarity indice	B1-B2	S1-S2-S3	Z1-Z2-Z3	O1-O2-O3-O4-O5	F1-F2-F3-F4-F5	S1-----S18
	40%	27%-40%	27-32%	5%-26%	11%-38%	0%-43%

The floristic composition of the three sectors, two bioclimatic floors, five orientations, five forest formations, three zones, and 18 stations was compared two by two using the Sorensen similarity index. The results indicated a low degree of similarity, with values ranging from 36% to 40% for the sectors, 40% for the two bioclimatic floors, 5% to 26% for the orientations, 11% to 38% for the forest formations, 27% to 32% for the three zones, and for the stations, the similarity was not particularly high, with values ranging from 0% to 43%.

The observed differences in floristic composition between the various sectors, orientations, forest formations, and stations may be attributed to the variable ecological conditions that these areas offer to the flora. These differences appear to be attributable to the distinctive compositions of the vegetation cover, as well as the type of soil and its physico-chemical characteristics, which exhibit notable differences between the two slopes and the different altitudes (Abedssemmed, 1981) at which they grow.

According to our results, the floristic composition of Belezma National Park is not uniform between sectors and forest formations; this is due to a series of factors, including variations in the bioclimatic type, altitude, slope, soil type, and the floristic succession of dominant species between two slopes. Furthermore, the dissimilarity is further increased by the difference in conditions between the two slopes of the park.

5.2. Bivariate Statistical Analysis

5.2.1. Statistical analysis of similarities (ANOSIM)

The study of the similarity by ANOSIM indicates a significant difference in floristic composition between the sectors, the two bioclimatic types. However, no significant differences were observed in the forest formation, the zones or orientations (Table 21).

Table 16. Result of Similarity Analysis (ANOSIM) of the botanical family composition between sectors, bioclimatic floors, forest formations, orientations and zoning.

	Mean rank within	Mean rank between	R	P (same):
Sectors	59,93	84,11	0,316	0,0001
Bioclimate	69.66	85.01	0.21	0.046
Orientations	72.77	67.06	-0.18	0.68
Forest formation	66.64	80.92	0.18	0.13
Zoning	74.72	78.07	0.04	0.315

The ANOSIM analysis revealed a significant difference in families composition between the sectors and between the two bioclimatic stages ($R = 0.31$; $R = 0.21$ respectively). However, the difference in the families composition of the formation turned out to be not significant ($R = 0.18$). The values of $R = 0.18$ and indicate that the families composition of the forest formations does not differ significantly. There is a certain degree of similarity. However, no significant differences in the families composition were found according to the zones and orientations ($R = 0.04$ and $R = -0.08$, respectively), indicating a noticeable similarity in the floristic composition of these zones and these orientations.

Variations in floral compositions, within a plant association, can have three different origins: the climate, the soil, human and animal action, factors acting in isolation or simultaneously" (Le Houérou, 1959)

5.2.2. Bivariate Analysis of Variance by Permutation "Permutational Multivariate Analysis of Variance" (PERMANOVA)

The PERMANOVA analysis indicates that there are significant differences depending on various variables, including sectors, forest formation and the bioclimatic types, and of the zoning and orientations, the difference is not significant (Table 22).

Table 17. PERMANOVA results for the different on botanical family composition between sectors, bioclimatic type, forest formations, orientations and zoning of Belezma National Park.

	Total sum of squares	Within-group sum of squares	F	p (same):
Sectors	2,051	1.562	2.51	0.0009
Bioclimat	2,051	1.77	2.51	0.0009
Forest formations	2.051	1.37	1.6	0.005
Orientation	1.88	1.3	1.0 6	0.36
Zoning	2,051	1,727	1.407	0.06

The results of Bivariate Analysis of Variance by Permutation (PERMANOVA), indicating a very highly significant difference in the families composition depending on the sectors (P = 0.0001). It is also show a significant difference on families composition depending on two bioclimatic types (P=0.009), While the difference in forest formation has a low statistical significance (P = 0.005) compared to the sector and the bioclimate.

No significant difference was indicated in families composition by PERMANOVA for zoning (P = 0.06) or in the orientation (P = 0.36).

Belezma National Park is a protected area dominated by the forest surface, contains many biological units, which are classified into ecosystem types for better habitat management. The majority of these units are composed of various forest forms (Boukerker, 2016). The presence of *Cedrus atlantica* (Endl.) Manetti ex Carrière (Pinaceae) on the northern slope of the park (includes the sectors of Oued Chaaba and Oued el Maa), which is adapted to different environmental conditions than that of *Pinus hapensis* Mill. (Pinaceae), which is found on the southern slope (fisdis sector). In addition, the presence of matorrals, including *Quercus ilex* (Fagaceae), *Juniperus*

oxycedrus (Cupressaceae), *Juniperus phoenicea* (Cupressaceae), and *Olea europaea* (Oleaceae), and the distribution of flora in the three sectors, as well as the change in bioclimate and the quality of the soil between the northern and southern slopes of the park, demonstrate that Belezma National Park presents a remarkable floristic diversity that varies not only between forest formations but also between sectors. The results of this study indicate that orientation and zoning do not affect the distribution of species.

5.3. Multivariate Statistical Analysis

5.3.1. Canonical correspondence analysis (CCA) of the botanical family distribution in Belezma National Park

The map of the canonical analysis of the correspondences (Figure 31) clearly associates the distribution of the 18 stations of the flora with the variables of the environment and the physiognomy of the vegetation. The plane formed by the first and second axes totals an inertia ratio of 44.26% (F1 = 25.64%; F2 = 18.62%).

