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Ethnobotanical study, phytochemistry, and biological activities of medicinal plants in the region of Boussaâda, M'sila (*Ruta montanaL.*).

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DEDICATION

TO WHOM BEEN MY SHADOW IN ALL CONDITIONS, TO MY SOUL MATE, MY HUSBAND "WESSAM AL MOMANI". THANK YOU FOR YOUR PRESENCE, PATIENCE AND EMOTIONAL SUPPORT.

TO MY SWEET HEART "RANIM".
TO WHOSE EXISTENCE HAS INSPIRED ME TO BE ALWAYS THE BEST….TO MY DARLING SON "WASSIM"

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TO MY WONDERFUL SISTER
TO MY BROTHERS

TO MY FAMILLY IN LAW

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Introduction

Introduction

Throughout history, the utilization of herbs for medicinal purposes can be traced back over 60,000 years, reaching as far back as the stone Age **(Jamshidi-Kia et *al*., 2017)**. Written records of medicinal plant usage in the preparation of remedies can be found on Sumerian clay tablets dating back approximately 5,000 years, as discovered in Nagpur **(Petrovska, 2012)**. The World Health Organization **(WHO)** in **2020** conducted an assessment and found that over 80% of the global populations, particularly those with limited financial resources, use alternative medicines. Several factors contribute to this significant percentage. These factors include limited access to modern healthcare due to geographical and economic constraints, insufficient and unequal distribution of healthcare professionals, as well as socio-cultural behaviors.The **WHO, (2020)** has highlighted that approximately 25% of modern pharmaceuticals have their origins in plants **(Verma and Singh, 2008)**. The exploration of plants as a source of novel drugs has been progressing slowly, despite significant scientific and technological advancements in the pharmaceutical industry. It is worth noting that out of the estimated 250,000 to 500,000 plant species, only a minor fraction of photochemical has been examined, and an even slighter percentage of them have been thoroughly examined for their pharmacological properties. In most cases, research has only touched the preliminary or primary stages **(Rates, 2001)**.

The interest in traditional medicine, remains relatively limited in Algeria, despite the country's vast expanse of approximately 2.382.741 million square kilometers and its rich natural resources found in diverse habitats such as coastal regions, hills and deserts, encompassing aextensive variety of plant life. Furthermore, the migration of rural farmers to urban areas poses an important risk of abandoning and losing the cultural heritage associated with plants used in traditional or alternative medicine **(Miara et *al*., 2019b)**. A significant proportion of the rural population in Bousâada traditionally relies on plants, primarily for nutrition and medicinal purposes. In current years, there has been agrowing interest among Algerian academics in the traditional usage of plants for medicinal treatments.

In developing countries exhibiting often inadequate primary healthcare system, and traditional medicine plays a critical role in meeting the healthcare needs **(Shrestha and Dhillion, 2003; WHO, 2020)**.

Similarly, Algeria faces challenges with insufficient and inaccessible modern healthcare services, leaving a significant portion of the population without adequate healthcare access. Many pastoral communities possess a wealth of traditional facts concerning medicinal plants **(Joshi and Joshi, 2000)**, which is preserved through generational transfer **(Tabuti et *al*., 2003)**. Herbal medicines are not only considered affordable and accessible but are also deeply rooted in the culture **(Karunamoorthi and Tsehaye, 2012)**. Given Algeria's varied socio-economic, ethnic, linguistic and cultural landscape, as well as its rich biodiversity, it's reasonable to expect a substantial repository of indigenous knowledge related to medicinal plants and their usage in treating various human illnesses. Additionally, due to high levels of illiteracy and poverty among the population in Bousâada, traditional phytomedicine remains a primary resource for addressing various health issues. The application of plants and their derivatives is a widespread exercise in many herbal medicine structuresuniversal **(Ishtiaq et *al*., 2010a, b)**.

Algeria, considered the richest in North Africa (Miara et *al*., 2018), is blessed with diverse and abundant natural vegetation, encompassing over 3.139 species **(Quezel& Santa, 1962)**. The exploration and documentation of this rich flora, comprising both endemic and exotic varieties, is of paramount importance due to the integral role of plants in traditional medicinal and nutritional practices, deeply rooted in historical and cultural perspectives across the nation. As reported by **Reguieg (2011)**, the Algerian people has depended onon medicinal and aromatic plants for times to address a numerous of health concerns.

Despite numerous studies conducted in Algeria on this subject, ethnobotanical investigations remain insufficient in comprehensively documenting the ancestral knowledge of plant use, primarily due to the vast expanse of Algeria.

The family of Rutaceae is a vast predominantly tropical and subtropical family, consisting of 150–162 genera and 1500–2096 species **(Kubitzki et *al*. 2011)**. Notably, it houses numerous essential medicinal plants and is primarily distributed across three key biodiversity hotspots **(Martyn, 2009; Groppo et *al*., 2012)**. *Ruta*, a prominent genus within the Rutaceae family, has held a significant place in the pharmacopoeia of North Africa for centuries. This plant protects against witchcraft, and serves as a defense against various sources of malevolence **(Ghazanfar, 1994)**.

Ruta exhibits widespread distribution in Algeria, particularly in mountainous areas **(Benkhaira et *al*., 2022)**.

R. montana is a species of Mediterranean chorology which is common in Algeria. It is present in all mountainous areas of the interior up to the Saharan Atlas**(Quézel et Santa, 1962-63)**.One of the most renowned species within this genus is *Ruta Montana* (*R. montana*),commonly recognized as "Fijel" in Algeria **(Mohammedi et *al*., 2020; Benkhaira et *al*., 2022)**. *R. montana* is widely employed for its versatile medicinal properties. It's known to contain various secondary metabolites confirmed by many phytochemical analyseswhich has revealed this richness. Algeria boasts a rich and diverse plant flora; however, the full potential of these plant resources and the diversity of their species remain only partially explored.The region of Bousâadalocated in the Tillian Atlas in the south of Chott-El Hodna, covers an area of 256 km^2 and serves as an entry to the desert. It is characterized by its rich botanical resources, diverse medicinal plants, and a prosperity of traditional healing practices. Bousâada stands as a valuable source of cultural inheritance, where the practice of using plants for folkloric medicinal purposes has been engrained since the dawn of human civilization.

Within this situation, the main objective of this study is to improve our understanding of traditional medication, particularly the employment of medicinal plants in the Bousâadaarea and itsneighboring areas, including Medjdel, Menaa, Tamsa and Slim. Then this research is dedicated to enhancing the valorization of Algerian plants from the region studied with the aim of identifying bioactive substances that hold promise for their biological and therapeutic properties. The focus of this part of our study includes a phytochemical investigation of *R. montana* and the evaluation of its pharmacological properties. Initially, the chemical constituents were extracted and identified, followed by an assessment of several biological activities.

Our manuscript will be divided in the subsequent way:

- An introduction of our study with the presentation of the objectives
- The first chapter which is devoted to the bibliographic synthesis data and literature review of our study.
- The second chapter defines the methodology, methods and materials of our work.
- The third chapter describes the gotten results, their explanations and discussions.
- Finally, we conclude this work with a general conclusion recapitulates and in highlighting of exciting results and future recommendation, then the list of references and annexes.

CHAPTER I:

LITERATURE REVIEW

1. Phytotherapy

1.1. Definition and generality of phytotherapy

Theterm "phytotherapy" is etymologically composed of two Greek roots: "phuton" and "therapeia", meaning "plant" and "treatment", respectively. Phytotherapy canthus be defined as a type of allopathic medicineaimed at thepreventionandtreatment of certain dysfunctions and/or certainpathologicalconditions using plants, plantparts or herbal preparations, whether eaten or used. Phytotherapy has been fully recognized by theAcademy of Medical Sciences since 1987 (**Wichtl and Anton, 2003**).

Phytotherapy is the practice of plants or botanicalmedicines to treatdisease (**Paul, 2005**); it is part of alternativemedicine (**Strang, 2006**).Traditional herbalremedieshavelongbeensoughtafter by those familiar with theircommon uses butunable to afford the consequences of modern medicine, this does not ignore there cent notabl ecomeback in alternative medicine (**Salhi et *al*., 2010**).

1.2. Different types of phytotherapy

1.2.1. Traditional phytotherapy

Traditionalmedicine or traditional phytotherapy is thecollection of practicalknowledge, whether explainable or not, for diagnosing, preventing or correctingphysical, mental or social imbalances, which is passeddownorally or internallysincegeneration to generation, relyingsolely on ancientandindigenouspracticesandobservations (**Zohoun and Flenon, 1997**). Traditionalmedicaltreatmentsinclude pharmacological treatmentsthat use herbal, animal and/or mineralmedicinesand non-drug treatmentsthat mostly do not use drugs, such as acupuncture, manualtherapyand psychotherapy. Traditionalmedicine is oftenreferred to as 'complementary', 'alternative' or 'unconventional' medicine (**Zohoun and Flenon, 1997**).

1.2.2. Modern medicine

Use products of plant origin obtained by extraction and dilution in a solvent. Doses of these extracts should be sufficient for long-lasting and quick results. They come in the form of syrups, drops, capsules, lyophilizates, etc.**(Bone& Mills, 2012**).

1.3. Methods of phytotherapy using

Medicinal plants become useful onlyafterundergoing a certainsum of transformations designed to release their active ingredients and make them available for absorption by thebody (**Hurabielle, 1981**). Therefore, the most common method of removing these active ingredients from herbal products is by using liquids that dissolve them (**Perrotis et *al*., 1999**)

1.3.1. Conservation and drying of medicinal plant

Plants can be kept in paper bags, cloth bags, iron, earthenware, or else glass pots in a dry, dark place, with labeling the container with the name and date of harvest or source. Always use the same material for one plant to avoid mixing flavors, generally speaking, the shelf life does not exceed one year.

They then lose their active ingredients, however, for the best method of preservation; this operation must be carried out quickly in order to avoid deterioration of the plants, fermentation and loss of some or all of the active ingredients, and with the aim of depriving the plants of the moisture they contain (**Ilina et al., 2002**).

1. Do not rinse with water except to clean the roots.
2. Remove thick wood and logs before and after drying.
3. Spread or hang the plants in a ventilated and dark place to dry and store, well-ventilated place.
4. Whole picked plants, such as laurel or lavender branches (shrub branches), can be hung in bunches from the ceiling. The roots must be well freed from the soil and dehydrated in an oven heated to 50°C or on a very hot radiator. Spread the flowers and leaves and roots evenly on a cloth or sieve to dry. So, plan some space. Berries and seeds dry easily in plain cardboard boxes or small boxes as long as they are shaken daily (**Ilina et al., 2002**).

For plants with stems, make small bouquets, hang on powder, and dry upside down in a dry, airy room such as a porch. Bouquets should dry quickly, evenly, and thoroughly. Especially pay attention to remove it immediately after it is completely dry to avoid dust and insects; this traditional method is not necessarily the most effective. These famous bouquets are used to prepare herbal teas and often become decorative elements. Oven drying is a particularly convenient method recommended for drying the roots and woody parts of aromatic plants. To do this, first clean the freshly picked vegetative organs thoroughly, and then dry them with a clean

dry cloth. Then cut them into crosswise slices or cubes. It takes two to three hours to dry in the oven (**Ilina et al., 2002**).

1.3.2. Preparation methods

- **Simple solution**:

Used when the substance dissolved is completely soluble in the solvent. Mixing and shaking are sufficient to obtain a homogeneous product (**Hurabielle, 1981**).

- **Maceration**:

This is the process of placing medicinal plants materials in a suitable solvent at room temperature for a period of time, overnight, sometimes days or even weeks, while not forgetting to stir frequently to facilitate the dissolution of soluble components (**Perrotis et *al*., 1999**). Water soaks should not last longer than half a day, as they favor the formation of a true microbial "broth", which is why alcoholic soaks are preferred (**Rodolphe et *al*., 2009**).

- **Infusion**:

Habitually used for herbs, leaves and fresh plants. Itspreparation is like to making a cup of tea. Boiling the water, then transfer over the herb (or mixture of herbs), cover and let steep for about 10-15 minutes (**Ogbonna et *al*., 2012**).

- **Decoction**:

Typically, this method is particularly effective when dealing with more robust and fibrous plant parts like bark and roots, especially those containing water-soluble compounds (**Taylor, 2004**). Instead of the simple infusion process involving steeping in warm water, the plant material is boiled over anlong period to become softer tougher, woody components and release their active ingredients, to prepare, take the desired quantity of herbs and place them in a ceramic saucepan with a well-fitting lid. Add cold water as required for the number of cups you intend to make. When using chopped herbs, strain the mix by pouring it through a tea strainer into your teacup (**Ogbona et *al*., 2012**).

- **Strong Decoctions:**

There are generally two primary methods for preparing these mixtures, and the choice between them depends on the type of plant material you're working with. In the first method, the mixture is cooked for an extended period, typically around 2 hours or more. This approach is well-suited for breaking down larger, tougher pieces of woody bark. On the other hand, if you're dealing with smaller wood pieces but still require a potent potion, you can follow the decoction method mentioned earlier (boiling for 20 minutes) and then allow the mixture to stand or soak overnight before straining the herbs. Additionally, during the straining process, it's important to press the chopped herb pieces into the sieve to extract as much water or broth as possible from them (**Taylor, 2004**).

- **Tincture**:

A tincture is a solution made from a combination of alcohol and water. It is employed when the plant material contains active compounds that don't easily dissolve in water or when large quantities are being prepared for the sake of convenience and long-term storage. With proper preparation, many botanical tinctures can be preserved for several years or even longer without any significant loss of their potency (**Ogbonna et *al*., 2012).**

- **Poultices and compresses**:

Poultices can be created using different methods, ranging from traditional practices like jungle shamans chewing fresh leaves or roots and applying them to the skin, to more manual methods like pounding fresh leaves or roots using tools like a mortar and pestle (**Ogbonna et *al*., 2012**). Typically, a soft cotton bandage is used to secure the poultice in position. In natural environments such as the jungle, a large supple leaf is often employed, held in place with twine.
Compresses involve soaking a piece of fabric in a prepared infusion, tincture, or decoction, then placing it onto the impacted area of the body or skin. For more precise adjustments and instructions, when relevant, refer to the primary plant section labeled "Traditional Remedy." In this section, you might come across directions to apply an infusion or decoction externally (**Taylor, 2004**).

- **Inhalation therapy**:

Inhalation therapy has brought about a transformative medical subspecialty, where therapeutic substances like powders, liquids, vapors, or gases are introduced into the inspired air. This field is now recognized as Inhalation Therapy, as highlighted by **(Ogbonna et *al*., 2012).** While some medical centers have adopted the term "Respiratory Therapy," it is a limited label that pertains solely to the treatment of respiratory issues, overlooking concerns related to other parts of the body. Presently, across various medical specialties, numerous procedures involve the inhalation of medications through various means to address a wide range of bodily ailments.

Various forms of drug inhalation are available, encompassing methods such as inhaling smoke from burnt reeds, plants, or minerals; using powders for snuffing or insufflation; inhaling liquids through droppers, sprayers, atomizers, or nebulizers; and breathing in vapors via inhalers, vaporizers, or humidifier gases, whether for therapeutic or anesthetic purposes (**Wollman and Smith, 1980**).

- **Leaching**:

Also known as percolation is a process characterized by gradually passing a substance through a suitable solvent to extract its soluble components. This technique, as outlined by **Hurabielle (1981),** involves the thorough removal of soluble constituents from a substance

Capsules: Finely powdered herbs are consumed in the form of capsules, which can also be added to food or ingested with water. Externally, they can serve as dusting powder for application on the skin. To prepare, dispense the powder onto a saucer and gently join the capsule halves, allowing the powder to accumulate. Once the capsule halves are filled, connect them carefully to avoid any spillage. Store the filled capsules in an airtight, dark glass container within a cool environment for duration of approximately 3-4 months, as outlined by **(Ogbonna et *al*., 2012).**

- **Medicated wines**:

Commonly referred to as medicated wines or tonic wines, present a pleasant method for consuming invigorating and tonic herbs, enhancing vitality, and alleviating indigestion. Wine, being less intense on the body compared to high-proof spirits, offers the advantage of being shipped in moderate amounts. These wines prove beneficial for addressing digestive concerns, as highlighted by **(Ogbonna et *al*., 2012).**

- **Syrups**:

These methods entail combining sugar with infusions, decoctions, expressed juices, fermented liquids, or plain water solutions. Honey and unrefined sugar act as natural preservatives and can be mixed with infusions or decoctions to create syrups and cordials. Syrups are made by blending equal parts of herbal infusions or decoctions with honey or unrefined sugar. Alternatively, tinctures can replace infusions or decoctions in syrup production. Notable syrup varieties include simple syrup, orange syrup, tolu syrup, raspberry syrup, wild cherry syrup, among othersas outlined by **(Ogbonna et *al*. 2012).**

- **Ointments**:

These fall under the category of semi-solid formulations intended for external application to the skin or mucous membranes. Ointments are designed to hydrate and protect the skin. An ointment can also be described as a soothing, therapeutic substance with a somewhat oily or fatty consistency that incorporates the healing properties of a medicinal plant. This combination is achieved by gently heating the fat or oil along with the plant material until the original color fades, and the oil or fat absorbs the healing chemical constituents. Ointment preparations are well-suited for addressing conditions such as leg sores, burns, scalds, and scabies (**Ogbonna et *al*., 2012).**

- **Creams**:

Creams are semi-solid preparations with a thick consistency, akin to ointments. They can exist in two forms: Oil-in-water (aqueous) creams or water-in-oil (oily) creams, as explained by **(Ogbonna et *al*., 2012).**

- **Aromatherapy:**

Aromatherapy is categorized as an alternative medicine approach, which involves the utilization of volatile plant substances, commonly referred to as essential oils, along with other aromatic compounds. The intention behind this practice is to influence an individual's mental state, mood, cognitive abilities, or overall well-being. The level of evidence supporting the effectiveness of aromatherapy in treating medical conditions is currently limited, mainly due to the scarcity of studies that adhere to rigorous research methodologies. Nonetheless, there is some

indication suggesting that essential oils might possess therapeutic possibilities, as noted by **(Edris, 2007).**

1.3.3. Steps involved in herbal formulations

The extraction of herbal extracts, phytodrugs, or bioactive botanical ingredients involves various methods or procedures, which can vary depending on the specific plant part utilized. The primary objective of any extraction process is to acquire a pure extract, devoid of any impurities that might influence the scent, physicochemical characteristics, or pharmacological attributes of the end product (**Ogbonna et *al*., 2012**):

- **Grinding**:

Dry and pulverize selected plant material using a hammer mill or a pan mill with a built-in screen. This helps break down the organs, tissues and cellular structures of the plant material, exposing the medicinal ingredients it contains to the solvents from which they were intended to be extracted. In the extraction process, the therapeutic compound is isolated from the plant material using an appropriate solvent. This may involve techniques such as hot water extraction (decoction), cold percolation, or solvent extraction with equipment like a Soxhlet extractor**(Alamgir& Alamgir, 2017**).

- **Filtration**:

Using this method, the resulting extract is isolated from the residue (spent plant material) by permitting it to filter through the internal partition of the extractor (covered with a filtering fabric) into a storage vessel (**Krakowska-Sieprawska et *al*., 2022**).

- **Concentration**:

The resulting extract is sent to a wiped film evaporator and concentrated in vacuo to get a concentrated extract (**Krakowska-Sieprawska et *al*., 2022**).

- **Spray drying**:

The highly concentrated extract is introduced into a spray dryer utilizing a high-pressure pump, which ensures a regulated feeding rate to obtain a powdered, dry form (**Krakowska-Sieprawska et *al*., 2022**).

- **Distillation**:

This technique stands as one of the earliest approaches employed to extract oil from plants. Essentially, it involves the application of heat to prompt the release of oil from plant tissue. The plant or its components must be in a state conducive to allowing steam and water to effectively permeate. Furthermore, essential oils, intricate blends of volatile secondary metabolites, can be isolated from plants through hydro or steam distillation (**Krakowska-Sieprawska et *al*., 2022**).

- **Expression**:

This method finds application, as seen in citrus oils. The term "expression" signifies that the oils are extracted or squeezed out from the peel of nearly ripe fruit. This is generally achieved by employing robust hydraulic presses positioned within a hollow cylinder, with perforated walls resembling a sieve. These perforations allow the juice and oils to emerge as pressure is applied. The liquid obtained through expression displays a milky appearance and is left to settle for several hours. During this period, the oil rises to the surface and can be segregated before undergoing the final filtration process. Furthermore, essential oils, intricate compositions of volatile secondary metabolites, can also be procured from plants through the expression technique, as observed in the case of citrus peel oils (**Krakowska-Sieprawska et *al*., 2022**).

- **Extraction**:

This method involves using carefully selected solvents to treat plants, with the aim of dissolving the essential components of the plant that include the desired active pharmaceutical ingredient. The choice of solvents and the methodology employed have been refined through experience to ensure both high yield and superior quality of the active component. This process results in the extraction of some of the most exquisite and delicate natural fragrances, as it

avoidspotential alterations caused by heat or steam, which can occur when using distillation techniques. Furthermore, this approach is especially suitable for polymeric materials, as the application of high temperatures during distillation could potentially affect the physicochemical properties of these materials. The process involves grinding them into a powder using a local grinding machine and subsequently immersing each substance in n-hexane, hot water, and ethanol (**Krakowska-Sieprawska et *al*., 2022**).

- **Enfleurage:**

This technique is known as enfleurage, a method employed to extract oils from plant materials. In this process, clarified lard acts as the solvent. It is spread evenly onto glass plates, which are then positioned over wooden frames and covered with flowers. These frames are organized in multiple tiers, and the setup is left undisturbed for a day or two. During this time, the oil is absorbed into the fat without the application of heat. Once the flowers have wilted, they are replaced with fresh ones, and this cycle is repeated. The process is continued until the fat has absorbed all the available fragrance and has achieved the desired level of aroma intensity (**Krakowska-Sieprawska et *al*., 2022**).

- **Maceration**:

Maceration serves as a method for extracting plant essences. It involves warming clarified lard or a blend of lard and vegetable oil in enameled iron pots to approximately 40-50°C. The plant matter is either gently incorporated into the warm fat or enclosed in linen bags and immersed in the fat. Stirring occurs over one or two days. Afterward, the spent material is replaced with fresh, and the cycle continues until saturation is achieved. The essential oils within the fatty pomade are then extracted by carefully mixing and agitating it with strong alcohol, which dissolves the oils and separates them from the fat. Following this, the alcoholic oil solution settles, and the alcohol is removed through low-temperature distillation (**Krakowska-Sieprawska et *al.*, 2022**).

- **Cold maceration with water**:

This method of preparation is unquestionably the most straightforward. Fresh or dried plant material is submerged in cold water and allowed to soak overnight. The herb is then

removed, and the resulting liquid is ingested. This method is ideal for extremely fragile plants, fresh botanicals, or those containing delicate compounds that could be affected by heat or might degrade in strong alcohol. Moreover, this technique can be easily adapted to Western practices, as it can be transformed into tablet or capsule forms (**Lasanta et *al*., 2023**).

- **Maceration with non-polar solvents**:

The maceration method is employed for oil extraction. It involves placing either lard or a mixture of lard and vegetable oil into enameled iron pots and gently heating it to approximately 40-50°C. Flowers are introduced into the warm fat either loosely or enclosed in linen bags, and the mixture is stirred over a period of one to two days. After this, the spent flowers are removed, fresh ones are added, and this cycle is repeated until the fat or oil blend becomes saturated. Following this, the essential oil is extracted from the fatty pomade through a gentle blending and agitation process using strong alcohol. This procedure causes the essential oils to dissolve and separate from the fat. The alcoholic oil solution is allowed to settle, and the alcohol is eventually separated through distillation conducted at a low temperature (**Wrona et *al*., 2019**).

