الجمهورية الجزائرية الديمقراطية الشعبية

DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC

وزارة التعليم العالي والبحث العلمي

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

Serial N° :

جامعة الشهيد حمه لخضر الوادي

ECHAHID HAMMA LAKHDAR UNIVERSITY OF EL-OUED

كلية العلوم الطبيعية والحياة

FACULTY OF NATURAL SCIENCES AND LIFE

قسم البيولوجيا الخلوية والجزيئية

DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY

Thesis

SUBMITTED TO OBTAIN 3rd CYCLE LMD DOCTORATE DEGREE

In biological sciences

Specialty: Plant biology and physiology

Theme

Contribution to the biological characterization of bioactive compounds from *Juniperus communis* and *Artemisia herba-alba* with stimulating effect on *Bacillus safensis* probiotic

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2024/2025

Dedication

Thanks to Allah for the success in completing this research

To my dear parents

To my husband

To me ….

Acknowledgment

Thanks to Allah for granting me the ability to finish this research.

I would like to thank **Dr. LAICHE Amamar Touhami**, my supervisor, from the beginning for letting me work under his guidance while I completed my doctorate; for his continuous support, invaluable efforts, and scientific guidance and encouragement throughout my research; and for his insightful remarks and discussion, which helped to shape and finish this thesis.

All thanks to **Dr. TLILI Mohamed Laid**, my co-supervisor for his help during the realization of this work and for the interest it brought to our knowledge.

Our sincere thanks go to the jury members who agreed to evaluate our work, and for taking the time to review this work.

I would like to express my deep gratitude to **Dr. Maha Mezghani Khemakhem** and **Chahnez Naccache** from Laboratory of Biochemistry and Biotechnology at the University of Tunis El Manarr, Tunisia for their valuable collaboration in carrying out this work necessary for the successful completion of some of my experiments.

My great gratitude to "**BABELHADJ Baissa**" the professor at the Higher Normal School of Ouargla, the discoverer of camel madness, for your precious and valuable efforts and for your scientific guidance.

LAYADI Ikram

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Abbreviations

IND Indol test
JAE *Juniperus communis* aqueous extract
Jc *Juniperus communis*
JHE *Juniperus communis* hydroethanolic extract
K_3Fe $(CN)_6$ Potassium ferricyanate solution
LAB Lactic acid bacteria
LDC Lysine decarboxylase test
Log logarithm
Na_2CO_3 Sodium carbonate
NaCl Sodium chloride
OD Optical density
ODC Ornithine decarboxylase test
ONPG O- Nitrophenyl- β- D-Galactopyranoside test
PBS Phosphate buffered saline
PCR Polymerase Chain Reaction
PLT Blood platelets
Q Quercetin
RBC Red Blood Cells
ROS Reactive Oxygen Species
Rpm Revolutions per minute
rRNA Ribosomal ribonucleic acid
TCA Trichloroacetic acid
TDA Tryptophan-deaminase activity
TFC Total flavonoid content
TPC Total phenolic content
URA Urease test
UV Use Value
WBC white blood cell (globules Blancs)
WHO World Health Organizat
AAE *Artemisia herba-alba* aqueous extract
AHE *Artemisia herba-alba* hydroethanolic extract
$AlCl_3$
ARA L-arabinose resistance test
ATCC American Type Culture Collection
BSA Bovine serum albumin
BSH Bile Salt Hydrolase / Hydrolase des sels biliaires
CFU Colony forming units
CIT Citrate test
CRP C - reactive protein
DMSO Dimethyl sulfoxide
DNA Deoxyribonucleic acid
DPPH 1,1-Diphenyl-2-picrylhydrazyl
EC_{50} Effective concentration 50%
EDTA Ethylenediaminetetraacetic acid
ESR Erythrocyte sedimentation rate
FAO Food and Agriculture Organization
FCR Folin-Ciocalteu reagent
FRAP Ferric reducing antioxidant power assay
GA Gallic acide
GLU Glucose test
H_2S Hydrogen Sulfide test
HCT Hematocrit
HPLC High performance liquid chromatography
IC_{50} The half maximal inhibitory concentration
ICF Informant Consensus Factor

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CHAPTER II

Comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions

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GENERAL INTRODUCTION

The human microbiota is a diverse community of microorganisms that live in different parts of the body, with the skin, mouth cavity, and gastrointestinal tract having the largest numbers. The bacteria of the colon are particularly important. There have been suggestions that this human body portion may be seen of as a bioreactor where a very dense microbial consortia is carrying out a number of vital tasks for overall health (Swidsinski et al., 2016)

The microorganisms that live or pass through the digestive tract are known as the gut microbiota. Distinct ecological situations (gencives, estomac, mucus, colon, etc.) and niches have distinct compositions. It is made up of organisms from the three domains of life-Bacteria, Archaea, and Eukarya -but a thorough description of the ecosystem is hampered by significant individual variances (Hsieh et al., 2021). Among their roles, It prevent diseases, immunomodulate, have metabolisms that do not ferment, and operate on the digestive tract's trophic and motility (Marteau & Doré, 2017).

Lactic acid bacteria (LAB) are advantageous bacteria that are well-known for improving health. The capacity of LAB to create significant quantities of bioactive chemicals during fermentation is well established. The most significant bioactive substances produced by LAB activity during fermentation include peptides, exopolysaccharides (EPS), bacteriocins, certain enzymes such as lipase, protease, and amylase, and lactic acid (Abdul Hakim et al., 2023). Because of its numerous health advantages, LAB is a widely used component in therapeutic nutrition. Probiotics are among the most popular forms of LAB used in therapeutic nutrition(Cory et al., 2018).

Probiotics get their name from the words pro (for) and bios (life). "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" is how the FAO defines probiotics (FAO/WHO, 2002). Probiotics must get beyond the biological barriers of the host's gastrointestinal system in order to have the desired health benefits (Khaneghah et al., 2020). However, a few characteristics, like the ability to withstand acidic and bile environments, the ability to lower cholesterol, the lack of hemolytic activity, the ability to combat bacteria, and the capacity to endure fermentation, should be taken into account when choosing novel probiotic isolates (Abushelaibi et al., 2017). Probiotics can occasionally contribute by strengthening the intestinal epithelial barrier and attaching to mucus and the cell surfaces of the epithelium. This process is highly dependent on the hydrophobic and hydrophilic characteristics of the microorganisms' cell membranes(Durazzo et al., 2020; Yeganehzad et al., 2007).

Recent studies have unequivocally demonstrated that the interplay between the gut microbiota and undigested dietary ingredients, medications, and other factors may produce a variety of metabolites that may either improve or endanger human health, despite its complexity and diversity (Koh & Bäckhed, 2020). The mystery of the relationships between gut microbiota, diet, and health is being solved by a variety of research methods, such as *in vitro* experiments, animal studies, and clinical trials. This is particularly significant since dysbiosis, or an imbalance in the makeup of gut microbes, has been linked to a number of disorders, including diabetes, obesity, inflammatory bowel disease, and colorectal cancer. A reduction in the quantity of helpful bacteria, an increase in harmful species, and a decrease in microbial diversity can all lead to dysbiosis (Anhê et al., 2015; Kumar Singh et al., 2019).

Plant metabolites called polyphenols may be found in fruits, vegetables, spices, and medicinal plants. They have a variety of biological properties, including antibacterial, anti-inflammatory, antioxidant, and anticancer effects (Cory et al., 2018; Santos-Buelga et al., 2019). There is a connection between their food consumption and the avoidance of some degenerative and chronic illnesses. Nevertheless, the precise mechanisms underlying the beneficial effects of polyphenols are yet unknown (Cory et al., 2018; Ruskovska et al., 2020). About 5–10% of the polyphenols that are consumed are thought to be reabsorbed in the small intestine, with the remainder making its way to the large intestine where the gut bacteria metabolizes them (Santos-Buelga et al., 2019). Polyphenols are a unique class of prebiotics because of their interaction with intestinal microorganisms, which is how their activity toward the human host is usually mediated due to their limited absorption in the digestive system (Thilakarathna et al., 2018). According to *in vitro* research, polyphenols may have a dual beneficial impact by simultaneously stimulating good bacteria and inhibiting pathogens (Ozdal et al., 2016).

The Algerian flora is characterized by a diverse range of spontaneous medicinal and fragrant plants, including *Artemisia herba-alba*, which belongs to the Asteraceae family (Djabou, 2012). Its composition of essential oils with antibacterial, vermifuge, and antispasmodic qualities, as well as its superior feed value, define it (Houmani et al., 2004). The various portions of *Artemisia herba-alba* have been utilized for menstruation discomfort, digestion issues, stomach ulcers, anticancer, febrifuge, and vermifuge purposes (Djeridane et al., 2007).

Since ancient times, *Juniperus communis* has been used in traditional medicine to treat a variety of conditions, including indigestion, diarrhea, tumors, bronchitis, and abdominal pain(Mansouri et al., 2011). This is because *Juniperus communis* is rich in phenolic compounds, which have been shown to have positive effects on a number of biological systems (Ait Said & Hameg, 2019). These compounds can also alter the microbiota in the intestine by moderating the growth of beneficial bacteria like lactobacilli and bifidobacteria while reducing the growth of pathogenic bacteria like clostridia (Tuck & Hayball, 2002).

The main aim of this study is to evaluate *in vitro* and *in vivo*, the effect of aqueous and hydroethanolic extracts obtained from common plant species used to treat gastrointestinal disoders by traditional healers (*Artemesia herba-alba*, *Juniperus communis*) on probiotic bacteria properties isolated from camel milk in the El-Oued region.

This thesis will be organized into three chapters contained in three articles:

Chapter 1: Medicinal plants used by traditional healers in the treatment of gastrointestinal disorders in Oued Souf region (southeast of Algeria)

Chapter 2: Comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions

Chapter 3: Effect of *Juniperus communis* and *Artemisia herba-alba* extracts on probiotic properties of *Bacillus safensis* isolated from camel milk in the region of El Oued (Algeria)

All experiments were conducted in:

- Laboratory of biodiversity and application of biotechnology in the agricultural field, Faculty of the Sciences of Nature and Life, University of El Oued, El-Oued, Algeria.
- Incubator of El Oued university.
- Laboratory of Biochemistry and Biotechnology (LR01ES05), Department of Biology, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunisia.

CHAPTER I:

Medicinal plants used by traditional healers in the treatment of gastrointestinal disorders in Oued Souf region (southeast of Algeria)

1.1. Introduction

The gastrointestinal tract, a highly sensitive human organ, is susceptible to a diverse range of diseases, including parasites, infectious disorders, gastroenteritis, reflux, bloating, constipation, and diarrhea (Kasper & Harrison, 2005; Mir et al., 2021). The prevalence of gastrointestinal illness is notably attributed to infections from various bacterial strains, causing millions of deaths each year in young children (Mafokwane et al., 2023). There is a growing interest in traditional medical systems, driven by the need for more efficient treatment. The demand for fundamental scientific research on medicinal plants used in indigenous medical systems has consequently increased. Recognizing the importance of traditional medicine, the World Health Organization (WHO) acknowledges it as the totality of knowledge, skills, and practices based on theories, beliefs, and experiences inherent to various cultures (Akerele, 1984; Lafi et al., 2022).

In Algeria, phytotherapy is deeply rooted in local culture, with indigenous knowledge accumulated over decades through practical study. The diverse flora, fostered by Algeria's geographic position and varied climate, has been extensively used to address numerous maladies, especially digestive system problems(Bouasla & Bouasla, 2017; Boutlelis et al., 2023). Despite lifestyle changes and industrialization, local communities in Algeria's Sahara, one of the world's largest deserts, still rely on traditional healers for medical needs(Benarba, 2016). Recognizing the declining transmission of this tradition, it has become crucial to record the historical applications of therapeutic herbs(Bouasla & Bouasla, 2017).

Ethnopharmacological studies play a vital role in acquiring and safeguarding ancestral medicinal history. Scientific investigations are necessary to confirm the efficacy claimed by conventional healers and to identify bioactive substances(Brahmi et al., 2023; Mushagalusa Kasali et al., 2021). While numerous ethnobotanical studies globally explore traditional remedies for gastrointestinal disorders(Heinrich et al., 1992; Sulaiman et al., 2022; Tetali et al., 2009; Vera-Ku et al., 2010), limited details are known about the traditional usage of therapeutic herbs in the Oued Souf region (North Southeast of Algeria) for treating digestive system diseases.

This study aims to document and analyze local knowledge of medicinal plants used by traditional healers to treat gastrointestinal disorders in the Oued Souf region. Specific details include the species employed in treatment, the types of gastrointestinal conditions addressed by certain plants, and the parts of the plant used as medication. Ethnomedical indexes are utilized to determine the most well-liked plants in the study area, with the data serving as a basis for additional phytochemical and pharmacological research.

1.2. Material and methods

1.2.1. Study area

The Oued Souf region is situated in the north-southeast part of Algeria, covering a total area of more than 54,573 km² with a population of 504,401 inhabitants. Geographically, it is positioned between latitudes 34° 17' 25" north and 7° 42' 41" east. The research location is bordered to the north by the wilayas of Biskra, Khenchela, and Tébessa, to the east by the Tunisian border, to the west by the wilaya of Djelfa, and to the south by the wilaya of Ouargla. The region comprises three sets, including a sandy region that spans the entire Souf region, as well as the eastern and southern parts of Oued-Righ. This area is part of the great eastern erg and holds minimal significance for agriculture (Figure 1)(Kholladi, 2005).

Figure 1. Location of study area (Oued Souf, southeast of Algeria)
(KHECHANA et al., 2010)

1.2.2. Data Collection

This study was conducted from May to September 2022, during which we carried out 20 interviews with traditional healers in the research region, obtaining their permission for participation. To identify medicinal plants used in the treatment of gastrointestinal disorders, we employed semi-structured questionnaires with open-ended questions. The questionnaire, divided into two parts, gathered socio-demographic information (address, age, sex, education level, and years of experience) and details about medicinal plants used for various digestive tract diseases (local name, scientific name, part used, mode of preparation, therapeutic uses, and usage warnings).

1.2.3. Plant Resources

During the collection of plant specimens, we sought validation from multiple specialists to ensure result accuracy. The identification of plant samples was confirmed by Professor Youcef Helis from the Scientific and Technical Research Center on Arid Regions

C.R.S.T.R.A, Campus of Mohamed Khider University. The scientific and popular names of the medicinal plants were verified using the web database (www.theplantlist.org) and botanical sources on Algerian flora(Aissa, 1991; Kaddem, 1990). Herbarium specimens of the identified plants were created and stored in the laboratory of Biodiversity and Application of Biotechnology in the Agricultural Field, Faculty of the Sciences of Nature and Life, University of El Oued, Algeria.

1.2.4. Data analysis

The obtained information was statistically examined using metrics such as Use Value (UV) and Informant Consensus Factor (ICF).

1.2.4.1. Use value (UV)

According to Phillips et al. (1994), UV assessed the relative relevance of a species compared to others and is calculated as: $\mathbf{UV = \Sigma U/N}$

where **U** is the number of reports of uses for a certain species, and **N** is the total number of informants. A high UV value implies significance, while a low UV value suggests lesser importance than other species (Musa et al., 2011).

1.2.4.2. Informant consensus factor (ICF)

ICF measures the degree of knowledge homogeneity among informants. The range is 0 to 1, calculated as: $\mathbf{ICF = (Nur - Nt) / (Nur - 1)}$

where **Nur** is the number of citations used in each disease category, and **Nt** is the number of species used(Khoja et al., 2022).

1.3. Results

1.3.1. Demographics data of the responders

In terms of age distribution, the majority of research participants fell within the 41 to 60 age range (50%). Among the total traditional healers, 15% were female, and 85% were male. The informants exhibited diverse educational backgrounds, ranging from 15% being illiterate to 85% being literate. The results indicate varying levels of expertise among traditional healers, with 45% having the highest proficiency (Table 1).

Table 1. Demographics of survey respondents on medicinal plants used in the treatment of gastrointestinal disorders in Oued souf region, algeria.

Variable	*Categories*	*Percentage*
Sex	Male	85%
	female	15%
Age(years)	<20	0%
	20-40	35%

	41-60	50%
	>60	15%
Educational level	illiterate	15%
	primary level	10%
	middle level	10%
	secondary level	40%
	University level	25%
Experience (years)	10-20	40%
	21-40	45%
	41-60	15%

1.3.2. The utilization of medicinal plants by traditional healers for the treatment of gastrointestinal disorders

According to our results, the traditional healers utilize 47 medicinal plant species from 22 botanical families to treat gastrointestinal disorders, as detailed in Table 2. Notably, 3 plant families stand out in significance - Lamiaceae with 9 species, Asteraceae with 7 species, and Apiaceae with 6 species, as illustrated in Figure 2.

Table 2. List of medicinal plants used by traditional healers to treat gastrointestinal disorders .

N°	Family	Local name	Scientific name	Growth form	Part used	Mode of preparation	Indication	Usage warnings	UV
1	Lamiaceae	Iklil jabel	*Rosmarinus officinalis* L.	Spontaneous	Leaves	Decoction	Gastrointestinal gases	-Is not advised for women who are pregnant or nursing - Causes blood pressure disorders	0.5
		khozama	*Lavandula angustifolia* Mill.	Cultivated	fruits	Infusion	-Gastrointestinal gases -Gastric disorders	- Is not advised for women who are pregnant or nursing	0.1
		khyata	*Teucrium polium* L.	Spontaneous	All plant	Decoction, Powder	-Gastric ulcer -Diarrhea	-Is not advised for women who are pregnant or nursing -Causes liver disorders	0.2
		Rihan	*Ocimum basilicum* L.	Spontaneous	Leaves	Decoction	-Gastrointestinal gases -Gastric disorders	-Increased bleeding -Causes liver disorders	0.55

		Zaater	*Thymus vulgaris* L.	Spontaneous	All plant	Infusion	- Gastrointestinal gases - Diarrhea	-Is not advised for women who are pregnant or nursing	0.25
		Mardakoch	*Origan marjolaine* L.	Spontaneous	Leaves	Decoction	-Gastric ulcer -Abdominal pain	-Is not advised for: children, women who are pregnant or nursing	0.05
		Maryot	*Marrubium vulgare* L.	Spontaneous	leaves	Infusion	-Indigestion - Gastrointestinal gases	-Is not advised for: women who are pregnant or nursing ,children	0.1
		Miramia	*Salvia officinalis* L.	Spontaneous	Stems, Leaves	Infusion	- Diarrhea -Abdominal pain	-Is not advised for: diabetics, women who are pregnant or nursing -Causes liver disorders	0.1
		Naanaa	*Mentha crispata* L.	Cultivated	Leaves	Infusion	- Gastrointestinal gases	-Is not advised for diabetics, women who are pregnant or nursing, children	0.25
2	Asteraceae	Babonj	*Matricaria chamomilla* L.	Cultivated	Flowers	Infusion	-Gastric disorders -Abdominal pain	- It is not permitted for surgical patients	0.3
		Chih	*Artemisia herba-alba*	Spontaneous	Leaves	Infusion	-Indigestion - Gastrointestinal gases -Irritable bowel syndrome	-Induces sleeplessness -leads to vomiting	0.85
		Keset hindi	*Saussurea costus* L.	Spontaneous	Roots	Infusion, powder	-Gastric ulcer	-Causes blood pressure disorders -Is not advised for women who are pregnant or nursing	0.05
		Kartofa	*Anacyclus valentinu* L.	Spontaneous	Seeds	Infusion	- Diarrhea -Irritable bowel syndrome -Gastric ulcer	-Is not advised for women who are pregnant or nursing	0.2
		Hindba	*Cichorium intybus* L.	Spontaneous	Roots, Leaves, Flowers	Infusion	-Constipation -Abdominal pain - Gastrointestinal gases	-leads to gallbladder problems -It is not permitted for surgical patients	0.05