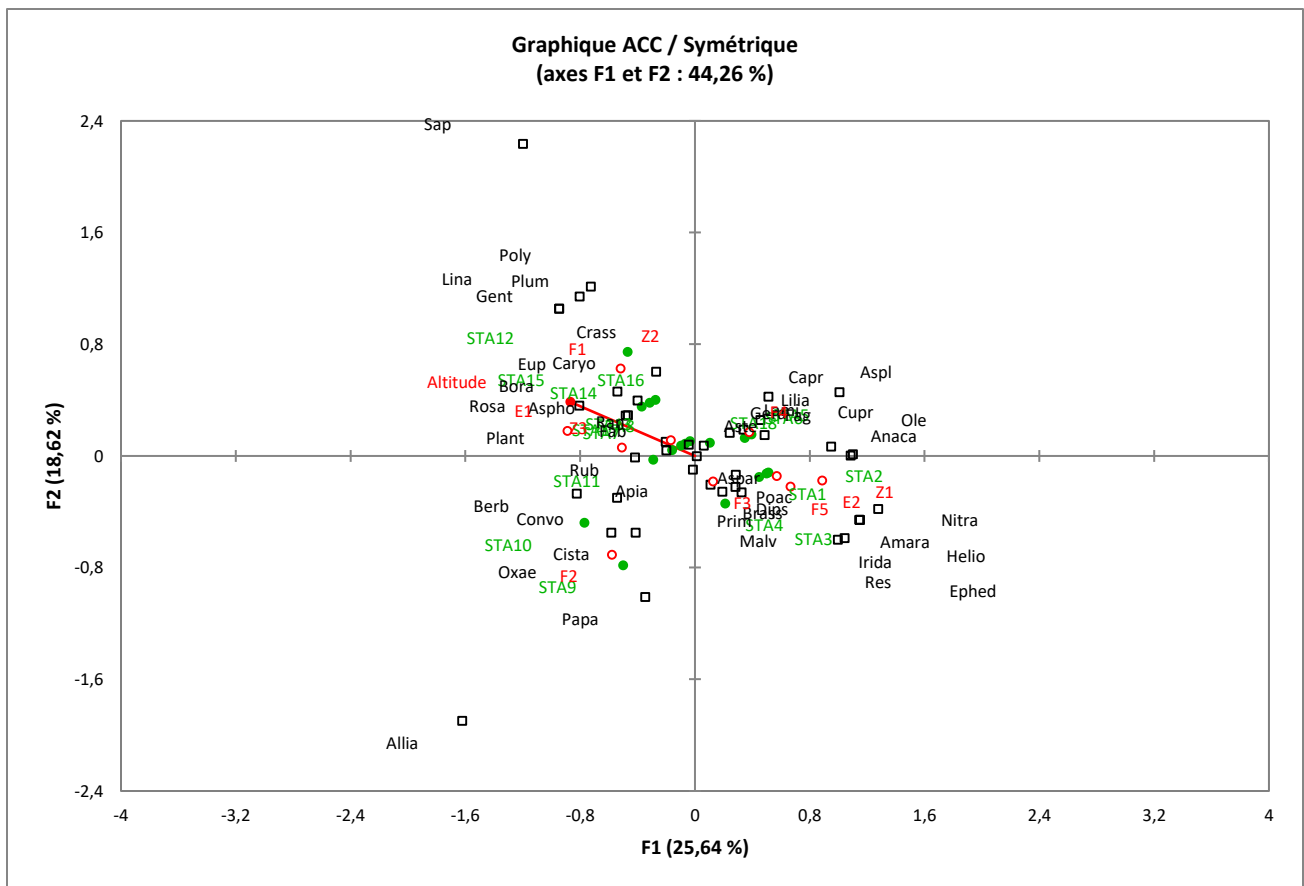


Figure 31. ACC map of the distribution of botanical families in the studied stations.

The canonical analysis of the correspondences makes it possible to highlight the relationships between the different families groups and ecological factors. According to the results of the canonical correspondence analysis, our stations can be classified into three groups.

The first group encompasses 4 stations, which are part of the cedar forest formation (F1) and the subhumid bioclimate (B1), in addition to the two peripheral and buffer zones (Z1, Z3). Altitude is a crucial factor in this group, exerting a notable influence.

The second group is not significantly affected by altitude and comprises four stations. The aforementioned stations exhibit characteristics that are the inverse of those observed in the first group. They classified as pine forest formations (F5), semi-arid bioclimate (B2), and peripheral zones (Z1).

The third group is distinguished by the presence of 10 stations at varying altitudes, distributed across different sectors, and supporting diverse bioclimatic types. These results indicate that altitude does not influence the distribution of species within these stations.

The Fisdis sector is located on the southern slope of the park and characterized by a semi-arid bioclimate with average annual rainfall. The flowering season depends on rainfall. It is distinguished by the presence of pinedes as the dominant forest formation (F5). This species requires specific environmental conditions.

Pinus halpensis Mill. (Pinaceae) known for its robust temperament and impressive power of expansion. It has demonstrated a high level of resistance to drought and disturbances and can found in various environments (Boudy, 1952). This species is most often found in semi-arid regions (Nahal, 1962). The growth of *P. halpensis* Mill. can be negatively affected by excessive humidity, especially when rainfall levels are below 700–800 mm. It is important to note that *Pinus halpensis* Mill. has different growth requirements than cedar (Boudy, 1952). In terms of soil, *P. halpensis* Mill. tends to grow best on loose or friable substrates such as marl, limestone, schists, or micaschists (Loisel, 1976). It is important to note that the presence of permanent aquifers can potentially asphyxiate the root system of the *P. halpensis* Mill. (Quezel, 1986).

The forest formation of pines is distinguished by the presence of numerous botanical families, among which the zygophyllaceae family stands out. This family is prevalent across all continents, with a particularly strong presence in arid, semi-arid, and saline regions in the warmer parts of the world (Quezel and Santa, 1963).

Oued Chaaba and Oued Lmaa sectors situated on the northern slope of the park, which is characterized by a subhumid bioclimate with an average annual precipitation that is higher than that of the Fisdis sector. The flowering season in this area extends from mid-April to the end of June. The slope is dominated by the Atlas cedar species.