1.4. Disadvantages of herbal medicine:

By and large, adhering to guidelines for prescribing herbal remedies entails minimal risk, often resulting in plants not delivering the expected benefits. Nonetheless, instances arise where specific medicinal plant species prove detrimental. Some may even conflict with conventional medications. While exceedingly uncommon, there have been cases of severe illness or fatalities linked to the consumption of herbal medicines. Typically, such exceptional occurrences can be attributed to disregarding safety protocols regarding the use of herbal remedies. If complications arise subsequent to the utilization of a medicinal plant, it is imperative to discontinue use, promptly seek advice from an herbalist or a medical professional (**Iserin, 2001**).

2. Ethnobotany

2.1. History, Definition and Importance of ethnobotany

The term 'ethnobotany' was first familiarized by the American botanist **John William Harshberger** in **1895**. Nevertheless, ethnobotanical studies have historical origins that predate the coining of this term. For example, the ancient Greek PedaniusDioscorides of Anazarbus documented the useful plants he collected from the Mediterranean region in his work "De Materia Medica" (**Pardo de Santayana et *al*., 2010**). Harshberger's initial definition of ethnobotany primarily revolved around 'the use of plants by indigenous peoples.' However, the concept of ethnobotany has evolved over time to encompass both anthropology and botany, involving the exploration of the dynamic relationship between humans and plants **(Martin, 1995; Paul, 2013)**. In **1940**, Conklin regarded ethnobotany as a component of ethnoscience, which is the study of indigenous knowledge (**Abdiche and Guergour, 2011**). The field of ethnobotany experienced significant growth in the late 1970s. In just 25 years, the number of scholarly articles dedicated to ethnobotany increased dramatically, growing by tenfold and now surpassing one hundred publications annually.

Over time, ethnobotany has witnessed significant transformations in its scope and definition. Its original emphasis solely on plant utilization and management has expanded to encompass broader dimensions, including sociocultural, economic, and local perspectives. This extension now involves the study of indigenous communities' perceptions, concepts, viewpoints, and values (**Cruz-Garcia, 2014**). As a result, ethnobotany has evolved into a multidisciplinary field that not only integrates anthropology and botany but also extends into areas like ecology, economics, linguistics, geography, agriculture, pharmacology, and more (**Cruz-Garcia, 2014; Martin, 1995**). Presently, ethnobotanical studies are guided by four main axes of objectives (**Malaisse, 2004**).

*Basic documentation of traditional botanical knowledge.

*Quantitative assessment of the application and management of plant resources.

*Experimental assessment of the contributions of plants, both in terms of substances and financial resources.

*Development of practical initiatives aimed at optimizing the utilization of local resources.

Ethnobotanical research facilitates the assessment of the indigenous population's understanding

and interaction with plants. It enhances this evaluation by incorporating ethnographic elements such as vernacular plant names, cultural insights, potential uses, and preparation methods. It essentially involves constructing and analyzing surveys focused on the traditional plant usage within a specific region. This process includes the establishment of an herbarium cataloging the most commonly employed medicinal plants (**Abdiche and Guergour, 2011**).

2.2. Ethnobotanical plants

A. Plants utilized for food purposes:

Encompass various categories, including fruit-bearing plants, edible leaf plants, starchy plants like roots, rhizomes, bulbs, and tubers, oilseed and oil-protein plants, as well as herbs, spices, and condiments (**Baba Aissa, 1999**).

B. Poisonous plants:

Are those that contain toxic substances, often organic compounds but sometimes minerals, in some or all of their parts, posing a risk primarily to humans and domestic animals. Poisoning typically occurs through ingestion of specific plant organs, but contact can also lead to toxicity.

C. Plants employed for industrial applications:

Serve artisanal and industrial purposes. These plants, either in their raw form or after processing, serve as renewable raw materials, including textile plants, dye sources, oilseeds, and hydrophilic materials like cotton. They contribute to various products ranging from fibers, essences, and resins to pharmaceuticals, cosmetics, and food items (**Baba Aissa, 1999**).

D. Aromatic plants:

Constitute a distinct category due to their production of volatile, fragrant substances known as essential oils (**Iserin, 2001**).

E. Medicinal plants and traditional medicine:

Involve plants or their parts with medicinal properties, as defined by the French pharmacopoeia and the European pharmacopoeia. These plants offer beneficial effects for human health and are employed through methods such as decoction, maceration, and infusion. Different

plant parts, such as roots, leaves, and flowers, can be utilized for their healing properties (**Adouane, 2016**).

Plants have held a central role in medicine for countless centuries (**Samuelsson, 2004**). In the beginning, medicinal treatments consisted of fundamental remedies such as tinctures, teas, powders, and poultices (Balick and Cox, 1997; Samuelsson, 2004). Knowledge regarding specific plants and their applications for various ailments was initially transmitted through oral tradition, eventually finding its way into herbal pharmacopoeias (**Balunas, 2005**).

The earliest written records in the Arabic tradition can be traced back to the Sumerians and Akkadians of Mesopotamia, originating from the same regions where archaeological findings at Shanidar IV were discovered (**Heinrich et *al*., 2004**). The oldest documented record related to medicinal plants can be dated back to 60,000 BCE and was uncovered in the burial site of a Neanderthal at Shanidar IV, an archaeological location in Iraq. Pollen from several plant species, likely used for medicinal purposes, was found, including Centaurea solstitialis (Asteraceae), Ephedra altissima (Ephedraceae), and Althea sp. (Malvaceae), among others. Although not directly associated with the Shanidar culture, these species or closely related ones from the same genus remain significant in Iraq's phytotherapy and other cultural traditions. These plants could have held symbolic importance for Neanderthals and might be part of a tradition documented for the first time at Shanidar IV (**Cragg and Newman, 2005**).

3. Phytochemistry and bioactivity of medicinal plants

3.1. Natural substances

Within the plant, there exists a metabolic process responsible for generating primary and secondary metabolites (**Hartmann, 2007**).

- ✓ **Primary metabolites:**

These are essential constituents crucial for the plant's survival and are uniformly present in all of its cells. They can be categorized into four main groups, including carbohydrates, lipids, amino acids (proteins), and nucleic acids (**Elshafie et al., 2023**).

- ✓ **Secondary metabolites:**

These originate from secondary metabolism, a process exclusive to the plant kingdom. They have a limited distribution and exhibit a wide variety of structural differences. Secondary metabolites can be broadly grouped into three key categories: polyphenols, terpenes, and alkaloids (**Abderrazak and Joël, 2007**).

3.2. Secondary metabolites

3.2.1. Polyphenols

The term 'Phenolic compounds' encompasses a wide variety of over 8000 molecules (**Bahorun, 1997; Garcia-Salas et *al*., 2010**), Polyphenols are produced by two biosynthetic ways: the shikimate pathway and the pathway resulting from acetate (**Bruneton, 2009**). The key structural element that distinguishes polyphenols is the direct linkage of at least one hydroxyl group and functional groups (such as Ester, Methyl ester, Glycoside etc.) to at least one benzene ring (**Bruneton, 1999**).Polyphenols are categorized according to the number of carbon atoms found within their fundamental structure (**Dacosta, 2003**). These encompass numerous polyphenol classes, including but not limited to simple phenolic acids, simple phenols, stilbenes, coumarins, tannins, quinones, flavonoids, lignans, lignins, and xanthones (**Table1)**.

a) Simple phenolic acids

Phenolic acid refers to compounds with as a minimum one carboxylic function and one phenolic hydroxyl. Hydroxybenzoic acids, which are benzoic acid derivatives having a general basic type of structure (C6-C1), can be distinguished from other phenolic acids. These molecules are commonly found as esters or glycosides. Hydroxycinnamic acids come from cinnamic acid and have a general basic structure of type (C6-C3). They commonly exist in combination with organic compounds such as chlorogenic acid. This is an ester of hydroxycinnamic acids, specifically caffeic acid and quinic acid that form monoesters, including caffeylquinic acid (ACQ). The list of phenolic acids occurring naturally in plants extends beyond those mentioned previously. Notably, methylgallic acid, rosmarinic acid, carnosic acid, and carnosol are also present (**Dacosta, 2003**). Phenolic acids are not commonly found in nature, with hydroquinone being a notable exception. Hydroquinone is present in various plant families, including Ericaceae and Rosaceae, often in the form of diphenol glucoside (arbutoside) or its monomethyl ether. In addition, alkenylphenols (such as Urushiol) and phenolic monoterpenes (like Thymol) can also

be identified (**Beddou, 2015**).

b) Quinones

Quinones are a group of dienes, hydrocarbons that have two double bonds, unlike benzoquinones that have a benzene ring (C6). Benzoquinones replace two hydrogen atoms with two oxygen atoms, forming two carbonyl bonds and cyclic conjugated ethylenic diketones. They are oxygenated compounds that result from the oxidation of aromatic derivatives with two ketone substitutions. Para-quinones are characterized by a 1,4-diketo cyclohexa-2,5-diene unit, while Ortho-quinones have a 1,2-diketo cyclohexa-3,5-diene unit (**Bruneton, 1993**). Quinones find their use in the manufacturing of dyes, drugs, and fungicides.

c) Tannins

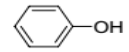
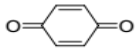
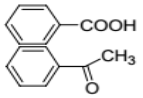
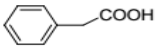
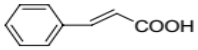
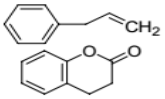
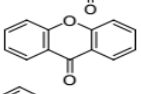
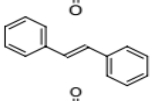
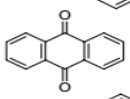
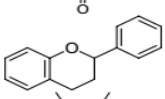
Tannins are highly prevalent in dicotyledonous angiosperms (hydrolysable tannins), as well as in gymnosperms (condensed tannins). They typically have a molecular mass ranging from 500 to 3000 PM (**Atefeibu, 2002**). They are commonly found in aged or diseased tissues. These compounds all share the characteristic of being able to tan skin by forming bonds with macromolecules (proteins, polysaccharides, etc.) that are resistant to fungal and bacterial attacks. Tannins are employed in the food industry for processing, as well as in the clarification of wines, beers, and fruit juices based on their biological properties. These complex molecular structures contain repeating monomeric units that are characterized by asymmetric centers. Tannins are typically composed of either polyol (or polyalcohols or glycols) such as glucose, or of catechins or triterpenoids to which galloyl units (or their derivatives) are attached, or of oligomers or polymers of flavanols. Two distinct tannin groups are commonly recognized, which differ in both structure and biogenic origin: hydrolysable tannins and gallic tannins (**Bruneton, 2009**) (**Table 1**).

d) Coumarins

Coumarins, which belong to the group of compounds known as benzo-α-pyrone (**O'Kennedy and Thornes, 1997**), are also derivatives of C6-C3 (**Table 1**), and all contain a hydroxyl substitution at position 7. They can be found in nature either in their free state or in combination with sugars. In response to either biotic or abiotic attacks, they are produced in

large quantities and function as a form of defense mechanism known as phytoalexins.

Table 1. Classification of the polyphenolfamily **(Garcia-Salas et *al*., 2010).**

Carbon number	Class	Basic structure
C6	Simple phenols	
C6	Benzoquinones	
C6-C1	Benzoic acid	
C6-C2	Acetophenones	
C6-C3	Phenylacetic acid	
C6-C3	cinnamic acid	
C6-C3	Phenylpropene	
C6-C3	Coumarins	
C6-C3	Chromones	
C6-C4	Naphthoquinones	
C6-C1-C6	Xanthones	
C6-C2-C6	Stilbenes	
C6-C2-C6	Anthraquinones	
C6-C3-C6	flavonoids	
(C6-C3)2	Lignans, neolignans	
(C6-C1) n	Hydrolyzable tannins	
(C6-C3) n	Lignins	

e) Lignans

These are compounds formed by the condensation of phenylpropane units (C6-C3)

(**Table 1**). They have a wide botanical distribution and are frequently present in the wood of gymnosperms and in lignified tissues of angiosperms (**Krief, 2003**). Lignans are primarily obtained from flax seeds, and to a lesser extent from lentils, white beans, cereal seeds, and certain vegetables (**Dacosta, 2003**).

f) Flavonoids

Flavonoids are distinguished by a core structure of C6-C3-C6, comprising two aromatic rings (referred to as Cycle A and B) connected by an oxygenated heterocycle (Cycle C). This arrangement constitutes the foundational framework of flavonoids (**Erlund, 2004**) (**Figure 1**). These compounds are widespread in all vascular plants and can be found in various plant organs, including roots, stems, leaves, and fruits (**Bruneton, 1999**). Polyphenols are synthesized at the chloroplast level and play a role in the light phase of photosynthesis by acting as electron carriers. Some of these polyphenols can move out of the chloroplasts and accumulate in the vacuoles (**Elicoh-Middleton, 2000**)

Flavonoids can exist either in their free form (aglycones or genins) or as C- or O-glycosides like hyperoside and rutin. Hyperoside is a glycoside formed between the flavonol quercetin and the disaccharide rutinose (**Dacosta, 2003**).

Flavonoids can be categorized into several groups, including flavones (such as apigenin and luteolin), flavonols (like quercetin, kaempferol, myricetin, and catechin), flavanones (for instance, naringenin), dihydroflavonols, flavans, flavanonols, flavan-3-ols (such as epicatechin), and flavylium compounds. Additionally, there are other groups like chalcones, aurones, and anthocyanins (including pelargonidin, cyanidin, and peonidin), chalcones (like butein and phloretin), isoflavonoids (including isoflavones and rotenoids), and coumaranochromones (**Bruneton, 2009**). These various groups of flavonoids originate from a common biosynthetic pathway, which involves both the malonate acetate pathway and the shikimate pathway (**Elicoh-Middleton, 2000**).

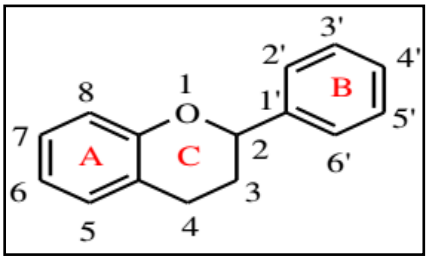


Figure 1. Basic skeleton of flavonoids (**Erlund, 2004**).

g) Stilbenes

The members of this family possess the C6-C2-C6 structure, as outlined in **Table** 1. These substances serve as phytoalexins, which plants produce when they are under attack by fungal, bacterial, or viral pathogens (**Krisa et al., 1997**).

h) Anthocyanins

Anthocyanin pigments impart color to flowers, fruits, and occasionally leave. They share structural and pharmacological similarities with flavonoids (**Catier and Roux, 2007**). These compounds are present in roots, stems, leaves, and seeds, often as glycosides referred to as anthocyanosides. The fundamental structure of anthocyanins involves a 'flavon' nucleus, with glucosylation commonly taking place at the C3 position.

3.2.2. Terpenes

Terpenes are naturally occurring hydrocarbons found throughout the plant kingdom and are synthesized through the mevalonic acid pathway (**Bhat et al., 2005**). Their unique structural feature is the inclusion of an isoprene unit consisting of 5 carbon atoms (C5H8), derived from 2-methylbutadiene **(Bakkali et *al.*, 2008)** (**Figure2**). This group of terpenes includes a range of substances, such as hormones like gibberellins and abscisic acid, carotenoid pigments like carotene and xanthophyll, sterols including ergosterol, sitosterol, and cholesterol, as well as sterol derivatives like digital glycosides, latex (which is a key component of natural rubber), and a multitude of essential oils responsible for the fragrances and flavors associated with plants (**Hopkins, 2003**).

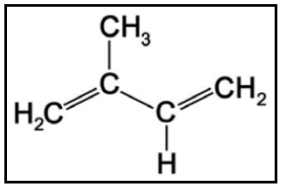


Figure 2. Structure of the isoprene unit (C5H8) (**Solène, 2012**)

As per **Hernandez-ochoa's (2005)** classification, these compounds are categorized based on the number of isoprene units in the following manner:

- Monoterpenes consist of two isoprenes (C10H16).
- Sesquiterpenes are composed of three isoprenes (C15H24).
- Diterpenes are formed from four isoprenes (C20H32).
- Tetraterpenes consist of eight isoprenes, which result in the formation of carotenoids.
- Polyterpenes are formed from (C5H8) n, where n varies between 9 and 30.

3.2.3. Alkaloids

a) **True alkaloids**: Arise from amino acids and contain at least one heterocycle. Proto-alkaloids: Stem from amino acids, yet their nitrogen isn't part of the heterocyclic structure.
b) **Pseudo-alkaloids**: Exhibit true alkaloid traits but aren't derived from amino acids. The following section outlines the most frequently occurring types of alkaloids: Pyrrolizidine alkaloids, tropane alkaloids, and quinoline alkaloids are amongst the most commonly found alkaloids.

Alkaloids, as noted by **Zenk and Juenger (2007),** belong to a class of heterocyclic nitrogen-containing compounds with potent physiological effects, even at low concentrations. They are intriguing natural substances identifiable by their common precipitation reactions and their ability to form compounds with metals. Alkaloids constitute one of the largest groups of natural products, with a vast array of structures, estimated at nearly 10,000 to 12,000 distinct compounds, as pointed out by **Stöckigt and colleagues (2002)**. Generally, alkaloids are categorized as follows, as outlined by **Beddou (2015).**

3.3. Techniques of extraction from plants

3.3.1. Conventionnel extractions techniques

Classical techniques include Soxhlet extraction, hydrodistillation and maceration. These techniques are based on the choice of solvent, temperature and agitation. Extraction with volatile organic solvents is still the utmost commonly used process. The most public solvents at this time used for extraction of essential oils are hexane, cyclohexane, ethanol, methylene chloride, and acetone (**Dapkevicius et *al*., 1998**). Solvents for example methanol, ethanol, acetone, water and

mixtures thereof, have also been used to extract bioactive compounds, although this is often time-consuming and requires organic solvents, which are expensive and hazardous to health (**Garcia-Salas et *al*., 2010**). The compounds extraction poses challenges due to their chemical structures and their interactions with other components in food. Numerous factors, counting the composition of the solvent, the duration of the extraction, temperature, pH, the ratio of solid to liquid, and particle size, can have a substantial impact on the process of solid-liquid extraction, as indicated by **Durling et *al*., (2007)**.

Polyphenols exhibit a spectrum of polarity, spanning from polar to non-polar, and studies have explored their extraction using different solvents like water, acetone, methanol, ethanol, or combinations thereof (**Wang et *al*., 2004**).

Consequently, modern extraction and separation methods have emerged as alternatives that effectively minimize solvent usage and expedite the extraction process. These modern approaches encompass supercritical fluid extraction and pressurized fluid extraction, as highlighted by **Klejdusa et *al*., (2009).**

Solid-liquid extraction (SLE) stands out as the most public and commonly employed practice for the phenolic compound'sextraction from a variety of plants, as outlined by **(Koleva et *al*., 2014)**. Typically, SLE needs the direct extraction of either fresh or freeze-dried plant materials employing numerous solvents, like the aqueous phase of methanol, ethanol, acetone, or solvent blends.

These solvents then necessitate further procedures to eliminate undesirable components, for example column chromatography or solid-phase extraction, as pronounced by **Gadkari et *al*.(2014).**

3.2.2. Modern extraction techniques

A. . Ultrasound Assisted Extraction

As **Yusof et *al*. (2019)** indicated in their report, Ultrasonic-Assisted Extraction (UAE) is recognized as an eco-friendly extraction method thanks to its efficiency, low solvent and time requirements, and its applicability for heat-sensitive compounds (**Figure 3a**).

Ultrasound operates within a frequency spectrum of 20 kHz to 100 kHz and is capable of generating substantial power (ranging from 10 to 1000 W/cm), which is adequate for disrupting intermolecular bonds. When the energy surpasses 10 W/cm, it triggers cavitation—a

phenomenon characterized by the formation and collapse of air bubbles. As these bubbles burst near cell walls, they produce high temperatures (reaching up to 5000 K) and pressures (up to 100 ATM), facilitating solvent penetration into cells and enhancing mass transfer. Beyond mass transfer enhancement, the elevated pressure and temperature can rupture cell walls and membranes, leading to particle size reduction and the release of intracellular components, thereby aiding the extraction process. To optimize ultrasonic-assisted extraction, it's essential to consider factors such as solvent selection, particle size, temperature, time, and solvent-to-solid ratio, akin to traditional methods. Additionally, optimizing ultrasonic parameters, including power and frequency, is crucial (**Bakin et *al*., 2021**) (**Figure 3b**).

Ultrasound operates within the frequency range of 20 kHz to 100 kHz, generating significant power (ranging from 10 to 1000 W/cm), which is ample for breaking intermolecular bonds. When the energy surpasses 10 W/cm, it triggers the cavitation effect, marked by the growth and collapse of air bubbles. When these bubbles burst near the cell wall, it results in the generation of high temperatures (reaching up to 5000 K) and high pressures (reaching up to 100 ATM). These conditions enable solvents to infiltrate the cells, thereby enhancing mass transfer. Moreover, the elevated pressure and temperature can disrupt cell walls and membranes, reduce particle size, release intracellular material, and facilitate the extraction process (**Krakowska et *al*., 2018; Yusof et *al*., 2019**).

To optimize ultrasonic-assisted extraction, it is imperative to choose the appropriate solvent, control particle size, manage temperature, duration, and solvent-to-solid ratio, as in traditional methods. Additionally, it's crucial to fine-tune ultrasonic parameters such as power and frequency (**Bakin et *al*., 2021**) (**Figure 3b**).

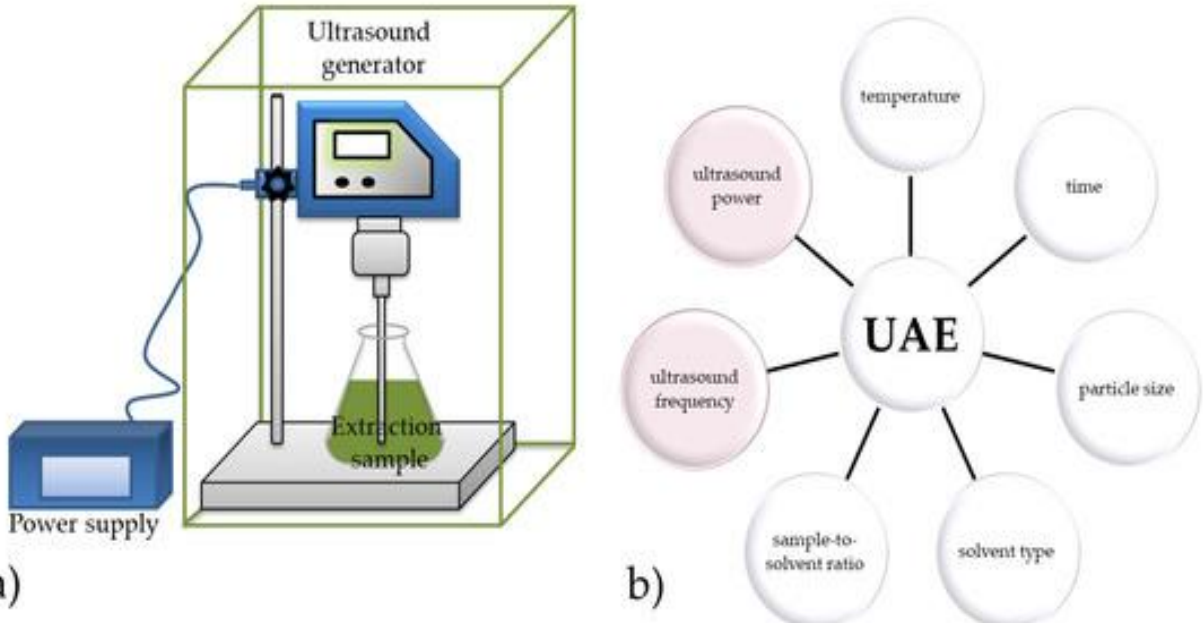


Figure 3. (**a**) Diagram illustrating the process of ultrasound-assisted extraction (UAE) and (b) Key variables that necessitate optimization for an effective UAE extraction (**Jurinjak Tušek et *al*., 2022**)

B. Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) (**Figure 4a**) uses microwaves to heat the matrix (**Yadav et *al*., 2020**). Their frequency range is 300 MHz to 300 GHz. The magnetic and electric fields in microwaves are perpendicular to each other. Electric fields cause heating through ionic conduction and dipole rotation (**Routray and Orsat, 2019**).