		Meraret henech	*Entyraea centarium* L.	Spontaneous	All plant	Decoction, Powder	-Gastrointestinal gases -Hemorrhoids	-Is not advised for women who are pregnant or nursing ,children -Causes gastric ulcers	0.1
		Dgeft	*Artemisia campestris* Scop.ex Steud	Cultivated	Leaves	Infusion	- Diarrhea -Abdominal pain	-Is not advised for women who are pregnant or nursing -Causes gastric ulcers	0.25
3	Apiaceae	Heltit	*Ferula assa –foetida* L.	Spontaneous	All plant	Decoction	-Gastric ulcer -Gastrointestinal gases -Irritable bowel syndrome	-Causes blood pressure disorders	0.05
		Deriga	*Ammodaucus leucotrichus* Coss. & Durieu	Spontaneous	Seeds	Infusion, decoction	-Irritable bowel syndrome -Abdominal pain gastrointestinal gases -Constipation	- The dosage must be followed	0.15
		Krefs	*Apium graveolens* L.	Cultivated	Seeds	Infusion	-Gastric ulcer -Abdominal pain -Constipation	-Causes blood pressure disorders	0.1
		Kesber	*Coriandrum sativum* L.	Cultivated	Leaves, Seeds	infusion	-Gastrointestinal gases	-Is not advised for Diabetics, women who are pregnant or nursing -Causes blood pressure disorders	0.1
		Kemun	*Cuminum cyminum* L.	Cultivated	Seeds	Decoction	-Gastrointestinal gases -Irritable bowel syndrome - Diarrhea	-Causes gastric ulcers -Is not advised for women who are pregnant or nursing	0.65
		Yenson	*Pimpinella anisum* L.	Spontaneous	Seeds	Infusion	-Gastric ulcer -Constipation	-Is not advised for Diabetics	0.45
4	Fabaceae	Helba	*Trigonella foenum-graecum* L.	Cultivated	Seeds	Decoction	-Gastric disorders -Constipation	-Is not advised for women who are pregnant or nursing,children	0.45

		Erek sos	*Glycyrrhiza glabra* L.	Spontaneous	Roots	Decoction, Powder	-Chronic inflammatory disorders -Gastric ulcer	-Causes blood pressure disorders -Is not advised for women who are pregnant or nursing	0.15
		Sena meki	*Senna alexandrina* Mill.	Spontaneous	Leaves	Infusion, decoction	-Constipation	-Causes severe diarrhea	0.5
5	Cupressaceae	Debegh	*Thuja occidentalis* L.	Spontaneous	All plant	Infusion	-Chronic inflammatory disorders	-Causes severe diarrhea	0.35
		Araar	*Juniperus communis* L.	Spontaneous	Leaves	Infusion	- Diarrhea -Gastrointestinal gases	-Leads to renal disorders -Causes blood pressure disorders -Is not advised for Diabetics	0.75
6	Lauraceae	Rend	*Laurus nobilis* L.	Spontaneous	Leaves	Infusion	-Abdominal pain - Diarrhea -Gastrointestinal gases	-Causes blood pressure disorders -Is not advised for women who are pregnant or nursing	0.15
		Kerfa	*Cinnamomum verum* J.Presl	Cultivated	Stems	Decoction	-Gastrointestinal gases - Diarrhea	-Increased bleeding -Causes liver disorders -Is not advised for women who are pregnant or nursing	0.05
7	Zingiberaceae	Zenjabil	*Zingiber officinale* Roscoe	Cultivated	Roots	Decoction	-Irritable bowel syndrome -gastric ulcer	-Not take it on an empty stomach	0.25
		Kerkum	*Curcuma longa* L.	Cultivated	Stems	Infision	-Chronic inflammatory disorders	-Increased bleeding -Causes blood pressure disorders -Is not advised for Diabetics	0.1
8	Chenopodiaceae	Ktef	*Atriplex halimus* L.	Spontaneous	Leaves, Seeds	decoction, Powder	-Constipation	-Causes gastric ulcers	0.05
		Demran	*Traganum nudatum* Delile	Spontaneous	All plant	Infusion, decoction	- Diarrhea -Hemorrhoids -Gastrointestinal gases	- The dosage must be followed	0.15

9	Phyllanthaceae	Amlej	*Phyllanthus emblica* L.	Spontaneous	Seeds	Infusion	-Constipation -Gastric ulcer	-Is not advised for women who are pregnant or nursing,Diabetics	0.05
10	Crassulaceae	Serra	*Centella asiatica*.L.	Spontaneous	Roots	Infusion	-Chronic inflammatory disorders	- The dosage must be followed	0.1
11	Amaryllidaceae	Besbess	*Foeniculum vulgare* Mill.	Spontaneous	Seeds	Infusion	-Constipation -Gastrointestinal gases	-Is not advised for women who are pregnant or nursing -Increased bleeding	0.7
12	Lythraceae	Henna	*Lowsonia inermis* L.	Cultivated	leaves , Flowers	Decoction	- Diarrhea	-Is not advised for women who are pregnant or nursing	0.2
13	Theaceae	Chee	*Camellia sinensis* L.	Cultivated	Leaves	Decoction	-Chronic inflammatory disorders	-Causes gastric ulcers -Induces sleeplessness	0.1
14	Pinaceae	Senober	*Pinus gerardiana* Wall. Ex D.Don	Spontaneous	Stems	Infusion	-Abdominal pain -Chronic inflammatory disorders -Gastrointestinal gases	- Weight gain	0.1
15	Verbenaceae	Louiza tizana	*Aloysia citrodora* Palau	Cultivated	Leaves	Infusion	-Gastrointestinal gases	-Causes thyroid malfunction	0.15
16	Anacardiaceae	Mestka hora	*Pistacia lentiscus* L.	Cultivated	All plant	Powder	-Gastric ulcer -Chronic inflammatory disorders	-Is not advised for women who are pregnant or nursing, children	0.1
17	Caesalpinioi-deae	kherob	*Ceratonia siliqua* L.	Cultivated	fruits	Infusion	- Diarrhea -Irritable bowel syndrome	-Is not advised for women who are pregnant or nursing	0.1
18	Apocynaceae	Karenka	*Calotropis procera* A.T.Aiton	Cultivated	leaves, Flowers, Roots	Powder	-Gastric ulcer - Diarrhea	- The dosage must be followed -Is not advised for women who are pregnant or nursing	0.05

19	Rutaceae	Fijel	*Ruta graveolens* L.	Spontaneous	Leaves	Infusion	-Gastrointestinal gases -Abdominal pain -Chronic inflammatory disorders	-Is not advised for women who are pregnant or nursing, children	0.2
20	Rhamnaceae	Sedra	*Ziziphus spina-christi* L.	Spontaneous	Leaves	Infusion	-Gastrointestinal gases - Constipation - Gastric ulcer	-Is not advised for women who are pregnant or nursing, children	0.2
21	Nitrariaceae	Hermel	*Peganum harmala* L.	Spontaneous	Seeds	Infusion	-Abdominal pain	-Is not advised for women who are pregnant or nursing -Causes gastric ulcers	0.15
22	Tamaricaceae	Terfa	*Tamarix aphylla* L.	Spontaneous	All plant	Infusion, decoction	-Constipation	-Is not advised for women who are pregnant or nursing, children	0.1

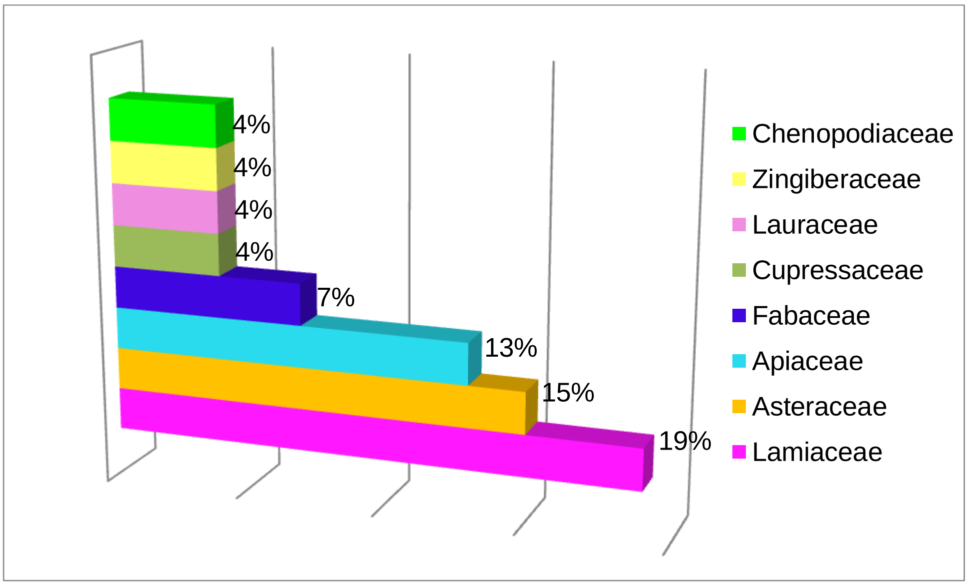

Figure 2. The majority of listed botanical families used by traditional healers for the treatment of gastrointestinal disorders.

1.3.3. Most frequently utilized species

The most frequently utilized species by traditional healers in the treatment of digestive system disorders include *Artemisia herba-alba* (17 mentions), *Juniperus communis* (15 mentions), *Foeniculum vulgare* (14 mentions), *Cuminum cyminum* (13mentions), *Ocimum* *basilicum* (11mentions), *Senna alexandrina*, and *Rosmarinus officinalis* (10mentions), as depicted in figure 3.

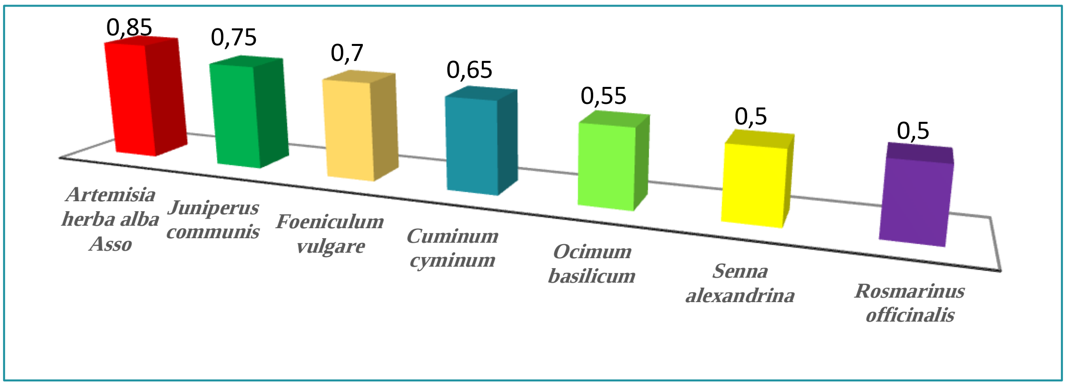

Figure 3. Most frequently utilized species used by traditional healers for the treatment of gastrointestinal disorders.

1.3.4. Used part

Results indicate that leaves are the most frequently utilized part (35%), followed by seeds (20%), the entire plant (15%), roots (11%), stems (8%), flowers (7%), and fruits (4%) (Figure 04).

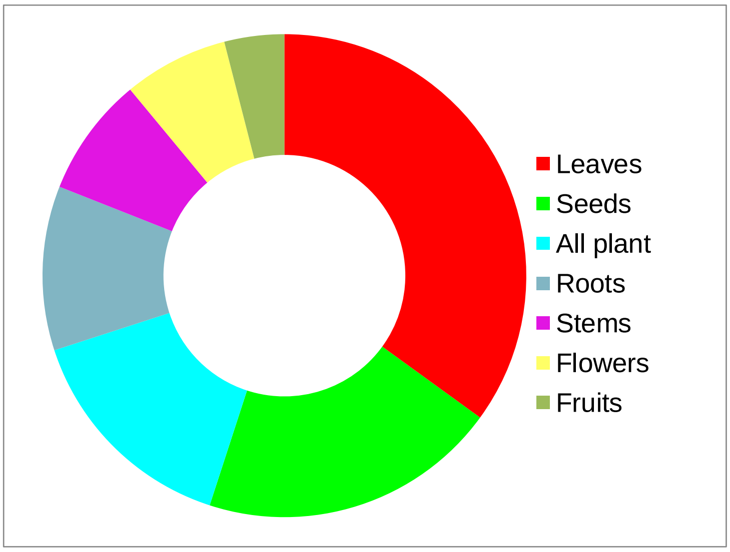

Figure 4. Frequency of plant parts used used by traditional healers for the treatment of gastrointestinal disorders.

1.3.5. Method of preparation

The study revealed that the infusion method was the most frequently indicated for herbal dregs preparation (55%), followed by decoction (32%) and powder formulations (13%) (Figure 5).

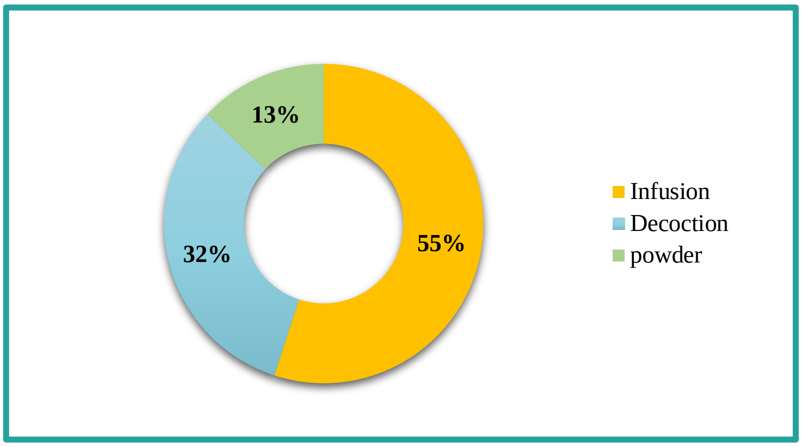

Figure 5. Frequency of several preparation methods used by traditional healers for the treatment of gastrointestinal disorders.

1.3.6. Therapeutic uses

Based on the information supplied by the respondents, ailments were classified into 10 disorders, with gastrointestinal gases (23%) being the condition most frequently treated with the indicated medicinal plants. This was followed by gastric ulcer and diarrhea (14% each), constipation and abdominal pain (12% each), chronic inflammatory disorders (9%), irritable bowel syndrome (8%), gastric disorders (4%), indigestion, and hemorrhoids (2% each) (Figure 6).

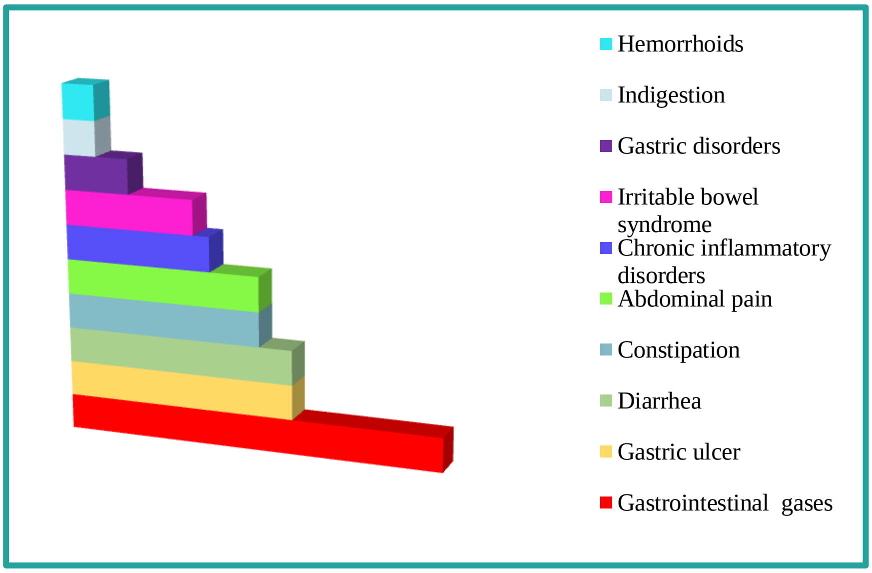

Figure 6. Gastrointestinal disorders treated by medicinal plants in the research area.

1.3.7. Usage warnings

The most crucial warnings provided by traditional healers emphasize that using medicinal herbs to treat digestive ailments is not advised for pregnant or nursing women (33%), children (11%), and individuals with diabetes and blood pressure disorders (9% each). Other cautions include the potential to cause gastric ulcers (7%), liver disorders, increased bleeding, and the importance of following specified dosages (5% each). It is not permitted for surgical patients, may cause severe diarrhea and induce sleeplessness (3% each), and should not be taken on an empty stomach. This may lead to vomiting, gallbladder problems, male breast development, weight gain, thyroid malfunction, and renal disorders (1% for each) (Figure 7).

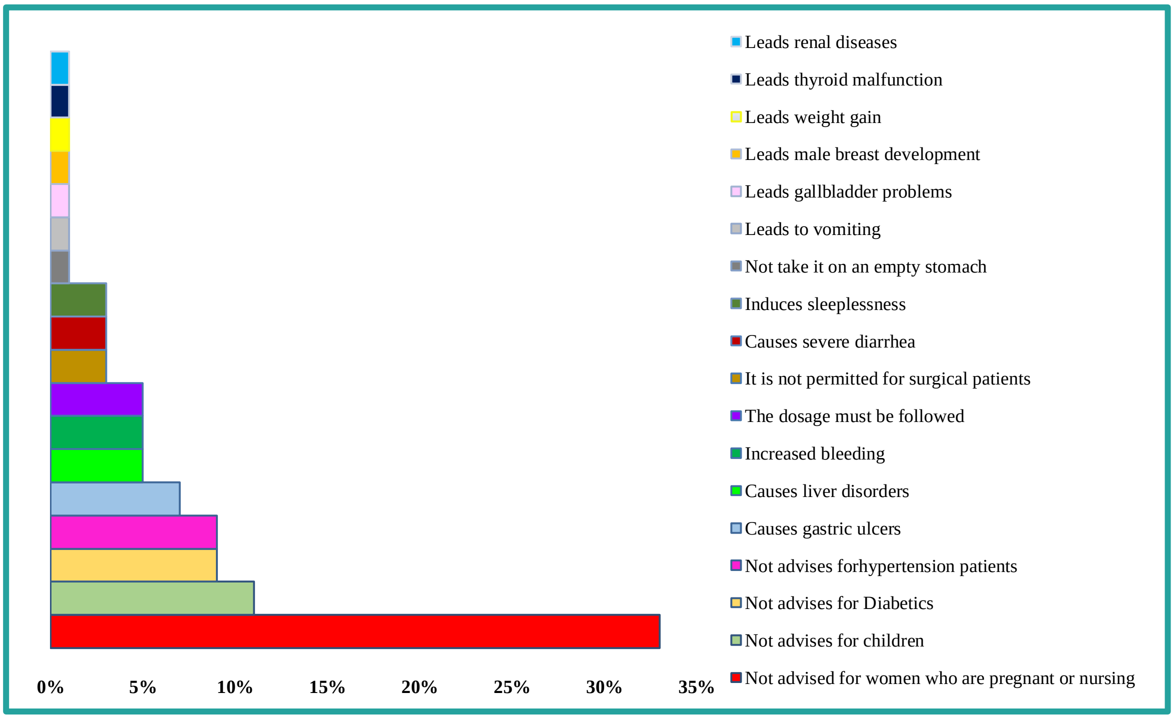

Figure 7. Usage warnings given by traditional healers

1.3.8. Statistical data analysis

1.3.8.1. Use value UV

The medicinal plants with the highest and lowest usage reports also exhibit the highest and lowest respective use values. In this study, *Artemisia herba-alba* demonstrated the greatest UV value (0.85), while several plants, including *Atriplex halimus* and *Calotropis procera*, had the lowest UV value (0.05) (Table 2).

1.3.8.2. Informant consensus factor (ICF)

The Informant Consensus Factor (ICF) was determined by categorizing all disorders into ten categories in the current study. Hemorrhoids and indigestion showed the highest ICF values (1 each), while gastrointestinal gases had a value of 0.55 (refer to Table 3).

Table 3. Value of informant consensus factor (ICF) for each disease category.

Disease categories	Nur	Nt	ICF
Gastrointestinal gases	21	10	0.55

Gastric disorders	4	3	0.33
Gastric ulcer	13	9	0.33
Diarrhea	13	8	0.41
Abdominal pain	11	7	0.4
Indigestion	2	1	1
Constipation	11	7	0.4
Irritable bowel syndrome	7	5	0.33
Chronic inflammatory disorders	8	5	0.42
Hemorrhoids	2	1	1

1.4. Discussion

Though traditional medicine is often associated with a particular gender, it is practiced by both men and women in some cultures. In the current study, a higher number of male respondents were interviewed compared to female respondents. A comparable study by Khoja et al. (2022) indicated that 34 male healers (70.83%) and 14 female healers (29.17%) were involved. Additionally, research in the M'sila region of Algeria revealed a predominantly male participation in traditional medicine(Boudjelal et al., 2013). The sociocultural framework of the society, actual circumstances, informants' commitment, and associated sociocultural boundaries are factors influencing ethnobotanical surveys.