Cedrus atlantica (Endl.) Manetti ex Carrière (Pinaceae) is subject to altitude-related influences due to the interplay of various climatic conditions, including precipitation and temperature. It is commonly observed that higher altitudes tend to receive more rainfall and experience lower temperatures. *Cedrus atlantica* (Endl.) Manetti ex Carrière is commonly found in cold, humid, or sub-humid environments (White, 1986). It is also a distinctive conifer found in mountainous regions, usually at altitudes ranging from 1,200 to 2,800 m (De-Vilmorin, 2003). Cedar growth is influenced by altitude due to various climatic conditions, including precipitation and temperature. It is sensitive to severe frosts and does not tolerate temperatures below -25°C . However, the Atlas cedar is able to tolerate severe drought and irregular water conditions, and can adapt to habitats with an average water balance, see deficit (Rameau, 1989). Cedar is known to grow on a variety of substrates and soils, including limestone, basalt, shale, marl, granite and sandstone (White, 1986). The plant species that coexist with cedar depend on ecological factors such as rainfall, altitude and soil conditions. For example, Azzahiri et al. (1994) have identified a specific plant grouping in the semi-arid zone of the Moroccan Middle Atlas on a basaltic substrate at an altitude of 1900–2000 m and in western exposure: *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Buplerum montanum*, *Acer monsepsulanum*, and *Rosa canina*. In compact calcareous soil and at an altitude of 1800–1900 m, the plant group becomes *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Quercus rotundifolia*, and *Paeonia coralina*.

The other matorrals formations, which not affected by altitude, are distributed across the various sectors of the park.

Second Part: the phytochemistry and biological activities of *Thymus algeriensis* Boiss. & Reut. and *Santolina rosmarinifolia* L.

As part of the enhancement of bioresources in Belezma National Park in Batna, a study was conducted on two plants, *T. algeriensis* Boiss & Reut. and *S. rosmarinifolia* L., commonly used in traditional medicine by the local population.

1. Plant material**1.1. Chemical characterization of the essential oils of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L.****1.1.1. Yield of essential oils (R)**

Essential oils extracted from the aerial parts of *T. algeriensis* Boiss & Reut. and *S. rosmarinifolia* L. by hydrodistillation gave the following yields: 0.43 g (0.43%) for *T. algeriensis* Boiss & Reut and 0.49 g (0.49%) for *S. rosmarinifolia* L. (Table 23).

Table 18. Quantity of essential oil of *T. algeriensis* Boiss. & Reut. And *S. rosmarinifolia* L. extracted by hydro distillation.

	Mass of the plant material used in g	Volume of distilled water used in ml	Mass of the extracted essential oils in g	Essential oil yield %
<i>T. algeriensis</i> Boiss. & Reut.	100 g	1000 ml	0.43 g	0.43%
<i>S. rosmarinifolia</i> L.	100g	1000ml	0.49	0.49%

The essential oils extracted from the aerial parts of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L. are distinguished by a strong aromatic odor and a yellow or pale yellow coloration, and the yields produced by these species can be considered relatively low.

A number of previous studies have calculated the average yield of *T. algeriensis* Boiss. & Reut. These include those by Dob et al. (2006) in the region of Media (a city in north-central Algeria), Zayyad et al. (2014) in the region of Aknoul (Taza region), and Toumi (2010) in the region of Ouest Guelma. The average yields of essential oils varied between 1.13% and 2.96%. It is

interesting to note that these results are superior to the yield of *T. algeriensis* Boiss. & Reut, which we harvested in Belezma National Park. The essential oil content turned out to be 0.43%. However, it should be noted that lower yields have been reported by Hazit et al. (2009) (0.04%), Kebbi et al. (2020) (0.05%), and Amarti et al. (2010) in Morocco (0.07%).

The yield of essential oil of *S. rosmarinifolia* L. from our study region is higher than that found in the M'sila region (0.15%) by Sarri et al. (2021) and Fodil (2021). This yield is lower than that found in the Aurès region (1.4%) by Chibani S. et al. (2013) and in Portugal (1.3%) by Jorge M. Alves Silva et al. (2023).

According to the literature, a variety of factors can influence the yield of essential oils (EO). These include geographical location, harvest period (Sid Larbi et al., 2016), extraction method and conditions (Khajeh et al., 2005), and plant material age (El Mokhtari et al., 2020).

1.1.2. Chemical composition of essential oils of *T. algeriensis* Boiss. & Reut.

The essential oils of the aerial parts of *T. algeriensis* Boiss. & Reut consist of 89 identified chemical elements, which represent 100% of the essential oil.

The main components of the essential oil from *T. algeriensis* Boiss. & Reut were identified as (+)-2-bornanone (12.54%), eucalyptol (8.65%), (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (8.48%), .alpha.-Terpinylacetate (6.58%), and linalool (6.23%) (Figure 32).

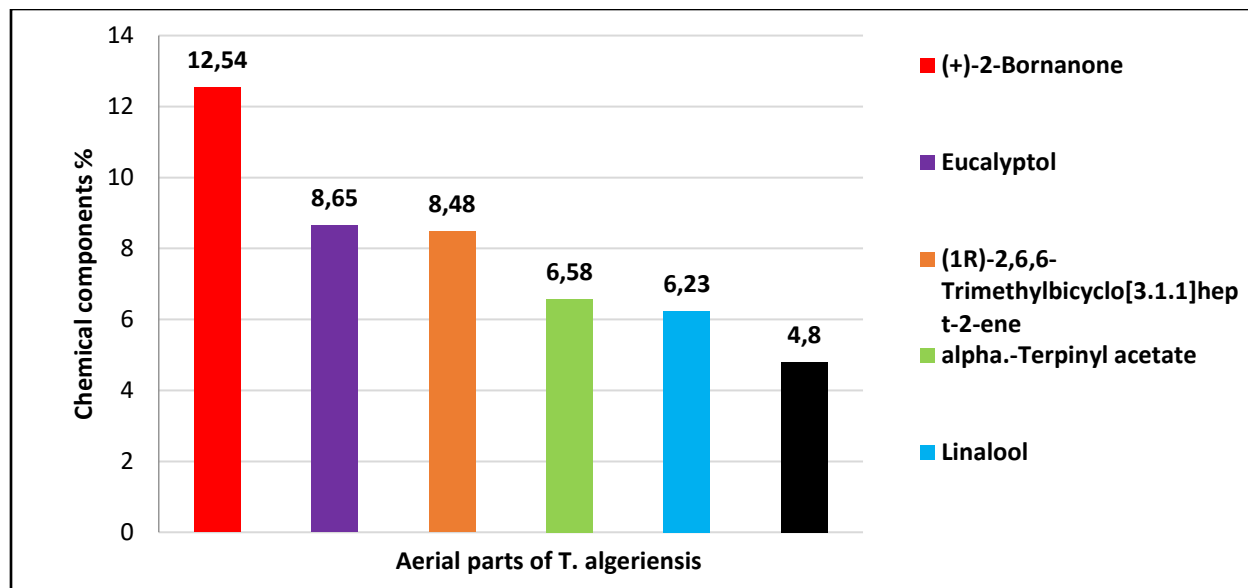


Figure 32. Contents in % of the major chemical components of the essential oils of aerial parts of *T. algeriensis* Boiss. & Reut.