Based on their dielectric properties, these components absorb microwave radiation. Irradiation promotes cell rupture and allows fluid to penetrate into the plant matrix. On the other hand, plant material from outside the cell enters the solution (**Cerdá-Bernad et *al*., 2022**). According to **Bagard et *al*. (2021),** the efficiency of microwave-assisted extraction is affected by (i) solvent type and volume, (ii) extraction time, (iii) microwave power, (iv) operating temperature, and (v) matrix and material properties.

The listed factors must be considered when optimizing the process (**Figure 4b**).

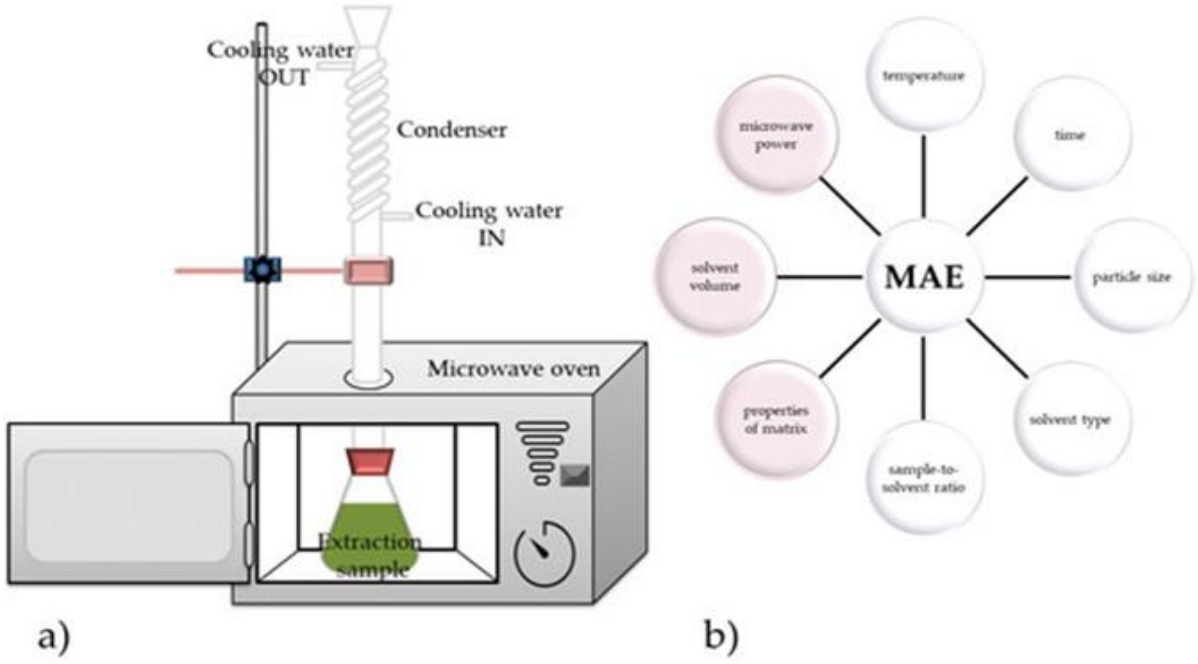


Figure 4. (**a**) Scheme of microwave assisted extraction. (**b**) Specific variables that should be optimized for UAE extraction **(Jurinjak Tušek et *al*., 2022)**

C. Pressurized Liquid Extraction

Accelerated solvent extraction (ASE), also referred to as pressurized liquid extraction (PLE), and enhances the dissolution, mass transfer, and extraction rates of bioactive molecules. It also improves the wet ability and permeability of the sample. This enhancement is achieved through the application of heat and pressure to the solvent system and sample, as depicted in **Figure 5a** (**Zhang and Wong, 2011**).

As described by **Soria et al. (2012),** PLE relies on traditional methods involving elevated temperatures and pressures of up to 200 bar. The elevated pressure ensures that the solvent remains in a liquid state, while the higher temperature increases the solubility of analyses and aids in the extraction process, thereby enhancing the material, as illustrated in **Figure 5b.**

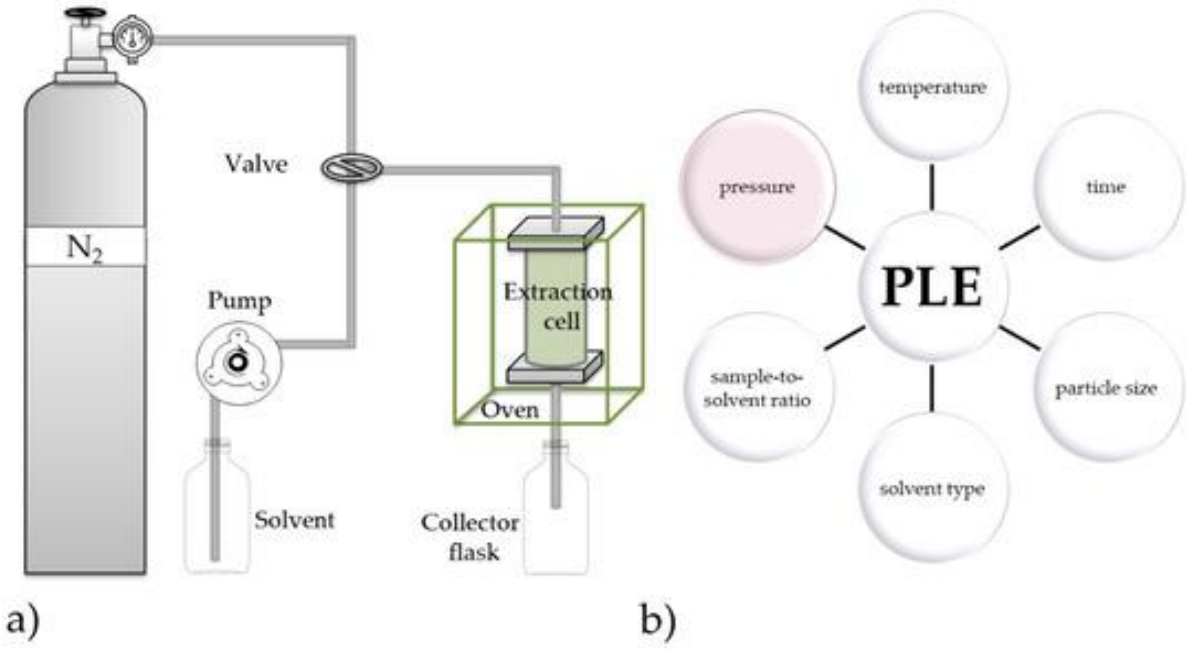


Figure 5. (**a**) Scheme for extracting liquid under pressure. (b) Specific variables that should be optimized for PLE extraction **(Jurinjak Tušek et *al*., 2022).**

D. **Supercritical Fluids Extraction**

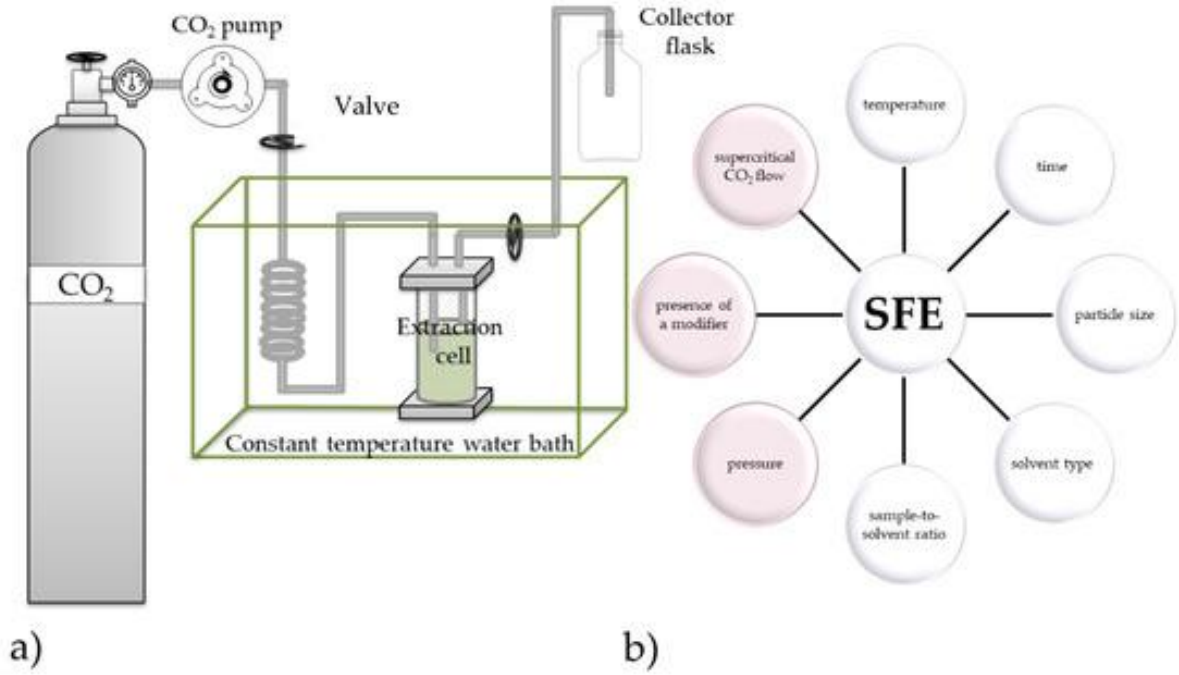


Figure 6. (**a**) Diagram illustrating Supercritical Fluid Extraction (SFE) (b) Specific parameters to be fine-tuned for optimal SFE extraction **(Jurinjak Tušek et al., 2022)**

Supercritical fluid extraction (SFE), depicted in Figure 6a, is regarded as an innovative

extraction technique that offers an environmentally friendly approach to obtain natural compounds from renewable sources like herbs, spices, aromatic plants, and medicinal plants. These compounds have a wide range of applications (**Uwineza and Vacivic, 2020**). SFE employs supercritical fluids to isolate and eliminate particular bioactive molecules (**Molino et *al*., 2020).**

3.3.3. Method of analysis of plant extracts

The quantification of phenolic compounds is contingent on multiple factors, including the compound's chemical properties, the chosen extraction technique, particle size, the standards selected, and the presence of any interfering substances or impurities. With advancements in analytical science, a range of methods has been utilized to measure phenolic compounds in plant materials, including spectrophotometry, HPLC, GC, and combinations of these techniques (**Zhang et *al*., 2022**).

A. Spectrophotometry

Spectrophotometry is a quick and simple method employed to quantify compounds in plant materials, primarily relying on distinct principles for assessing the various structures present in phenolic compounds. The Folin-Ciocalteu assay is a widely utilized technique for detecting compounds in plants. It operates through chemical reduction facilitated by reagents containing tungsten and molybdenum (**Gogia et *al*., 2014**). The Folin-Ciocalteu method represents a modification of the Folin-Denis assay, involving slight adjustments to the reagent composition. Typically, spectrophotometry is employed to measure flavonoid content (**Pouraboli et *al*., 2016**), as well as to estimate phenolic content and condensed tannin content (**Sankhalkar et *al*., 2016**). Due to its simplicity and cost-effectiveness, spectrophotometry is a prevalent technique for quantifying various classes of phenolic compounds.

Table 2. Different Extraction Methods of Phenolic Compounds (**Zhang et *al*., 2022**).

Extraction method	**Advantages**	**Disadvantages**	**Affectingfactors**	**Application**	**Ref.**
Solid–liquid extraction	Simple; well-established and widely employed; easily adaptable for industrial-scale applications.	High solvent consumption; extended extraction time.	Solvents, extraction time, temperature, stirring mode, plant materials used, powder size, solvent-to-solid ratio.	Catechin syringic acid p-coumaric acid.	**Koleva et *al*., 2014**
Ultrasound-assisted extraction	Simple to implement; utilizes affordable equipment; requires minimal solvent usage; rapid extraction process; yields high extraction rates; minimal environmental footprint.	Generation of excess hydroxyl radicals cause degradation of active compounds.	Solvents, plant materials, ultrasound properties, extraction time, and temperature, solvent-to-solid ratio.	Gallic acid and rutin pmanthocyanidin	**Ameer et *al*., 2017**
Supercriticalfluid extraction	High selectivity; cheaper solvent; easily controlled extraction conditions; low operating temperature; environmental friendliness; easy separation of solvent from solutes.	Low total yield; not suitable for extraction of polar active compounds.	Pressure, temperature, co-solvents, solvent flow rate, time, and plant materials.	Anthocyanin gallic acid and protocatechuic acid.	**Pimentel-Moral et *al*., 2019**
Microwave-assisted extraction	Short extraction time; low solvent consumption.	Degradation of thermally sensitive compounds.	Solvents, solvent-to-solid ratio, microwave power, temperature, extraction time, and plant materials.	3-caffeoylquinic acid, 5-caffeoylquinic acid, ellagic acid, and ferulic acid	**Dahmoune et *al*., 2015**
Pressurizedliquid extraction	Consumes fewer organic solvents; fast and efficient; possibility to avoid organic solvents by using water only.	The need for optimal extraction temperature.	Solvents, solvent-to-solid ratio, temperature, extraction time, pressure, and plant materials.	Rutinquercetin	**Fernández-Ponce et *al*., 2016**
Enzyme-assisted extraction	Safe and green; do not require complex paraphernalia.	Long extraction time; low-efficiency	Type and concentration of enzyme, temperature, pH, solvents, extraction time, and substrate concentration.	Proanthocyanidinnaringinhesperidin	**Kitryte et *al*., 2017**

B. Gas Chromatography (GC)

GC is a valuable technique for the separation, identification, and quantification of specific phenolic compounds in plants, including tannins, flavonoids, and anthocyanins. It capitalizes on the distinctive vaporization temperatures of individual compounds, separating the sample from the solution by passing it through a heated column located between a pressurized inert gas and a thin layer of a non-volatile liquid containing an inert matrix (**Balas and Popa, 2007**). The derivatization and volatility of phenolic compounds are key components of GC detection. GC coupled with Mass Spectrometry (MS) detectors is widely employed for the analysis of complex compounds, leveraging its exceptional selectivity and quantitative sensitivity. For instance, the low molecular weight fraction, predominantly lignans, in the hydrophilic extract from Knotweed spruce, was characterized using GC-MS (**Smeds et al., 2016**).

C. High-Pressure Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is the predominant method for separating and detecting phenolic compounds. This versatile instrument offers numerous advantages, including exceptional selectivity, sensitivity, resolution, precision, and sample handling capabilities (**Naczk and Shahidi, 2006**). The fundamental principle of this method involves the separation of compounds from complex mixtures based on their solubility and/or the interaction between a less polar stationary phase and a more polar mobile phase (**Coskun, 2016**). Consequently, various factors can influence the HPLC analysis of phenolic compounds, such as the type of column used, the detector employed, the mobile phase composition, and the characteristics of the compound being tested. To obtain information about a specific phenolic compound, it's essential to compare its retention time with a standard. However, one notable limitation of HPLC techniques is the lack of standards for certain classes of polyphenols, particularly flavonoid glycosides and proanthocyanidins, which poses a significant challenge (**Ignat et *al*., 2011**).

D. HPLC–Mass Spectrometry

Phenolic compounds can undergo analysis using High-Performance Liquid Chromatography (HPLC) coupled with tandem Mass Spectrometry (MS). The combination of

HPLC and MS detection represents an advanced analytical method known for its remarkable sensitivity and selectivity. This approach is particularly valuable for gathering structural information about unidentified compounds present in either raw or partially purified samples of natural origin (**Mocan et *al*., 2014**). Recent research endeavors focused on assessing various techniques that involve the integration of HPLC and MS for the analysis of phenolic compounds. Mass spectrometry has gained popularity in recent years for such analyses due to its exceptional sensitivity and selectivity. Furthermore, it has the capability to provide valuable structural insights into unknown compounds. Currently, this method stands as the most superior analytical approach for investigating phenolic compounds across a range of biological resources, offering unparalleled effectiveness in structural analysis. Nevertheless, it's important to note that one of its major drawbacks is the high cost of the required equipment.

E. HPLC–Diode Array Detector

HPLC coupled with a diode array detector (HPLC-DAD) is another widely employed method for the analysis of phenolic compounds in plant materials (**Da Silva et *al*., 2016**). Among all the detectors paired with HPLC for phenolic compound determination, Mass Spectrometry (MS) is the costliest and less commonly used, whereas Diode Array Detection (DAD) is the most practical and prevalent (**Zhang et *al*., 2013**). DAD detectors have the ability to simultaneously scan the full UV/Vis spectrum of an analyte, offering insights into specific spectral properties that aid in compound identification. Overall, HPLC-DAD is recognized as a straightforward, cost-effective, reliable, and adequately sensitive analytical platform for assessing constituents in food materials (**Rejczak&Tuzimski, 2017**)

F. Other Analytical Methods

HPLC with fluorescence detection (HPLC-FLD) has also been utilized for the examination of phenolic compounds (**Wulandari et *al*., 2015**). HPLC-FLD is effective in detecting substances that exhibit fluorescence or can be induced to fluoresce through suitable excitation. In contrast, thin-layer chromatography (TLC) is a relatively cost-effective chromatographic method (**Males et *al*., 2013**). This technique allows for the rapid separation of phenolic compounds in crude plant extracts, with multiple substances detectable on a single TLC plate. Furthermore, capillary electrophoresis represents an advanced analytical method for the

quantification of phenolic compounds in plant materials. It is especially well-suited for the separation and measurement of polar and charged compounds with low to moderate molecular weights (**Boiteux et *al*., 2014**).

4. Biological Activities

Natural plants serve as a crucial reservoir of bioactive compounds, which hold significant value across a diverse range of applications, particularly within the realm of biological activity. Bioactive compounds are instrumental in delivering natural antioxidant, antibacterial, and anti-inflammatory effects, contributing substantially to the treatment of conditions like obesity, cancer, and diabetes. A few noteworthy biological activities associated with these bioactive compounds are outlined below:

4.1. Antioxidant Activity

The exploration of plant extract antioxidant potential stands as a prominent subject within the scientific community **(Avello et *al*., 2013)**. In recent decades, assessing antioxidant activity has become a focal point in studies related to medicinal plants and their constituent components. The concept of measuring the collective antioxidant capability within a single test has garnered widespread attention. This method allows for the incorporation of the individual antioxidant effects of various compounds, about their additive, synergistic, or antagonistic interactions **(Sadowska-Bartosz et *al*., 2022).** Numerous techniques have been proposed to gauge the overall ability of biological materials to counteract reactive oxygen species, neutralize free radicals, or house reducing substances. While these terms are closely related, they aren't entirely synonymous, which has introduced some level of ambiguity. A reducing substance contributes an electron or a hydrogen atom to a compound with a higher redox potential. This group includes free radicals and other oxidative agents present in biological systems. It's important to note that not all reactive oxygen species (ROS) are free radicals. Hydrogen peroxide, among others, doesn't fall into the category of free radicals **(Sadowska-Bartosz et *al*., 2022)**. The majority of total antioxidant capacity (TAC) tests are founded on the interaction of antioxidants with stable free radicals or free radicals generated in situ (**Table 3**).

Table 3. Most popular assays of antioxidant capacity.

Method	Principle of Measurement	References
$ABTS^{\bullet}$ Reduction	Decrease in absorbance of solution of pre-formed $ABTS^{\bullet}$ radical (usually at 734 or 414 nm)	**Kut et *al*., 2022**
$DPPH^{\bullet}$ Reduction	Decrease in absorbance of solution of the stable $DPPH^{\bullet}$ radical (around 517 nm)	**Blois, 1958 ; De Menezes et *al*., 2021**
FRAP	Increase in absorbance of Fe^{2+}-TPTZ complex upon reduction of Fe^{3+} to Fe^{2+} (at 593 nm)	**Benzie et *al*., 1996**
CUPRAC	Increase in absorbance of bis(neocuproine) copper (+) upon reduction of Cu^{2+} to Cu^{+} (at 540 nm)	**Apak et *al*., 2004**
ORAC CL assay	Inhibition of fluorescence decrease of R-phycoerythrin or fluorescein induced by a source of peroxyl radicals Inhibition of chemiluminescence of a detector induced by an oxidant	**Ou et *al*., 2001**

In addition to the well-established methods, various other Antioxidant Capacity assays are in use. The Hydroxyl Radical Antioxidant Capacity (H-ORAC) test relies on the oxidation of a fluorescent probe by hydroxyl radicals generated through a Fenton system, typically involving Co2+ and H2O2 (**Ou, Hampsch et *al*., 2002**). The Potassium Ferricyanide Reducing Power (P-FRAP) assay is based on the reduction of ferricyanide to ferrocyanide by antioxidants (**Berker et al., 2010**). The Total Reactive Antioxidant Potential (TRAP) test measures the capacity of antioxidants to inhibit the reaction between peroxyl radicals and a target molecule, originally represented by the O2 consumption during the peroxidation process triggered by the thermal decomposition of AAPH (**Munteanu et *al*., 2021**). The Total Radical-Trapping Antioxidant Parameter assay also gauges the ability of antioxidants to interfere with the reaction between peroxyl radicals and a detector (**Ghiselli et *al*., 1995**). The Total Oxyradical Scavenging Capacity (TOSC) test is based on the inhibition of the formation of ethylene from α-keto-gamma-methiolbutyric acid by antioxidant compounds (**Winston et *al*., 1998**). The β-Carotene Bleaching assay (**Marco et *al*., 1968**) employs an aqueous emulsion of linoleic acid and β-carotene, which undergoes discoloration due to the radicals generated through the spontaneous oxidation of the fatty acid at elevated temperatures. Another assay involves the reduction of galvinoxyl, a radical that's commercially available, oxygen-centered, and more reactive toward polyphenols than DPPH (**Kotora et *al*., 2016**). The Folin-Ciocalteau assay is also considered among the TAC assays. Originally developed for the assay of tyrosine and tryptophan, it was later modified for the analysis of total polyphenols (**Singleton et *al*., 1965**). Additionally, there

are TAC assays that don't rely on optical measurements but instead utilize voltammetry, amperometry, chromatography, electrophoresis, or direct measurement of radical scavenging through electron paramagnetic spectroscopy, often employing nanoparticles.

4.2. Antibacterial Activity

In addition to antioxidant effects, plant phenolic compounds also focus on antibacterial effects. Further analysis revealed a strong relationship between antimicrobial activity and phenol content (**Jang et *al*., 2018**).

Numerous polyphenols, with a focus on flavonols and tannins have demonstrated antibacterial, antiviral, and antifungal properties. These compounds can disrupt bacterial physiology through various mechanisms. Typically, they interfere with membrane functions or suppress key virulence factors, including enzymes, toxins, signal receptors, and the formation of bacterial biofilms. Additionally, some polyphenols have been found to synergize with antibiotics, such as catechins, which can modulate β-lactam resistance in multi-resistant strains like Staphylococcus aureus and Extended-Spectrum Beta-Lactamase (ESBL)-producing Escherichia coli. Several common assays are employed to assess polyphenol activity, including broth micro and macro dilution, disk diffusion assays, and the agar dilution technique. It's important to consider specific variables like the size of the inoculum, the size of wells or paper disks (in the case of the broth microdilution assay or disk diffusion assay, respectively), and the duration of incubation. Standardization of in vitro susceptibility tests and the establishment of tentative breakpoints for the most promising polyphenols are necessary. Breakpoints are crucial for classifying common pathogens as susceptible or resistant to a particular antibacterial agent. Standardizing susceptibility methods is vital for comparing in vitro results obtained by different research laboratories. Following the completion of the in vitro stage, further in vivo studies are required to assess the activity, toxicity, and metabolic fate of selected polyphenols within the body before they can be used as therapeutic agents for human infections. In conclusion, the research suggests that polyphenols hold promise as potential sources of antimicrobial agents, and further exploration is essential in an era challenged by antibiotic-resistant "superbugs (**Coppo and Marchese, 2014**).