The majority of participants in the present study were aged 41-60 (50%), with the majority of healers (85%) being literate. It appears that the number of reported species is correlated with the informants' ages. Younger individuals, exposed to modern education, may have decreased interest in learning about and applying ethnomedical practices. Simultaneously, as science and technology advance rapidly, younger generations are adopting new customs(Abdulsalami et al., 2020). Additionally, 45% of healers have between 21 and 40 years of experience. Bouasla and Bouasla (2017) emphasize that experience gained with age provides older individuals with more knowledge. Furthermore, Kadir et al. (2013) found that the majority of healers (33.36%) have 10–20 years of relevant expertise from their ethnopharmacological assessment.

This study reports the utilization of 47 medicinal plant species from 22 families by traditional healers for treating gastrointestinal disorders in the Oued Souf region. Lamiaceae, with 9 species, emerges as the most utilized family, followed by Asteraceae (7 species), Apiaceae (6 species), and Fabaceae (3 species). These findings contrast with those of Kadir et al. (2013), who claimed that the Fabaceae family accounted for the majority of medicinal plants used by traditional healers in Bangladesh. This discrepancy highlights the considerable taxonomic diversity of medicinal plants in our study area, underscoring the wealth of knowledge regarding their application in traditional gastrointestinal treatment(Chekole, 2017). Furthermore, Lamiaceae is notable for its high content of phenolics and flavonoids, contributing to its elevated antioxidant levels, as demonstrated in previous research(Khaled-Khodja et al., 2014).

The most cited species in our study are *Artemisia herba-alba* and *Juniperus communis* (17-15 mentions). The widespread usage of these species by respondents for various ailments can be attributed to their familiarity and frequent employment. However, it's crucial to note that intensive usage and overuse of these species may jeopardize their survival, impacting the region's biodiversity(Bouasla & Bouasla, 2017).

Results indicate that leaves are the most frequently utilized plant part (35%). This aligns with several investigations emphasizing the significance of leaves in developing remedies, as highlighted by Abdulsalami et al. (2020). The effectiveness of leaves in treating illnesses may be attributed to the various bioactive components they contain, as leaves actively participate in photosynthesis, making them a crucial component of several herbal remedies(Shosan et al., 2014).

The study revealed that the infusion method was most commonly indicated (55%). Traditional healers believe that infusion is the most effective method for preparing medicinal extracts to treat gastrointestinal diseases because it maintains the therapeutic characteristics of the extract, allowing for the secure extraction of active ingredients(Dextreit, 1987). The simplicity of preparation and administration makes decoction and infusion in water popular techniques, as highlighted in previous research(Benítez et al., 2010; Hammiche & Maiza, 2006).

Gastrointestinal gases (23%) represent the most frequently treated condition with the indicated medicinal plants. Several plants have demonstrated robust biological defenses against a range of digestive illnesses. Consequently, delving into the biological research of some medicinal plants used by Oued Souf healers to treat gastrointestinal disorders becomes particularly intriguing. Traditional healers emphasize a crucial piece of advice by prohibiting the use of medicinal herbs for pregnant women (33%). It is erroneous and deceptive to assume that herbal treatments are exceptionally safe and devoid of side effects, as is commonly believed. Herbs have been shown to induce various unpleasant or unfavorable responses, some of which have the potential to be lethal or cause severe injuries and other life-threatening conditions. It is crucial to remember that larger dosages of therapeutic plants can occasionally have detrimental consequences(Ekor, 2014).

In the current study, *Artemisia herba-alba* and *Juniperus communis* have the highest UV value (0.85) due to their diverse therapeutic characteristics. Conversely, low usage values (UV) for medicinal plants suggest that access to or knowledge of those particular plants may be at risk(Chaudhary et al., 2006; Mahmood et al., 2013). Hemorrhoids and indigestion each have the highest ICF values (1). A high ICF score indicates the usage of a relatively small number of plant species by a significant majority of informants(Heinrich et al., 1998).

Most frequently, plants such as *Foeniculum vulgare*, *Cuminum cyminum*, *Coriandrum sativum*, *Ammodaucus leucotrichus*, *Rosmarinus officinalis*, *Ocimum basilicum*, *Mentha crispata*, *Entyraea centarium*, *Aloysia citrodora*, *Ruta graveolens*, and *Traganum nudatum* were claimed to be helpful for treating gastrointestinal gas. The informants' consensus on using a specific plant species to treat various ailments is reflected in the high ICF value. This suggests that these plants may contain physiologically active components(Cakilcioglu & Turkoglu, 2010). *Foeniculum vulgare* (Apiaceae) is a well-known plant with significant therapeutic value, particularly for treating gastrointestinal disorders(Verma et al., 2021). Cumin seeds are believed to have carminative properties, and according to tradition, the plant may be effective in treating a variety of conditions, including diarrhea, jaundice, indigestion, and stomach discomfort(Singh et al., 2021; Tahir et al., 2016).

1.5. Conclusion

The aim of the present study was to identify potential medicinal plants in the Oued Souf region (North Southeast Algeria) that traditional healers may use to treat various gastrointestinal disorders. Our survey yielded a wealth of data, clearly demonstrating that traditional healers in the Oued Souf region employ numerous medicinal plants for treating various digestive system ailments. A total of 47 medicinal plant species from 22 families were documented in this research, with Lamiaceae and Asteraceae being the most common families. Plant leaves were predominantly used to treat gastrointestinal related problems, and infusion emerged as the most widely employed conventional preparation technique in the area. It is crucial to document the preservation of traditional knowledge before it diminishes from the region, where it is disappearing at an alarming rate. While preliminary research on these medicinal plants has shown their effectiveness, further investigation is necessary, particularly to ensure the safe use of these plants in therapeutic procedures.

CHAPTER II:

Comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions

2.1. Introduction

Pharmaceutical industries, agri-food and cosmetics are all interested in aromatic plants (Daira et al., 2016). Because the various active compounds they contain —alkaloids, flavonoids, tannins, saponins, and essential oils —give them a variety of medical benefits in addition to their aromatic and culinary properties. They serve as an endless supply of the most potent folk cures and serve as the natural source of the majority of the pharmaceuticals that are currently prescribed (Larayetan et al., 2019).

Because Algeria is known for the diversity of its medicinal flora, which comprises hundreds of plant species, the use of herbal medicine is firmly ingrained in our culture (Dahmane et al., 2023). The so-called secondary metabolites that are accumulated by this plant material are a significant source of compounds like polyphenols and flavonoids, which are frequently utilised in medicine as antioxidants, anti-inflammatories, antibacterials, etc.(Boudjema et al., 2021)

Juniper falls under the Cupressaceae family, which has various species. The common Juniper (*Juniperus communis*) is a fragrant evergreen shrub with great therapeutic potential for treating both human and animal illnesses (Raina et al., 2019). The leaves of *J. communis* were found to be rich in a variety of chemical components, including Camphene, Verbenene, Myrcene, Limonene, Sabinene, α-Pinene, α-Thujene, and α-Terpinene, which were in charge of exhibiting varied pharmacological activity and treating a variety of ailments (Kirti Dhaka & Amit Mittal, 2021). Numerous previous investigations support the biological effects of *Juniperus communis* (Šojić et al., 2017). Their berries revealed a variety of pharmacological properties including an antioxidant (Höferl et al., 2014), antimicrobial (Peruč et al., 2018) and anti-inflammatory potential (Raina et al., 2019). In conventional medicine they are utilized as rheumatic, antiseptic, stomachic, diuretic, and cardiac medications (Stoilova et al., 2014).

The Asteraceae family includes the species *Artemisia herba-alba* (white mugwort), an aromatic and therapeutic plant (Nedjimi & Beladel, 2015). It was distinguished by a significant morphological diversity in respect to the regional ecological factors. *Artemisia herba-alba has* been shown to contain a number of secondary metabolites, the most significant of which are sesquiterpene lactones, including eudesmanolides and germacranolides. The flavonoids found in mugwort also exhibit a structural diversity, ranging from common flavonoids (flavonesglycosides and flavonols) (Ivănescu et al., 2021). In traditional medicine, *Artemisia herba-alba* is frequently used to treat stomach issues like diarrhea and abdominal pain. It is also used as a remedy for inflammation of the gastrointestinal tract (Benyahia et al., 2021). Previous scientific studies have also proven the effectiveness of white mugwort as an antioxidant and antibacterial agent (Bordean et al., 2023).

In light of our interest in the therapeutic effects of plants. This study's objective was to examine the antioxidant, antibacterial, and anti-inflammatory properties in case of *Artemisia herba-alba and Juniperus communis* extracts, which were prepared using a variety of solvents.

2.2. Material and methods

2.2.1. Plant material

Artemisia herba-alba was collected in septembre 2022 from the Kchida region (Batna, Algeria, 35°33´21" N 6° 10´ 26" E), and *Juniperus communis* in novembre 2022 from Wanza region (Tebessa, Algeria, 35° 57′ 00" N, 8° 08′ 00" E).

2.2.1. 1.Extraction method

The aerial parts are cleaned, dried, and ground. Then, it was stored at ambient temperature in a glass box until use. To obtain the extracts, the leaves (10 g) were macerated in 200 ml of distilled water and a hydroalcoholic solution of (ethanol : water= 80: 20) for 24 h at ambient temperature with fresh solvent every 24 hours, this procedure was carried out three times. The resultant mixture was then filtered, concentrated by evaporation under decreased pressure for at least 48 hours at a temperature below 40 °C, and kept until use.

2.2.2. Total phenolic contents (TPC)

500 µl of newly made Folin-Ciocalteu reagent (10 times diluted) and 400 µl of 7.5% sodium carbonate (Na_2CO_3) are combined with 100 µl of the extract. After 30 minutes in room temperature incubation, the absorbance is measured at 765 nm using a spectrophotometer against a blank (100 µl of the solvent is mixed with 400 µl of 7.5% sodium carbonate and 500 µl of Folin-Ciocalteu reagent). Concentrations of polyphenols were inferred from the range of the linear calibration curve created using gallic acid (Jaradat et al., 2024).

2.2.3. Total flavonoid contents (TFC)

A 1 ml of the $AlCl_3$ solution (2% in methanol) was combined with 1 ml of each sample (made in methanol or distilled water). A spectrophotometer was used to measure the absorbance at 430 nm following ten minutes of incubation. With reference to the quercetin calibration curve, the findings are given in mg QE/g of plant extract(Mourad & Sihem, 2018).

2.2.4. Antioxidant activity

2.2.4.1. DPPH cavenging

The DPPH technique was performed to conduct the antioxidant test. A 1 ml of various concentrations of extract is mixed with 1 ml of DPPH methanolic solution (0.1×10^{-3} M). A negative control is simultaneously created by mixing 1 ml of methanol with a methanolic solution of DPPH. After 15 minutes of dark incubation at room temperature, the absorbance measurement is taken in comparison to a blank that has been created at 517 nm for every concentration. The test is done three times for each concentration. The positive control is a solution of ascorbic acid, whose absorbance was measured under the identical conditions as the samples. The findings were presented as an inhibition percentage (I%) (Țicolea et al., 2024).

$$I\% = [(\text{Abs controle} - \text{Abs test})/\text{Abs controle}] \times 100$$

Through linear regression, the IC_{50} values were visualized.

2.2.4.2. FRAP assay

200 µl of each extract solution at various concentrations are combined with 2.5 ml of potassium ferricyanate solution ($K_3Fe\ (CN)_6$) produced in distilled water and 500 µl of phosphate buffer solution (0.2 M; pH = 6.6). After 20 minutes of incubation at 50°C, the mixture is taken out of the test tubes. After adding 2.5 ml of 10% trichloroacetic acid (TCA), centrifugation is carried out for 10 minutes at 650 rpm. Add 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ to 500 µl of supernatant. The wavelength at which the absorbance is measured is 700 nm. The solvent is substituted for the extract to create a blank. Under the same circumstances, ascorbic acid is utilized as a positive control. The results were represented by the EC_{50} value (Asbabou et al., 2024).

2.2.5. Antimicrobial activities

A 20 ml of Muller-Hinton agar is added to sterile Petri plates, and the dishes are incubated for 20 minutes. Following solidification, a 1 ml bacterial suspension containing 10^8 CFU/mL (Colony Forming Unit) was applied evenly across the surface of each culture medium (Shunying et al., 2005). Paper discs of 6 mm in diameter, sterile Whatman N°1, are impregnated with a volume of 5 µl of varying concentrations (2.5, 5, 10, and 20 mg/ml) and positioned on the solidified medium's surface. After that, the Petri plates are incubated for 24 to 48 hours at 37°C in an oven. *Escherichia coli* ATCC 25922 ($Gram^-$), *Pseudomonas aeruginosa* ATCC 27853 ($Gram^-$), *Klabsiella pneumoniae* ATCC 13883 ($Gram^-$), and *Staphylococcus aureus* ATCC 25923 ($Gram^+$), are the tested strains. For anti-fungal activity, *Candida albicans* ATCC 14053 ($Gram^+$) is examined. The following formula is utilized to determine the inhibition diameter Zi:

Zi en (mm) = Inhibition zone diameter (mm) - Disc diameter (6 mm)

2.2.6. Anti-inflammatory activities (Albumin denaturation method)

For every combination, a reaction vessel was made up of 1 ml of the test extract, 1.4 ml of phosphate buffered saline solution, and 200 µl of egg albumin. As a negative control, distilled water was utilized rather than extract. After that, the mixtures were heated for five minutes at 70°C after being incubated for fifteen minutes at 37°C. The absorbance was measured at 660 nm following cooling (Osman et al., 2016). Under the same operational circumstances, diclofenac sodium is utilized as a positive control. Three duplicates of the experiment were conducted. Following is the calculation of the percent inhibition of protein denaturation:

Percentage protection against denaturation = [(1 – Abs Sample)/Abs Control] × 100

2.2.7. Statistical analysis

Triplicate tests have been conducted on antioxidant capacity, antibacterial potential, and anti-inflammatory action. The findings are displayed as mean ± standard deviation (SD).

2.3. Results and discussion

2.3.1. Total phenolic and flavonoid contents

The *Artemisia herba-alba* TPC results showed that the hydroethanolic extract had a significant polyphenol concentration with 104.54 ±0.35 mg GAE/g AHE compared to aqueous extract (58.89 ±0.22 mg GAE/g AAE). For *Juniperus communis* the aqueous extract showed a greater value (103.80 ±0.30 mg GAE/g JAE) than the hydroethanolic extract (78.11 ±0.27 mg GAE/g JHE) (Table 1).

The overall flavonoid concentration was found to be lower in the *Artemisia herba-alba* aqueous and hydroethanolic extracts (4,88 ±0,075 mg QE/gAAE and 2,06 ±0,03 mg QE/g AHE, respectively) than the two *Juniperus communis* extracts (15.85 ±0.80 mg QE/g of aqueous extract and 17.05 ±0.13 mg QE/g of hydroethanolic extract) (Table 1).

These results are highly correlated to those obtained by (Megdiche-Ksouri et al., 2015) with high contents of total polyphenols and flavonoids of *Artemisia campestris* were found in the crude methanolic extract compared to the ethyl acetate fraction and water fraction and accounting for (158. 23 ± 7.2 mg EAG/g DM versus 94.17 ± 12.14 and 10.63 ± 2.16 mg EAG/g DM) total polyphenol and (175.23 ± 7.2 mg EC/g DM versus 67.45 ± 2.28 and 63.81 ± 0.52 mg EC/g DM) flavonoids, respectively. However, Abdallah et al. (2015) have reported that the extraction of *A. herba -alba* using 70% ethanol showed a high total polyphenol content (248.6 ± 20.4) mg GAE/g dry extract and flavonoids (62.15 ± 5.8) mg rutin/g dry extract. *Jc* showed a significantly higher phenolic and flavonoid content ($p > 0.05$), respectively, at 3.04 ± 0.09 mg GAE/g DW and 1.14 ± 0.36 mg QE/g DW.

The polarity of the extraction solvents utilized can be responsible for the modest differences in measured phenol and flavonoid levels that were noticed. Because phenols, flavonoids, and other secondary metabolites have varied degrees of polarity, different solvents extract diverse compounds from the plant matrix in various ways (Kaczorová et al., 2021). The drying and extraction conditions in terms of method, duration, temperature, particle size, solvent, number of extraction steps, expression of results, and geographic origin all contribute to the heterogeneity of the polyphenol content (Idoudi et al., 2023).

2.3.2. Antioxidant characteristics

The results are shown in Table 1 as the IC_{50} value for the Antioxidant characteristics of the investigated extracts. The findings demonstrated the capacity of several extracts to lessen DPPH radicals. Compared with the standard, the aqueous extracts of two studied plants showed weak antioxidant effects with IC_{50} 151.235 ±0.14 µg/mL for *A.herba-alba* and 101.06 ±0.19 µg/mL for *J.communis*, compared with hydroethanolic extracts (*Aha*: 69.44 ±0.17 µg/mL, Jc: 97.96 ±0.22 µg/mL).

FRAP assay was another antioxidant activity test used in this research. The results (Table1) showed that *J.communis* has the strongest ferric reducing antioxidant power, especially the hydroethanolic extract (49.49 ±0.14 µg/mL), while *A.herba-alba* exhibited a weak antioxidant effects where the hydroethanolic extract recorded the lowest EC_{50} value (72.46 ±0.09 µg/mL).

These results are comparable to those of the study conducted by Abdul Qadir et al. (2017) and his associates, who found that an increase in the concentration of antioxidant chemicals increased the reducing power. According to a different study, the crude methanolic extract of A. campestris showed a higher reducing power than the other two extracts, with EC50 values of 110 ± 2.01 µ g/ml vs 230 ± 5.22 and 340 ± 7.51 µ g/ml, respectively, for the ethyl acetate fraction and water fraction. Mohammed et al. (2021) results showed the abilities of different fractions of *A. herba-alba* to reduce DPPH radicals.According to Khlifi et al. (2013) research**,** when comparing the antioxidant activity of extracts from *Ruta chalepensis L*. and *Peganum harmala L,* the extract from *A. herba-alba* showed the highest value (IC_{50}: 20.64 ±0.84 mg/L). Further, Younsi et al. (2016) reported an IC_{50} value of 100 µg/mL for methanolic *A. herba-alba* extracts. *J. communis* plant has been widely employed in the food and beverage, pharmaceutical, and other industries because of its strong antioxidant action and polyphenol content (Tang et al., 2019). Owing to their repeatability and ease of operation, the antioxidant strength of pure molecules or unprocessed plant extracts is typically assessed using the DPPH and FRAP experiments. Other techniques are also employed, and they rely on the reactive oxygen species (ROS), which are oxidizing agents found in biological systems. It should be noted that antioxidants, whether they be natural or synthetic, can restrict the creation of reactive oxygen species, limit their ability to spread, or even destroy them, allowing for the prevention of diseases brought on by oxidative stress (Apak et al., 2007). The greatest antioxidant effects are often found in polyphenols with a high hydroxyl group count because these compounds can contribute more atoms to stabilize free radicals (Heim et al., 2002). Along with the previously mentioned factors, it should be noted that the antioxidant activity also depends on the concentration of the extracts, the components' structures, the antioxidants' nature, the evaluation method, how sensitive the antioxidants are to the test's temperature, and whether they are water- or fat-soluble (Falleh et al., 2008).

Table 1.Values of total phenolic, flavonoid contents, and *in vitro* antioxidant potential of different extracts of *Artemisia herba-alba* and *Juniperus communis*.

Plant extracts	***Total phenolic contents*** (mg GAE/g E)	***Total flavonoid contents*** (mg QE/g E)	***DPPH*** IC_{50} (µg/mL)	***FRAP*** EC_{50}(µg/mL)
Artemisia herba-alba				
Aqueous extract	58.89 ±0.22	4,88 ±0,075	151.23 ±0.14	60.24 ±0.12
Hydroethanolic extract	104.54 ± 0.35	2,06 ±0,03	69.44 ±0.17	72.46 ±0.09
Juniperus communis				
Aqueous extract	103.80 ±0.30	15.85 ±0.80	101.06 ±0.19	59.54 ±0.12
Hydroethanolic extract	78.11 ±0.27	17.05 ±0.13	97.96 ±0.22	49.49 ±0.14

G: Galic acid **Q**: Quercetin **E** :Extract

2.3.3. Antimicrobial activity

It emerges from the results obtained (presented in Table 2) that all these extracts prove to be active against some microbial strains tested with different degrees, the increase in concentration of the extracts increases the diameter of the zone of inhibition. Significant antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus* was demonstrated by the hydroethanolic extracts of the plants, as well as anti-fungal activity against *Candida albicans* with a super inhibition at concentrations of 20 mg/mL. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were highly resistant to *A.herba-alb* aqueous extract, while *Escherichia coli* resisted the hydroethanolic extract of the same plant. *J. communus* aqueux does not possess antibacterial activity against *Pseudomonas aeruginosa* and *Candida albicans.* The results of a test using DMSO as a solvent (negative control) indicate that the solvent is appropriate and has no impact on the typical growth of any microbial strains.