The major chemical classes of the essential oil of *T. algeriensis* Boiss. & Reut. are predominantly oxygenated monoterpenes (50.94%) and monoterpene hydrocarbons (30.32%). Furthermore, the oxygenated sesquiterpenes account for approximately 11.12% of the oil, while the non-terpene derivatives represent 5.38%. The sesquiterpene hydrocarbons are present in smaller quantities, representing approximately 2.24% of the oil (Figure 33).

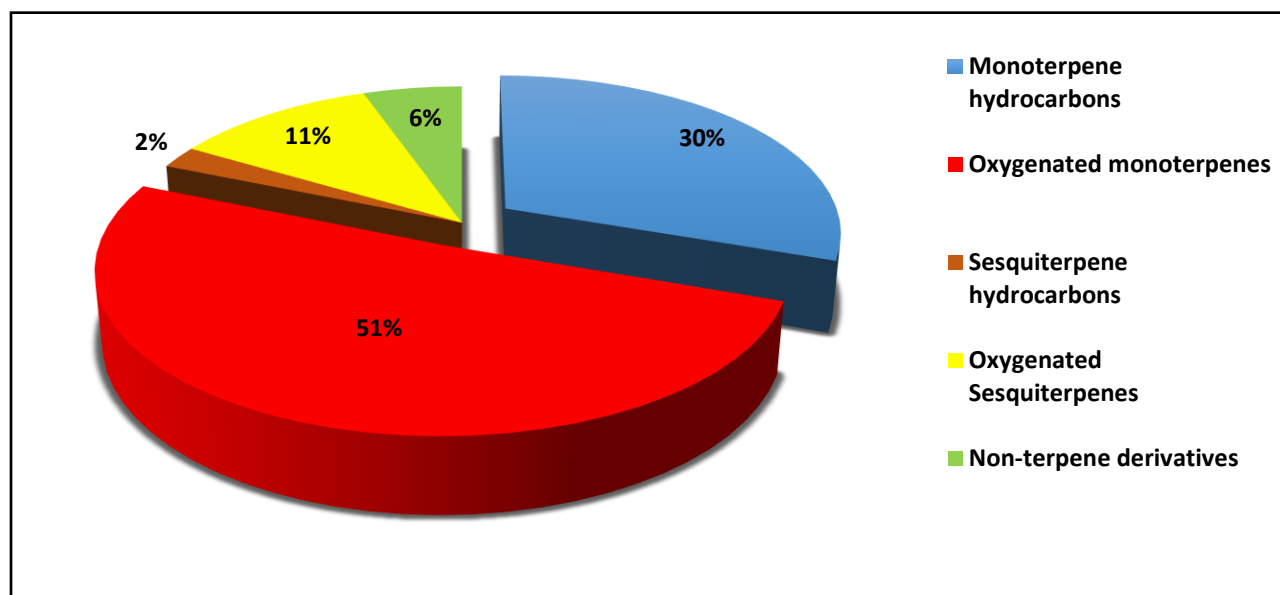


Figure 33. The chemical classes of the essential oil of *T. algeriensis* Boiss. & Reut.

According to previously reported data, it seems that the chemical profiles of the essential oils of *T. algeriensis* Boiss. & Reut. may vary depending on the region. It should be noted that there are differences in chemical composition between the samples. The main components of our sample are (+)-2-bornanone, .alpha.-Terpinylacetate et linalool, which belong to the chemical class of oxygenated monoterpenes. Eucalyptol (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene is part of the chemical class of monoterpene hydrocarbons. . According to various studies, the main components of the oil of *T. algeriensis* Boiss. & Reut of the area of the Souk El Haras and Media are linalool (47.3%) and alpha-pinene (25.5% to 27.1%) (Dob et al., 2006; Giordian et al., 2008). In the Aurès region, the sample was found to contain a high concentration of germacrene D (29.6%) and β -caryophyllene (11.0%) (kebbi et al., 2020). Conversely, the samples of *T. algeriensis* Boiss. & Reut. oil purchased from various herbalists in Blida were found to be rich in linalool (78.8%) and thymol (62.7%) (Houmani et al., 2002). Thymol has proven to be the dominant component of the oils of the Alouane Hammam in Blida, Algeria (Chemat et al., 2012) and in a sample from Libya (Giweli et al., 2013). However, Benjilali et al. (1987) reported that the predominant compounds were thymol and carvacrol, while Ait-Ouazzou et al. (2011) discovered that borneol was the main compound present in the Moroccan samples. However, according to Amarti et al. (2010) and Zouari et al. (2011), camphor (27.7%) and 1,8-cineole (7.55 to 22.07%) have been reported as the main components of *T. algeriensis* Boiss. & Reut. obtained, respectively, from Morocco and Tunisia.

1.1.3. Chemical composition of essential oils of *S. rosmarinifolia* L.

The chemical composition of the essential oil of *S. rosmarinifolia* L. The oil is composed of 96 volatile components, which constitute 100% of the total composition.

The main components of the essential oil from *S. rosmarinifolia* L. were identified as Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene (18.39%), Eucalyptol (15.78%), 2(10)-Pinene (10.67%), (-)-Germacrene D (5.88%) (Figure 34).