4.3. Cytotoxic Activity

Over the past decades, cell culture methods have been utilized to evaluate the cytotoxicity of plants materials. The development of these methodologies has relied on original research and modified applications of existing techniques. Various parameters are employed to assess cytotoxic effects, including inhibition of cell growth, cell lysis, the presence of membrane or cytoplasmic markers, and alterations in metabolic activity. These methods also employ diverse approaches to establish contact between cells and materials, ranging from direct contact to the use of permeable spacers such as agar, dentine, or molecular filters. This includes the use of material extracts or particulate materials. One major challenge in comparing results across these various methods is the accurate assessment of the concentration and identity of the substances to which the cells are exposed (**Canga et *al.*, 2022**).

The ratio of specimen surface area to the volume of cell culture medium is a crucial parameter in most testing systems, and this ratio can vary significantly from one method to another, often selected to induce a cytotoxic effect. Advanced methodologies for cytotoxicity studies are readily available for both routine investigations and research projects. However, a more comprehensive understanding of the dissolved components or degradation products would substantially enhance the long-term significance of the results. The use of cell culture methods for toxicity testing of dental materials has been proposed as a relatively simple, reproducible, cost-effective, and relevant alternative to animal experiments. Additionally, these methods have been suggested for toxicity screening of new materials, identifying cytotoxic substances, and for ensuring the biological quality control of production batches. While these claims have been made, most lack robust substantiation through research data. There may be some validity to each of these assertions, but further correlation studies that bridge in vitro tests, physical/chemical data, and in vivo studies are clearly warranted (**Hensten- Pettersen, 1988**).

5. Plant Monographs

5.1. Rutaceae family: description, chemical composition and therapeutic use

Rutaceae is a large family mainly distributed in the tropics and subtropics, is a rich source of natural products and exhibits a wide range of structural diversity, three main centers of diversity: Tropical America, Southern Africa and Australia (**Groppo et *al.*, 2012**). In Algeria, Rutaceae is widely distributed, especially in mountainous areas. Many species of this family are used in traditional medicine because they contain a variety of molecules with therapeutic properties.

They are herbaceous plants with alternate, odorous, simple, well-regulated leaves. Terminal inflorescence. Hermaphroditic flowers. Sepals and petals 4-5. Stamens 8-10 inserted on disc with 8-10 nectar-bearing dimples. The ovary has 4-5 cells separated by an inner border (**Quezel and Santa, 1962**).

Comprehensive studies on this plant family have been conducted, revealing the potential use of these natural compounds in managing a wide array of conditions, including Alzheimer's disease, depression, cancer, and various infections, owing to their antibacterial, antifungal, anti-leishmanial, and anti-plasmodial properties (**Adamska-Szewczyk et *al.*, 2016**).

The *Ruta* genus comprises ten species, with the most frequently documented ones being *R. chalepensis*and *R. graveolens*. These *Ruta* plants are perennial shrubs and have a long history of use in traditional folk medicine, primarily for addressing a range of female reproductive health issues (**Coimbra et *al.*, 2020**).

5.2. The species *Ruta montana* L.

5.2.1. Botanical, systematic description and Geographical repartition of Ruta montana

R. montana is a naturally occurring species native to the Mediterranean and Middle East (**Benkhaira et *al.*, 2022**). One of the best-known species is *R. montana*, whose botanical name is synonymous with "*Ruta Graveolens var. montana* L." (**Benkhaira et *al.*, 2022**). It is commonly called Fijel in Algeria (**Benkhaira et *al.*, 2022**; **Mohammedi et *al.*, 2020**). Fijel, Aourmi in Morocco (**Drioiche et *al.*, 2020**) and Fijelel-djbel in Tunisia (**Khadhri et *al.*, 2014**). *Ruta montana* is a perennial shrub, typically reaching heights of 20 to 60 cm, featuring slender triangular leaves, the plant's small yellow flowers are bisexual, with two whorls of stamens

(Figure 2c), and its fruits take the form of capsules with four rounded lobes, notably, *Ruta montana* is distinguished by its pungent and unpleasant odor, attributed to the presence of a substantial quantity of essential oil stored within large secretor glands (**Figure 7: a,b,c**and **d**) (**Benkhaira et *al*., 2022**).

5.2.2. Previous investigation, therapeutic uses and composition of *Ruta montana*.

The plant is known for its anti-abortion and anti-fertility, antispasmodic, analgesic, hypoglycemic, diuretic, anthelmintic, antirheumatic, menstrual and antiparasitic, antifungal, anti-inflammatory, disinfectant, antipyretic, laxative and antiepileptic characteristics are widely used. In addition, it is used in the treatment of skin diseases (**Kambouche et *al*., 2008**; **Abdelwahab et *al*., 2011**; **Mohammedi et *al*., 2020**; **El-Ghazouani et *al*., 2021**; **Belhaj et *al*., 2021)**. In the Hodna region of Algeria, it is used to treat high blood pressure. Recent studies have shown that *R. montana*has various biological activities such as: antioxidant (**Mohammedi et *al*., 2020; Kara, 2016**; **Khadhri et *al*., 2017**); **Benali et *al*., 2020;**Mergem and Dahamna, 2020). Antibacterial and antifungal activity (**Gibka et *al*., 2009**; **Hammami et *al*., 2015**; **Allouni, 2018**; **Mohammedi et *al*., 2020**; **Benali et *al*., 2020),** antidiabetic (**Farid et *al*., 2017**), anticancer (**Ali et al., 2016**), antifertility (**Merghem andDahamna, 2020**), hypotensive (**El-Ouady et *al*., 2021**), insecticidal and larvicidal properties (**Boutoumi et *al*., 2009**; **Bouzeraa et *al*., 2019**).

Some studies have found that *Ruta montana* contains multiple secondary metabolites such as alkaloids, combined anthracenes, sterols, triterpenes, coumarins, and tannins (**Benkiki, 2006;Daoudi et *al*., 2016**; **Khadhri et *al*., 2017**; **Allouni, 2018**).

Phytochemical studies have shown that this species is rich in coumarins and rutins such as: Chalepensin, Chalepin, Rutamarin, Umbelliferone, Daphnoretin, Scopolamine, Psoralen, Rutolide, Daphnoretin, Isopinpinillin, Heraclenol, Rutamontin, Xanthotoxin and Bergapten (**Agullo Martinez et *al*., 1969**); **Kuffner et *al*., 1973; Sepulveda et *al*., 1973; Del Castillo et *al*., 1986; Abyshev et *al*., 1992; Tuati et *al*., 2000; Kabouche et *al*., 2003**), lignans (sesamin) (**Ulubelen et *al*., 1990**).According to other existing reports, *R. montana* crude extract contains other bioactive compounds, including alkaloids such as leucocyanidins. On the other hand, small amounts of flavonoids, catechols, sterols, triterpenes, sugars, hologlycosides and C-heterosides were also identified (**Daoudi et *al*., 2016**). Quantitative phytochemical analysis of ether air fraction extracts using silica gel column chromatography revealed the presence of a new alkaloid called montane and two known alkaloids: 1,2-dimethyl-4-quinolin phenone and leucantine

(**Ulubelen et *al*., 1990**).

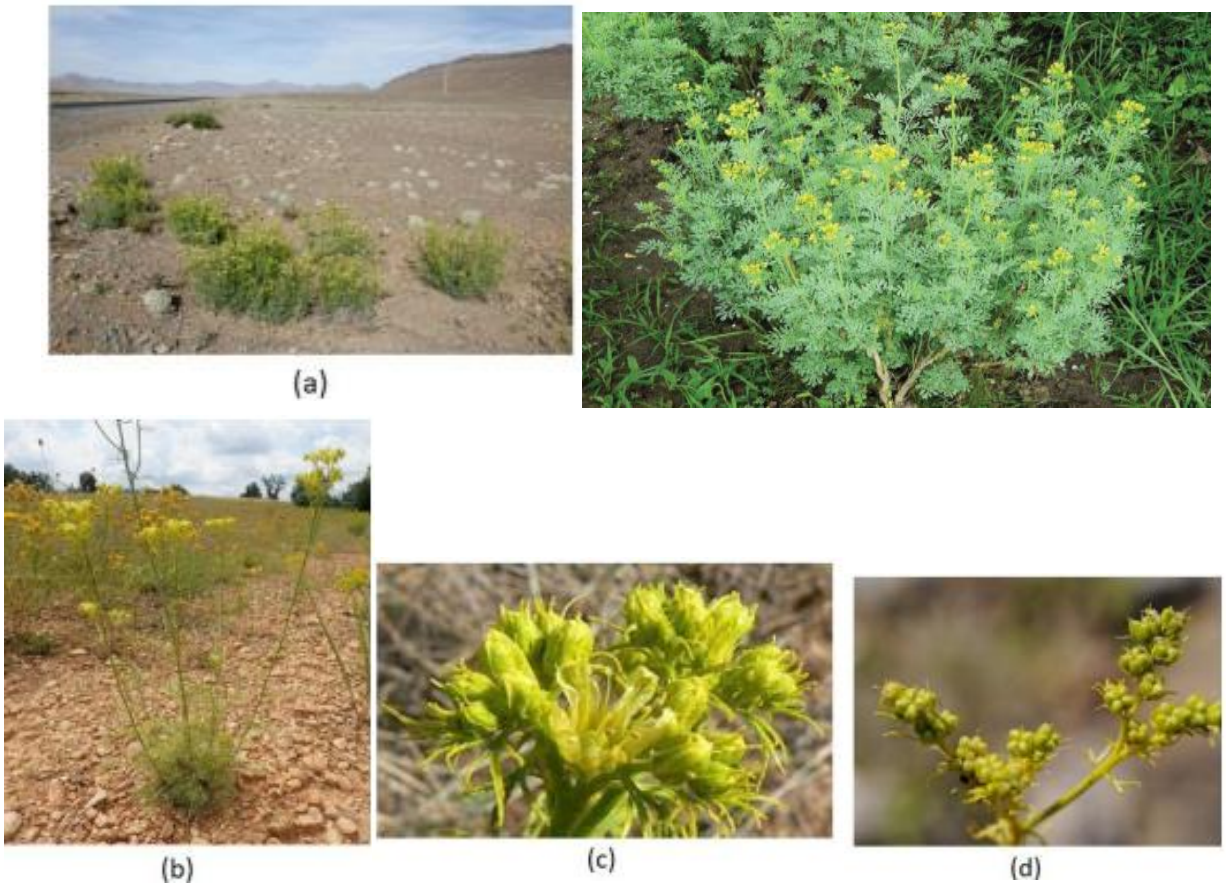


Figure7.*Ruta montana* L. habitat (a); whole plant (b); flowers (c); and fruits (d). (**Choucha et *al*., 2017; Benkhaira et *al*., 2022**)

In Algeria, *Ruta montana* is employed to alleviate various health issues, including digestive disorders, toothaches, joint pains, and to assist in challenging childbirth (**Adli et *al*., 2021**). Additionally, the essential oil extracted from *Ruta montana* holds great significance in the fragrance industry (**Hammicheand Azzouz, 2013; Miara et *al*., 2019**). In Persian traditional medicine, powdered *R.montana* leaves have been a remedy for epilepsy (**Abolhasanzadeh et *al*., 2017**). This powder is also used as a culinary spice; the leaves' bitterness enhances appetite, and their aroma adds a pleasant flavor to fish dishes. Furthermore, in Italy, R. *montana* leaves are utilized to flavor vinegar and a type of alcohol known as "Grappa" (**Hammiche and Azzouz, 2013**).

CHAPTER II:

EXPERIMENTAL STUDY

1. Ethnobotanical study

1.1. Study area

The study area (Bousàada) is located in the province of M'sila, southeast of Algiers (36°42'13" N, 6°51'23" E), situated 260 km above sea level. It includes five cities: Mdjedel, Temsa, Menaa, Slim and Bousâada (**Figure8),** with an area of 2257 km^2 and a world population of 210181 people (**A.S.M.** (**Annuairestatistique de M'Sila, 2019**)). The Saada region is bounded by the Hodna Mountains to the north, the Ziban Mountains to the south, the Berezma Mountains to the east, and the Ould-Nagel Mountains to the west (**A.N.A.T.** (**Agence National pour l'Aménagement du Territoire, 2004**). The natural structure of the entire province is very heterogeneous. Busada's local economy is based on farming, animal husbandry and tourism and basically dates back to French colonial times (**D.S.A., 2020**).

The study area exhibits two distinct natural regions: the first is the steppe, primarily composed of *Stipa tenacissima* L. and *Artemisia herba alba Asso*.

This region is characterized by sparse plant cover, which serves as an indicator of the extent of degradation. The second region is the mountain area, designated for extensive mountain farming, where green oak (*Quercus ilex* L.) is notably present on the slopes.

Earlier research has indicated that the M'sila region boasts a relatively diverse flora with therapeutic applications, including prominent plant families such as Lamiaceae, Asteraceae, Fabaceae, and Zygophyllaceae (**Benkheira et *al*., 2005**).

The climate in the Bousàada region is of a continental type, influenced in part by Saharan factors. It is characterized by scorching summers, frigid winters, and limited, irregular precipitation, averaging around 260 mm per year. The annual mean rainfall measure 11.90 mm per year, with the highest monthly average temperature occurring in August at 33°C and the coldest month being January with an average of 8°C.

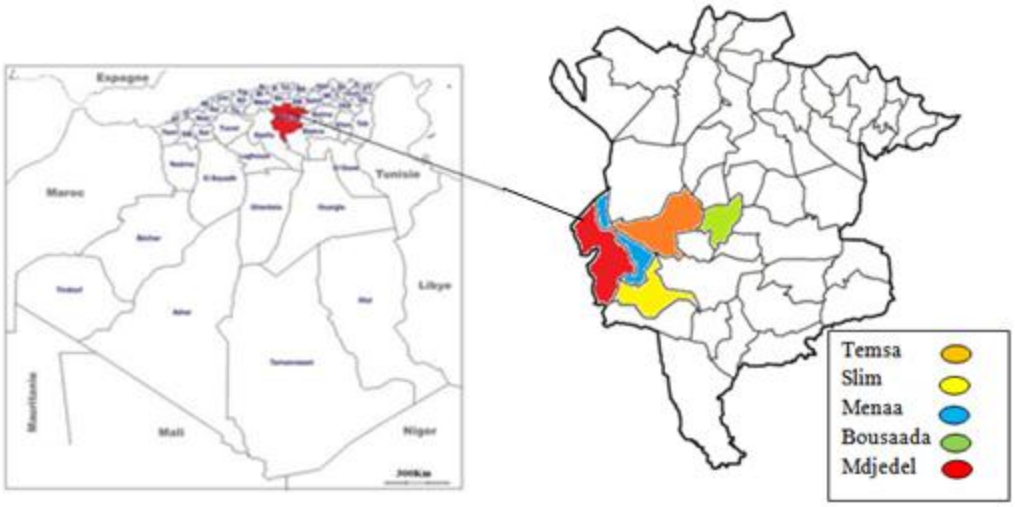


Figure 8. Localization of study area (Rural regions of Bousaada; South Weast of M'sila province, Algeria), (Municipalities of Mdjedel, Temsa, Menaa, Slim and Bou Saàda).

1.2. Ethnobotanical surveys

An ethnobotanical survey was conducted in the rural towns of Bousàada over two years **(2020-2022)** by interviewing users of medicinal plants through 534 structured questionnaires (**Annexe 01**). The interviews were carried out face-to-face following the guidelines presented by **Martin (1995).** The selection of users was based on a simple stratified random sampling approach. The interviews adhered to the ethical guidelines outlined by the International Society of Ethnobiology (ISE), ensuring that participants felt no pressure, enabling them to respond candidly (**Akerreta et *al*., 2007**).

The questionnaire employed in this study encompassed two main sections. The first part gathered information regarding the background of the participants, covering aspects such as educational level, gender, age, monthly income, the source of their knowledge, and preferred healthcare practices. The second section was dedicated to the plants and their uses, including vernacular names. To validate the information obtained from local informants, data was collected on the uses of the plants, preparation methods, therapeutic applications, and the specific plant parts utilized.

Permission from local knowledge holders to participate in the questionnaire was obtained, aligning with the principles of the International Society of Ethnobiology **(ISO, 2006)**. The botanical identification of plant specimens was performed by Professor Miara (M.D.), a consulting botanist at Tiaret University, who referred to related floras by [**(Battandier and**

Trabut, 1895), (Maire,1952), (Quézel and Santa,1962), (Kaddem, 1990), (Baba Aissa,1991), and **(Dobignard and Chatelain, 2010)].** To confirm scientific names and synonyms of the plants, an online database **(www.theplantlist.org)** was consulted.

1.3. Data analysis

The recorded information was input into our in-house database utilizing commonly available software (Excel). Subsequently, this data was subjected to a comprehensive analysis and comparison by referencing numerous national and international ethnopharmacology sources, including articles, books, and reviews available on electronic databases such as Science Direct, PubMed, and Google Scholar. The aim was to identify both commonalities and disparities, as well as to unearth new applications for both familiar and unfamiliar medicinal plants.

To perform this analysis, three indices, which have been frequently used in prior studies (**Abu-Irmaileh and Afifi, 2003; Uddin and Hassan, 2014; Benarba et *al*., 2015; Miara et *al*., 2019 a, b**), were applied to the collected data:

1.3.1. The use-value of species (UV)

A quantitative approach for assessing the significance of plant species known to the local community is computed using the following formula: $UV = \Sigma U/n$, where "U" represents the total number of mentions made by each interviewee regarding a specific plant species, and "n" is the total number of interviewees surveyed at a particular site.

UV values are employed to measure the effectiveness of plants in treatment illnesses. UV scores are higher when there is a greater number of reference to a plant's usage and lower when there are fewer mentions of its use, as previously outlined by **Abu-Irmaileh and Afifi (2003).**

1.3.2. Fidelity Level (FL)

This metric is utilized to identify the plant species that respondents in the study area most frequently use to address specific categories of diseases. It's calculated using the formula established by **Martin (1995)**: $\mathbf{FL = (Np / N) \times 100}$, where "Np" represents the count of usage reports linked to a particular species and disease category, and "N" stands for the total number of usage reports connected to that species.

Typically, high FL values are associated with plants for which nearly all usage reports pertain to a single method of application, while low FL values are found for plants employed for

various purposes, as indicated by **Heinrich et *al*. (1998).**

1.3.3. The Informant Consensus Factor (ICF)

Used to express the degree of information homogeneity. The calculation method is as follows: ICF= (only – Nt)/ (only – 1)

Where: "only" is the number of quotes used in each category. “Nt” represents the number of species reported in each category. ICF values were lower (closer to 0) when plants were selected randomly or when informants did not share information about their uses. Values are high (close to 1) when there are clearly defined selection criteria in the community and/or information is exchanged between information providers (**Kaya, 2006**).

This metric is employed to measure the level of consistency in information among informants. The calculation method is as follows: ICF = (only - Nt) / (only - 1), where "only" stands for the number of citations used within each category, and "Nt" represents the number of species reported in each category.

ICF values tend to be lower (closer to 0) when plants are chosen randomly or when informants do not share information about their uses. Conversely, values are higher (nearer to 1) when there are clear selection criteria within the community or when information is actively exchanged among providers, as observed by **Kaya (2006).**

2. Phytochemical and biological study

2.1. Plant material

The aerial parts of *R. montana* were harvested from naturally growing individuals in Bousaàda region in northeastern Algeria (100*Meters* above mean *sea level*, 35° 42' 20.99 N, 4° 32' 30.98" E) in **November 2020**. The botanical determination was carried out by **Pr. Rebbas. K**fromM'sila University used existing literature (**Quezel and Santa, 62-1963**),and then the voucher was deposit in the herbarium of the University of M'sila in Algeria, the dry stems and leaves in a ventilated chamber for several weeks before extraction. Light preservation, crushed and stored in glass bottles.

2.2. Extraction

A total of 10 g of the plant powder are macerated in 200 mL of 80% ethanol/H2O for 24 h in the dark room temperature with constant stirring. This process is repeated three times. The extracts were collected by filtration through filter paper and evaporated using a rotary evaporator at 40°C and stored at 4°C.

The aqueous phase obtained in the first step underwent a series of liquid-liquid extractions using organic solvents of increasing polarity, such as chloroform, ethyl acetate, and n-butanol, in a separatory funnel. This extraction process was repeated three times for each solvent to ensure maximum recovery of the desired compounds. The organic phase obtained after each extraction was concentrated using a rotary evaporator, while the aqueous phase underwent another extraction with a solvent of higher polarity than the previous one in the following order: chloroform, ethyl acetate, and finally n-butanol (**Benchadi et *al*., 2020**). At the end of this step, three distinct fractions were obtained: a chloroform fraction, an ethyl acetate fraction, and a butanol fraction. Finally, the extraction yield of different extracts (crude, chloroformic acid, ethyl acetate, butanol) was calculated.

2.3. Chromatography analysis

2.3.1. Thin layer chromatography (TLC)

TLC analysis of ethyl acetate and butanol extracts was performed using TLC precoated plates (silica gel 60F254) using the one-way rise technique and a solvent system; methanol: hexane (9:1). Phytochemical analysis of emerging spots was performed using liquid chromatography coupled to mass spectrometry (LC-MS).

2.3.2. LC-MS analysis

Phenolic compounds in the crude extract (80% ethanol/water) of R. Montana were subjected to LC-MS analysis. The phenolic components were provisionally identified by comparing the results with existing literature. The LC-MS analysis was carried out using the Agilent 6420 series triple quadrupole dual MS instrument, equipped with a state-of-the-art high-performance liquid chromatography system (HPLC 1260 Infinity LC). This setup included a vacuum seal degasser, infinity 1260 autosampler, a dual piston pump, and an exceptionally sensitive UV diode array detector (DAD). Separation was achieved using a Zorbax Eclipse Plus C18 column (1.8 μm, 150 mm × 4.6 mm) from Agilent Technologies, Palo Alto, CA, USA. The mobile phase consisted of a gradient elution with eluent A being water containing 0.1% formic acid and eluent B being acetonitrile. The multi-step linear gradient was as follows: 0 min, 15% B; 35 min, 95% B; 40 min, 95% B; 55 min, 15% B. Subsequently, a 5-minute conditioning period under the same conditions was applied for the next analysis. The injection volume was 10 μL, the mobile phase flow rate was 0.4 mL/min, and the column temperature was maintained at 40 °C. The mass spectrometer operated in negative ion mode with a capillary voltage of 4000 V, a drying gas flow (nitrogen) pressure of 25 psi, and a drying gas flow rate of 7 L/min (**Braca et *al*., 2002**).

2.4. Biological activities

2.4.1. Antioxidant activity

The antioxidant power was evaluated using two different techniques: DPPH (2,2 Diphenyl-1-picryl hydrazine) and total antioxidant assays.

A. 2,2 Diphenyl-1-picryl hydrazine (DPPH) test.

The assessment of anti-free radical activity followed the methodology outlined by **Braca et *al*. (2002).** This test is notable for its capacity to generate stable free radicals, owed to the

electron delocalization within the molecule, resulting in their stability. In a nutshell, 1 mL of DPPH dissolved in methanol (0.004%) was combined with 1 mL of diverse test extracts spanning a concentration range of 10–160 µg/mL. The resulting mixture was then shielded from light and left at room temperature for 30 minutes. Subsequently, the absorbance was gauged at 517 nm. The percentage of inhibition was calculated using the subsequent formula: I (%) = [(At0 - At30) / At0] × 100.