Mohammed et al. (2021) found that the fractions of extracts of *A. herba-alba* showed varying degrees of antibacterial activity against the bacteria (*Staphylococcus aureus, Escherichia coli*). By using the disc diffusion method, crude leaf organic extracts of the Himalayan plant *J. communis* in the solvents chloroform, methanol, hexane, and ethanol, as well as aqueous extracts, revealed antibacterial potential against a variety of drug-resistant bacteria species, including *Agrobacterium tumefaciens, Escherichia coli* (Sati & Joshi, 2010). According to Shamsudin et al. (2022), phenolic chemicals, flavonoids, and steroids have the ability to prevent the growth of microbes at specific concentrations. It has been demonstrated that lipophilic flavonoids break down microbial membranes by making membrane lipids more fluidity. Microorganism toxicity can be caused by the quantity and location of hydroxyl groups on the aromatic ring of phenolic substances (Cowan, 1999). Crude extracts can be employed in food systems to stop the growth of foodborne bacteria, extending the shelf life of processed foods. These extracts have broad-spectrum antibacterial activity against positive and negative gram bacteria.

Table 2 . Zone inhibition diameters of *Artemisia herba-alba* and *Juniperus communis*.

Plant extract	Concentrations (mg/mL)	Inhibition zone (mm)				
		E.coli	***P. aeruginosa***	**K. pneumoniae**	***S. aureus***	***C.albicans***
Aq(*Aha*A)	20	10 ±1.04	NA	12 ±1.25	NA	12 ±0.76
	10	8 ±0.28	NA	18 ±1.5	NA	11 ±1.32
	5	NA	NA	8 ±0.76	NA	NA
	2.5	NA	NA	NA	NA	NA
Hydroeth (*Aha*A)	20	NA	11 ±2.02	9 ±1.15	13 ±1.5	13 ±2.84
	10	NA	7 ±0.28	9 ±0.5	12 ±2.51	12 ±2.36
	5	NA	NA	8 ±0.5	9 ±1.15	12 ±1.32
	2.5	NA	NA	NA	9 ±0.86	10 ±1.04
Aq (Jc)	20	11 ±2.08	NA	10 ±1.52	10 ±1.32	NA
	10	10 ±1.73	NA	10 ±0.57	NA	NA
	5	NA	NA	NA	NA	NA
	2.5	NA	NA	NA	NA	NA
Hydroeth (Jc)	20	7 ±0.28	15 ±1.60	11 ±1.73	21 ±1.15	13 ±2.02
	10	NA	10 ±1.04	8 ±0.5	15 ±1.52	12 ±0.76
	5	NA	NA	7 ±0.28	12 ±2.08	10 ±1.73
	2.5	NA	NA	NA	10 ±1.32	8 ±0.57
DMSO		0	0	0	0	0

NA = no activity

2.3.4. Anti-inflammatory activity

Proteins may get denatured when they are subjected to heat or chemicals. As a result, their physiological qualities are lost and their molecular structure is altered. Inflammatory disorders are known to be associated with tissue protein denaturation (Ruiz-Ruiz et al., 2017). In the current study, *A. herba alba and J. communis* extracts' *in vitro* anti-inflammatory efficacy was estimated by measuring the denaturation of egg albumin. According to Table 3, for both the aqueous and hydro-ethanolic extracts, the percentage inhibition of egg albumin denaturation increased in a concentration-dependent manner. Maximum inhibition provided by diclofenac sodium is greater than 97.5%. The hydroethanolic extract of *J. communis*, with IC_{50}: 23.58 ±0.02 µg/mL, might have the strongest ability to prevent albumin from becoming denaturated, followed by the aqueous extract of *A.herba alba* (IC_{50}: 32.24 ±0.02 µg/mL). While the hydroethanolic extract of the same plant (IC_{50}: 58.22 ±0.02 µg/mL) showed the lowest inhibitory activity. In comparison with diclofenac (IC_{50}: 21.7 µg/mL), All of the extracts exhibited a lesser ability of preventing albumin denaturation (Figure 1).

The ability for some extract constituents, such as flavonoids, phenolic acids, and tannins, to interact with the aliphatic areas around the lysine residues on albumin molecules and prevent it from denaturing owing to heat has been demonstrated by research (Williams et al., 2008). More investigation is necessary, nevertheless, to completely comprehend the precise mechanisms by which these chemicals are able to inhibit denaturation. Kirti Dhaka and Amit Mittal (2021) and Khlifi et al. (2013) confirmed that *A.herba-alba* and *J. communis* extracts possess anti-inflammatory activities .

Table 3. The percentages of protein denaturation inhibition by *Artemisia herba-alba* and *Juniperus communis*.

Concentration (µg/mL)	**Inhibition of protein denaturation (%)**				
	Aq(*A*)	**Hydroeth(*A*)**	**Aq(JC)**	**Hydroeth(Jc)**	**Diclofenac sodium**
10	40.83	26.66	34.16	44.16	-
50	55	55	59.16	56.66	54.16
100	73.33	67.5	74.16	73.33	76.66
150	78.33	79.16	82.5	82.5	90.83
200	89.16	85.83	92.5	91.66	97.5

A : *Artemisia herba-alba* JC: *Juniperus communis*

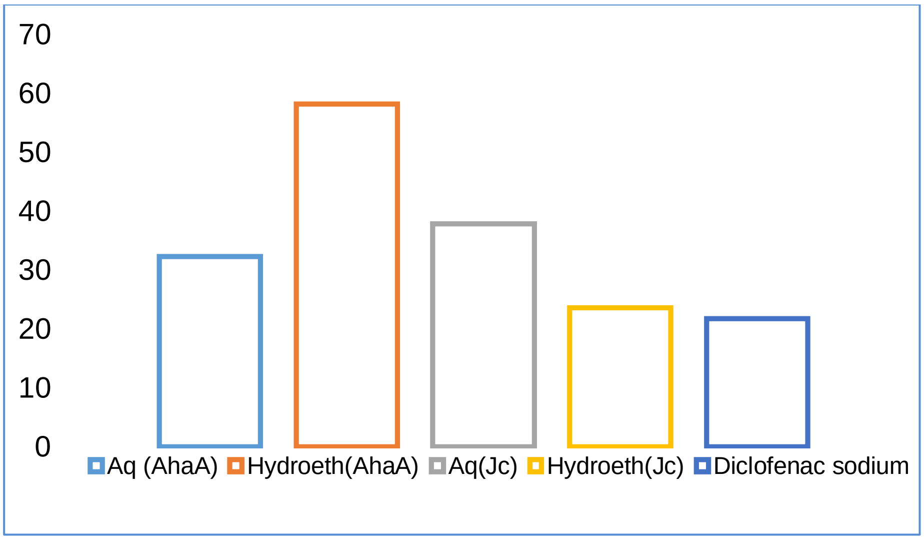

Figure 1. IC_{50} values of anti-inflammatory activity *of Artemisia herba-alba and Juniperus communis* extracts.

2.4. Conclusion

The findings of this study prove that hydroethanolic extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions have significant *in vitro* antioxidant, antibacterial, anti-inflammatory effects. The results discovered the presence of total phenolics and flavonoids, which enhance its various biological activities. These conclusions support the traditional use of mountainous *Artemisia herba-alba* and *Juniperus communis* in the treatment of inflammatory diseases. They may allow the development of pharmaceutical drugs to treat all disorders associated with the studied activities, such as inflammatory gastrointestinal diseases. However, supported *in vivo* research is required to understand the exact mechanisms of inhibiting free radicals, antibacterial and anti-inflammatory activities.

CHAPTER III:

Effect of *Juniperus communis* and *Artemisia herba-alba* extracts on probiotic properties of *Bacillus safensis* isolated from camel milk in the region of El Oued (Algeria)

3.1. Introduction

According to FAO and WHO, probiotics are "live microorganisms that, when administered in adequate amounts, confer health benefits on the host". Probiotics can be found in meals and supplements as well as in the intestines (Hill et al., 2014). Using probiotics has been demonstrated to enhance immune function by changing the composition of the intestinal microbiota and interacting with different immune cells. Probiotics' capacity to enhance immunity and promote health is therefore widely acknowledged (Mazziotta et al., 2023)**.**

The probiotics used had an impact on how these bacteria were produced. Biological sources include things like the human large and small intestines, breast milk, food sources like raw or fermented milk, and animal origins (Shewale et al., 2014).

Dairy products are the primary source of probiotics, which produce their enzymes and other beneficial elements. The safety and quality of dairy products are crucial assurances for the dairy industry's recovery. Because of its significance, bioactive components have drawn the attention of the government, researchers, and citizens among the numerous concerns pertaining to dairy safety (Xiong, Chen, et al., 2022; Xiong, Wen, et al., 2022).

Similarly, probiotics and whey fermentation have been extensively utilized in the creation of hypoallergenic milk in recent years (Zhao et al., 2023) .

Plant foods are frequently a good source of antioxidants, which are essential for avoiding disease, and they have a variety of therapeutic effects. To increase the activity of probiotic strains and shield them from the harmful effects of oxygen and its active derivatives, the concept of adding probiotics to a plant matrix is still being explored as a crucial element (Spacova et al., 2020).

The impact of plant extracts high in polyphenolic compounds on the development of probiotic bacteria and other microorganisms has been the subject of numerous investigations. It has been demonstrated that plant extracts inhibit the growth of bacteria linked to food deterioration as well as pathogenic and physiological gut microbiota (Haddadin, 2010).

Since ancient times, *Juniperus communis* has been utilized in traditional medicine and is regarded as a significant medicinal plant (K. Dhaka & A. Mittal, 2021), This covers the treatment of bronchitis, stomach pain, diarrhea, indigestion, and malignancies (Mansouri et al., 2011) , this is because phenolic compounds, which have been shown to have positive impacts on a variety of biological systems, are present in significant amounts in it (K. Dhaka & A. Mittal, 2021). Furthermore, these substances can alter the intestinal microbiota by favoring the growth of *bifidobacteria* and *lactobacilli* while suppressing the growth of harmful bacteria like *clostridium* (Tuck & Hayball, 2002).

Artemisia herba-alba has reportedly played a significant part in folk medicine across numerous civilizations, including North Africa, Arabic traditional medicine, and European medicine (Moufid & Eddouks, 2012) . Among these, it demonstrated numerous medicinal qualities, such as anti-diabetic, antimicrobial, antioxidant, antiradical, antispasmodic, antihypertensive, antimalarial, anthelmintic, antileishmanial, nematicidal, neurological pesticidal, allelopathic, and cytoprotective activities (Abou El Hamd et al., 2010).

The initial idea of this study was to separate and purify lactic acid bacteria from camel milk, which has probiotic properties. Additionally, to look into how the extracts from *Juniperus communis* and *Artemisia herba-alba* affects the probiotic properties of these strains.

3.2. Materials and Methods

3.2.1. Biological material

In January 2023, a sample of milk was taken from eleven-year-old camels (Camelus dromedarius) in the Al-Nakhla area (Al-Wed). The camels were fed olive pomace (Fatura) and son (Bran).

3.2.2. Qualitative phytochemical analysis

Based on color and/or precipitation reactions, a phytochemical examination is required to pinpoint the major families of secondary metabolites found in the studied plant's leaves, including polyphenols, tannins, alkaloids, saponins, flavonoids, terpenoids, free quinones, and reducing sugars. based on the experimental guidelines of Evans (2009).

3.2.3. Chromatographic analysis by (HPLC)

Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 µm), a Shimadzu type analytical column was utilized in a high performance liquid chromatography (HPLC) system, which was equipped with a universal injector (Hamilton 25 µL). It was a Shimadzu UV-VIS detector SPD 20A (Thammana, 2016) .

The analysis was carried out according to the method described by Jun et al. (2014). A solution of plant extract in the volume of 20 µL was injected into the mobile phase flow. Detection at = 268 nm was used to identify the separated phenols using the column for 40 to 50 minutes.The standards used in HPLC analysis are: gallic acid, chlorogenic acid, vanilic acid, caffiec acid, vanilin, p-coumaric acid, rutin, naringin, quercetin.

3.2.4. Isolation and identification of Probiotics strains

3.2.4.1. Isolation of Probiotic strains

In sterile tubes with a capacity of 10 ml each, the milk samples were dispersed. One milliliter of the parent suspension was used to dilute the milk in decimal amounts (10^{-1}, 10^{-2}, and 10^{-3}) in physiological saline solution. Into the mass of M17 agar, 1 ml of each dilution was inoculated, and the plates were then incubated in the oven at 37°C and 45°C (Ayyash et al., 2018). In order to maintain the purity of the cultures after isolation, colonies were subcultured on M17 medium and incubated at 30 or 45°C. The streak technique was used to purify strains on agar medium, and this was followed by microscopic examination (Laiche et al., 2019).

3.2.4.2. Phenotypic and molecular identification of probiotics strains

Macroscopic and microscopic examinations were performed followed by biochemical and physiological tests including the catalase test, the analytical profile index 10s gallery (API 10s gallery is a classification system for bacteria, its strips consist of wells containing dehydrated substrates such as the redox substrates, electrogenic substrates and luminogenic substrates to detect enzymatic activity, usually related to the fermentation of carbohydrate or catabolism of proteins or amino acids by the inoculated organisms), growth at different temperatures (10°C and 45°C), and NaCl sensitivity (2%, 4% and 6.5%).

The NucleoSpin Soil Kit (Macherey-Nagel, USA) was used to extract the genomic DNA from the bacterial isolates in accordance with the manufacturer's instructions. Thermo ScientificTM™, USA provided the Nanodrop spectrophotometer, which was used to measure the amount and purity of the DNA extracts. Using the universal bacterial primers for 16S rRNA 27F (5'- AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), a 1500-bp fragment of the 16S rRNA gene was amplified by PCR. (Jansen et al., 2006). The thermal cycling conditions were as follows: a 4-minute initial denaturation at 96 °C, 35 cycles of 30 s at 94 °C, 30 s at 57 °C, and 30 s at 72 °C, and a 5-minute final extension at 72 °C. The PCR products were processed for 45 minutes at 100 V in a gel electrophoresis system using 1% agarose gel 1xTE buffer. The Wizard SV Gel and PCR Clean-up System (Promega, New England) was utilized to purify the 16S rRNA PCR products, and an ABI-PRISM 3700 DNA automated sequencer (Applied Biosystems) was utilized for sequencing. The sequences were first edited using 4peaks V1.8 to ascertain their identity. They were then submitted to BLASTN (National Center for Biotechnology Information; http://blast.ncbi.nlm.nih.gov/Blast.cgi) for comparison with sequences that had been published on GenBank based on identity ranking (>97%) and E-values (0.0).(Nguyen et al., 2016)

3.2.4.3. Selection of probiotic strains

3.2.4.3.1. Acidity tolerance

The resistance of bacteria to acidic pH was determined according to the method described by Das et al. (2016) with some modifications. Bacterial strains were activated at 37 °C for 16 to 18 hours in a volume of 4 mL of sterile nutritional broth. The bacterial cultures were then centrifuged at 2500 for 10 minutes before being cleaned with PBS solution. The recycled pellets were centrifuged at 1,000 rpm for 5 minutes, then washed with PBS buffer. The pellet was divided into two tubes each containing 4 mL of feed broth, then HCl and NaOH were used to bring the pH value of each tube to 2 or 3. Successive dilutions were made up to 10^{-2} after exposure to acidic pH at t = 0 and t = 3 hours. After inoculating the mass onto nutrient agar, these dilutions were incubated at 37 °C for 24 to 48 hours. At t=0 and t=3, the viable count was calculated. The quantity of live cells is then measured.

3.2.4.3.2. Bile Tolerance

One of the essential probiotic selection characteristics is a strain's capacity to tolerate conditions comparable to those found in the human small intestine (Ren et al., 2014).In 4 ml of sterile nutritional broth, bacteria were activated at 37 °C for 16 to 18 hours. The pellets were then centrifuged once more for five minutes at a speed of 1000 rpm, followed by a PBS buffer wash. Divide the granules into 3 tubes, each of which contains 3 ml of nutritional broth and 1%, 3%, or 5% bile, respectively. Following a bulk inoculation on nutrient agar, they were incubated for 24 to 48 hours at 37 °C. Decide the relevant number at t = 0 h and at t = 3 h.

3.2.4.3.3. Antimicrobial Activity

Using the Barefoot and Klaenhammer (1984) well diffusion method, sterile Petridishes were filled with a volume of Mueller-Hinton agar media. After the medium has solidified, the pathogenic strain suspension is injected onto the plates, and then sterile 6-mm-diameter wells are excavated using a cookie cutter. (Durham bell) on the M17 agar, which will be filled with 60 to 80 µl of filtered and neutralized supernatant after a lactic strain culture was centrifuged at 4000 rpm for 15 min. Zones of inhibition surrounding the wells indicate antibacterial activity after the dishes have been incubated at 37°C for 24 hours. The bathogenic bacteria tested were: *Enterococcus faecalis* ($Gram^{+}$), *Staphylococcus haemolyticus* ($Gram^{+}$), *staphylococcus aureus* ($Gram^{+}$), *Klebsella sp* ($Gram^{-}$), *Salmonella sp* ($Gram^{-}$), *Escherichia coli* ($Gram^{-}$). For anti-fungal activity, *Candida albicans* ($Gram^{+}$) strain was examined.

3.2.4.3.4. Antibiotic sensitivity

This test was performed using the method of Bazireh et al. (2020), but with some minor changes. On M17 agar medium, the lactic strains were grown and kept at 30°C for 24 hours. Each strain's bacterial colonies were then put into physiological salin solution after having their OD adjusted to 0.5 McFarland. The antibiotic discs, which were comprised of Gentamycin (10 µg), Penicillin (10 IU), Oxacilline (10 µg), Amoxicilline (10 µg), and Ciproflaxacine (5 µg), were then put on the surface (four per box) of the dishes containing M17 agar. The zones of inhibition were determined by including the antibiotic disk's (6 mm) diameter into the zone's breadth after the dishes had been incubated at 30°C for 48 hours.

3.2.5. *In vivo* evaluation of *Juniperus communis* and *Artemisia herba-alba* extracts on probiotic properties

In this experiment, the effect of the aqueous extract on *Bacillus safensis* strain was chosen to be studied, as the aqueous extract showed better biological activities and *Bacillus safensis* strain had high probiotic properties.

3.2.5.1. Induction of intestinal inflammation

The rats were divided into 5 groups of 5 individuals each: The first group received distilled water by gavage . The second group received a dose of 0.5mL of castor oil after fasting for 16 hours every day by gavage, for a week. The third group received a dose of 0.5mL of castor oil after fasting for 16 hours every day by gavage, for a week, then it treated by 0.5mL of a probiotic lactic acid bacteria (*Bacillus safensis)* diluted in 5ml of physiological salin solution, for one week. The fourth group was received a dose of 0.5mL of castor oil after fasting for 16 hours every day by gavage, for a week, then it treated daily with mixture of *Bacillus safensis* solution and 200 mg/kg of *Juniperus communis* aqueous extract (JAE). The fifth group was received a dose of 0.5mL of castor oil after fasting for 16 hours every day by gavage, for a week, then it treated daily with mixture of *Bacillus safensis* solution and 200 mg/kg of a *Artemisia herba-alba* queous extract (JAE).

3.2.5.2. Blood samples

Blood sampling is done at the time of sacrifice of the rats (the rats were fasted for 24 hours before being sacrificed), the blood collected in EDTA tubes to be used for the biochemical parameter assays (Blood count: BCN).Then the samples were centrifuged for 15 min at 4000 rpm. The plasma was collected and frozen until it was used for the determination of certain inflammatory assessment parameters: C - reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR).

3.2.5.3. Histological study

After washing the organs with physiological saline solution (NaCl 0.9%), the intestine was fixed in alcoholic Bouin. The sections were kept at cold temperature, then the incisions were made using a Leica microtome to a thickness of 6 µm. The haematoxylin-eosin stain was used on the cuts, with the haematoxylin giving the nucleus a blue-purple color. Conversely, eosin is causing the cytoplasm and other fundamental cellular components to become pink (Tlili et al., 2017).

3.3. Results

3.3.1. Qualitative phytochemical analysis

A phytochemical screening was performed (Table 1) to determine whether any chemical groups were present in *Juniperus communis* and *Artemisia herba-alba* extracts.