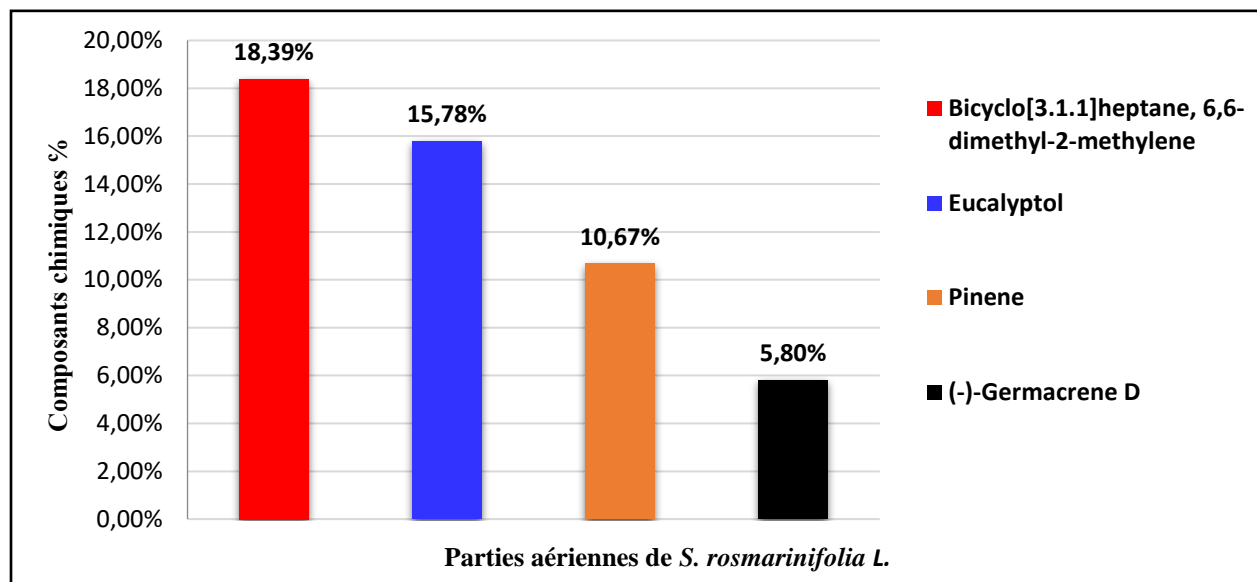


Figure 34. Contents in % of the major chemical components of the essential oils of *S. rosmarinifolia* L.

The constituents of the sample were classified into five groups, as follows: oxygenated monoterpenes (38.19%), hydrocarbon monoterpenes (34.33%), oxygenated sesquiterpenes (12.72%), and hydrocarbon sesquiterpenes (11.74%), and non-terpene derivatives (3.02%) (Figure 35).

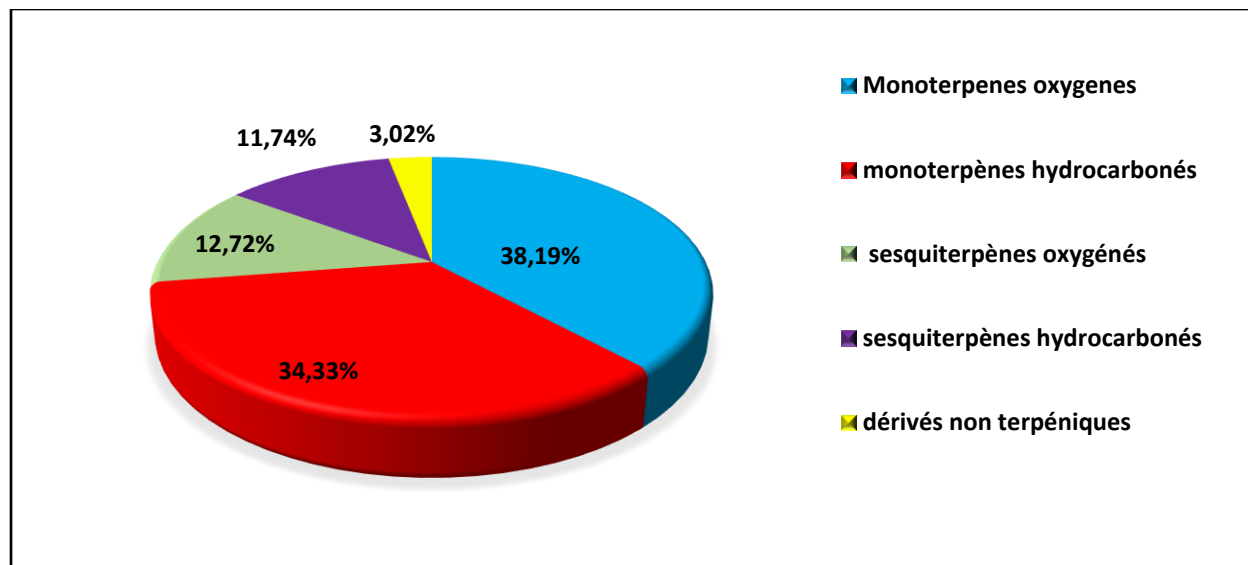


Figure 35. The chemical classes of the essential oil of *S. rosmarinifolia* L.

The species of the genus *Santolina*, in particular *S. rosmarinifolia* L., clearly present remarkable variability in the chemical composition of their essential oils. This variability is very clear by comparing the main compounds of *S. rosmarinifolia* L. collected in a forest biotope (Belezma National Park), which is the subject of this study, to those collected in another forest biotope (the Aurès) and a steppe biotope (Hodna region). These last samples revealed that capillene was a major component, representing 35.2% in the Aurès region (Chibani et al., 2013) and 32.79% in the Honda region (Fodil, 2021; Sarri et al., 2021). However, it should be noted that the capillene was absent from our sample. The phytochemical analysis of *S. rosmarinifolia* L. collected in Portugal (M. Alves Silva et al., 2023) showed differences in the main compounds compared to our sample, whose major component is β -pinene (29.6%). Other previous reports on various species of *Santolina* indicated the presence of certain components, including 1,8-cineole (Villar et al., 1986; Giner et al., 1993; Cherchi et al., 2001; Flamini & Cioni, 2007; Tirillini et al., 2007; Grosso et al., 2009), β -myrcene (Poli et al., 1997; Zaiter et al., 2015), and capillene (Malti et al., 2019).

Therefore, certain climatic factors in regions with a semi-arid climate had an effect on the quality and composition of the essential oil. (Mehalaine & Chenchouni, 2018).

1.2. Determination of total phenols and flavonoids

The total phenolic and total flavonoid contents of the extracts were determined as pyrocatechol (PEs) and quercetin (QEs) equivalents, respectively.

Table 19. Total phenol and flavonoid contents of the aerial parts of the methanolic extracts of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L.