I (%): Inhibition percentage; At0: Absorbance of control (absorbance at time 0); At30: Absorption of test extract after incubation for 30 minutes. Results are expressed as IC50. Ascorbic acid was used as a control. Each experiment was performed in triplicate.

B. Total antioxidant capacity

The overall antioxidant capacity or molybdate reducing activity of our extracts was assessed following the procedure introduced by **Prieto (1999).** This technique depends on the reduction of molybdenum (in the form of molybdate-MoO_4^{2-}) to molybdenum-MoO^{2+} ions in the presence of antioxidants, leading to the creation of green phosphate complexes in an acidic environment. Specifically, 0.3 mL of the test extract was blended with 3 mL of a reagent comprising sulfuric acid (H2SO4; 0.6 M), sodium phosphate (Na3PO4; 28 mM), and ammonium molybdate ((NH4)6Mo7$O_2$4; 4 mm). After incubating at 95 °C for 90 minutes, allowing it to cooland the solution's absorbance was gauged at 695 nm relative to the control. The total antioxidant capacity was quantified in micrograms of ascorbic acid equivalents per milligram of extract (microgram equivalents AA/mg E).

2.4.2. Antibacterial activity

The antimicrobial activity of *R. montana* different extracts and fractions was determined by disc diffusion test according to a modification method described by **Nicoletti et *al.* (2012),** following the Clinical and Laboratory Standards Institute (CLSI) guidelines (**CLSI, 2009**), and all the equipment used was sterilized in an autoclave at 121°C for 15 min. The test was screened against three pathogenic bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa*(ATCC 27853), and *Staphylococcus aureus* (ATCC 25923). Bacterial strains were subjected to a continuous overnight subculture in nutrient agar and incubated at 37°C for 24 h to optimize their growth, streaked to ensure purity in order to obtain a young culture and isolated colonies.

Briefly, cells were resuspended in saline (1–2x10^8 cells/mL for bacteria (0.5 Mc Farland) and spread on the petri dishes of Mueller-Hinton Agar (MH). Sterile Whatman paper discs (6 mm in diameter) were placed on the surface of inoculated Petri dishes and spotted with 20 µl of 30 mg/ml test extract solubilized in dimethylsulfoxide (DMSO). The Petri dishes of Mueller-Hinton Agar (MH) were incubated 24 h at 35±1°C. The activity was done in triplicate and it was determined by measuring the diameter of the growth inhibition zone (IZD) visible around the paper disc and comparing it with reference diameters related to the antibiotics used. Negative controls were set using Wattman disks impregnated with DMSO and the antibiotics gentamicin served as controls.

2.4.3. Cytotoxicity activity

The cytotoxic activity of all tested extracts was evaluated using the "Brine shrimp" test based on the cytotoxic effects of shrimp larvae (Artemia). The experiment was carried out according to the method developed by **Vanhaecke (1981).** It consists in determining the concentration that kills 50% of *Artemia salina* within 24 h under standardized conditions (T = 28 °C, pH = 8, light, ventilation). Shrimp eggs hatch in a salt water solution. Larvae recover after 24 hours and are ready for use 48 to 72 hours after hatching begins. Dissolve a total of 4 mg of each test extract in a solution containing 950 µL of saline and 50 µL of DMSO. Test concentrations are 4, 2, 1, 0.5 and 0.25mg/ml. Place a total of 10 Artemia larvae into test tubes containing 100 µL of each dilution and 4900 µL of saline. Control tubes were prepared with DMSO under the same conditions. Each experiment was performed in triplicate. After 24 hours of incubation, count dead larvae using a binocular magnifying glass and determine percent mortality (M%) using the following formula:M% = (NDM / NLT) * 100, M%: Percentage of mortality; NDM: Number of Dead Larvae; NLT: Number of Larvae Tested. Results are expressed in LC_{50}.

2.5. Statistical Analysis

The collected data were statistically analyzed using analysis of variance (ANOVA) using GraphPad Prism 8 software. Differences among treatment means were separated using the Least Significant Differences (LSD) at $p \leq$ **0.05.**

CHAPTER 3: RESULTS AND DISCUSSION

1. Ethnobotany study

1.1. User's sociodemographic profile

According to our results, both men and women are involved in traditional medicine, but there is a notable predominance of females at 76% (as depicted in **Figure9**). This observation can be rationalized by the active role of women in processing and concocting herbal remedies, not only for their own well-being but also for the entire family. Consistent with prior research conducted in Algeria, various scholars have consistently found that Algerian women exhibit a deeper familiarity with the use and treatment of medicinal plants; like the work of **Souilah et *al*. (2018)**, in El Cala National Park (El Taref), of **Bouziane (2017)** in the Adjara region (Tlemcen), **Bendif et *al*. (2018),** Bordj Bou Arreridj, **Oulebani et *al*. (2016)** in the Constantine and Mila regions, **Adouane (2016)** in the southern Ores regions and**Aribi (2013)** in Jijel region. This work was also done in Morocco by **Eddouks et *al*. (2017)** and **El Hafian et *al*. (2014).**

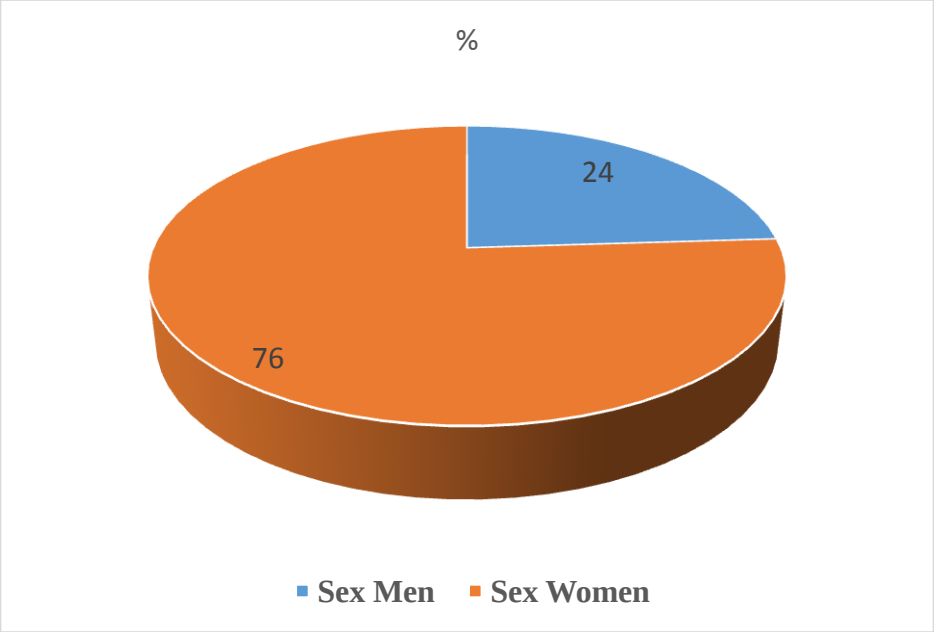


Figure 9. Use frequency of medicinal plants (%) according sex in Bousaada region

In Bousaada, the utilization of medicinal plants is widespread across all age groups, as illustrated in **Figure 10**. Individuals over 60 years of age constitute the largest group at 36%, followed by the age bracket of 50-59 years, accounting for 28%. The age categories of 40-49, 30-39, and 20-29 represent 15%, 10%, and 9%, respectively. The youngest age group, under 20 years, makes up only 2%. These findings suggest that older adults possess a more profound

knowledge of traditional medicine compared to their younger counterparts. Our results align with previous Algerian studies, notably the work of **Souilah et *al*. (2018)** in the Elkala region, where individuals over 60 years of age were found to be the primary users of medicinal plants. A comparison of our findings with studies conducted outside Algeria reveals similarities with the results reported by **Gonzales-Tejero et *al*. (2008)** in Morocco and Egypt, where the prominent age groups were 55 and 50 years, respectively. Generally speaking, experience is accumulated with age, the transfer of knowledge from one person to another, and through reading and social networking.

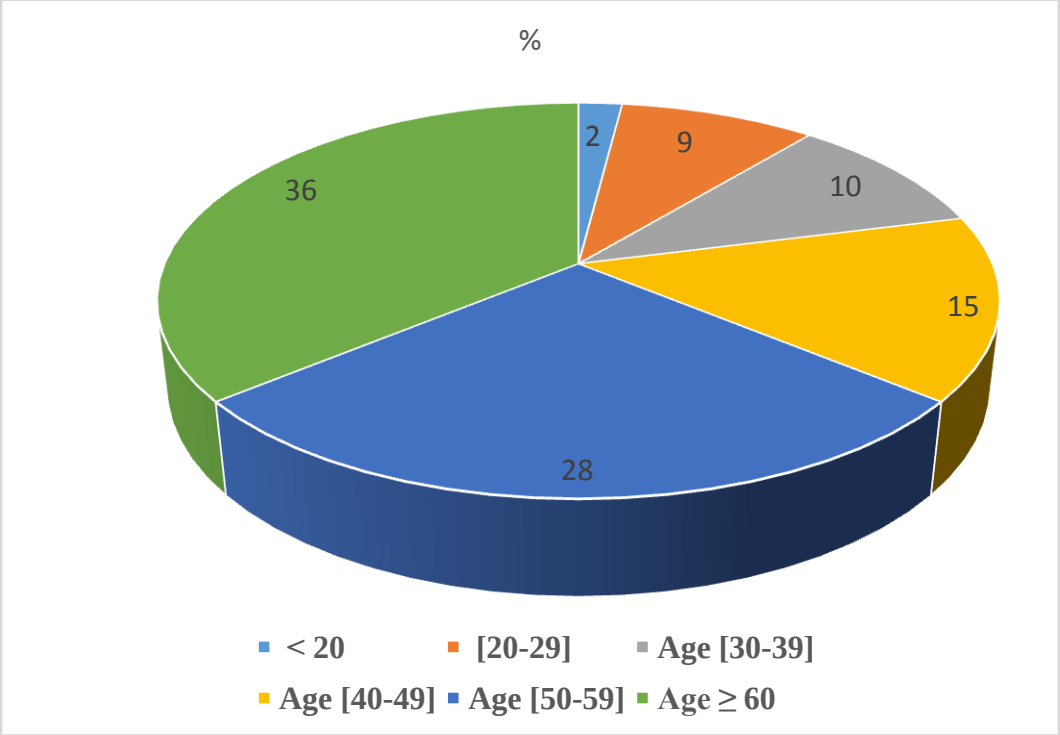


Figure 10. Use frequency of medicinal plants (%) according age in Bousaada region

The largest proportion of individuals utilizing medicinal plants had a moderate level of education, accounting for 31.9%. This was followed by individuals with no formal education and those with primary school education, constituting 21.8% and 21.6% of the total, respectively. Conversely, individuals with higher levels of education, specifically secondary and university education, showed lower reliance on medicinal plants, making up about 12.9% and 11.8%, respectively. This pattern could be attributed to the inclination of younger individuals towards experimenting with natural remedies, as depicted in **Figure11**. These findings align with studies conducted by other Algerian researchers, such as **Amrouni (2009)** in Serradi (Annaba), **Miara**

et *al*. (2013) in Tiaret, **Bendif et *al*. (2018)** in El Mansourah (Bordj Bou Arreridj), and **Souilah et *al*. (2018)** in El Kala National Park (Eltaref), all of which revealed that the majority of individuals relying on medicinal plants had limited or no formal education.

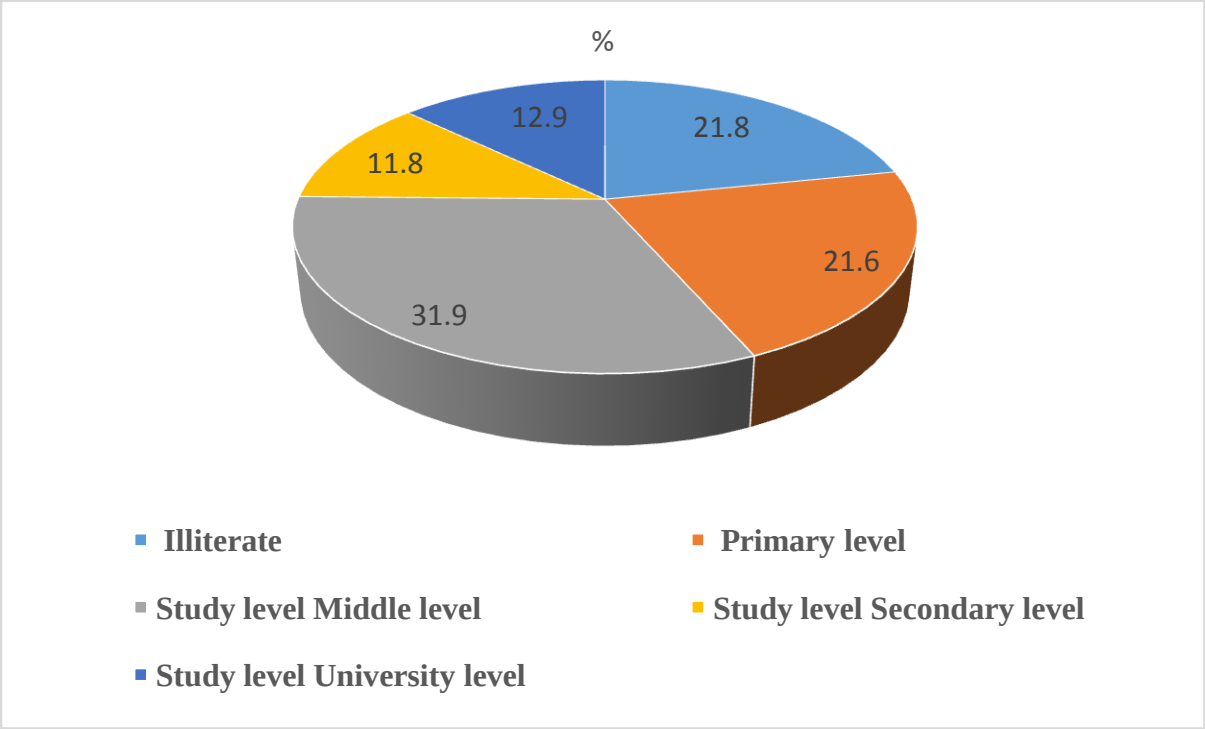


Figure 11. Use frequency of medicinal plants (%) according study level in Bousaada region

The local economy in the Bousâada region primarily revolves around traditional agriculture and intensive livestock farming for local consumption. Consequently, it's not surprising that a substantial portion of the unemployed population, approximately 34.28% of all users of medicinal plants, as indicated in **Figure 12**, turns to phytotherapy.

These findings suggest that individuals who are unemployed are inclined to explore alternatives in order to reduce or avoid the expenses linked to medical consultations and the purchase of pharmaceuticals. Notably, even among individuals with incomes below 15,000 DA, the percentage of medicinal plant users is considerable at 18.93%. In contrast, the lowest rate of usage (7.14%) was observed among those with incomes exceeding 50,000 DA. Users with incomes ranging between [25,000-35,000], [15,000-25,000], and [35,000-50,000] AD exhibited varying usage rates of medicinal plants, which were 16.43%, 13.93%, and 9.28%, respectively. These results align with those documented by **Amrouni (2009)** in Serradi (Annaba), **Bendif et**

***al*. (2018)** in El Mansourah (Bordj Bou Arreridj), and **Souilah et *al*. (2018)** in El Kala National Park (Eltaref).

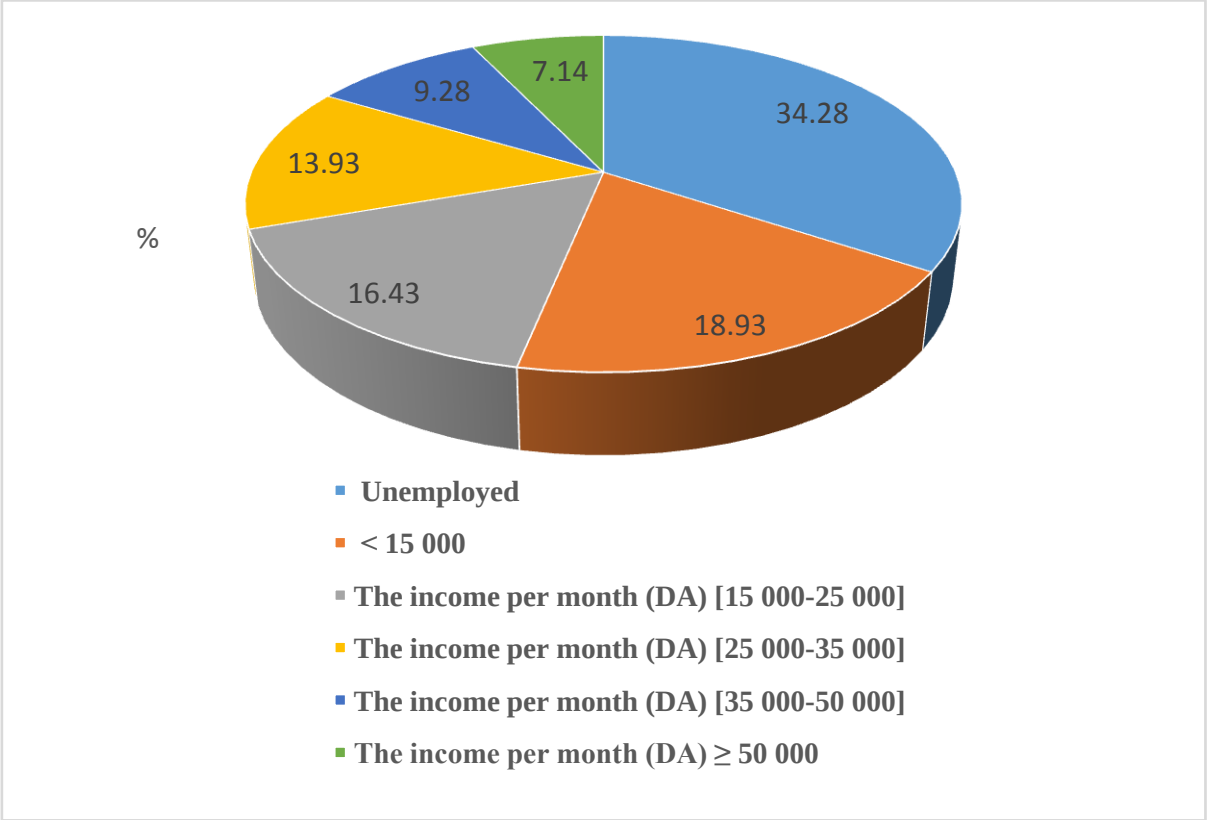


Figure 12. Use frequency of medicinal plants (%) according income per month (DA) in Bousaada region

When examining the sources of information about medicinal plants within the study area, it's evident that a significant portion of the population relies on familial experiences, accounting for 33.57%, and the knowledge shared by other community members, comprising 30.36%. These frequencies underscore the intergenerational transfer of traditional wisdom and practices. Furthermore, 20% of individuals occasionally seek information through external sources, while 15.07% rely on consulting traditional medical literature themselves, as depicted in **Figure 13**. These findings echo those of previous studies in Algeria conducted by **Amrouni (2009)** in Serradi (Annaba) and **Souilah et *al*. (2018)** in El Kala National Park (Eltaref).

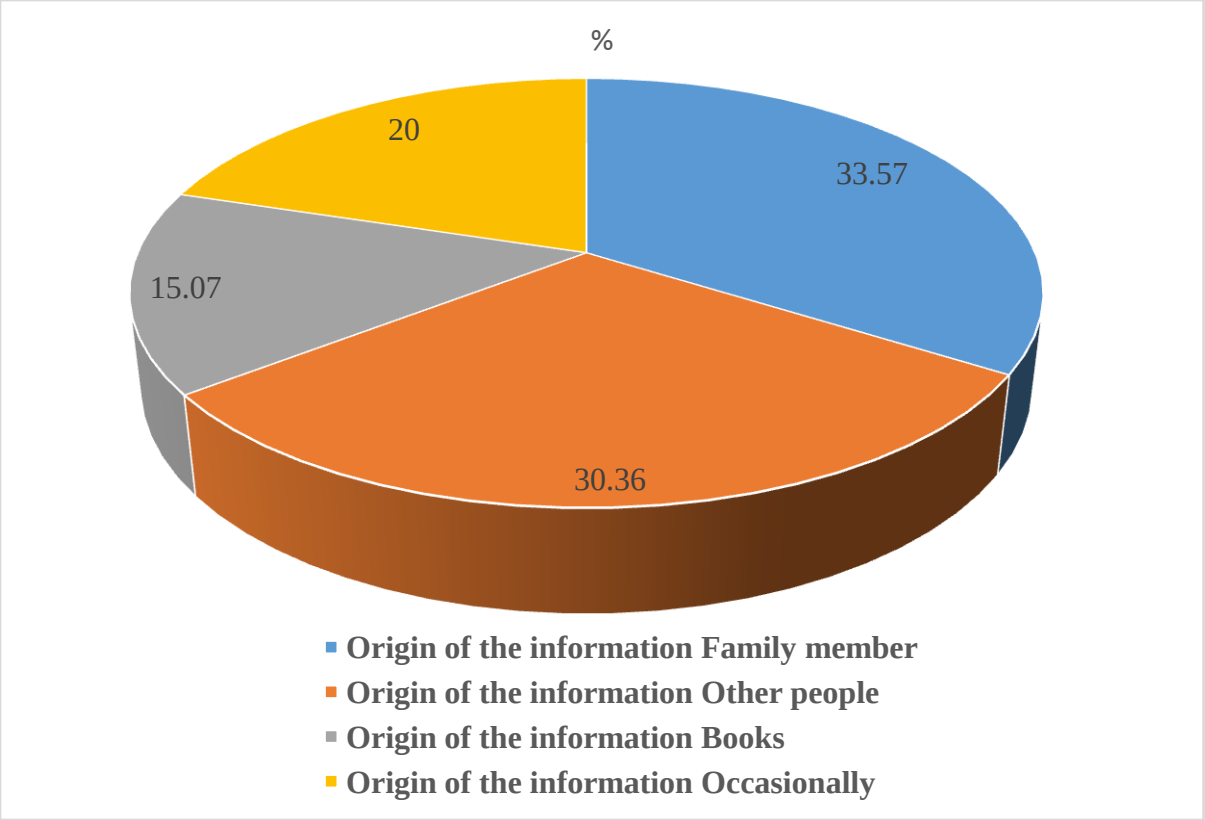


Figure 13. Use frequency of medicinal plants (%) according origin of information in Bousaada region

Household circumstances are indicative of medicinal plant usage. We observed that the largest segment of medicinal plant users consists of married individuals with 52%. This inclination might be attributed to their efforts to reduce expenses related to medical consultations and the purchase of medications for their families. Subsequently, singles accounted for 44% of users, with divorcees and widowers exhibiting the lowest percentages at 3% and 1%, respectively (see **Figure 14).**

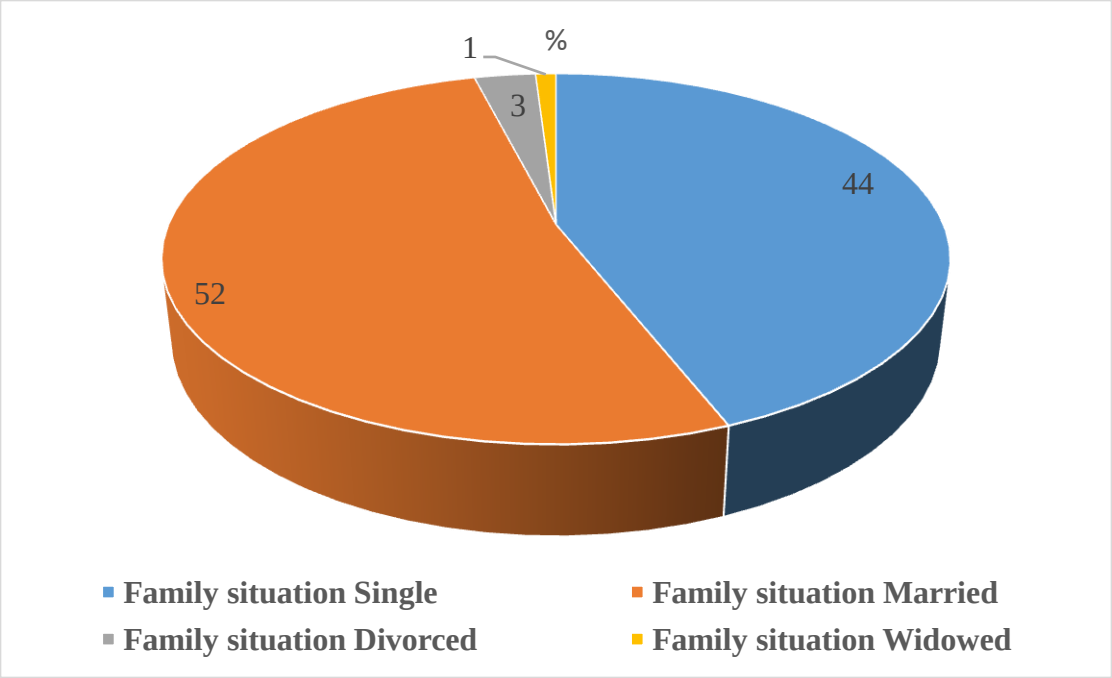


Figure 14. Use frequency of medicinal plants (%) according family situation in Bousaada region

Upon analyzing the compiled data, it became evident that a majority of the local residents preferred conventional medical treatment (53.93%), while 46.07% leaned towards traditional treatments (see **Figure 15**). These outcomes may shed light on why a substantial number of people harbor concerns about the side effects and toxicity associated with medicinal plants.