The table indicates that the aqueous extract of *Artemisia herba alba* include the following: flavonoids, polyphenols, reducing sugars, free quinones, and cardiac carbohydrates, saponins. But alkaloids was found to be missing.

According the results, the most components present in *Juniperus communis* aqueous extract: polyphenols, tannins, terpenoids, and quinones release in high quantities, on the other hand flavonoids, saponins, reducing sugars and cardiac glycosides presented by a small quantity, while the alkaloids are absent.

Table 1: Phytochemical qualitative analysis of *Juniperus communis* and *Artemisia herba-alba* aqueous extracts.

Secondary Metabolites	Reagent	*Artemisia herba-alba*	*Juniperus communis*
polyphenols	$FeCl_3$	++	++
Tannins	$FeCl_3$	++	++
Flavonoids	Mg^{2+}	+	+
Alkaloids	Dragendorff	-	-
Terpenoids	H_2SO_4	++	++
Saponins	The foam	+	+
Quinones bast	NaOH	++	++
Reducing sugars	Fehling	-	+
Cardiac glycosides	H_2SO_4	+	+

+: Present compound ; -: Absent compound

3.3.2. Chromatographic analysis (HPLC)

Comparison of retention times and peak areas of standards with those recorded in the chromatogram (figure 1) allowed the identification and quantification of 9 probable phenolic compounds in aqueous extract of *Artemisia herba-alba*.The results show the presence of flavonoids in significant concentrations (Rutin, Naringin, Quercetin), and gallic acid compound presented in low concentration.

HPLC detected seven phenolic components in *Juniperus communis* aqueous extract. the results show the presence of quercitin in significant concentrations, while the rest compounds were separated at little concentration. The concentration of these compounds and their retention time are presented in the table 2.

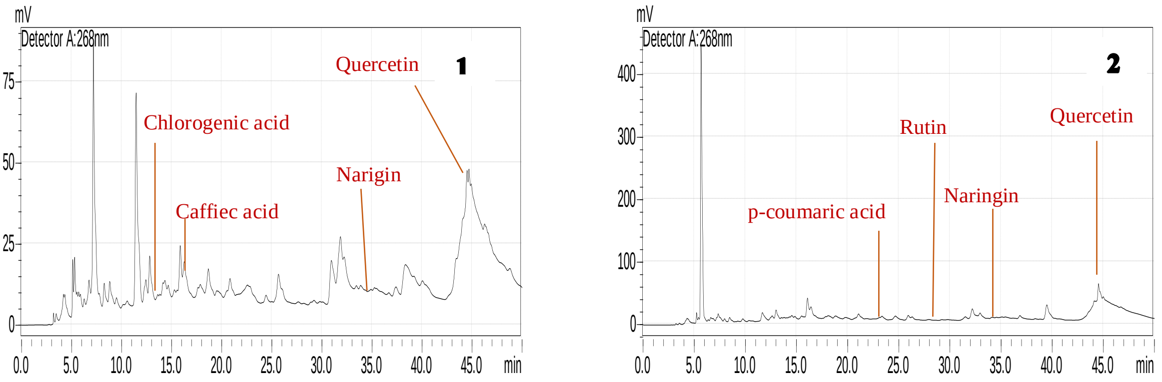

Figure 1: HPLC chromatographic profile of *Juniperus communis* (1) and *Artemisia herba-alba* (2) aqueous extracts

Table 2: Some phenolic compounds identified by HPLC in *Juniperus communis* and *Artemisia herba-alba* aqueous extracts

Artemisia herb-alba aqueous extract			*Juniperus communis* aqueous extracts		
Retention Time (min)	Compounds	Concentration (µg/mg extract)	Retention Time (min)	Compounds	Concentration (µg/mg extract)
5.378	Gallic Acid	1.087	5.296	Gallic Acid	0.827
13.556	Chlorogenic Acid	1.446	13.610	Chlorogenic Acid	1.270
15.532	Vanilic Acid	1.362	15.538	Vanilic Acid	0.294
16.420	Caffiec Acid	1.721	16.244	Caffiec Acid	1.642
21.169	Vanilin	1.667	-	Vanilin	-
23.723	p-Coumaric Acid	4.186	-	p-Coumaric Acid	-
28.748	Rutin	5.505	28.175	Rutin	0.375
34.897	Naringin	6.297	34.897	Naringin	1.331
44.759	Quercetin	8.706	44.897	Quercetin	8.794

3.3.3. Isolation and identification of probiotic strains

3.3.3.1. Isolation of Probiotic strains

Based on biochemical tests and morphological characteristics, eight colonies were isolated after gram staining, but we chose only four to study their probiotic properties. They are symbolized by SM45°c, 10^{-1} 45°c, 10^{-1} 37°c, 10^{-2} 37°c.

3.3.3.2. Phenotypic and molecular identification of probiotic strains

From a macroscopic examination of the cultures on M17 agar, the isolates were both big and small, white and creamy. The homogeneous form of bacteria reflects their purity, this indicates that every strain had a Gram positive. Microscopic observation revealed that the form of cells is ovoid or spherical cocci form, arranged in pairs or in chains (Figure 2).

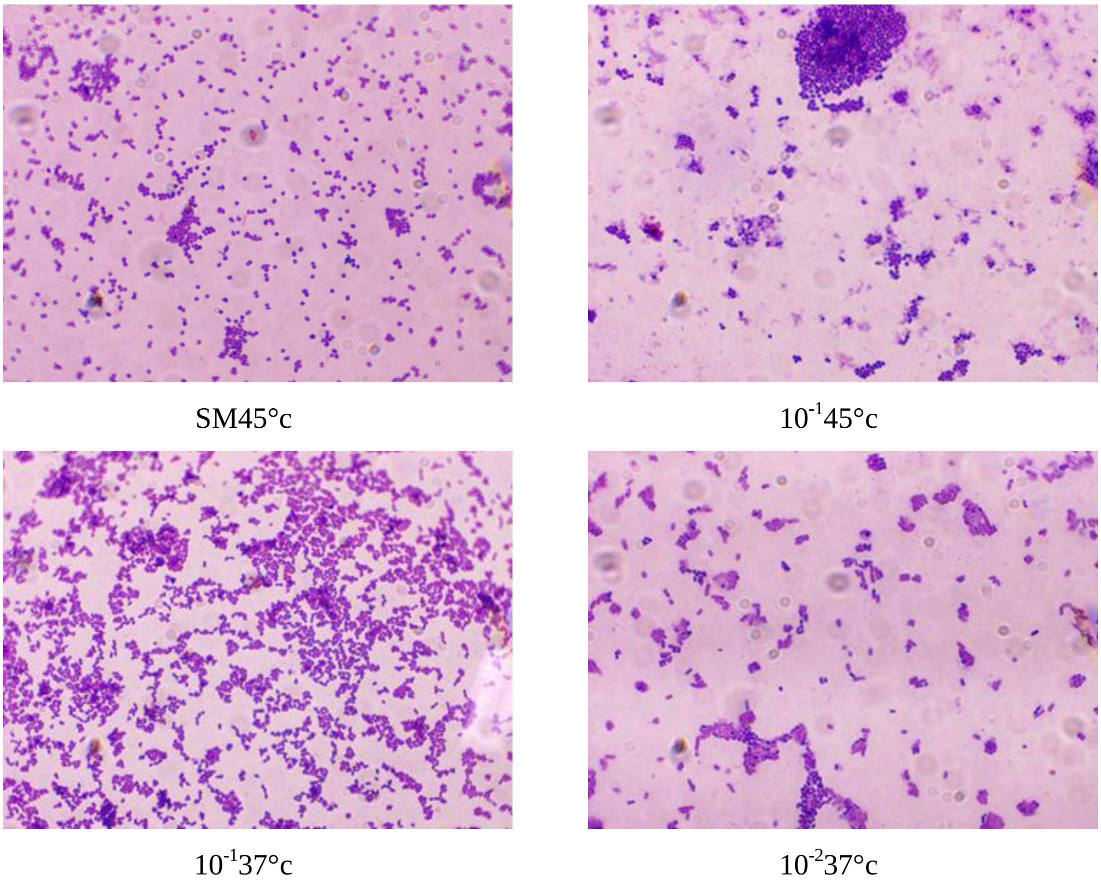

Figure 2: Colonies' microscopic appearance (Gx100)

The strains are generally catalase (+) positive, showed better growth at different concentrations of NaCl (2%, 4% and 6.5%). Probiotic strains demonstrated similarity in growth at the incubation temperature of 10°C, a significant increase in growth is observed for most of the strains at 45°C. Based on macroscopic, microscopic aspects, as well as on certain biochemical and physiological tests (Table 3), 50% of the isolates were identified as *Streptococcus thermophiles* and these included SM45°C, 10^{-1}45°C. Fifty percent of the isolates belonged to *Lactococcus lactis* (10^{-1}37°C, 10^{-2}37°c).

The DNA sequencing results were analyzed using NCBI-BLAST. The analysis revealed that the 16S rDNA sequence of *Streptococcus thermophilus* exhibited the highest degree of identity with *Bacillus pumilus* (accession number: NZ_PTXV01000013.1, 99%), while *Lactococcus lactis* showed identity with *Bacillus safensis* (accession number: NZ_CP043404.1, 100%).

Table 3: Biochemical and physiological criteria of probiotics isolated from camel milk

Tests		Strains			
		SM45°c	**10^{-1}45°c**	**10^{-1}37°c**	**10^{-2}37°c**
Catalase		+	+	+	+
T	45 C°→10 C°	-	-	/	/
	37 C°→10 C°	/	/	++	++
	37 C°→45 C°	/	/	+++	++
NaCl	2%	+++	+	++++	++++

	4%	++	++	++++	++++
	6.5%	+++	++	++++	++++
	PH	+++	+++	/	/
Api 10 sGallery	ONPG	+	+	+	+
	GLU	+	+	+	+
	ARA	+	+	+	+
	LDC	+	+	+	+
	ODC	+	+	+	+
	CIT	+	+	+	+
	H2S	+	+	+	+
	URE	-	-	-	-
	TDA	+	+	+	+
	IND	+	+	+	-

T: Temperature; NaCl: Sodium chloride; ONPG: O- Nitrophenyl- β- D- Galactopyranoside test; GLU: Glucose test; ARA: L-arabinose resistance test; LDC: Lysine decarboxylase test ; ODC: Ornithine decarboxylase test ; CIT: Citrate test; H_2S : Hydrogen Sulfide test; URA: Urease test; TDA: Tryptophan-deaminase activity; IND: Indol test.

3.3.3.3. Selection of probiotic strains

3.3.3.3.1. Acidity tolerance

The growth of *Bacillus safensis* decreased after incubating at pH =2 conversely at pH= 3 it increased and reached its greatest value. The growth of *Bacillus pumilus* is increased in all pH values after incubation. The highest values were after incubation at ph=3 (Table 4).

Table 4: Effect of acidic pH on *Bacillus pumilus* and *Bacillus safensis* viability in (log CFU/ml)

Strains	**PH=2**		**PH=3**	
Bacillus pumilus	t=0h	t=3h	t=0h	t=3h
10^{-1}	2.710	2.173	2.767	5.136
10^{-2}	3.158	3.822	3.843	3.915
	2.934± 0.316	2.997± 1.166	3.305± 0.760	4.525± 0.863
Bacillus safensis	**t**=0h	**t**=3h	**t**=0h	**t**=3h
10^{-1}	1.806	1.643	2.675	2.853
10^{-2}	2.643	2.799	3.694	3.880
	2.224±0.591	2.221±0.817	3.184±0.720	3.366±0.726

3.3.3.3.2.Bile Tolerance

The findings, which are presented in table 5, indicate that all strains are tolerant to bile salts at concentrations of 1%, 3%, and 5%, with the majority exhibiting better survival rates at the latter.

Table 5: Effect of bile salts on *Bacillus pumilus and Bacillus safensis* viability in (log CFU/ml)

Strains	**Bile concentration %**					
	1%	1%	3%		5%	
	t:0h	t:3h	t:0h	t:3h	t:0h	t:3h
Bacillus pumilus	1.363	1.416	1.912	1.567	1.547	1.625

Bacillus safensis	1.852	1.713	1.404	1.660	1.519	1.641

3.3.3.3.3. Antimicrobial Activity

According to the results, the strains of probiotics selected do not represent any activity antimicrobial against *Enterococcus faecalis, Staphylococcus haemolyticus, staphylococcus aureus, Candida albicans, Klebsella sp, Salmonella sp, Escherichia coli.*

3.3.3.3.4. Antibiotic sensitivity

According to table 6 can be concluded that *Bacillus safensis* is highly resistant to Oxacillin, Penicillin, and Amoxicillin, however it is susceptible to Gentamicin and Ciproflaxacine. While *Bacillus pumilus* was susceptible to Gentamicin, Ciproflaxacine, and Amoxicillin, it was resistant to Oxacillin and Penicillin.

Table 6: Antibiotic test results of *Bacillus pumilus* and *Bacillus safensis*

Antibiotics	*Bacillus pumilus*	*Bacillus safensis*
GEN 10	S (12mm)	S (15mm)
AX 10	S (24mm)	R (6mm)
CIP 5	S (18mm)	S (24mm)
OX 10	R (6mm)	R (6mm)
P 10	R (6mm)	R (6mm)

S: Susceptible, R: Resistant

3.3.4. *In vivo* evaluation of the *Juniperus communis* and *Artemisia herba-alba* aqueous extracts on probiotic properties

3.3.4.1. Macroscopic observation

The results of the macroscopic study (Table 7) showed that group 2 rats which did not receive any treatment, had signs of inflammation accompanied by diarrhea compared to first group rats. These symptoms disappeared in group 4 and 5 faster than group 3 after the administration of treatment solutions . Macroscopic observation provisionally confirms the protective effect of the studied plant with *Bacillus safensis* strains.

Table 7. Macroscopic results of experimental animals during *in vivo* study

	Group 1	**Group 2**	**Group 3** (*Bacillus safensis* Probiotic treatment)	**Group 4** (*Bacillus safensis* +JAE treatment)	**Groupe 5** (*Bacillus safensis* +AAE treatment)
In inflammation period					
Diarrhea indicator	-	++	-	+	-
Body weight					

(g)					
Befor	220.4±4.56	203.2±19.11	210.2±23.76	211.2±15.35	201.8±25.99
After	224±4.63	188.4±13.31	206.2±23.88	203.8±13.17	196.2±24.50
After dissection					
Intestine colour	Normal	dark brown	Brown	Normal	Brown
Intestine shape	Normal	Irritated	Normal	Normal	Normal
Intestine diameter(cm)	0.3 -0.4	0.2 - 0.3	0.2 - 0.4	0.3 - 0.5	0.2 - 0.5

3.3.4.2. Microscopic observation

Analysis of the histological sections was done under the supervision of Dr. Djellali Noudjoud from Debakh said hospital – El meghier. The intestinal section of the first group reveals a healthy structure with a healthy intestinal wall made up of the mucosa (epithelium, lamina propria, and muscular mucosa), the submucosa, the muscularis, and the sub-serous. It also has healthy and normal villi and is completely free of inflammation, tissue/cellular damage, and necrosis. As a result, the tissue appearance in the histological section is more or less regular. After the castor oil-treated rats were dissected, microscopic examination of the histological sections showed that the intestinal cell structure had been destroyed. Additionally, there were indications of a very serious inflammtion that led to lymphocytic cell grouping, a decrease in the height of the villi, and some villi that appeared necrotic, along with cellular lesions and a completely destroyed lamina propria. These findings demonstrated that the rats had diarrhea.

In comparison to the villi in group 2, the rats that received the probiotic treatment following the inflammation exhibit a partial return of the height of the villi along with the absence of necrosis, and the histological sections reveal a less damaged intestinal structure and mucosa that appears less damagesd. There is also a decrease in the rate of inflammation. Rats that treated with *Bacillus safensis* and and studied plants *(Juniperus communis, Artemisia herba-alba)*extracts responded more effectively to the treatment; all of the symptoms seen in group 2 vanished, the inflammation signs vanished entirely, and the villi's height nearly entirely returned, where we discovered a healthy intestinal wall construction with its constituent parts in contrast to the damaged one, as well as a reduction in the degree of inflammation and the lack of necrotic cells (Figure 3).

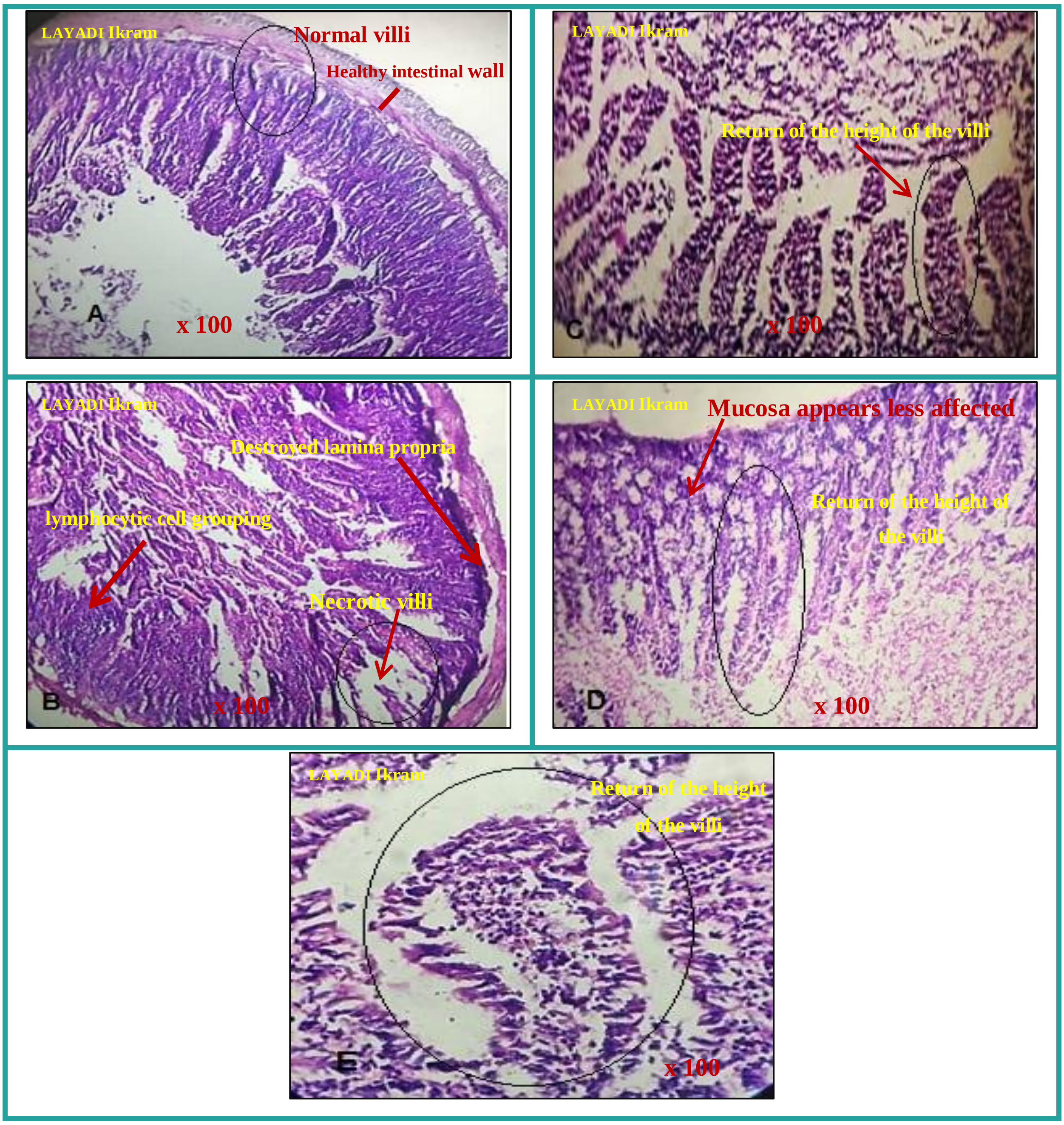

Figure 3. Microscopic observation of the intestine histological section in rats of different groups (x 100). (A) first group; (B) second group ; (C) third group (*Bacillus safensis)* ; (D) fourth group (*Bacillus safensis* + JAE); (E): fifth group ((*Bacillus safensis* + AAE)