Extracts		Phenolic content ($\mu\text{g AGE/mg of extract}$) ^b	Flavonoid content ($\mu\text{g EQ/mg of extract}$) ^c
Methanol extracts of <i>T. algeriensis</i> Boiss. & Reut.	Aerial parts	256.18 \pm 0.33	74.12 \pm 0.34
Methanol extracts of <i>S. rosmarinifolia</i> L.	Aerial parts	278.50 \pm 0.66	106.54 \pm 0.33

The analysis of the results found that the methanol extract of the aerial part of *T. algeriensis* Boiss. & Reut. Contained 256.18 \pm \pm 0.33 μg of AGE/mg of phenol and 74.12 \pm 0.34 μg of QE/mg of flavonoid. These values are comparable to those reported in the literature for other samples of *T. algeriensis* Boiss. & Reut. in other regions. For example, the work of Jaouadi et al. (2019) in Tunisia shows that the phenolic content of the methanol extract was 9.1 \pm 0.1 mg GAE/g leaves dry weight (DW) of phenol and 2.8 \pm 0.1 mg ER/g leaves DW for the flavonoid extract. Additionally, the work of Rezzoug et al. (2019) in Algeria (Saharan Atlas) revealed that the phenolic content of the MeOH extract was 125 \pm 1 mg/g extract, while that of the flavonoid content was 118 \pm 1 mg/g of extract.

The methanol extract from the aerial part of *S. rosmarinifolia* L. contained 278.50 \pm 0.66 μg AGE/mg of phenol content and 106.54 \pm 0.3334 μg QE/ mg of flavonoid extract. Schmidt et al. (2024) found that the phenolic content of the MeOH extract was 70.74 \pm 0.02 $\mu\text{g/ml}$.

The contents and composition of total phenols and flavonoids differ according to genotype, geographical, and ecological factors (Liu et al., 2018).

2. Biological activities

2.1. Evaluation of the antibacterial activity

Table 27 presents the results of the antibacterial activity of the essential oils and the methanolic extract of *T. algeriensis* and *S. rosmarinifolia* L. It is observed that the essential oil and the methanolic extract of *T. algeriensis* and *S. rosmarinifolia* exerted moderate antibacterial activity at dilution 8mg/ml and strong antibacterial activity at dilutions of 16mg/ml. This dilution was sufficient to inhibit the growth of *E. coli* and *S. aureus*. In contrast, *P. aeruginosa* exhibited reduced sensitivity to all inhibition dilutions.

Table 20. Antibacterial activity of the aerial part of *T. algeriensis* Boiss. & Reut and *S. rosmarinifolia* L.

Bacteria	Dilution (mg /mL)	Extracts	Diameter of the inhibition zone (mm)	
			<i>T.algeriensis</i> Boiss. & Reut	<i>S. rosmarinifolia</i> L.
<i>Escherichia coli</i> ATCC 8739	2	EO	7.5 ± 0	9 ± 0
		ME	8.5 ± 0	10 ± 0
	4	EO	11.5 ± 0	10.5 ± 0
		ME	9 ± 0	12 ± 0
	8	EO	13 ± 1	12 ± 1
		ME	16 ± 1	18 ± 1
	16	EO	16.5 ± 0.33	15 ± 0.66
		ME	18 ± 1	21 ± 1
<i>Pseudomonas aeruginosa</i> ATCC 27853	2	EO
		ME	..	7
	4	EO
		ME	7 ± 1	8.5 ± 1
	8	EO
		ME	7.5 ± 1	11.5 ± 1

	16	EO
		ME	7.5 ± 1	13 ± 1
<i>Staphylococcus aureus</i> ATCC 25923	2	EO	8 ± 0	7 ± 0
		ME	6.5 0	..
	4	EO	10 ± 0	8 ± 0
		ME	9.5 ± 0	7.5 ± 0
	8	EO	14± 1	11 ± 1
		ME	12 ± 0	9.5 ± 0
	16	EO	16.5 ± 1	14.5 ± 1.15
		ME	15.5 ± 1.15	13 ± 1

EO: essential oil / ME: methanol extract

The results indicate that the essential oils and the methanolic extract of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L. from the PNB show antibacterial activity against the selected microorganisms. However, it is important to note that the sensitivity of the strains tested varies according to the dilutions.

At dilutions of 8 mg/ml, essential oils extracted from *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L. represent significant antibacterial activity against the gram-negative bacterium *E. coli* and the gram-positive bacterium *S. aureus*. In addition, these oils showed strong antibacterial activity against the same bacterial strains at a dilution of 2 mg/ml, with inhibition zones of 15 ± 0.66 mg/ml and 16.5 ± 0.33 mg/ml, respectively.

However, the essential oils of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L. did not affect *P. aeruginosa* at any of the four dilutions examined (2, 4, 8, or 16 mg/ml). The bacterial strain has demonstrated resistance to *T. algeriensis* and *S. rosmarinifolia* L.

At a dilution 8 mg/ml of Methanol extracts of *T. algeriensis* Boiss. & Reut. and *S. rosmarinifolia* L., the bacterial strains of *E. coli* and *S. aureus* exhibits significant sensitivity. However, the methanolic extract demonstrated high activity at a dilution of 16 mg/ml against both *E. coli* and *S. aureus* bacteria, with inhibition zones of 18 ± 1 and 21 ± 1 mg/ml, respectively. Nevertheless, the methanolic extract demonstrated relatively low activity against *P. aeruginosa* compared to its activity against other bacteria.

Amati et al. (2010) conducted a study in Morocco that demonstrated the weak inhibitory effect of *Thymus algeriensis* essential oil on *Escherichia coli* and *Staphylococcus aureus*. In contrast, the study by Zayad et al. (2014) in the same region showed that *Thymus algeriensis* has a high activity against *E. coli* (1/2000 v/v) and a sensitivity against *S. aureus*. Furthermore, Messaoudi et al. (2019) demonstrated that the methanolic extract of *T. algeriensis* exhibited potential antibacterial activity against *E. coli* and *P. aeruginosa*, while the thymus methanol extract demonstrated no effect on *P. aureus*.

Chibani et al. (2013) show that the essential oil of *S. rosmarinifolia* L. has good antibacterial activity against pathogens almost of food origin, in particular *Escherichia coli*.

2.2. Evaluation of the antioxidant activity

2.2.1. Free radical scavenging test DPPH (2,2-diphenyl-1-picrylhydrazyl)

The test is based on the principle that the extracts will reduce the violet-colored free radical DPPH to a yellow color, which can be measured by spectrophotometry.