Furthermore, when scrutinizing the gathered data, it was apparent that 70% of medicinal plant users hailed from rural environments, followed by 22% from urban areas, while the urban population comprised merely 8% (see **Figure 16**). These findings imply that the choice of habitat significantly influences the utilization of medicinal plants, as the proximity of these areas facilitates the accessibility of such plants in their natural surroundings.

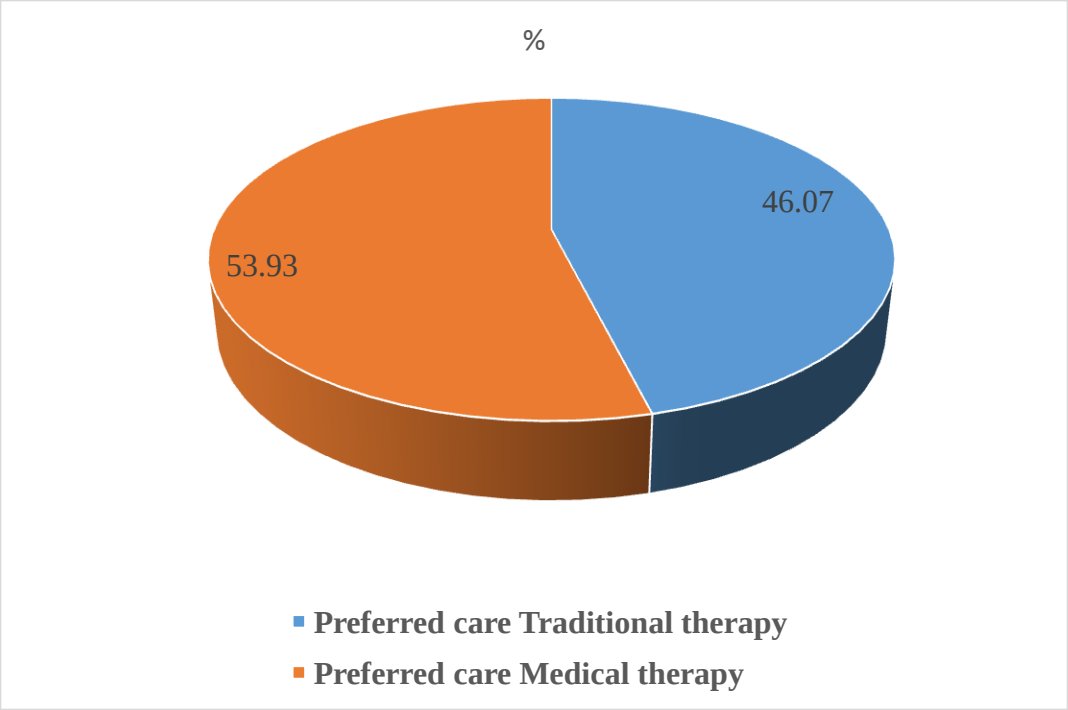


Figure 15. Use frequency of medicinal plants (%) according preferred care in Bousaada region

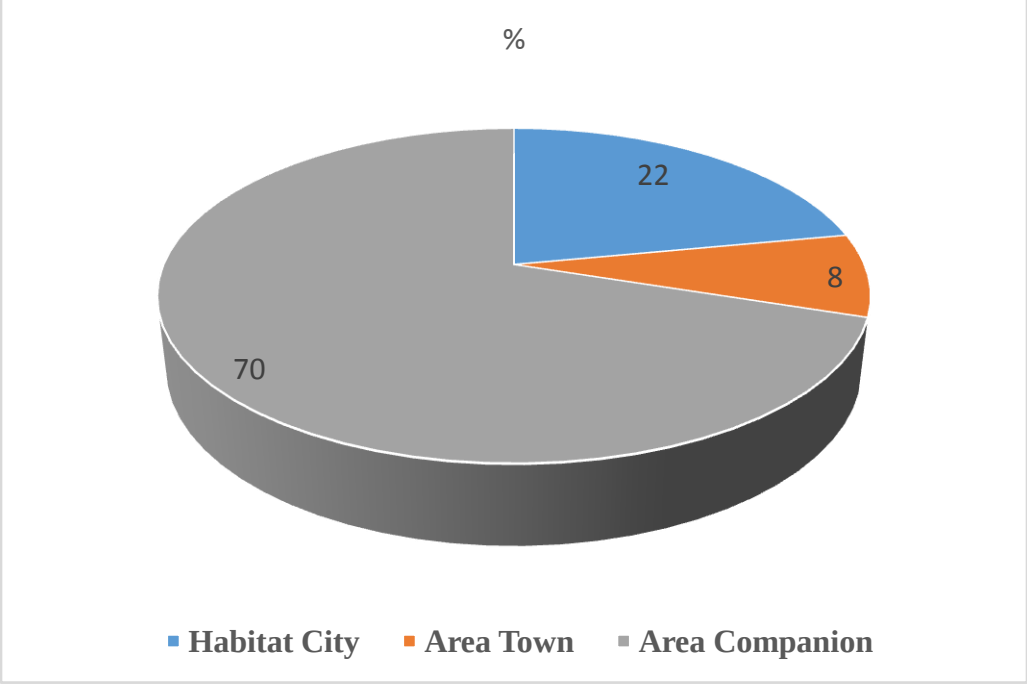


Figure 16. Use frequency of medicinal plants (%) according population habitat in Bousaada region

1.2. Parts used in the plant

The findings of this investigation highlight that multiple methods are employed for the preparation of certain plants. Based on the results, leaves appear as the most frequently used plant component, constituting roughly 33% of preparations. Followingby leaves, seeds and fruits which, are used in 13% of cases, while stems and aerial parts are employed in 12% and 10% of herbal formulations, respectively. Additionally, whole plants, flowers, bark, roots, rhizomes, and other plant components collectively account for approximately 1% to 6% of herbal preparations (see **Figure17**).

Several authors also detected leaf preparations in different regions of Algeria (**Amrouni (2009)**; **Chermat and Gharzouli, (2015); Ouelbani et *al*. (2016); Bouasla and Bouasla, (2017); Bendif et *al*. (2018-2021); Miara et *al*. (2018); Souilah et *al*. (2018); Miara et *al*. (2019)** and other studies in the Mediterranean region **Parada et al. (2009); Tuttolomondo et *al*. (2014 a et b), Carrió and Vallès, (2012); Guzel et *al*. (2015); Eddouks et *al*. (2017).** The high importance of using leaves can be explained by the comfort and speed of harvesting (**Bitsindou, 1996; Giday et *al*., 2009**); abundance compared to other parts (**Yemele et *al*., 2015**) and ease of preservation (**Kadir et *al*., 2012**), but also because of its incompetence as a site of photosynthesis and sometimes storage of secondary metabolites responsible for these properties of plant life (**Bigendako-Polygenis et *al*., 1990**).

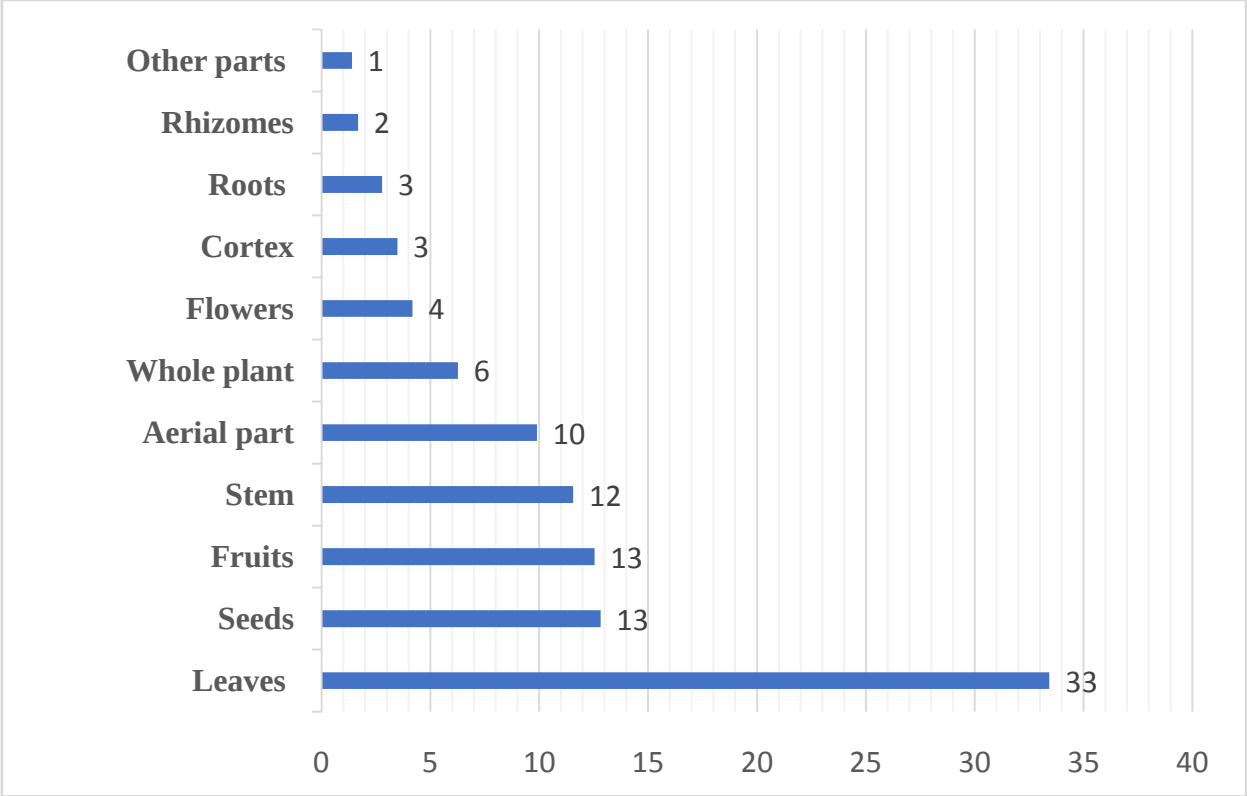


Figure 17. Use frequency of different parts of medicinal plants (%) in Bousàada region.

1.3. Methods of preparation

For the therapeutic applications of the numerous symptoms declared above, we encountered diverse dosage forms, the most common of which were infusions, accounting for 23%, followed by decoctions, accounting for 20%, and infusions and powders, accounting for 19%. In order: 18%, 11% for other methods, and less important preparations such as juices, ointments and pastes: 5%, 4% and 3% **(Figure 18).** The predominance of infusion preparation methods has also been detected in earlier ethnobotanical reporsts in Algeria and other parts of the world: **Parada et *al*. (2009); Tuttolomondo et *al*. (2014 a et b); Hammiche and Maiza, (2006);Guzel et *al*. (2015);Ouelbani et *al*. (2016).** Several others, such as **Eddouks et *al*. (2017)** Morocco stated that decoctions were the main preparation method. The best use of the plant is to retain all its properties whereas allowing the extraction and assimilation of the active constituents (**Dextreit, 1984**).

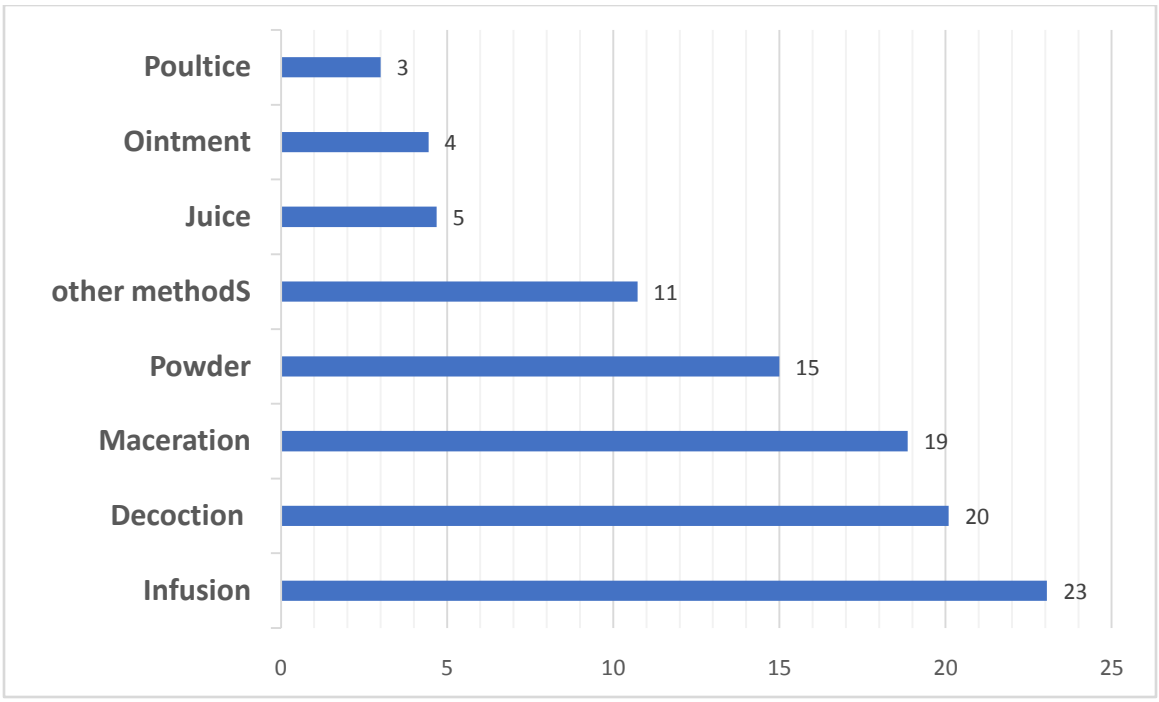


Figure 18. Use of medicinal plants according to the method of preparation (%).

1.4. Therapeutic applications

Ethnobotanical investigation has acknowledged several illnesses that can be preserved with medicinal plants (**Figure 19**). Many diseases are treated by medicinal plants. In general, the results obtained showed that the most commonly treated diseases were gastrointestinal diseases, accounting for 31.2%. These results are comparable to those observed in many ethnobotanical reports in the Mediterranean region. Cardiovascular diseases, ranked second, accounting for 13.4%, followed by skin and urinary tract diseases, accounting for 10.6% each, respiratory diseases accounting for 10.4%, and finally rheumatism, related gland diseases, and digestive system diseases and neurological disorders.

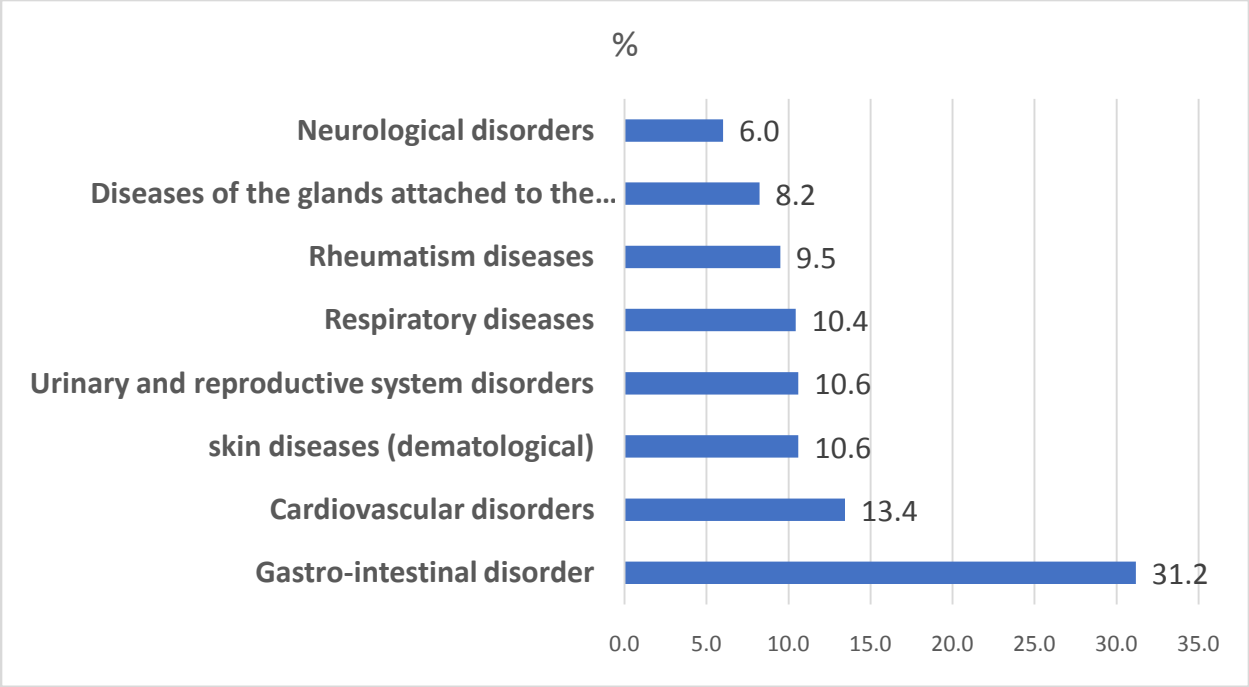


Figure 19. Use of medicinal plants according to the therapeutic applications (%).

1.5. Diversity of species used

Floristic examination carried out in the Bousaâda region using 534 questionnaires permitted us to understand the richness of some plant species. The study area contains a total of 193 taxa, dispersed in 69 families (**Annexe 1)**.

The distribution of these families is quite uneven, with four main plant families: Lamiaceae (sixteen species), Fabaceae (fourteen species), Asteraceae (thirteen species), Apiaceae (twelve species), and Rosaceae (twelve species) and Apiaceae (ten species) (**Figure20**, **Annexe 1**).

Our results support **Hendel et *al*. (2012),Madani et *al*. (2014), Souilah et *al*. (2018)** reports among which Lamiaceae is the most main family. Giving to our results, *Artemisia herba-alba*, *Juniperus oxycedrus*, *Mentha viridis*, *Thymus vulgaris*, and *Artemisia vulgaris*are the plants most commonly used by local populations in traditional medicine, depending on the total number of uses reported for a specific species (**Figure 21**).

This result supports the findings of **Chermat and Gharzouli, (2015)** in Djebel Zdimm (Setif region in Algeria), where *Artemisia herba-alba* was the most commonly used plant. On the other hand, some herbs are rarely used due to their toxicity, such as *Nerium oleander* and *Thapsia garganica*. Compared to other studies conducted in Algeria (Mascara 141, Ilizi 118, Elkara 112,

Constantin and Mila 102, Bourdj Bou Arreridj 83, Kabylia 98, 80 in TassiliNajjer, 78 in El Mansourah, Bourdj Bou Arreridj, 66 in Tiaret, 53 in Wed Righ and 37 in Ourgla by **Ouelbani et *al.*(2016),Bendif et *al*. (2019),Souilah et *al*. (2018),** (**Meddour and Meddour-sahar, 2015**), **Miara et *al*. (2019),(Hammache and Maïza 2006),Benarba et *al*. (2015),Bendif et *al*. (2018),Miara et *al*. (2013),Lakhdari et *al.* (2016)** and (**Ould El Hadj et *al*. 2003**) respectively, our significant results (193) may be very interesting in his region.