3.3.4.3. Determination of blood biochemical parameters

3.3.4.3.1. Compmet blood count (CBC)

From CBC results (Figure 4), it was observed that the first group had an increase in red blood cell levels, hematocrit, platelet count and White blood cell values compared to the other groups. While it was observed that there was a decrease in these values after treatment with *Bacillus safensis* strains, they returned to almost normal values for the group that received the combination of *Bacillus safensis* and *plant extracts (Juniperus communis, Artemisia herba-alba).*

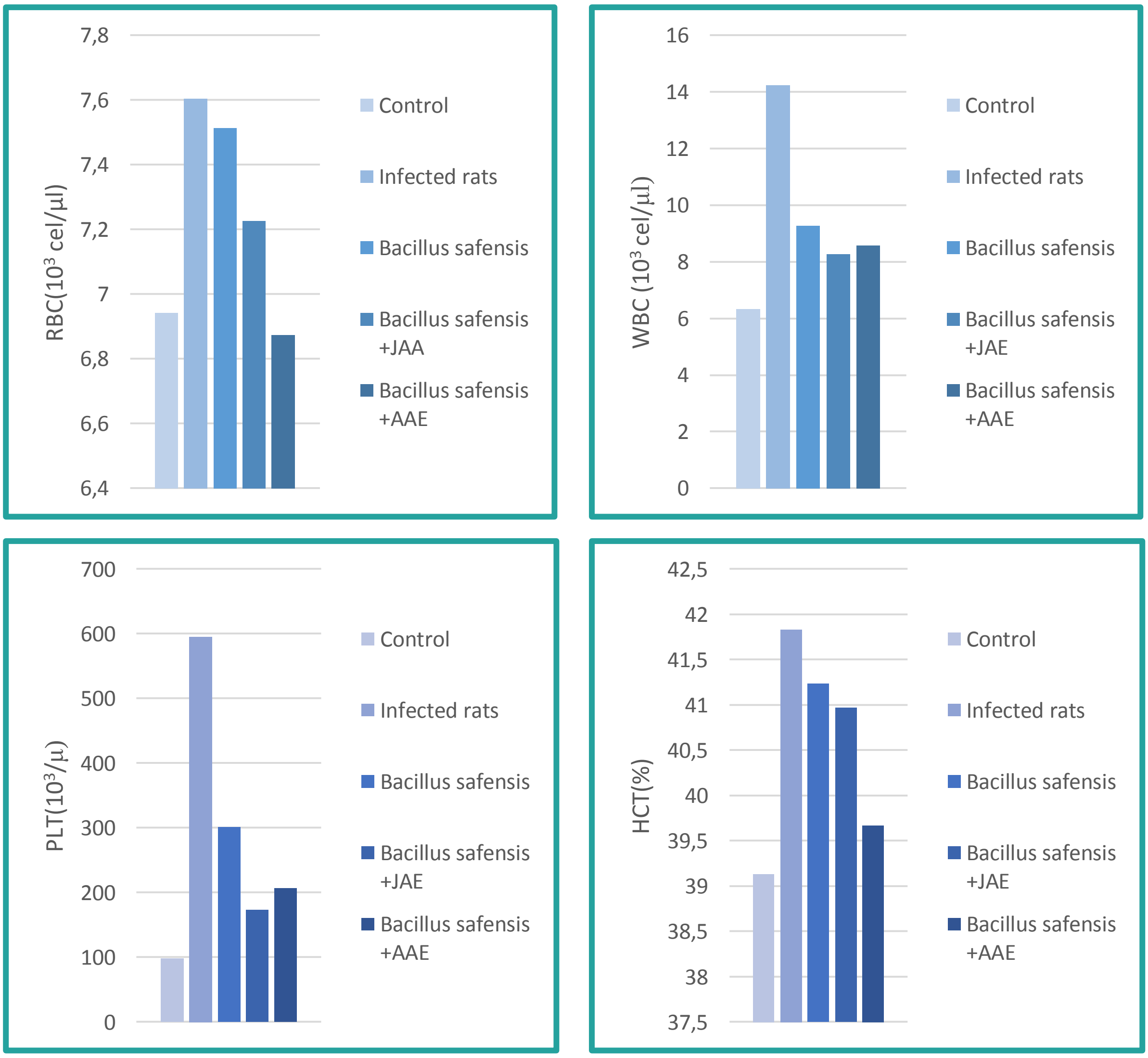

Figure 4. Determination of hematological parameter (CBC) of experimental rats

3.3.4.4. Inflammatory assessment paramaters

3.3.4.4.1. Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate (ESR) is a commonly performed hematology test that may indicate and monitor an increase in inflammatory activity within the body caused by one or more conditions such as autoimmune disease, infections, or tumors (Tishkowski & Gupta, 2020). The ESR results (Table 8) showed that there was a significant increase in the ESR parameter in the second group compared to others, while it decreased in the treated groups, with more effectiveness recorded in the fourth and fifth groups.

3.3.4.4.2.C-reactive protein (CRP)

The findings demonstrated the presence of severe inflammtions in group 2, and treatment with *Bacillus safensis* probiotics in group 3 reduced the severity of inflammation, also the effectiveness of the mixture (*Bacillus safensis* +JAE or AAE treatment) showed a higher anti-inflammatory effect in groups 4 compared to group 3 (Table 8).

Table 8. determination of inflammatory assessment parameters (CRP, ESR) in experimental rats

	Group 1	Group 2	Group 3 (Bacillus safensis treatment)	Group 4 (Bacillus safensis +JAE treatment)	Group 5 (Bacillus safensis +AAE treatment)
ESR (mm/h)	1.53±0.29	5.09±0.32	4.37±0.38	3.96±0.19	4.13±0.15
CRP (mg/dl)	3.65±0.35	9.93±1.81	6.26±0.71	5.15±0.24	6.29±0.5

3.4. Discussion

The aqueous extract of *Artemisia herba alba* include the following: flavonoids, polyphenols, reducing sugars, free quinones, and cardiac carbohydrates, saponins. Benyahia et al. (2021) state that numerous investigations have demonstrated that *Artemisia herba alba* plant is rich in phytochemicals like polyphenols, alkaloids, flavonoids, lactones, tannins, etc., yet additional research has shown that this plant lacks several of these beneficial substances.

the most components present in *Juniperus communis* aqueous extract: polyphenols, tannins, terpenoids, and quinones release in high quantities, on the other hand flavonoids, saponins, reducing sugars and cardiac glycosides presented by a small quantity, while the alkaloids are absent.The results obtained by Garg (2010a), are similar to the results of the qualitative phytochemical analysis of *Juniperus communis* extracts. Also Fernandez and Cock (2016) found in their screening chimique of aqueous extract the same secondary metabolites but without the Free quinones . On the other hand, Živić et al. (2019) conducted an analysis of ethanolic extract of the studied plant and obtained similar results.These levels may be influenced by a variety of elements, including storage conditions, extraction techniques, genotype, growth and maturation circumstances (Benhammou et al., 2007).

In *Artemisia herba-alba* aqueous extract, the results show the presence of flavonoids in significant concentrations (Rutin, Naringin, Quercetin), and gallic acid compound presented in low concentration. The similar results was determinated by Bourgou et al. (2017) and Younsi et al. (2016). EL Hajli et al. (2024) reported that Six chemicals are included in the aqueous extract: rutine, gallic acid, caffeic acid, hydroxybenzoic acid, and syringic acid.

The aqueous extract of *Juniperus communis* contained seven phenolic components identified by HPLC. It was also observed that the extract contained a high concentration of quercetin, which facilitates many physiological processes in plants, such as photosynthesis, pollen development, antioxidant machinery, and seed germination. The findings of the qualitative phytochemical study of the extract of *Juniperus communis* are comparable to those of Garg (2010b). Numerous factors, including as storage conditions, extraction methods, genotype, and growth and maturation conditions, may have an impact on these levels (Benhammou et al., 2007). The cells of all isolated strains are ovoid or spherical cocci, and they are all Gram positive.This outcome closely resembles Hamed and Elattar (2013) findings. The fact that the strains are typically catalase (+) positive indicates that the bacterial production of the catalase enzyme, which breaks the link and eliminates the toxicity of H_2O_2, is what causes the bubbles to occur (Andhikawati & Permana, 2022). Every isolate grew more effectively at varying NaCl concentrations (2%, 4%, and 6.5%).As the concentration of NaCl rose (from 2% to 6.5%), the growth of *L. paracasei* L2 decreased, and the maximum growth rate (61.74%) was observed at 2% NaCl, according to M'hamed et al. (2022). Furthermore, Qin et al. (2022) verified that probiotics can thrive in both high-saline and high-alkaline environments. The majority of the strains exhibit a notable increase in growth at 45°C, suggesting that lactic acid bacteria may proliferate at elevated temperatures. Laiche et al. (2019) also reported the isolation of *Bacillus pumilus* and *Bacillus safensis* from camel's milk. . After comparing the 16s rDNA sequenced in this study with the NCBI database, we discovered that the strains identified are members of the *Bacillus pumilus* and *Bacillus safensis* genera. At pH = 3, *Bacillus safensis* and *Bacillus pumilus* grow more. In their study on the tolerance of several probiotic bacteria to artificial intestinal gastrics and juices, Yasmin et al. (2020) found that the isolated strains could survive for two hours at pH values of 2 and 3. The results show that at 5% bile salt concentrations, all strains show improved survival rates. Numerous studies have found that intestinally derived probiotics, such as lactobacilli, have developed a resistance against the detergent impact of bile salts (Begley et al., 2006; Zhang et al., 2023). The ability of the isolates to manufacture the intracellular bile salt hydrolase enzyme (BSH) may be connected to this (Horáčková et al., 2018). The poisonous effects of castor oil seeds *(Ricinus communis*, Euphorbiaceae), which contain ricin and are one of the relatively well-studied members of the phytotoxins are the cause of the emergence of inflammation signs in rats. Therefore, the first sign is a decrease in body weight, which appears about 12 hours after a ricin dosage is administered. Diarrhea with inflammation is frequently observed, this is most likely due to an extensive intestinal lesion (Balint, 1974).

The reason for the reduction in inflammatory symptoms following *Bacillus safensis* treatment is that this probiotic strain benefits humans, primarily at the gut level(Heyman & Ménard, 2002). Moreover, consuming doses of *Bacillus safensis* helps them regain equilibrium because diarrhea may be linked to alterations in the gut bacterial flora(Ko et al., 2011). When Bacillus safensis strains and medicinal plants *(Juniperus communis, Artemisia herba-alba)* were combined, the symptoms in groups 4 and 5 vanished. Research indicates that plant biocompounds such phenols improve the anti-inflammatory effects of probiotics.

Microscopic examination of the histological sections of the rats given castor oil showed that the intestinal cells had been damaged and that there were indications of a very significant inflammations. Due to Most body tissues and organs are frequently affected by ricin. There has been observed variation in the extent of anatomical and functional changes brought on by extended exposure of a particular organ or tissue to ricin. Out of all the internal organs, the intestines are the most affected by ricine since it frequently enters and leaves the body through them. This effect is also associated with a few minor changes in the mitochondria and mainly impacts the smooth endoplasmic reticulum of intestinal cells. It also prevents the production of proteins (Balint, 1974; Guo et al., 2024).

Because probiotics have the ability to strengthen the intestinal barrier by preserving tight junctions and stimulating mucin synthesis, group 3's histological sections display less damaged intestinal structure and a mucosa that appears less affected and has a lower rate of inflammation than group 2. The rats who received a combination of *Bacillus safensis* and the extracts from the examined plants responded to treatment more well than group 3, where all inflammation symptoms vanished. This is because the extracts' active ingredients regulated the intestinal flora, which in turn generated active metabolites with anti-inflammatory and potentially intestinal barrier-passing qualities during intestinal transit. In healthy conditions, this would increase the inhibitory tone of the intestinal milieu, and in pathological conditions, it would help reduce inflammation (Menard et al., 2004).

Similar to those seen in inflammation disorders, the group 2 elevated red blood cell, hematocrit, platelet count, and white blood cell values were noted. Because probiotics enhance blood biochemical parameters, group 3's hematological indices have decreased(Biswas et al., 2022). In group 4 and 5, however, it was discovered that *Juniperus communis* and *Artemisia herba-alba* improved the qualities of *Bacillus safensis*, including its positive effects on rats' hematological.

Because of the harmful effects of the ricin component in castor oil, group 2's elevated CRP and ESR test results show the presence of serious intestinal infllammations. It has been shown that *lactobacilli*, *bifidobacteria*, or probiotic combinations can treat inflammatory bowel diseases in humans (Borruel et al., 2002; Gionchetti et al., 2000; Gupta et al., 2000), and digestive disorders in experimental animals (K. Madsen et al., 2001; K. L. Madsen et al., 1999). The decrease in CRP and ESR test values in group 3 rats signifies the end of inflammatory symptoms. In neonatal rats with stress-induced enterocolitis, necrotizing enterocolitis was avoided by early *Bifidobacterium* infantis therapy.

Compared to group 3, group 4 and 5's administration of a *Bacillus safensis* and studied plants mixture was more successful in removing inflammatory markers. This was demonstrated by research by Palhares Campolina et al. (2021) on how the extracts' active ingredients alter intestinal flora, hence lowering the incidence of digestive disorders. Probiotic strains produce substances that can cross the intestinal barrier and have an anti-tumor necrosis factor-alpha effect (Menard et al., 2004). The balance of intestinal flora then causes macrophages and T cells from the inflamed colon to produce and secrete more interleukin 10 (Pathmakanthan et al., 2004).

3.5. Conclusion

This research's goal was to examine how *Juniperus communisa* and *Artemisia herba-alba* aqueous extracts affect on probiotic properties of *Bacillus safensis* isolated from camel milk in the region of El Oued (Algeria). The extracts is high in quercetin, according to the chromatographic profiles of the phenolic components. Probiotic strains are isolated from camel milk, it is rich in *Bacillus safensis* and *Bacillus pumilus*. The results of this study showed that *Juniperus communis* which contains a significant amount of polyphenols and flavonoid can play a major role in the activity of probiotic bacteria such as *Bacillus safensis*. This demonstrates that it has a favorable impact on the digestive system.

GENERAL CONCLUSION AND RECOMMENDATIONS

The Algerian flora is one of the richest in the world and contains many endemic species. Eastern Algeria is home to several of them and most are used medicinally. These medicinal plants have very important biological properties linked certainly to the therapeutic virtues attributed to an extraordinary range of bioactive molecules synthesized by plants not only as chemical agents against diseases, herbivores and predators but also as medicinal agents such as antioxidants and antibacterials.

The current study focuses on two fragrant medicinal herbs, *juniperus communis* and *Artemisia herba-alba*, that have been used for a long time in Algeria to treat gastrointestinal ailments. The research's primary objective is evaluation of *in vivo* effect of aqueous extracts obtained from common plant species used to treat gastrointestinal disoders by traditional healers (*Artemesia herba-alba*, *Juniperus communis*) on probiotic bacteria properties isolated from camel milk in the El-Oued region.

Ethnobotanical survey investigation lists 47 medicinal plant species from 22 botanical families that traditional healers in the Oued Souf region use to treat digestive system ailments and the most often used species are *Artemisia herba-alba* (UV value =0.85), *Juniperus communis* (UV value =0.75).

HPLC analysis dimonstrated that *Artemisia herba-alba* and *Juniperus communis* aqueous extracts have a significant concentration of flavonoids while the quercetin is the majority compound.

Qualitative phytochemical screening highlighted the presence of polyphenols, tannins, flavonoids, terpenoids, free quinones, cardiac glycosides, in studied medicinal plants and both extracts with the absence of alkaloids in both extracts. Saponins, reducing sugars, are present in one extract and absent in the other.

The quantitative estimation of total polyphenols of *Artemisia herba-alba* indicated that hydroethanolic extract exhibited a high content of polyphenols with 104.54 ± 0.35 mg GAE / g compared to aqueous extract (58.89 ± 0.22 mg GAE / g). Whereas *Juniperus communis* aqueous extract demonstrated a greater polyphenols value (103.80±0.30 mg GAE/g) than the hydroethanolic extract (78.11 ±0.27 mg GAE/g).

Regarding the total flavonoid content, the aqueous and hydroethanolic extracts of *Artemisia herba-alba* showed lower content (4,88±0,075 and 2,06±0,03 mg QE/g respectively). But *Juniperus communis* hydroethanolic extract had the highest amounts of total flavonoids (17.05±0.13 mg QE/g) than aqueous extract (15.85±0.80 mg QE/g).

Lactic acid bacteria strains are isolated from camel milk. It is rich in probiotic bacteria (*Lactococcus lactis*, *Streptococcus thermophilus*) which have a role in preserving the digestive system. According to p henotypic and molecular identification, the analysis revealed that *Lactococcus lactis* shown identity with *Bacillus safensis* whereas *Streptococcus thermophilus* had the highest degree of identity with *Bacillus pumilus.*

when *Bacillus safensis* strains and medicinal plant *(Juniperus communis* or *Artemisia herba-alba)* were combined, the macroscopic inflammation symptoms vanished, revealing an almost complete return of the villi's height, also there is a reduction in the degree of inflammation and the absence of necrotic cells, as well as the presence of a healthy intestinal wall structure with its constituent parts as opposed to the damaged one. The mixture improved the qualities of *Bacillus safensis* including the positive effects on the hematological of rats, and showed a higher anti-inflammatory effect by lowering ESR and BCR values.

In light of this, it will be required to:

- ✓ Make investments in phytochemical screening of plant extracts from various of *Artemisia herba-alba* and *Juniperus communis* sections in order to quantify important secondary metabolites for biotechnological uses.
- ✓ Explaining the exact mechanism by which plants enhance probiotics.
- ✓ Exploiting other therapeutic properties of probiotics, namely: improving the immune system.

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APPENDICES

Appendix 01. *Juniperus communis* and *B. Artemisia herba-alba*

A. *Juniperus communis* (Bais et al., 2014)

B. *Artemisia herba-alba* (Abderrhmane, 2022)

Appendix 02. In vitro effect of the *Juniperus communis* and *Artemisia herba-alba* extracts on probiotics

1. Bile salt resistance

- ***Juniperus communis***

The findings demonstrate that both aqueous and hydroethanolic extracts increased the number of developing colonies when compared to the growth that occurred before to utilizing the extract; however, aqueous extract produced a faster rate of colony development than hydroethanolic extract.

- ***Artemisia herba-alba***

The results obtained showed that before incubation, the growth of *Bacillus safensis* with *Artemisia herba-alba* aqueous extract was higher than its growth with hydroethanolic extract at most bile salt concentrations. Following incubation, it was shown that the hydroethanolic extract at most doses causes the growth to reach its maximum value.

Table a. Effect of acid bile salt on the viability of *Bacillus safensis* in (log CFU/ml) in the presence of different concentrations of *Juniperus communis* and *Artemisia herba-alba* extracts.

			1%		3%		5%	
			t: 0h	t: 03h	t: 0h	t: 03h	t : 0h	t: 03h
Juniperus communis	Aqueux concentrations (mg/ml)	2.5	4.41	4.38	4.38	4.63	4.48	4.64
		5	4.62	4.54	4.35	4.68	4.68	4.64
		10	4.92	4.69	4.42	4.40	4.56	4.68
	Hydroethanolic concentrations (mg/ml)	2.5	4.30	4.18	4.29	3.94	4.29	4.28
		5	4.25	4.43	4.28	4.32	4.40	4.44
		10	4.33	4.14	4.14	4.02	4.17	4.18
Artemisia herba-alba	Aqueux concentrations (mg/ml)	2.5	4.561	4.48	4.23	4.38	4.72	4.35
		5	4.37	4.50	4.30	4.54	4.44	4.06
		10	4.54	4.72	3.80	4.57	4.62	4.46
	Hydroethanolic concentrations (mg/ml)	2.5	4.25	4.63	4.36	4.65	4.32	4.62
		5	4.32	4.55	4.27	4.29	4.17	4.44
		10	4.34	4.29	4.42	4	4.21	4.32

2. Acidity tolerance test

- ***Juniperus communis***

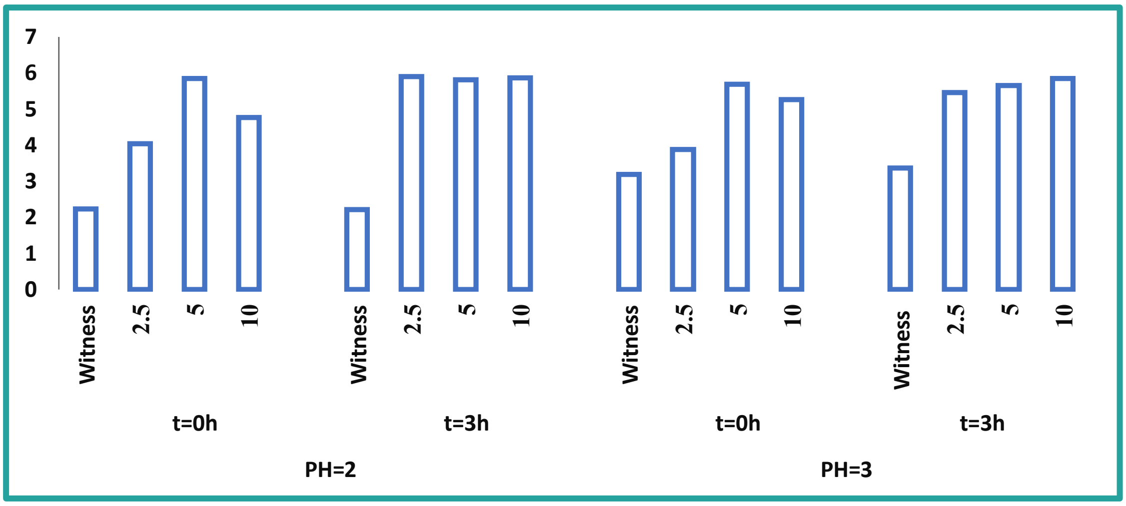
The findings of the acidity tolerance test showed that in pH= 2 or pH= 3 after incubation in 10 mg/ ml concentrations of aqueous extract, there is an overall rise in the number of colony growths.