The results presented in Table 28 indicate that the essential oils and methanolic extracts of *T. algeriensis* and *S. rosmarinifolia* exerted significant antioxidant activity.

The IC₅₀ values (µg/ml) of the EO and the ME of the plants studied and of the BHT standard in the DPPH test are in (µl/ml)

Table 21. Results of the antioxidant activity of the essential oils of *T. algeriensis* Boiss. & Reut, *S. rosmarinifolia* L.

Species	Extract	IC ₅₀ (µg /mL)
		DPPH
<i>T. algeriensis</i> Boiss. & Reut	OE	223.38 ± 0.18
	ME	83.18 ± 0.61
	BHT	68.07 ± 0.23
<i>S. rosmarinifolia</i> L.	OE	198.66 ± 0.33
	ME	72.33 ± 0.15
	BHT	68.07 ± 0.23

analysis of the results represents that the IC₅₀ values for the extracts were as follows: for the essential oils, 83.18 µg/mL (*T. algeriensis* Boiss. & Reut) and 72.33 ± 0.15 µg/mL (*S. rosmarinifolia*); for the methanolic extract, 223.38 µg/mL (*T. algeriensis* Boiss. & Reut) and 198.66 ± 0.33 µg/mL. These values are significant. BHT exhibited the highest radical-scavenging activity.

Previous literature has suggested that the trapping of DPPH radicals by Algerian essential oils of *Thymus* species is due to the presence of phenolic constituents, in particular thymol or carvacrol, which are major components (Hazzit et al. 2009; Sokmen et al. 2004). However, our results demonstrate that the essential oil of *T. algeriensis* exhibited DPPH radical scavenging activity despite the absence of thymol. Therefore, our results are consistent with those of another report, which demonstrated that the essential oil of *T. algeriensis* contains a high concentration of oxygenated monoterpenes, which have the potential to function as radical scavengers (Zouari et al. 2011, Salhi et al. 2016).

The studies of Bendjabeur et al. (2018) in Algeria, Gadhoumi et al. (2020), and Ghorbel et al. (2021) in Tunisia are consistent with our current study because they found that the methanolic extract and the essential oils of *T. algeriensis* Boiss. & Reut showed lower activity than the reference standard BHT.

The study Schidmt et al. (2024), on the antioxidant activity of *S. rosmarinifolia* L. extracts by the in vitro ABTS test, where the antioxidants present in the samples act as reducing agents of ABTS•+, revealed that the flower extracts exerted a significantly lower antioxidant activity in the aqueous extracts as well as in the extracts containing 60% EtOH. The aqueous root extracts and 60% EtOH showed the lowest antioxidant capacity.

In comparison with *S. chamaecyparissus* L., chirane et al. (2020) found that the essential oil and methanolic extract of gerne *Santolina* subsp. *S.chamaecyparissus* L. had very low antioxidant capacities to reduce DPPH radicals compared to BHT, which remained less than 3.47±0.25 at a concentration of 1000 mg/L. However, the ethanol extract showed significant activity compared to BHT at a concentration of 400 mg/L (1.129±0.016 and 1.161±0.0).

The high levels of polyphenols and total flavonoids present in our methanolic extracts suggest that the high free radical scavenging activity of DPPH* may be attributed to the number of hydroxyl groups in the flavonoid nucleus or to the high levels of phenolic compounds, acids, and their

derivatives. These compounds have been shown to act as reducing agents, hydrogen donors, and singlet oxygen deactivators (Miguel, 2012).

Conclusion

The present study is being conducted in Belezma National Park to conduct a floristic inventory, define plant groups, and value some bioresources in the park.

Belezma National Park situated within the wilaya of Batna, home to a very diverse flora, including many aromatic and medicinal plants that contain therapeutic and pharmacological compounds.

The floristic inventory has identified a total of 288 taxa, the majority of them having a Mediterranean to cosmopolitan distribution. The most represented families at the specific and generic levels are the Asteraceae, Fabaceae, Apiaceae, Brassicaceae, Poaceae, and Lamiaceae. In addition, the floristic list includes a significant number of rare species. Among these endemic species, we have rediscovered the presence of *Tulipa sylvestris* subsp. *primulina* (Baker) Maire & Weiller, an endemic Algerian-Moroccan species.

Moreover, it should be noted that of the 288 species inventoried, hemicryptophytes are the most abundant biological type with 37.5%, followed by therophytes with 37.15%. Additionally, chamephytes, phanerophytes, and geophytes are also present. The disturbance index value is 49.30%.

The biogeographic spectrum analysis indicates that the Mediterranean distribution has the largest number of species, with 51%. Additionally, the endemic species are represented by 29 taxa, accounting for 10.06% of the total flora identified.

The use of specific richness indices, the similarity index, and the disturbance index enables an evaluation of the diversification and ecological balance of each sector, forest formation, orientation, zone, and bioclimatic stage within the study area.

The bi-variate analyses ANOSIM and PERMANOVA permit an evaluation of the dissimilarity rates within the study area, as well as a characterization of the vegetation through the study of the gradients that regulate the organization and distribution of plant species.

A canonical correspondence analysis (CCA) was conducted on the floristic inventories to ascertain the influence of altitude and the bioclimatic floor on the floristic composition at the 18 stations of Belezma National Park. This analysis identified three distinct groups of species: the first group; which is characterized by the presence of *Cedrus atlantica* (Endl.) Manetti ex Carrière, is associated with the support of high altitude and subhumid bioclimat; the second group, which is characterized by the presence of *Pinus halpensis* Mill., is associated with low altitude and semi-arid bioclimat; and the third group, which is not affected by either altitude or bioclimate.

The second part of this study involved a phytochemical analysis of *T. algeriensis* and *S. rosmarinifolia*. This entailed a chemical analysis to ascertain the components of their essential oils, and we studied their antibacterial and antioxidant properties.

The essential oils were extracted from *T. algeriensis* and *S. rosmarinifolia* L. by hydrodistillation, resulting in yields of approximately 0.43% and 0.49%, respectively.