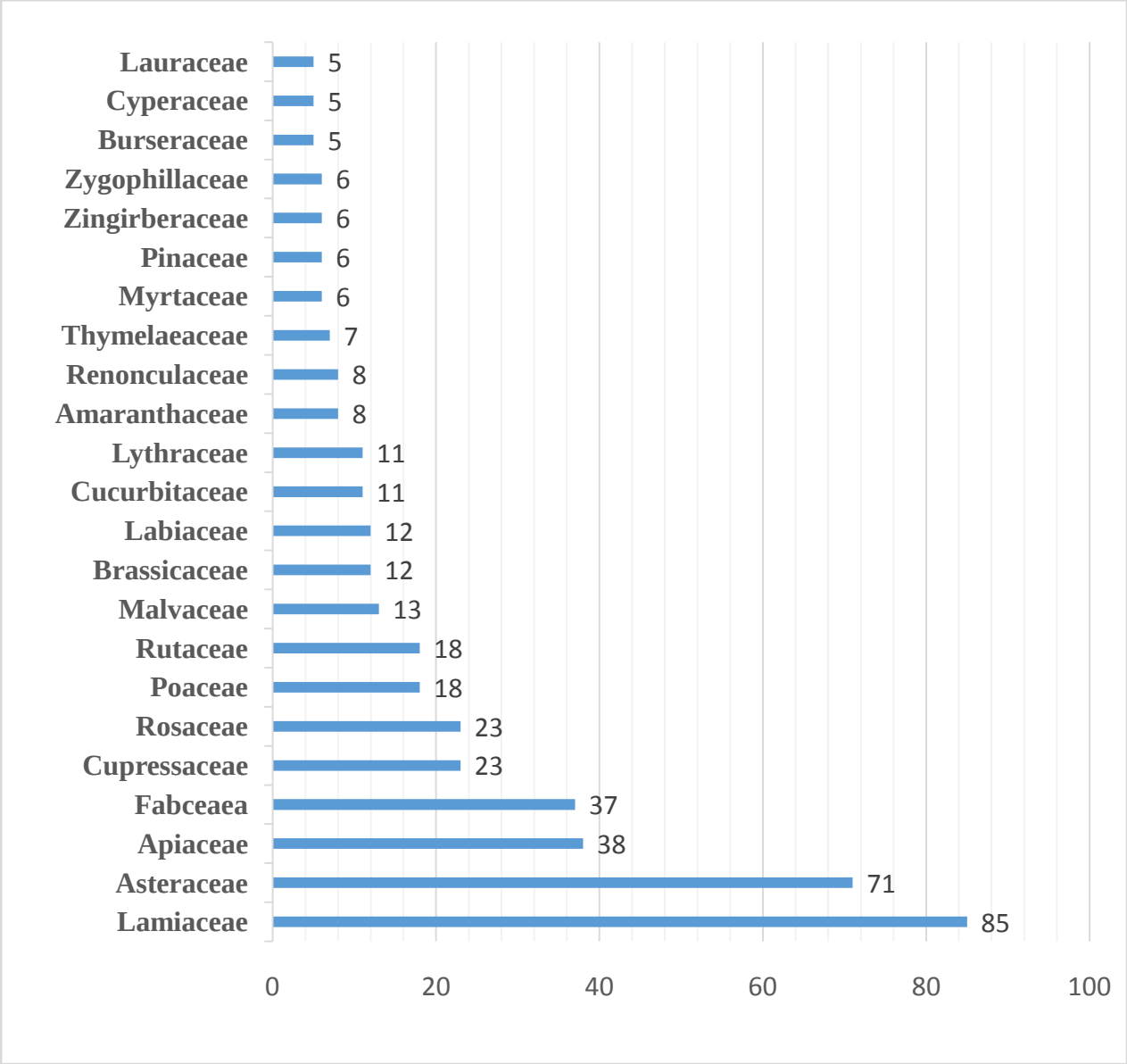


Figure 20. Number of medicinal species according to botanical families

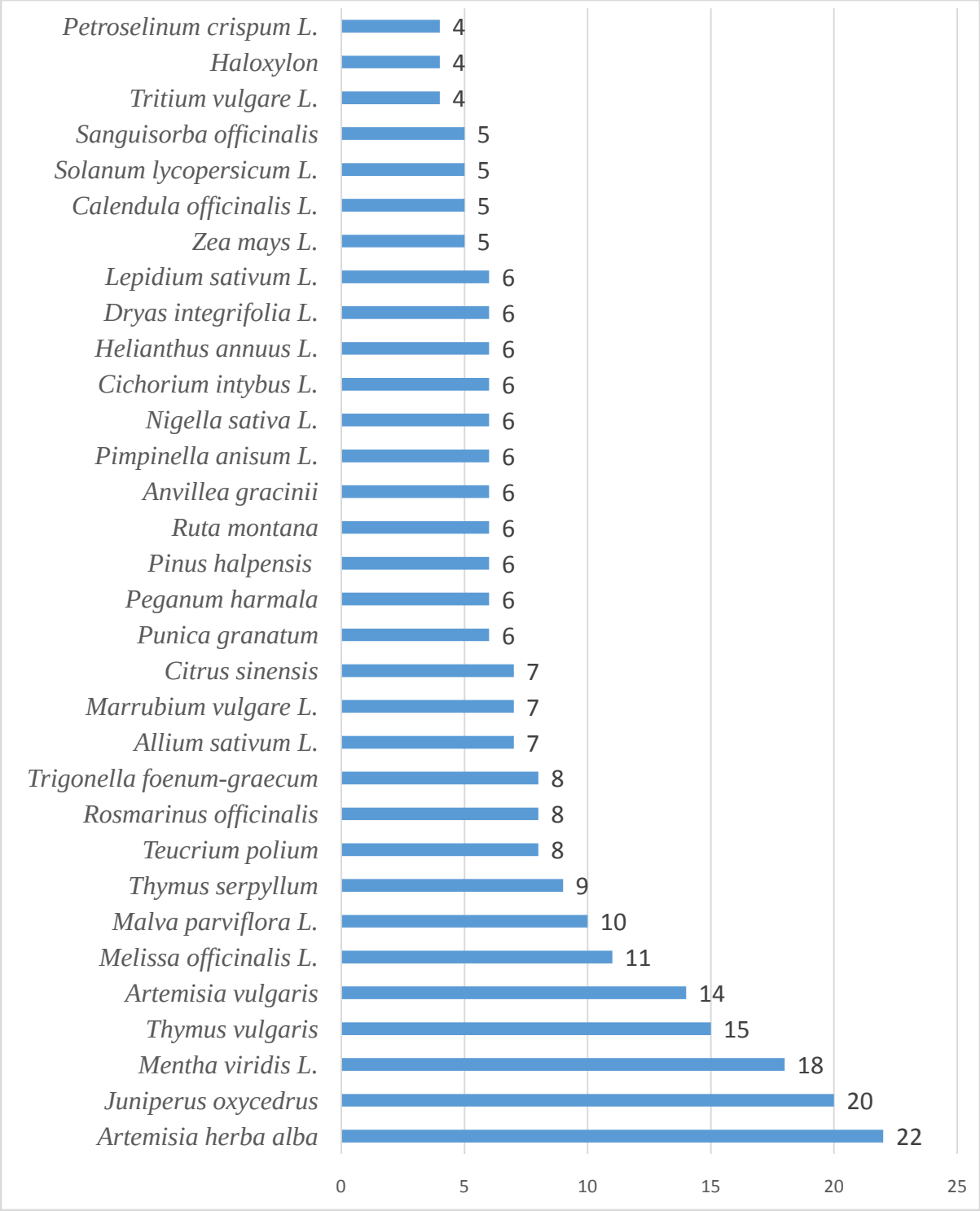


Figure 21. The most commonly used plants according to the total number of reported uses cited for a given species by the local population.

When we also compared our results with other studies from the Mediterranean region outside Algeria, we found 148 and 159 species in Morocco (**Fakchich and Elachouri, 2021**;

Teixidor-Tile et *al*. 2016), whereas **Carrió and Vallès, (2012)** found 121 species in the Balearic Islands and 88 species in the Italian Alps by **Pieroni and Giusti, (2009).** On the other hand, the number of species is lower than in other studies focusing on larger areas but reporting much higher species numbers, such as 222 species in Turkey by **Güzel et *al*. (2015),** 335 and 224 species of Spanish **Parada et *al*. (2009)** and **Benitez et *al*. (2010),** 406 species from the Mediterranean by **González-Tejero et *al*. (2008)**. These differences may be affected by geographic location, climate and soil conditions.

1.6. Use of herbal plants according to the harvesting season

In terms of accessibility, 64% of reported plants were existing only in spring, 15% were forever available year-round (all seasons), 9% in summer, 8% in winter and only 4% in autumn (**Figure 22**). The residual species are only partially available, contingent on advantageous rainfall conditions. These results are dependable with those from Ouargla (Algeria) (**Chehma and Djebar, 2008**), which found the highest percentage (72%) in spring.

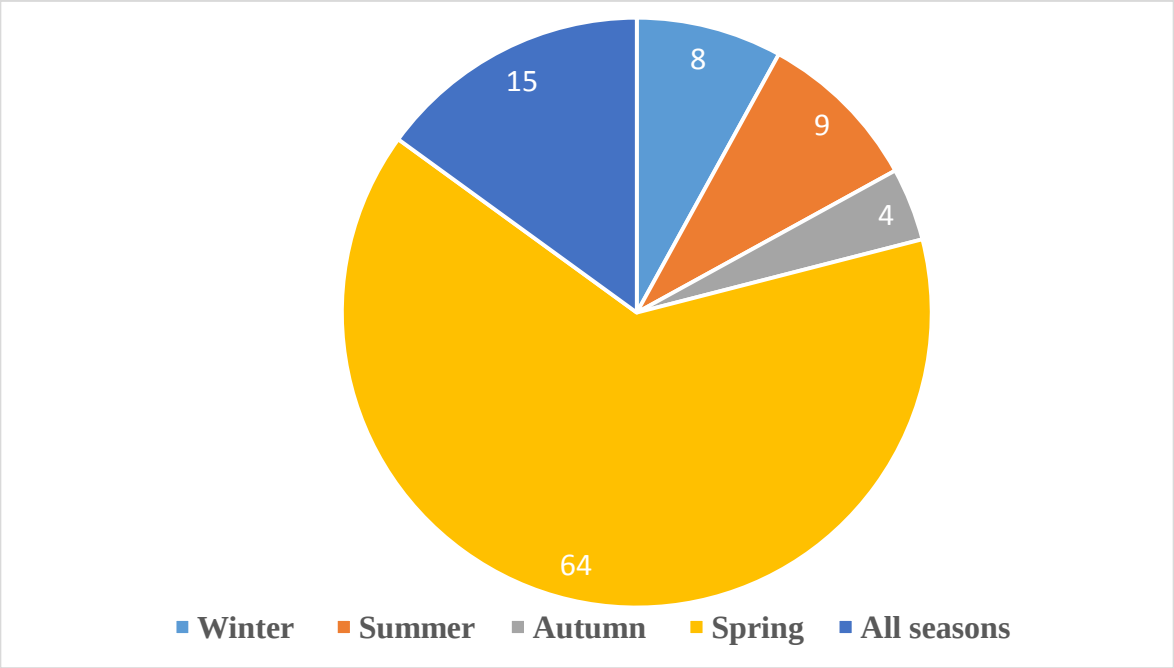


Figure 22. *Use of herbal plants according to the harvesting season*

1.7.Use of herbal plants according to the type of plant

Based on the kind of plants used, wild plants were found to have the highest proportion at 53%, followed by cultured plants at 45%, whereas, exotic plants had the lowest percentage (**Figure 23**).

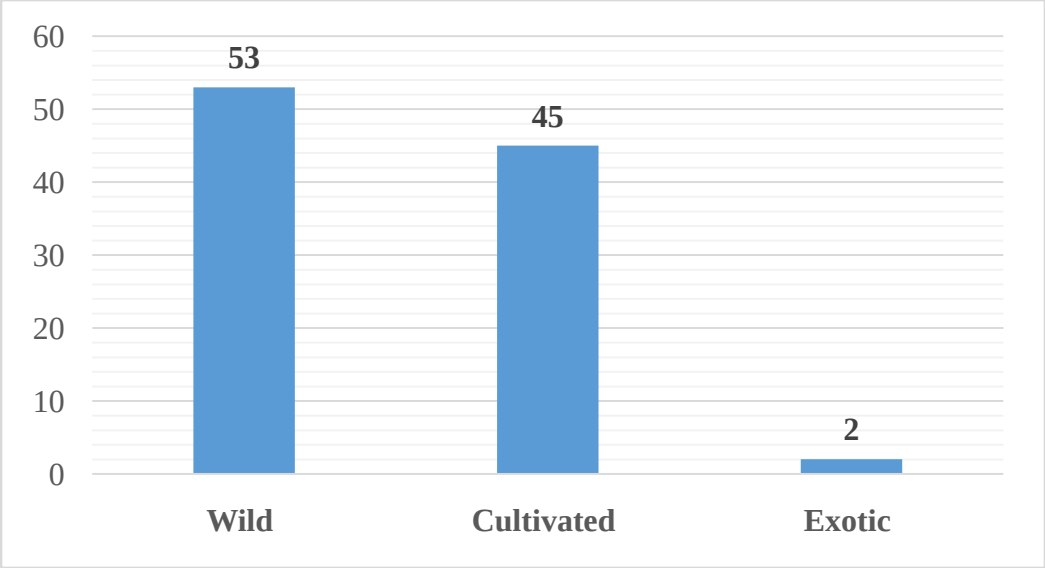


Figure 23. Use of herbal plants according to the type of plant in Bousâada

1.8. The Use-Value (UV)

Regarding the utilization value of the varieties listed in **Table 4, Annex 1,** it was observed that *Citrus Lemon* (L.) Burm. f., *Ficus carica* L., *Moringa oleifera* Lam., and *Olea europaea* L. are the most commonly employed by local residents, with utilization values as high as 5.

Following closely are nine species with a utilization value of 4, including *Acanthus mollis*L., *Diospyros kaki* L., *Brassica oleracea var. Capitata*, *Buxus sempervirens* L., Corchorus olitorius L., *Diospyros kaki Thunb.*,*Iris germanica* L., *Narcissus tazetta* L., and *Narcissus senegal* L.

A higher utilization value suggests that the local population employs the plant for various purposes in the treatment of different categories of ailments (**Barnert and Messmann, 2008**). On the other hand, the lowest utilization values, at a value of 1, were attributed to 43 species

Table 4. The most Use-Value (UV) of species in Bousaàda region

Espèce	UV	Espèce	UV
Olea europaea L.	5	*Hyssopus officinalis L.*	3
Moriga oleifera L.	5	*Humulus lupulus* L.	3
Citrus lemon L.	5	*Senegaliasenegal* L.	3
Ficus carica L.	5	*Globularia alypum L.*	3

Diospyros kaki L.	4	*Fraxinuse xcelsior* L.	3
Phoenix dactylifera L.	4	*Artemisia dracunculus L.*	3
Narcis sustazetta L.	4	*Ecballium elaterium* L.	3
Anagalisarvensis L.	4	*Dipsacus fullonum* L.	3
Panicum virgatum L.	4	*Tritium vulgare* L.	3
Harpagophytum procumben L.	4	*Avenasativa* L.	3
Cyperusesculentus L.	4	*Solanum melongena* L.	3
Corchorusolitorius L.	4	*Arum creticum* L.	3
*Brassicaoleracea*var capitata	4	*Synaracardunculusvar.scolymus*	3
Valerianaofficinalis L.	3	*Mentha viridis L.*	2.66
Thujaoccidentalis L.	3	*Cucurbitapepo* L.	2.66
Tamarindusindica L.	3	*Glycyrrhizaglabra* L.	2.5
Salva officinalis L.	3	Syzygiumaromatic L.	2.5
Rubiatin ctorum L.	3	*Cacumissativus* L.	2.5
Thymelae amill.hirsita L.	3	*Punica granatum L.*	2.33
Junglans regia L.	3	*Sesamumindicum* L.	2.25
Ziziphus vulgaris L.	3	*Marrubium vulgar* L.	2.14
Jasminum poyanthum L.	3	*Artimisia herba-alba* L.	2.04

1.9. Fidelity level (FL)

FL values reaching up to 100% were recorded for 73 species, among them, 27 species are utilized for various diseases, including *Allium cepa, Anvilleagarcinii subsp. radiata, Aquilaria malaccensis, Astragalus gummifer, Ceratonia siliqua, Chrysanthemum pacificum, Cinnamomum verum, Cyperus diffusus, Cyperus esculentus, Elettaria cardamomum, Foeniculum vulgare var. dulce, Linum usitatissimum, Melissa officinalis, Opuntia ficus-indica, Origanum majorana, Panax ginseng, Prunus persica, Rhamusalternus, Salvadora persica, Senna alexandrina, Sesamum indicum, Sinapis arvensis, Solanum lycopersicum, Thymelaea hirsute, Vitex agnus-castus, Vitis vinifera, Ziziphus lotus*. Additionally, 21 species are used in the treatment of gastro-intestinal diseases, including *Apium graveolens, Artemisia herba-alba, Artemisia vulgaris, Citrus sinesis, Commiphoramyrrha, Cuminum cyminum, Curcuma longa, Cutrulluscolocynthis, Hammda scoparia, Juniperus communis, Juniperus phoenicia, Laurus nobilis, Mentha viridis,*

Nigella sativa, Ocimumbasilicum, Pimpinella anisum, Pinus halpensis, Quercus ilex, Ruta montana, Triticum aestivum, Vachellianilotica.

These results are not the highest observed, as previous studies in Algeria have reported 100% FL, such as **Souilah et *al*. (2018)** with 38 species in El Kala National Park, **Benaraba et *al*. (2015)**listing 7 species in Mascara, and **Ouelbani et *al*. (2016)** in Constantine and Mila identifying only one species. Certain plants are also suitable for treating gastrointestinal diseases, including *Ajuga iva, Globularia alypum, Juglans regia, Opuntia ficus-indica, Trigonella foenum-graecum*.

In general, the highest FL values are associated with species widely used by the local population to treat a specific disease, while the lowest FL value (4%) was found for *Olea europaea*, indicating its potential use in treating various diseases.

1.10. Informant Consensus Factor (ICF)

The**Table 5** displays the calculated Informant Consensus Factor (ICF) values for the 10 disease categories, which vary from 0.33 to 0.6. The disease category related to gastrointestinal diseases and glandular diseases of the digestive system has the highest ICF value of 0.6. This category includes 6 species such as *Juniperus sp, Artemisia herba-alba, Mentha viridis, Artemisia campestris, Pinus halpensis,* and *Malva parviflora*. Five of the most frequently used species within this category are *Juniperus communis, Mentha viridis, Marrubium vulgare, Thymus vulgaris,* and *Artemisia herba-alba.* Neurological diseases follow with an ICF value of 0.5 and involve four species (*Mentha viridis, Trigonella foenum-graecum, Calendula officinalis, and Melissa officinalis*).

Table 5. Informant Consensus Factor (ICF) for different disease categories

Categories of diseases	Nur	Nt	ICF	Most usedspecies	Nbr of species
Dermatologicaldisorders	72	48	0.33	*Teucriumpolium* L.	5
				*Punicagranatum*L.	5
Respiratorydiseases	69	40	0.42	*Thymus vulgaris* L.	12
				Artimisia herba halba L.	7
Kidney and reproductive system disorders	88	47	0.47	*Thymus serpyllum* L.	8
				Artimisia herba halba L.	7
				Allium sativum L.	6
				Mentha viridis L.	5
Cardiovasculardiseases	64	48	0.25	*Cichoriumintybus* L.	5
Bone and joint pain	62	39	0.37	*Peganum harmala* L.	6
				Lepidium sativum L.	5
Gastrointestinal diseases	202	81	0.6	*Juniperus communis*L.	20
				Artimisia herba halba L.	15
				Mentha viridis L.	13
				Artimisia campestris L.	9
				Pinushalpensis Mill.	6
				Malva parviflora L.	6
Diseases of the glands attached to the digestive system	56	23	0.6	*Juniper usphoenicia*L.	9
				Mentha viridis L.	6
				Marrubiumvulgar L.	5
				Thymus vulgaris L.	4
				Artimisia herba halba L.	4
Neurologicaldiseases	41	21	0.5	*Mentha viridis* L.	8
				Trigonella foenum-graecum L.	5
				Calendula officinalis L.	5
				Melissa officinalis L.	4
Other diseases	272	165	0.39	*Thymus vulgaris* L.	10
				Artimisia herba halba L.	9
				Mentha viridis L.	7
				Malvaparviflora L.	6
				Melissa officinalis L.	6
				Artimisia campestris L.	5
				Anvillea gravinii L.	5
				Thymus serpyllum L.	5
				Marrubium vulgar L.	4
				Allium sativum L.	4
				Thymelaea, mill. hirsita L.	4

Nur: refers to the number of use-reports for a particular disease category; **Nt**: refers to the number of taxa for a particular disease category by all informants

This is succeeded by plants used for treating kidney and reproductive diseases (ICF of 0.47) featuring four species, respiratory diseases (ICF of 0.42) with two species, other diseases (ICF of 0.39) involving 10 species, bone and joint pain (ICF of 0.37) with two species, and skin diseases (ICF of 0.33). Plants employed for cardiovascular diseases display a lower IFC value (0.25) compared to other disease categories.

These results align with findings from other regions in Algeria (Be**narba et *al*. 2015, Bendif et *al*. 2017, Souilah et *al*. 2018**), as well as studies in Morocco (**El-Hilaly et *al*. 2003**), Tunisia (**Leporatti and Ghedira, 2009**), Italy (**Dei Cas et *al*. 2015, Tuttolomondo et *al*. 2014**), and Spain (**Benítez et *al*. 2010**). These studies collectively highlight the prevalence of gastrointestinal disorders as the primary concern, as evidenced by their high ICF values.

2. Phytochemical and biological study

2.1. Extraction

The extraction yield of each *R. Montana* extract tested is expressed as a percentage. The results are listed in **Figure 24**.

The crude extract (80% ethanol/H2O) yielded the highest yield of 21.3%, while the yields of butanol extract and ethyl acetate extract were 4.28% and 0.64%, respectively. Finally, the chloroform extract gave the lowest yield of 0.58%. Our results are higher than those reported in the literature. **Alomi et *al*., 2018** Yields of raw leaf and seed extracts were reported to be reduced by approximately 6.46% and 5.52%, respectively.

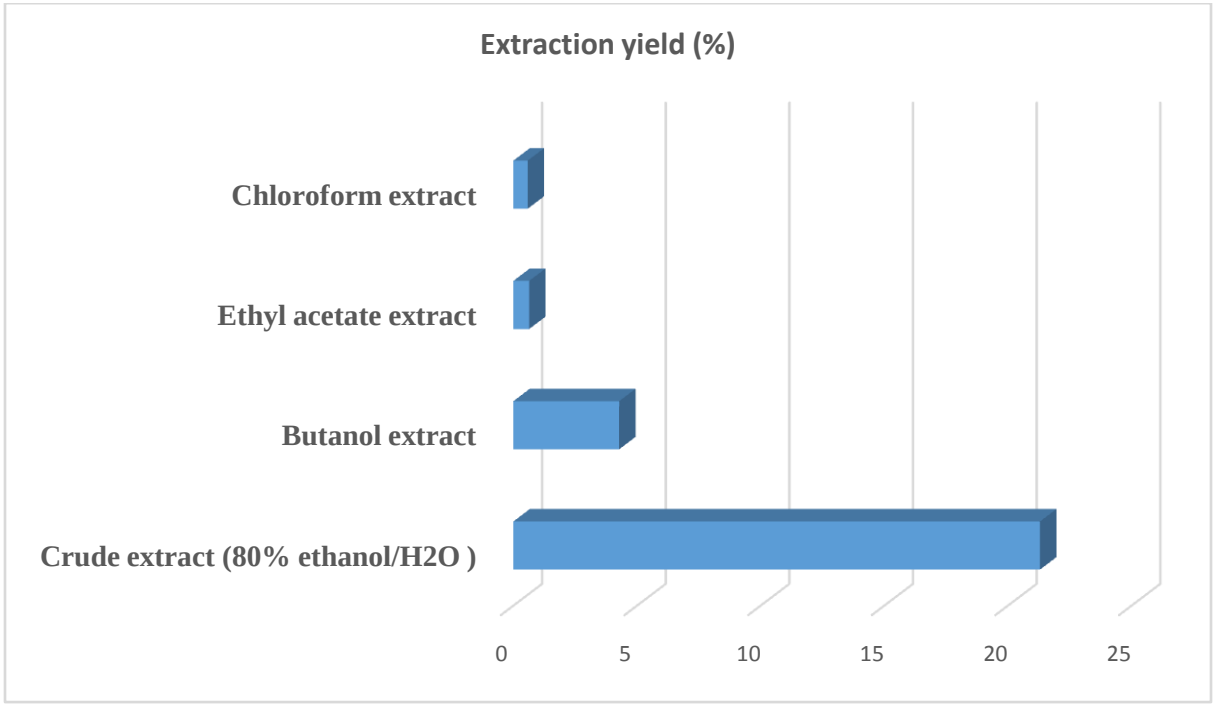


Figure 24. Extraction yield, and antioxidant activity of *R. montana*extracts

2.2. Chromatography analysis

2.2.1. Thin layer chromatography (TLC)

Thin layer chromatography analysis of ethyl acetate and butanol extracts is shown in the **Figure 25.**

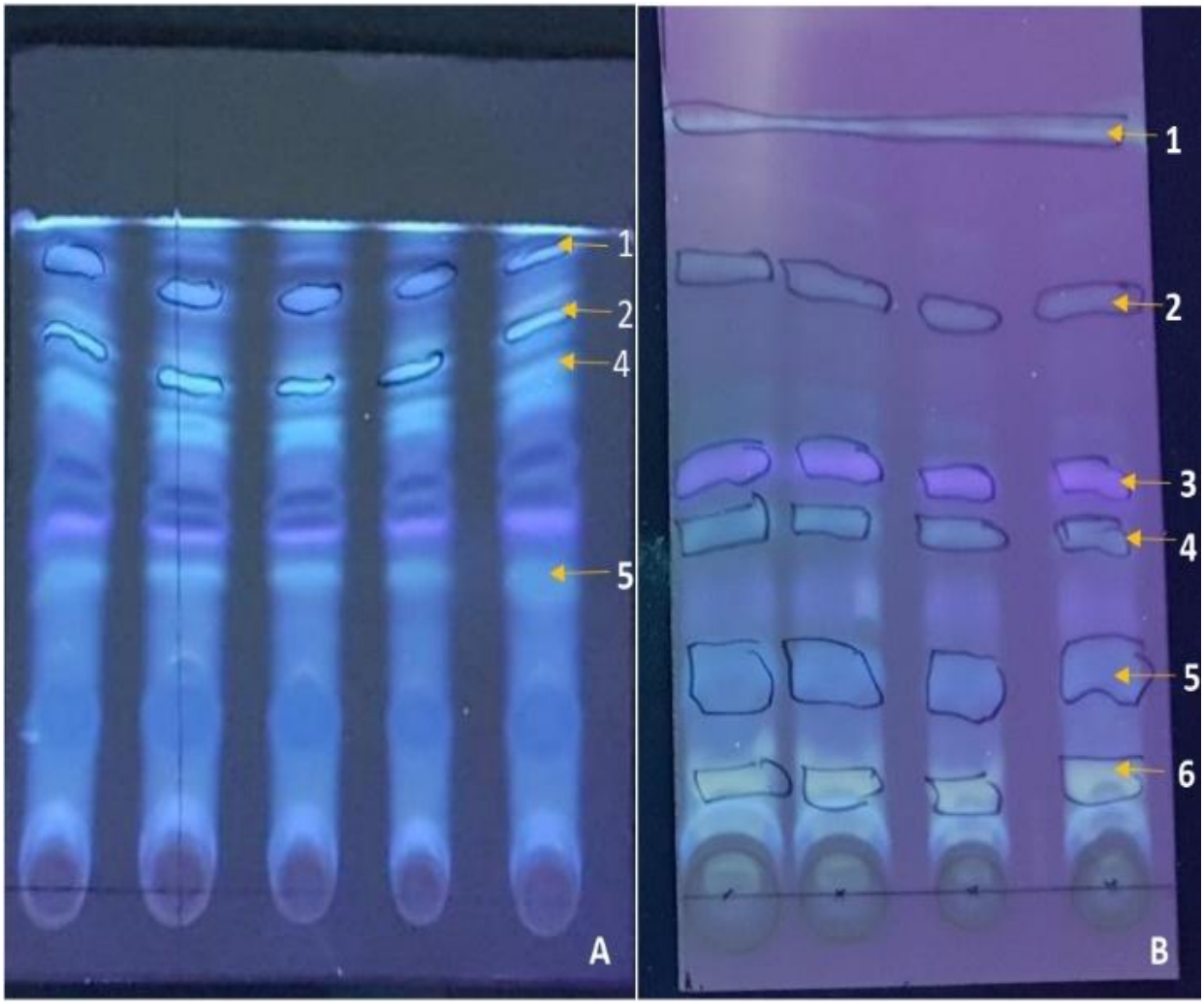


Figure 25. TLC chromatograms of ethyl acetate and butanol extract of *R. montana*; (A: Ethyl acetate, B: butanol).