As for the hydroethanolic extract, there is a direct proportion between the increase in acidity values and the rate of bacterial reproduction at all required concentrations.

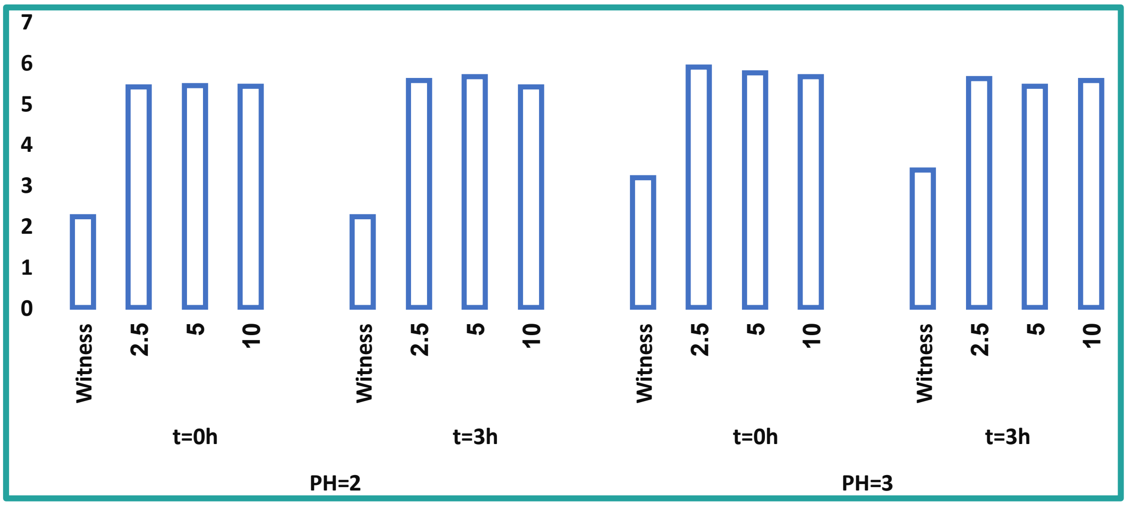
- ***Artemisia herba-alba***

In pH= 2 or pH= 3: After the incubation period, it is observed that there is a slight increase in the growth of *Bacillus safensis* compared to before incubation, at various concentrations [2.5; 5 ; 10 mg/ml] of *Artemisia herba-alba* hydroethanolic extract.

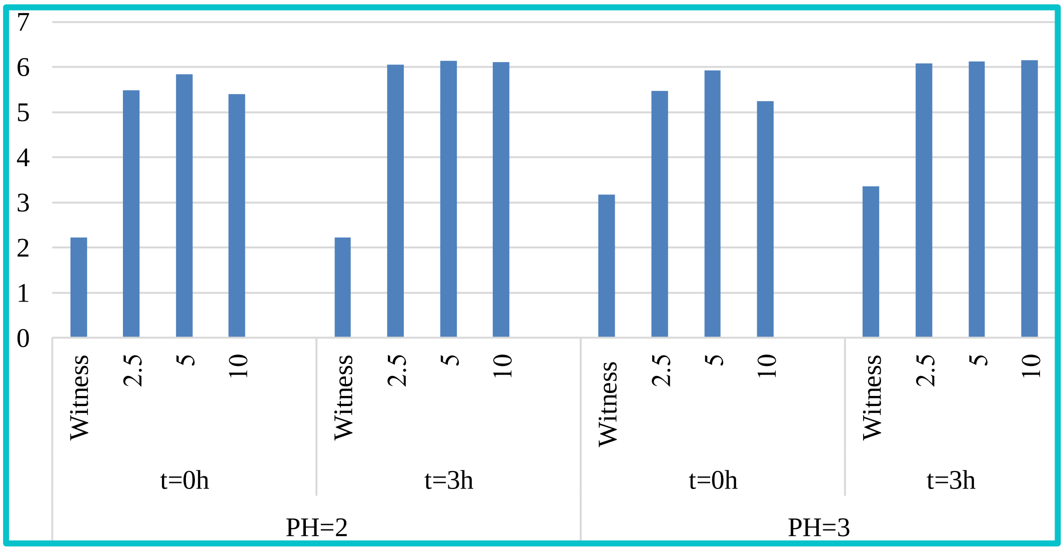
Based on our results, we can affirm that there is a clear correlation between the increase in the concentration of the aqueous extract and the rate of bacteria development.



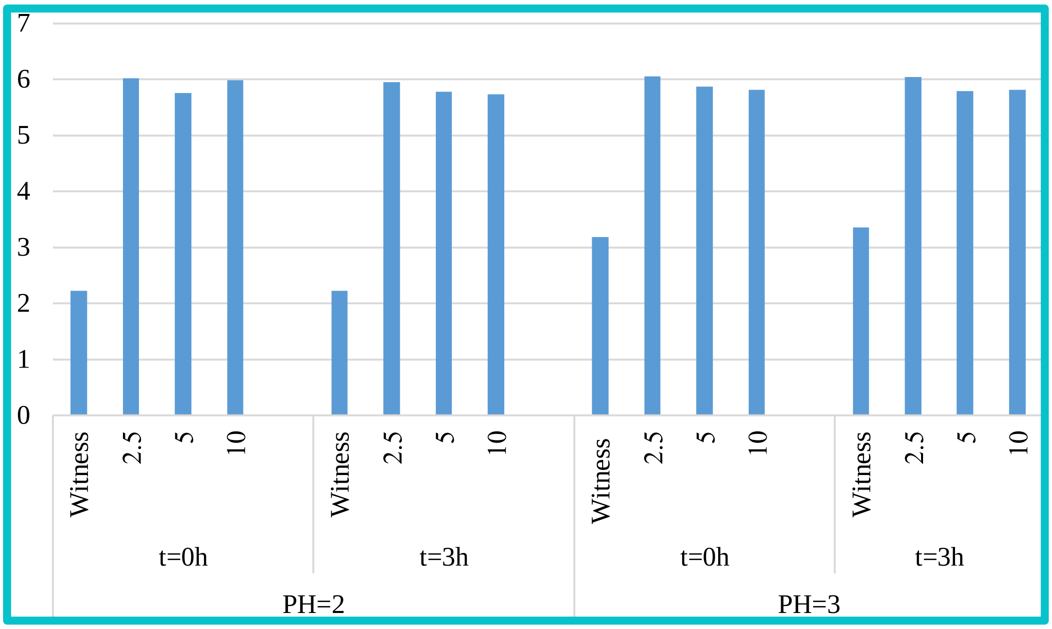
A. Effect of acid pH on the viability of *Bacillus safensis* in (log CFU/ml) in the presence of different concentrations *of Juniperus communis* aqueous extract.



B: Effect of acid pH on the viability of *Bacillus safensis* in (log CFU/ml) in the presence of different concentrations of *Juniperus communis* hydroethanolic extract.

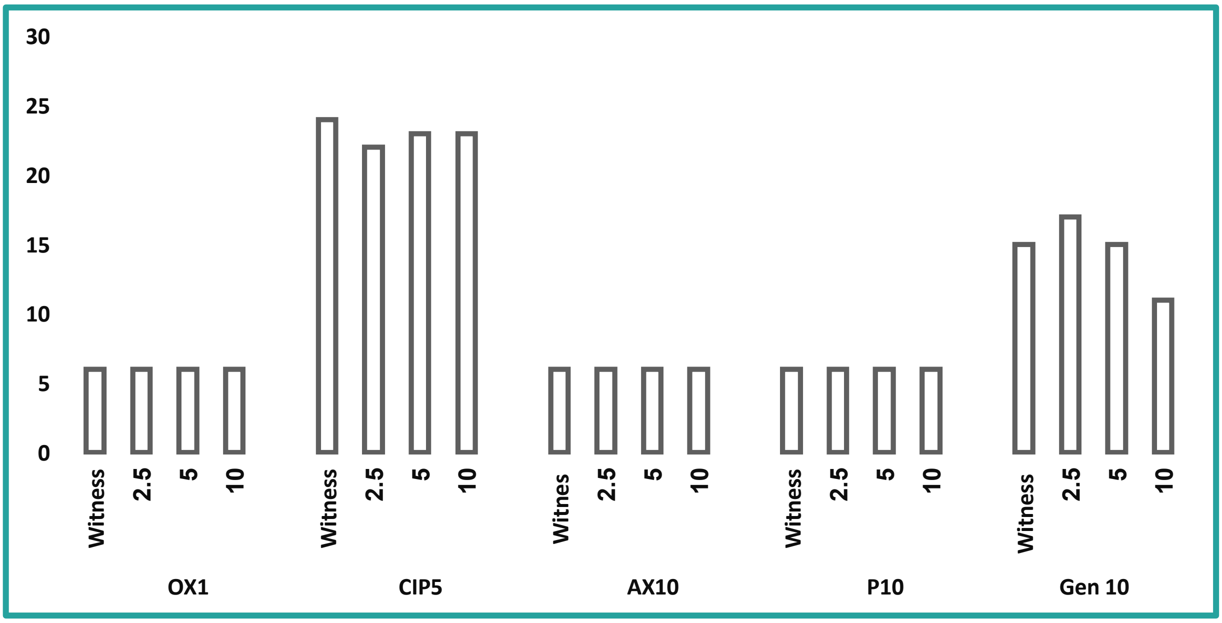


C. Effect of acid pH on the viability of *Bacillus safensis* in (log CFU/ml) in the presence of different concentrations of *Artemisia herba-alba* aqueous extract.

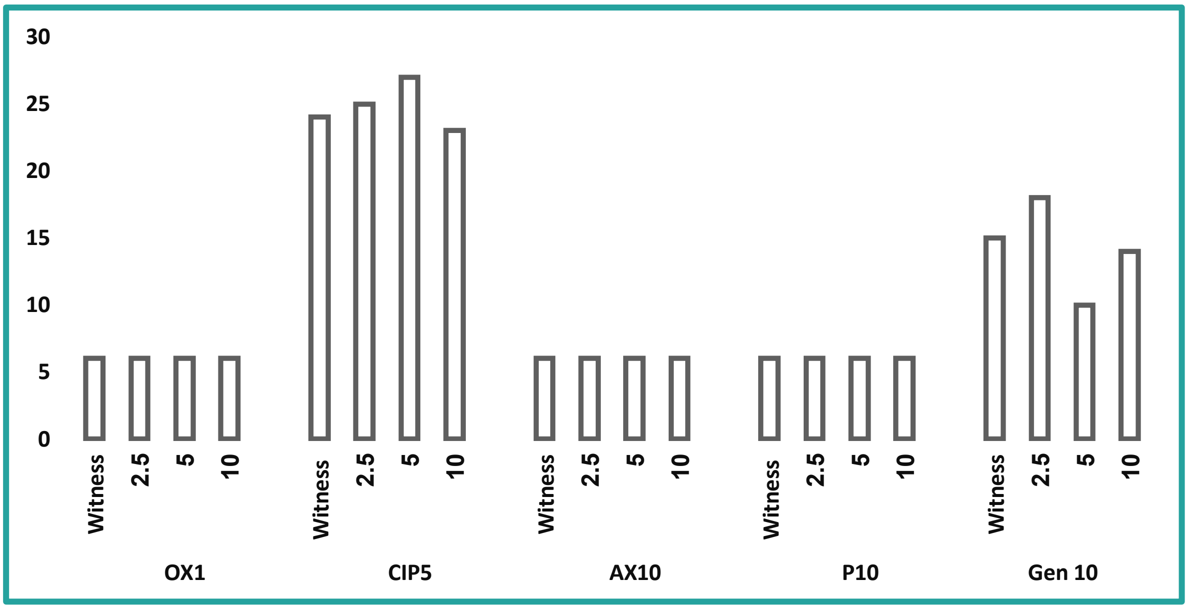


D. Effect of acid pH on the viability of *Bacillus safensis* in (log CFU/ml) in the presence of different concentrations of *Artemisia herba-alba* hydroethanolic extract.

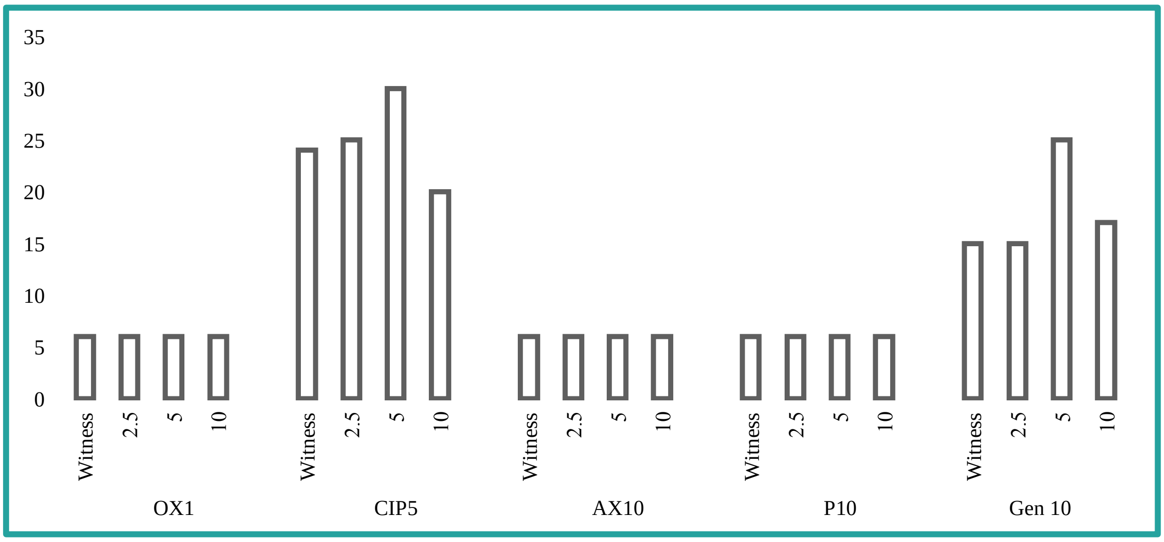
3. Antibiotic Test



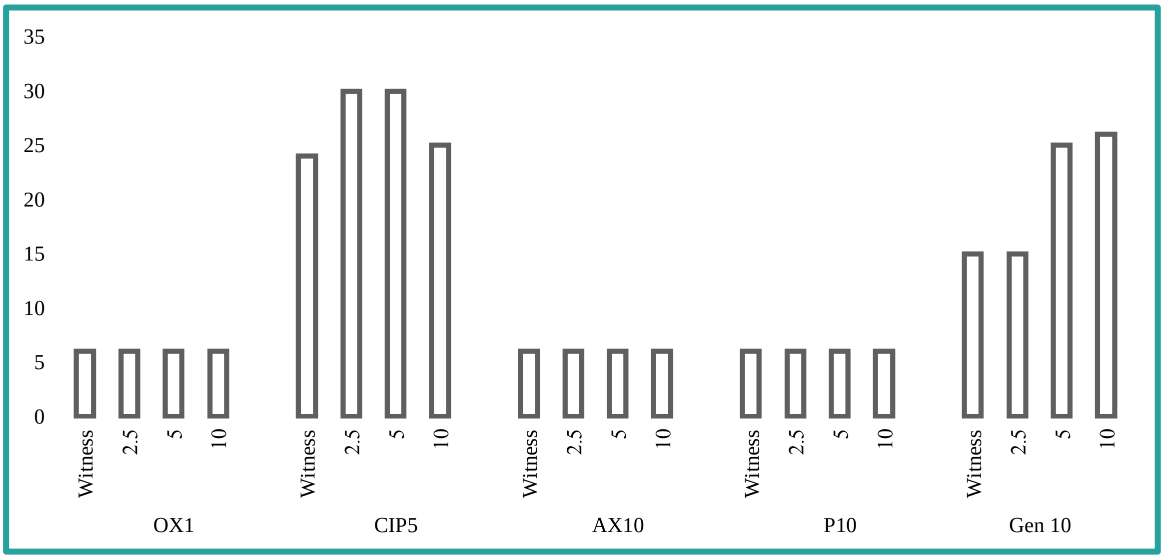
E. Effect of different concentrations of *Juniperus communis* aqueous extract on the antibiotic susceptibility of *Bacillus safensis*.



F. Effect of different concentrations of *Juniperus communis* hydroethanolic extract on the antibiotic susceptibility of *Bacillus safensis.*



J. Effect of different concentrations of *Artemisia herba-alba* aqueous extract on the antibiotic susceptibility of *Bacillus safensis*.



H. Effect of different concentrations of *Artemisia herba-alba* hydroethanolic extract on the antibiotic susceptibility of *Bacillus safensis.*

4. Antimicrobial test

Because the pathogenic strains utilized may have a high resistance, neither the substrate nor other bacteria were able to alter the pathogenic bacteria found in the extracts of *Juniper communis* and *Artemisia herba-alba*. The antibiotic test for these harmful bacteria confirms the findings.

Table b. Antibiotic test of pathogenic strains.

Pathogens Antibiotics	Enterococcus Faecalis	Staphylococcs haemolyticus	Canadida albicans	Klebsella sp	Salmonella sp	Staphylococcus aureus	Eschericha coli
Penicillin G	R	R	R	R	R	R	R
oxacillin	R	R	R	R	R	R	R
Amoxicillin	R	13	R	R	R	29	R
Gentamicin	R	15	29	18	15	24	18
Ciproflaxacin	R	19	35	24	23	28	24

R: Resistant

Appendix 03. The ethnobotanical survey

الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

جامعة الشهيد حمه لخضر

كلية علوم الطبيعة والحياة

قسم البيولوجيا

إستبيان حول:

حالة النباتات الطبية المستخدمة في الطب البديل

لعلاج أمراض واضطرابات الأمعاء

إعداد الطالبة:
العياضي اكرام

تحت إشراف الدكتور:
عمار التهامي العايش

السنة الجامعية : 2021/ 2022

يهدف هذا الإستبيان إلى معرفة حالة النباتات الطبية التي تستخدم لعلاج أمراض و اضطرابات الأمعاء من خلال تتبع أكثر النباتات شيوعا وإستعمالا، وذلك بعد طرح العديد من التساؤلات على أهل الإختصاص (المعالجين التقليديين) قصد التعرف عن كثب عن الحالة العامة وكيفيات ومجالات إستخدام هذه النباتات مع التركيز على فهم أوسع للعديد من المعلومات المقدمة من طرف أهل الإختصاص للرقي بهذا المجال في المنطقة.

1. معلومات عامة:

الإسم واللقب: ....**الجنس:**...................... **السن:** ....

المستوى التعليمي:................................ **الأقدمية في المهنة (بالسنوات):** ....