The analysis of the leaves and flowers of *Thymus algeriensis* by gas chromatography and mass spectrometry (GCMS) identified 89 volatile compounds, representing 100% of the sample. The most abundant compound was (+)-2-bornanone (12.54%), followed by eucalyptol (8.65%) and (1R)-2,6,6-trimethyldicyclo[3.1.1]hept-2-en-4-ol (8.48%).

S. rosmarinifolia L., 96 volatile components have been identified, which represent 100%. Among the important components were [3.1.1]-heptane, 6,6-dimethyl-2-methylene, which represented 18.39% of the sample, eucalyptol (15.78%), 2(10)-pinene (10.67%), and (-)-Germacrene D (5.88%).

Previous research has also identified the majority of the chemicals found in our sample, but at various concentrations. Furthermore, certain molecules were lacking when compared to our findings. The qualitative and quantitative changes in chemical composition observed in essential oils can be attributable to one or more external or intrinsic variables.

The antibacterial activity of the essential oils of *T. algeriensis* and *S. rosmarinifolia* was evaluated by the diffusion method. All the species of bacteria tested showed a moderate and significant sensitivity to the essential oils of *T. algeriensis* and *S. rosmarinifolia*, with the exception of *Pseudomonas aeruginosa*, for which a low antibacterial activity was observed.

The antioxidant activity of the essential oils of *T. algeriensis* and *S. rosmarinifolia* evaluated with the DPPH test. The results indicated that both oils had low inhibition percentages compared to BHT, which suggests that BHT is the most effective. However, the antioxidant activity of the essential oils of the two plants studied was only low.

It would be beneficial to conduct further research on various aspects of biodiversity in Belezma National Park. The current disturbances affecting biodiversity could be analyzed in order to predict future ecosystem situations and develop preservation strategies in order to ensure the continuity of life and its evolution. It is crucial to consider all perspectives and collaborate with stakeholders to achieve optimal results.

The exploration of the potential benefits of two medicinal plants constitutes the first step in the search for biologically active substances of natural origin. To further expand the range of biological properties that essential oils can possess, it is recommended to extract the oils from various parts of plants and to study and identify in depth the responsible molecules and their corresponding biological activities. This approach will help to clarify their mechanism of action and will potentially serve as a substitute for synthetic substances used in therapy or in industry. In addition, it is advisable to evaluate other activities, such as antifungal, insecticidal, antitumor, and antiviral activity, through *in vitro* and *in vivo* studies.

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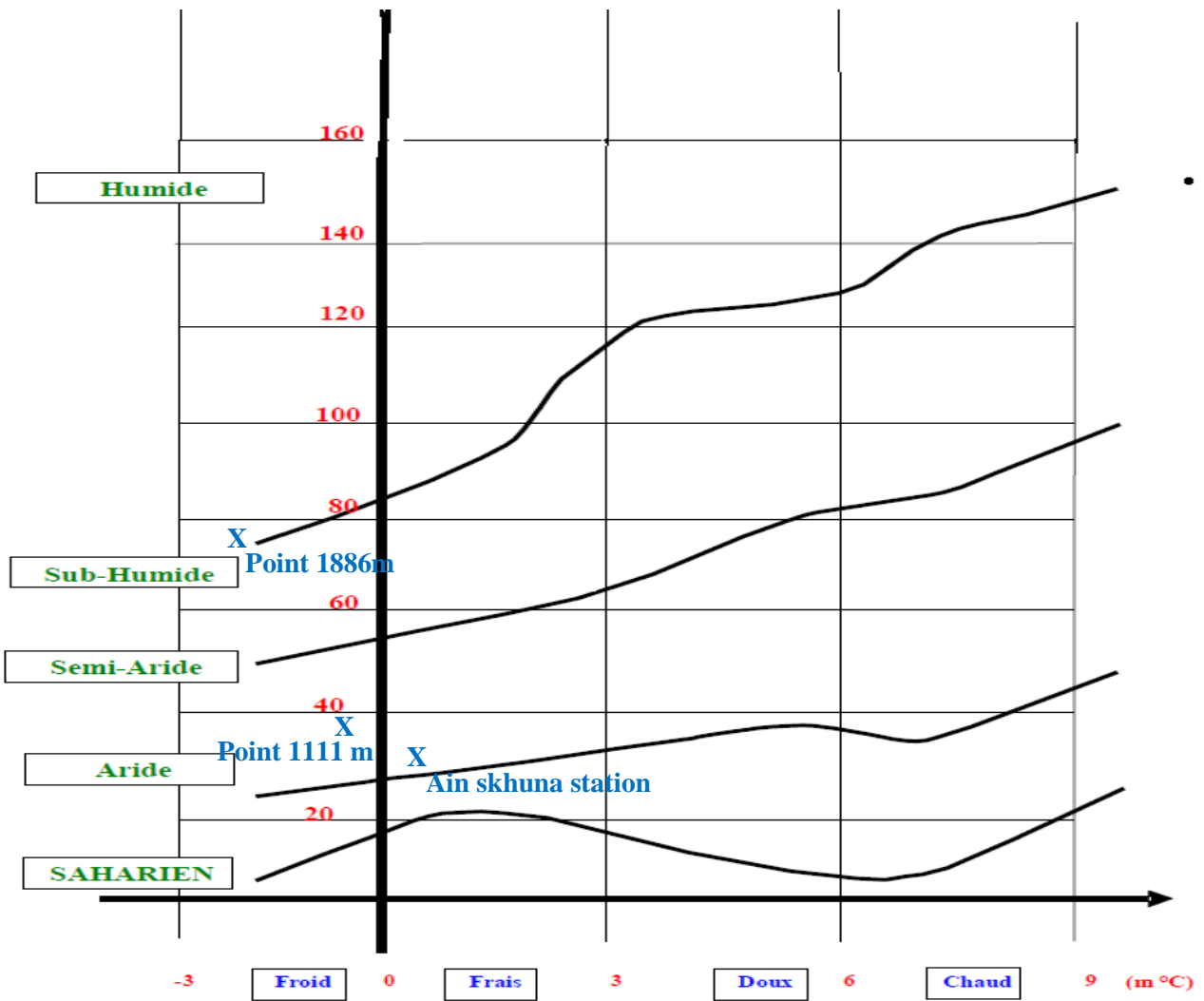
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Annexes

Annexe 1



Annexe 1. Climatgramme d'Emberger