2. قائمة النباتات الطبية الصحراوية التي تستخدم في علاج أمراض و اضطرابات الأمعاء :

- النباتات الطبية التي تستخدم في علاج الاسهال
- النباتات الطبية التي تستخدم في علاج الامساك
- النباتات الطبية التي تستخدم في علاج سوء الامتصاص
- النباتات الطبية التي تستخدم في علاج القولون العصبي
- النباتات الطبية التي تستخدم في علاج الالتهابات المعوية
- النباتات الطبية التي تستخدم في علاج سرطان القولون و الامعاء

	الاسم الشائع	الاسم العلمي	مايعالجه النبات في الجهاز الهضمي	الجزء المستعمل	شكل الاستعمال	طريقة االتحضير	طريقة الاستعمال	مدة الاستخدام	طريقة نمو النبات	تحذيرات الاستعمال	الأعراض الجانبية
النبتة 1											
النبتة 2											
النبت 3											

3 . الاسئلة:

1. هل اختيارك للمهنة عن؟ □اهتمام □تجاري □ علمي
2. كيف ترى علاج الجهاز الهضمي و الأمعاء بالنباتات الطبية الصحراوية؟ □مكمل □بديل
3. كيف ترى اهتمام الزبائن بهذا المجال؟

....

....

4. هل ترغب في تطوير هذه المهنة خارج المنطقة؟ □ نعم □ لا

....

....

5. ما مصدر معلوماتك: الإعلام □ المعالج بالنباتات □ عن طريق الخبرة

6. من أين تتحصل على النبات؟: □ مناطق زراعية □ من البرية □ مصادر أخرى

7. هل توجد النباتات يجب التعامل معها بحذر؟ اعطي مثال

....

....

8. كيف تفرق بين الاعشاب المتشابهة؟

....

....

9. ما هي النباتات الجديدة التي دخلت المجال؟

....

....

10.ما هي المعايير التي تحدد على اساسها ثمن العشبة؟

....

....

11.ما هي الطرق التي تستعملها للتفريق بين الاعشاب الاصلية والأعشاب المغشوشة؟

....

....

12.هل تقدم نصائح وإرشادات للزبائن مع ذكر مثال؟ □ نعم □ لا

....

....

13.هل هناك خلطات عشبية خاصة ؟ مع ذكر مثال

....

....

14.هل هناك خلطات لها اثار جانبية اذا استعملت بشكل خاطئ ؟

....

....

15.هل هناك صلاحية قانونية لبيع خلطات النباتات الطبية المستعملة في علاج الجهاز الهضمي ؟

□ لا □ نعم

16.ما هي أبرز الصعوبات التي توجه بائعي الاعشاب المستخدمة في هذا المجال ؟

....

....

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17. أسئلة أخرى مقترحة:

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Appendix 01. Bublications

Jordan Journal of Pharmaceutical Sciences, Volume 17, No. 2, 2024

Medicinal Plants Used by Traditional Healers in the Treatment of Gastrointestinal Disorders in Oued Souf Region (southeast of Algeria)

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[3] Laboratory of biogeochemistry of Desert Environments laboratory, University of Ouargla, Algeria.
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ABSTRACT

This study aims to analyze indigenous knowledge of medicinal plants used by traditional healers to treat gastrointestinal disorders in the Oued Souf region. Data were collected through open-ended, semi-structured interviews. Various statistical indices, such as UV and ICF, were employed to evaluate quantitative data. The findings reveal that traditional healers utilize 47 medicinal plant species from 22 families for treating gastrointestinal disorders. Lamiaceae and Asteraceae emerge as the most dominant families, with 9 and 7 species, respectively. The most frequently used plant parts were leaves (35%), and the predominant method of preparation was infusion (55%). Among the most popular plants used by local healers were Artemisia herba alba Asso (UV = 0.85) and Juniperus communis (UV = 0.75). The study highlights the significant number and variety of medicinal plants employed by traditional healers to address digestive disorders. Consequently, this research can aid scientists in identifying plants with medicinal properties that may contribute to the development of new medications.

Keywords: Gastrointestinal disorders, Traditional healers; Oued Souf; Medicinal plants; Indigenous knowledge.

INTRODUCTION

The gastrointestinal tract, a highly sensitive human organ, is susceptible to a diverse range of diseases, including parasites, infectious disorders, gastroenteritis, reflux, bloating, constipation, and diarrhea[1,2] . The prevalence of gastrointestinal illness is notably attributed to infections from various bacterial strains, causing up to 3 million preschooler deaths annually[3]. There is a growing interest in traditional medical systems, driven by the need for more efficient treatment. The demand for fundamental scientific research on medicinal plants used in indigenous medical systems has consequently increased. Recognizing the importance of traditional medicine, the World Health Organization (WHO) acknowledges it as the totality of knowledge, skills, and practices based on theories, beliefs, and experiences inherent to various[4,5].

In Algeria, phytotherapy is deeply rooted in local culture, with indigenous knowledge accumulated over decades through practical study. The diverse flora, fostered by Algeria's geographic position and varied climate, has been extensively used to address numerous maladies, especially digestive system problems[6,7]. Despite lifestyle changes and industrialization, local communities in Algeria's Sahara, one of the world's largest deserts, still rely on traditional healers for medical needs[8]. Recognizing the declining transmission of this tradition, it has become crucial to record the historical applications of therapeutic herbs[6].

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Received: 23/9/2023 Accepted: 21/12/2023.
DOI: https://doi.org/10.35516/jjps.v17i2.1783

Received: 15 April 2024 | Revised: 22 May 2024 | Accepted: 27 May 2024

DOI: 10.1002/fsn3.4262

ORIGINAL ARTICLE

Food Science & Nutrition Open Access WILEY

Effect of *Juniperus communis* extract on probiotic properties of *Bacillus safensis* isolated from camel milk in the region of El Oued (Algeria)

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Funding information
King Saud University, Riyadh, Saudi Arabia, Grant/Award Number: RSPD2024R709

Abstract

The current study focuses on the effect of *Juniperus communis* extract on the probiotic properties of lactic acid bacteria isolated from camel milk in the region of El Oued (Algeria). Chromatographic analysis by HPLC was carried out to detect the most important compounds extracted from the plant. The total phenolic and flavonoid contents were determined using the colorimetric procedures Folin–Ciocalteu and aluminum chloride. The probiotic properties were studied and evaluated in vivo with *Juniperus communis* extracts after isolating strains from camel's milk and identifying them using 16S rRNA gene sequencing. Chromatographic profiles of the phenolic compounds revealed that *Juniperus communis* extract is rich in quercetin. After conducting chemical analyses of polyphenols and flavonoids, the results demonstrated a high content of phenolic compounds in *Juniperus communis* extracts (polyphenols: 103.80 ± 0.30 mg GAE/g E. flavonoids: 15.85 ± 0.80 mg QE/g E). Sequencing and phylogenetic analysis showed that the isolates belong to *Bacillus pumilus* and *Bacillus safensis* strains. The combination of *Juniperus communis* and *Bacillus safensis* restored the healthy intestine

JOURNAL OF AGRICULTURE AND APPLIED BIOLOGY
2025, Vol. 6, No. 1, 114 - 123
http://dx.doi.org/10.11594/jaab.06.01.09
E-ISSN: 2723-5106

Research Article

Comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions

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Article history:

Received 06 August 2024
Revised 31 August 2024
Accepted 04 September 2024
Published 25 January 2025

Keywords:

Antimicrobial characteristics
Protein denaturation
Antioxidant activity
Pharmaceutical products
Phenolic compounds

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Abstract

This research reports a comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions. The plants extracts total phenolic acid content was ascertained using the Folin-Ciocalteu method, and their total flavonoid content was ascertained using the aluminum chloride colorimetric test. By estimating their ability to remove free radicals using the DPPH method and the ferric reducing antioxidant power (FRAP) assay, their antioxidant capacity was assessed. Disk diffusion was used to measure the antibacterial activity against five strains of bacteria, and a protein denaturation assay was performed to examine the anti-inflammatory activity. A high polyphenol content of 104.54 ±0.35 mg GAE/g was observed in the hydroethanolic extract of *Artemisia herba-alba*, while a high flavonoid content of 17.05 ±0.13 mg QE/g was found in the hydroethanolic extract of *Juniperus communis*. Both species extracts showed important antioxidant activity in two separate tests, where the hydroethanolic extracts demonstrated effective action. The crude extracts showed positive antibacterial activity, especially the hydroethanolic extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae* with a super inhibition on concentration 20 mg/ml. In the anti-inflammatory assay, the hydroethanolic extract of *J. comminus* with IC_{50}: 23.58 ±0.02 µg/mL, has the most potent anti-denaturation effect on albumin. According to the study's findings, the hydroethanolic extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions are possible sources of phenolic compounds with important natural antioxidant, antimicrobial and anti-inflammatory characteristics that may be employed in pharmaceutical products.

How to cite:
Layadi, I., Tlili, M. L., & Laiche, A. T. (2025). Comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions. *Journal of Agriculture and Applied Biology, 6*(1): 114 - 123. doi: 10.11594/jaab.06.01.09

Abstract

Contribution to the biological characterization of bioactive compounds from *Juniperus communis* and *Artemisia herba-alba* with stimulating effect on *Bacillus safensis* probiotic

Incorporating probiotics within a plant matrix is still being thought of as a crucial element for improving probiotic strain activity. In this regard, the main objective of this study is the evaluation of the *in vitro* and *in vivo* effects of aqueous and hydroethanolic extracts obtained from common plant species used to treat gastrointestinal diseases by traditional healers (*Artemesia herba-alba*, *Juniperus communis)* on probiotic bacteria properties isolated from camel milk in El-Oued region. A series of surveys were carried out in the study area among traditional healers with the aim of acquiring the maximum amount of information on medicinal plants used for gastrointestinal disorders treatment. Standard operating protocols were applied for the extraction of phenolic components and for quantitative and qualitative analysis. However, HPLC analysis was used to identify and quantify each unique phenolic component. The probiotic properties were evaluated *in vitro* and *in vivo* with *Artemesia herba-alba* and *Juniperus comminus* extracts, after isolating strains from camel milk and identifying them using 16s rRNA gene sequencing.

Based on an ethnobotanical survey, the most often used species by traditional healers in Oued Souf region are *Juniperus communis* (UV value = 0.75) and *Artemisia herba-alba* (UV value = 0.85). The HPLC analysis revealed that quercetin makes up the majority of the compounds in the hydroethanolic extract and the crude extracts of *Juniperus communis*. The quantitative estimation of total polyphenols of *Artemisia herba-alba* indicated that hydroethanolic extract exhibited a high content of polyphenols with 104.54 ± 0.35 mg GAE/g AHE compared to aqueous extract (58.89 ± 0.22 mg GAE/g AAE). Whereas *Juniperus communis* aqueous extract demonstrated a greater polyphenol value (103.80±0.30 mg GAE/g JAE) than the hydroethanolic extract (78.11±0.27 mg GAE/g JHE). Regarding the total flavonoid content, the aqueous and hydroethanolic extracts of *Artemisia herba-alba* showed lower content (4.88±0,075 mg QE/g AAE and 2.06±0,03 mg QE/g AHE, respectively). But *Juniperus communis* hydroethanolic extract had the highest amounts of total flavonoids (17.05±0.13 mg QE/g JHE) than aqueous extract (15.85±0.80 mg QE/g JAE). The isolates lactic acid bactria are related to strains of *Bacillus pumilus* and *Bacillus safensis*, according to sequencing and phylogenetic analysis. *In vitro* results of the effect of different extracts of *Artemisia herba-alba* and *Juniperus communis* on the bacterial probiotic properties showed an increase in the growth of bacteria and their resistance to antibiotics with their inability to resist pathogenic strains, due to the strong resistance of the latter to dairy strains. The combination restored the healthy intestinal wall structure and returned the blood's biochemical properties to normal levels. Additionally it reduces the symptoms of intestinal inflammation by lowering the erythrocyte sedimentation rate and C-reactive protein levels.

Finally, the aqueous and hydroethanolic extracts of *Artemisia herba-alba* and *Juniperus communis* have a positive effect on *Bacillus safensis* properties, which contribute to the use of medicinal products for improving the nutritional value of dairy products.

Keywords: *Artemisia herba-alba, Juniperus communis,* gastrointestinal disoders, probiotics, gene sequencing.

Résumé

Contribution à la caractérisation biologique des composés bioactifs de *Juniperus communis* et d'*Artemisia herba-alba* ayant un effet stimulant sur le probiotique *Bacillus safensis*

L'incorporation de probiotiques au sein d'une matrice végétale est encore considérée comme un élément crucial pour améliorer l'activité des souches probiotiques. À cet égard, l'objectif principal de cette étude est l'évaluation des effets *in vitro* et *in vivo* d'extraits aqueux et hydroéthanoliques obtenus à partir d'*Artemesia herba-alba* et *Juniperus communis* qui utilisées pour traiter les maladies gastro-intestinales par les guérisseurs traditionnels sur les propriétés probiotiques des bactéries isolées à partir du lait de chamelle dans la région d'El-Oued. Une série d'enquêtes ont été réalisées dans la zone d'étude auprès des guérisseurs traditionnels dans le but d'acquérir le maximum d'informations sur les plantes médicinales utilisées pour le traitement des troubles gastro-intestinaux. Des protocoles opératoires standard ont été appliqués pour l'extraction des composants bioactifs et pour l'analyse quantitative et qualitative. Cependant, l'analyse HPLC a été utilisée pour identifier et quantifier chaque composant phénolique. Les propriétés probiotiques ont été évaluées *in vitro* et *in vivo* avec des extraits d'*Artemesia herba-alba* et de *Juniperus comminus*, après avoir isolé les souches du lait de chamelle et les avoir identifiées par séquençage du gène de l'ARNr 16 s. D'après une enquête ethnobotanique, les espèces les plus souvent utilisées par les guérisseurs traditionnels de la région d'Oued Souf sont *Juniperus communis* (UV = 0,75) et *Artemisia herba-alba* (UV = 0,85). L'analyse de HPLC a révélé que la quercétine constitue la majorité des composés présents dans l'extrait hydroéthanolique et les extraits bruts de *Juniperus communis*. L'estimation quantitative des polyphénols totaux *d'Artimisia herba-alba* a indiqué que l'extrait hydroéthanolique présentait une teneur élevée en polyphénols avec 104,54 ± 0,35 mg GAE/g par rapport à l'extrait aqueux (58,89 ± 0,22 mg GAE/g). Alors que l'extrait aqueux *de Juniperus communis* a démontré une valeur de polyphénols plus élevée (103,80 ± 0,30 mg GAE/g) que l'extrait hydroéthanolique (78,11 ± 0,27 mg GAE/g). Concernant la teneur totale en flavonoïdes, les extraits aqueux et hydroéthanoliques *d'Artemisia herba-alba* ont montré une teneur plus faible (4,88 ± 0,075 et 2,06 ± 0,03 mg QE/g, respectivement). Mais l'extrait hydroéthanolique de *Juniperus communis* contenait les quantités les plus élevées de flavonoïdes totaux (17,05 ± 0,13 mg QE/g) que l'extrait aqueux (15,85 ± 0,80 mg QE/g). Les isolats sont apparentés aux souches de *Bacillus pumilus* et *Bacillus safensis*, selon le séquençage et l'analyse phylogénétique. Les résultats *in vitro* de l'effet de différents extraits *d'Artemisia herba-alba* et de *Juniperus communis* sur les propriétés probiotiques bactériennes ont montré une augmentation de la croissance des bactéries et de leur résistance aux antibiotiques avec leur incapacité à résister aux souches pathogènes. Grâce à cette combinaison, les caractéristiques biochimiques du sang ont été ramenées à des valeurs normales, ce qui a également permis de réparer la structure saine de la paroi intestinale. Et réduire les symptômes d'inflammation intestinale en diminuant les niveaux de protéine C-réactive et la vitesse de sédimentation des érythrocytes.Enfin, les extraits aqueux et hydroéthanoliques *d'Artemisia herba-alba* et de *Juniperus communis* ont un effet positif sur les propriétés de *Bacillus safensis*, ce qui ouvre de nouvelles voies d'utilisation de médicaments tout en améliorant la valeur nutritionnelle des produits laitiers.

Mots clés : *Artemisia herba-alba, Juniperus communis,* troubles gastro-intestinaux, probiotiques, séquençage du gène.

الملخص

دراسة الخصائص البيولوجية للمركبات النشطة بيولوجيًا من *Juniperus communis* و *Artemisia herba-alba* ذات التأثير المحفز على سلالة البروبيوتيك *Bacillus safensis*

لا يزال يُنظر إلى دمج البروبيوتيك مع النباتات الطبية كعنصر فعال لتحسين نشاط سلالة البروبيوتيك. إن الهدف الرئيسي من هذه الدراسة هو تقييم التأثيرات في المختبر وفي الجسم الحي للمستخلصات المائية والإيثانولية التي تم الحصول عليها من الأنواع النباتية الشائعة المستخدمة لعلاج أمراض الجهاز الهضمي من قبل المعالجين التقليديين (*Artemesia herba-alba*، *Juniperus communis*) على خصائص البروبيوتيك للبكتيريا المعزولة من حليب الإبل بمنطقة الوادي. تم إجراء سلسلة من الدراسات الاستقصائية في منطقة الدراسة بين المعالجين التقليديين بهدف الحصول على أكبر قدر ممكن من المعلومات عن النباتات الطبية المستخدمة لعلاج اضطرابات الجهاز الهضمي. تم تطبيق البروتوكولات القياسية لاستخلاص المكونات النشطة بيولوجيا وللتحليل الكمي والنوعي. ومع ذلك، تم استخدام تحليل HPLC لتحديد وقياس كل مكون فينولي فريد من نوعه. تم تقييم خصائص البروبيوتيك في المختبر وفي الجسم الحي باستخدام مستخلصات *Artemesia herba-alba*و*Juniperus communis*، بعد عزل السلالات من حليب الإبل وتحديدها باستخدام التسلسل الجيني ARNr 16s. استنادا إلى المسح العرقي النباتي، فإن الأنواع الأكثر استخداما من قبل المعالجين التقليديين في منطقة واد سوف هي نبات العرعار (UV= 0.75) و نبات الشيح (UV= 0.85). كشفت نتائج تحليل HPLC أن الكيرسيتين يشكل غالبية المركبات الموجودة في المستخلص الهيدروإيثانولي والمستخلصات الخام ل *Juniperus communis*. أشار التقدير الكمي لإجمالي البوليفينول في *Artimisia herba-alba*إلى أن المستخلص الهيدروإيثانولي أظهر محتوى عاليًا من البوليفينول (104.54 ± 0.35 مغ من حمض الغاليك مكافئ / غ من المستخلص) مقارنة بالمستخلص المائي (58.89 ± 0.22 مغ من حمض الغاليك مكافئ / غ من المستخلص). في حين أظهر المستخلص المائي ل *Juniperus communis* قيمة بوليفينول أكبر (103.80 ± 0.30 مغ من حمض الغاليك مكافئ / غ من المستخلص) من المستخلص الهيدروإيثانولي (78.11 ± 0.27 مغ من حمض الغاليك مكافئ / غ من المستخلص). فيما يتعلق بمحتوى الفلافونويد الإجمالي، أظهرت المستخلصات المائية والهيدروإيثانولية لعشبة *Artemisia herba-alba*محتوى أقل (4.88±0,075 و2.06±0,03 مغ من الكرستين مكافئ / غ من المستخلص ، على التوالي). لكن مستخلص *Juniperus communis* الهيدروإيثانولي يحتوي على أعلى كميات من إجمالي مركبات الفلافونويد (17.05±0.13 مغ من الكرستين مكافئ / غ من المستخلص) مقارنة بالمستخلص المائي (15.85±0.80 مغ من الكرستين مكافئ / غ من المستخلص). وترتبط العزلات بسلالات *Bacillus pumilus* و*Bacillus Safensis*، وفقًا للتحليل التسلسلي والتطوري. أظهرت النتائج المخبرية لتأثير مستخلصات مختلفة من نبات *Artemisia herba-alba*و*Juniperus Communis* على خواص البروبيوتيك البكتيرية زيادة في نمو البكتيريا ومقاومتها للمضادات الحيوية مع عدم قدرتها على مقاومة السلالات المسببة للأمراض. تم إرجاع الخصائص البيوكيميائية للدم إلى قيمها الطبيعية من خلال هذا المزيج، مما أدى أيضًا إلى إصلاح بنية جدار الأمعاء الصحي. وخفض أعراض عدوى الأمعاء عن طريق خفض مستويات بروتين C التفاعلي ومعدل ترسيب كرات الدم الحمراء. و في الاخير، فإن المستخلصات المائية والهيدروإيثانولية لنبات *Artemisia herba-alba* و *Juniperus communis* لها تأثير إيجابي على خصائص *Bacillus Safensis*، مما يساهم في استخدام المنتجات الطبية لتحسين القيمة الغذائية لمنتجات الحليب.

الكلمات المفتاحية: *Artemisia herba-alba*، *Juniperus communis* ، أمراض الجهاز الهضمي، البروبيوتيك، تسلسل الجينات.