This chromatography allowed us to obtain several fractions, among which fractions A1, A2, A4, A5 from the ethyl acetate extract and fractions B1, B2, B3, B4, B5, B6 from the butanol extract passed LC-MS analysis.

2.2.2. LC-MS analysis

All tested extracts and fractions (crude extract (80% ethanol/H2O), chloroform, ethyl acetate, and butanol) as well as fractions obtained by TLC were analyzed using an LC-ESI-MS instrument. The obtained chromatograms were processed using Agilent Mass Hunter Workstation Qualitative Analysis B.06.00 software. Our investigation identified 16 compounds. The identified compounds are shown in **Table 6.**

Table 6: Phenolic compounds identified using LC-MS analysis in crude extract (80% ethanol/H2O) of *R. montana*

Peak number	Compounds	Retention time (min)	Molecular formula	Experimental m/z	Ionization mode
1	Umbelliferone	17.7	$C_9H_6O_3$	161.00	Neg
2	Scopoletin	17.9	$C_{10}H_8O_4$	191.00	Neg
3	Rutaretine	31.7, 32, 32.5	$C_{14}H_{14}O_5$	261.00	Neg
4	6,7,8-trimethoxy coumarine	22.4	$C_{12}H_{12}O_5$	235.00	Neg
5	5hydrox-6,7,4'-trimethoxyflavone	23.6	$C_{18}H_{16}O_6$	327.00	Neg
6	Chalepin	31, 32.633.8	$C_{19}H_{20}O_4$	311.00	Neg
7	4-O-p-cumaroylquinic acid	15.4	$C_{16}H_{18}O_8$	337.00	Neg
8	4-O-feruloylquinic acid	17	$C_{17}H_{20}O_9$	367.00	Neg
8	Cnidioside A	17	$C_{17}H_{20}O_9$	367.00	Neg
9	Sinapoylferuloyldihexoside (e.g.1-sinapoyl-2-feruloyl gentiobioside)	19.1	$C_{33}H_{40}O_{18}$	723.00	Neg
10	Daphnoretin Methyl ether	19.3	$C_{20}H_{14}O_7$	365.00	Neg
11	Suberenon	19.6	$C_{14}H_{12}O_4$	243.00	Neg
11	Dimethylallyl-herniarin	19.6	$C_{14}H_{12}O_4$	243.00	Neg
12	Rutamontin	25.8	$C_{19}H_{12}O_7$	351.00	Neg
12	Daphnoretin	25.8	$C_{19}H_{12}O_7$	351.00	Neg
13	6,8-C-dihexosyl-apigenin	17.8	$C_{27}H_{30}O_{15}$	593.00	Neg
14	Rutin	19.7	$C_{27}H_{30}O_{16}$	609.00	Neg
15	Isorhamnetin-3-O-rutinoside	13.8	$C_{28}H_{32}O_{16}$	623.00	Neg
16	Disinapoyldihexoside (e.g. 1,2-Disinapoylgentiobioside)	21.1	$C_{28}H_{32}O_{14}$	753.00	Neg

Among all identified compounds, umbelliferone (1), scopolamine (2), chalepin (6), daphnetin methyl ether (10), daphnetin (12), rutamundin (12) and Ding (14) was previously reported from *R. montana* (**Benkhaira et *al*., 2022**). While, Rutaretin(3), 6,7,8-trimethoxycoumarin (4), 4-O-p-coumaroylquinic acid (7), 4-O-feruloylquinic acid (8), ostioside A (8), sinapinoyl feruloyl digexoside (9), hematoxylin (already described in Rutaceae 11), dimethylallyl herinin (11), 6,8- C-dihexose apigenin (13), isorhamnetin-3-O-rutinoside (15) and diserpinoyldihexoside (16) (**Li et *al*., 2006**). 5 Hydrox-6, 7, 4'-trimethoxyflavone (5) was identified in the Rutaceae family (**Moretti et *al*., 2012**). Several phytochemical studies have been conducted on the phenolic composition of *Cornusmontana*.

The reported studies are consistent with our phytochemical studies. **Kabush et *al*.** in **2003** isolated and identified rutamundine. **Abhishev et *al*.** in **1992**identified, umbelliferone, daphnetin and daphnetin methyl ether. **Aguro Martinez et *al***. in **1969**identified scopolamine and daphnetin. while **Sepulveda et al. (1973)** identified Chalepin. In comparison with the study on *Ruta graveolens* by **Pacifico et al.** in **2016,** our study allowed the identification of rutarin, 6,7,8-trimethoxycoumarin, 4-O-p-coumaroylquinic acid, 4- O-feruloylquinic acid, ostholeside A, threonin, dimethylallylherinin, 6,8-C-dihexoseapigenin, isorhamnetin-3-O-rutin and sinapinyl Ferulic acid xlosapoyhex for the first time. Compared with the study of by**LI et al.** in **2006,** 5-hydroxy-6,7,4'-trimethoxyflavone was also identified for the first time.

The above-mentioned compounds show significant antioxidant and antibacterial effects. In addition, these compounds also have various biological effects, such as: B. Antitumor, vasodilator, antibacterial, anti-inflammatory, antidiabetic, and cardioprotective effects (**Guimaraes et *al*., 2009**). Their beneficial effects are attributed to their ability to reduce oxidative stress and more easily capture hydrogen atoms to scavenge free radicals (**Gonzalez et al., 1977**). Furthermore, the compounds umbelliferone (7-hydroxycoumarin) and scopolamine identified by LC-MS in this study are known to have antibacterial activity (**Lindelöf et *al*., 1991**; **Houria et *al*., 2015**). In fact, they are able to damage the plasma and outer membranes of Gram bacteria, disrupting their permeability and cell death (**Dai et *al*., 2010**).

2.3. Biological activities

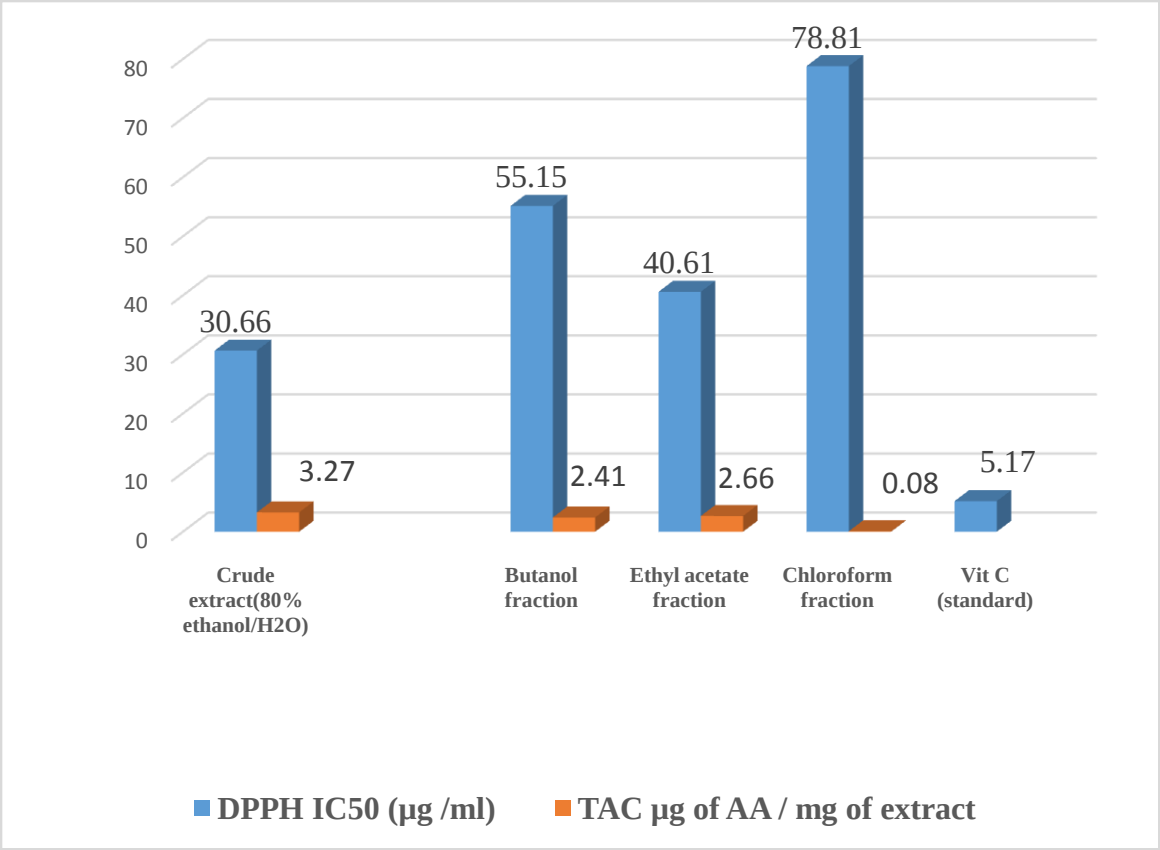
2.3.1. Antioxidant activity

The antioxidant activity of different concentrations of crude extracts (80% ethanol/H2O), fractions and ascorbic acid (Vitamine C) against DPPH free radicals was evaluated spectrophotometrically. According to our results expressed as IC_{50} (µg/ml) and summarized in **Figure 26,** all extracts of *R. montana*showed dose-dependent antiradical activity. To compare our results, ascorbic acid was used as standard. The tested extract was less active than the standard extract used, which showed higher antiradical activity with an IC_{50} value of = 5.17 µg/ml.

Our results show that the crude extract (80% ethanol/H2O) has strong anti-radical activity compared to other extracts, with an IC_{50} value of 30.66 µg/ml. The efficacy of the tested extracts was in the following order: vit C> crude extract (80% ethanol/H2O) > ethyl acetate fraction > n-butanol fraction > chloroform fraction.

The anti-free radical effects of various extracts of *R. Montana* have been the subject of several studies which reported different results. According to **Allouni**in **(2018)**, *R. montana* extract showed significant free radical activity against DPPH radicals, with IC_{50} values of 38.61 ± 0.9259 µg/ml and 44.1±4.397 µg/ml respectively for the crude ethanol extract of leaves and seeds, our results are close to reported studies.**Khadhri et al. *in*(2017)** reported similar results, the ethanolic extract of the leaves was found to possess significant antioxidant activity with a very low IC_{50} value of approximately 1.47 ± 0.1 µg/ml. Meanwhile, **Kara**in**(2016)** examined ethanol extracts of aerial parts of *R. montana* and reported IC_{50} = 0.12 mg/ml. **Guimaraes** in **(2009)** suggested that polyphenols have stronger antioxidant properties because they can more easily donate hydrogen atoms to scavenge free radicals formed by DPPH.

The total antioxidant capacity "TAC" of the tested extract and fractions is expressed in micrograms of ascorbic acid equivalents per milligram of plant extract. The results obtained are shown in **Figure 26**. Our results showed that the crude extract (80% ethanol/H2O) was the most active extract with the highest antioxidant capacity (3.27 ± 0.29 µg AA/mg extract). In comparison, the ethyl acetate and butanol extracts were less active, 2.66 ± 0.05 µg AA equivalent/mg and 2.41 ± 0.2 µg AA equivalent/mg, respectively. In comparison, the chloroform extract had the lowest reducing activity, with AA equivalents of 0.54 ± 0.08 µg/ mg of extract.



TAC: total antioxidant capacity
DPPH: 2,2 Diphenyl-1-picryl hydrazine
Values are represented as mean ± standard deviation (*n*=3);

Figure 26. Antioxidant activity (IC_{50} value µg/ml) of *R. montana*extracts

This study shows that the crude extract (80% ethanol/H2O) has strong antioxidant capacity. The observed activity may be due to the presence of phenolic compounds, which are known for their reduced electron donor potential. The chemical structure of phenols with the presence of hydroxyl groups determines the scavenging capacity. In addition, hydroxyl groups can donate hydrogen atoms or electrons to radicals and stabilize aromatic systems through resonance (**Dai et *al*., 2010**).

Among polyphenols, flavonoids usually have the strongest antioxidant properties due to the presence of 3',4'-dihydroxy (di ortho-OH) groups on the aromatic nucleus B. They have the characteristics of electron donors. The presence of 3-OH in the C ring, coupled with the presence of the C2-C3 double bond conjugated with the 4-keto group, is responsible for the delocalization of electrons from the B nucleus, further improving the anti-free radical activity (**Amić et *al*., 2003**). Rutin is one of the flavonoids identified in our qualitative analysis, this is a flavonoid similar to flavonol (quercetin diglycoside). This compound exhibits strong free radical scavenging activity in vitro and may help prevent certain types of cancer (**Sawa et *al*.,**

1999).

2.3.2. Antibacterial activity

The results of the antibacterial activity of the tested extracts against Gram+ and Gram- strains are shown in **table 7** and **Figure 27-29.**

Table 7. Antibacterial activity of *R. montana*crudeextract and fractions

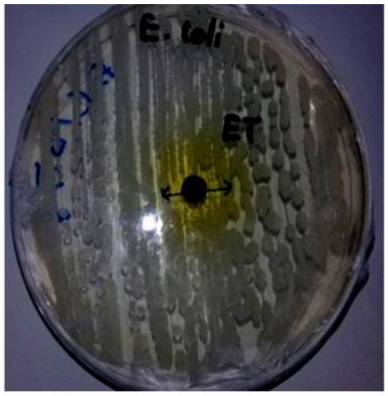
Bacteria	**Zone of inhibition (mm)**				
	Crudeextract (80% ethanol/H2O)	Chloroform fraction	Ethylacetate fraction	n-butanol fraction	**Antibiotic**
Escherichia coli	11.00±4.73	18.66±3.81	[a]NE	NE	30
P. aeruginosa	11.66±0.30	18.33±0.33	NE	NE	36
S. aureus	11.00±2.00	14.33±4.90	NE	NE	33

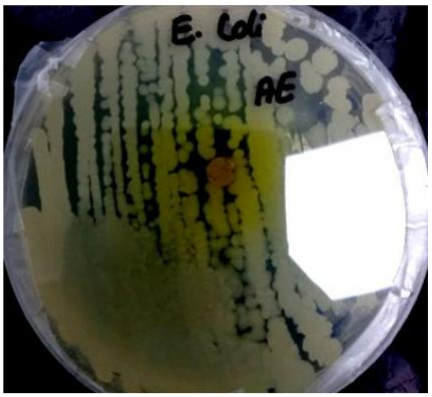
[a]NE: No effect

The results obtained represent the overall appearance of the different inhibition zones observed for different extracts and antibiotics of the strains tested. Antimicrobial activity was assumed to be 6 mm or greater based on the critical zone of inhibition determined by **Chifundera (1999), i**t is classified as follows: stretch-resistant: diameter ≤ 9 mm, insensitive stretch: diameter between 10-15 mm, sensitive stretch: diameter between 16-22 mm, very stretch-sensitive: diameter > 22 mm.

The results show that the antibiotic vancomycin has a strong inhibitory effect on Gram + Staphylococcus aureus (33mm), and gentamicin has a strong inhibitory effect on Gram-P strains. *Pseudomonas aeruginosa* (36 mm) and *Escherichia coli*(30 mm). Among the tested extracts, butanol and ethyl acetate extracts had no inhibitory effect on all tested bacteria. Evaluation of the inhibitory potential of the crude extract (80% ethanol/H2O) showed a low antibacterial capacity of 11-11.66 mm.

The **Figures (27-28-29)** represent the general appearance of the different zones of inhibition observed for the different extracts and antibiotics against the strains tested.

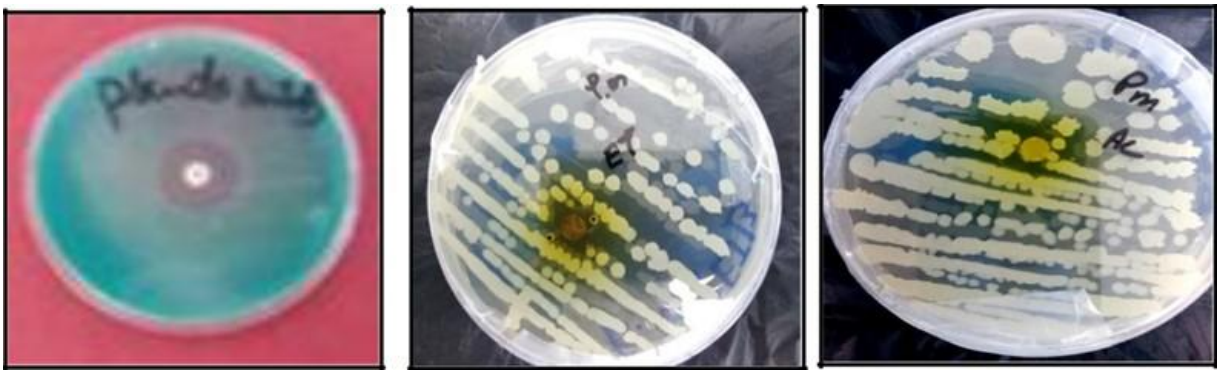




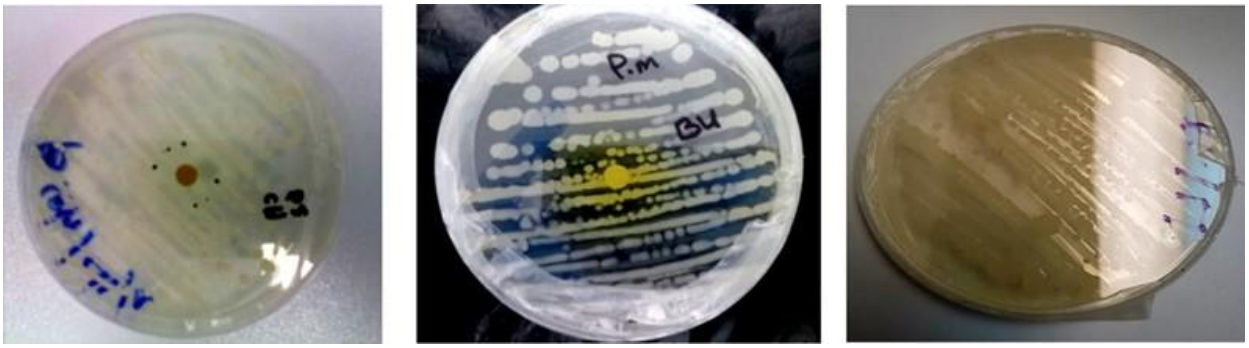
ATB:antibiotic CE: crudeextract AE: ethylacetate fraction

BU: Butanolic fraction CH: chloroform fraction CN: negative control

Figure 27. The results of the antibacterial evaluation of the crude extract and fractions against *Escherichia coli*



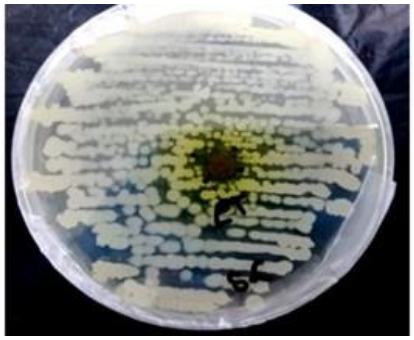
ATB:antibiotic CE: crudeextract AE: ethylacetate fraction



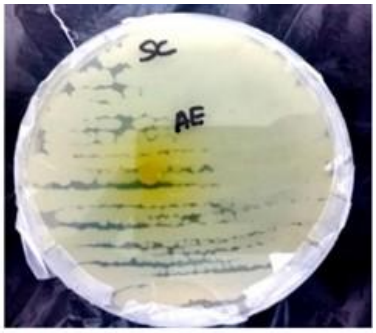
CH: chloroform fraction BU: Butanolic fraction CN: negative control

Figure 28. The results of the antibacterial evaluation of the crude extract and fractions against *Pseudomonas aeruginosa* strain

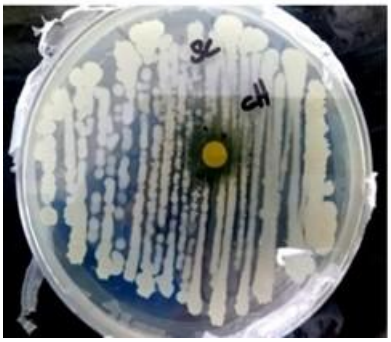
ATB:antibiotic



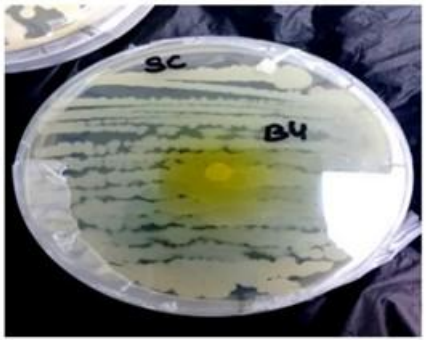
CE: crudeextract



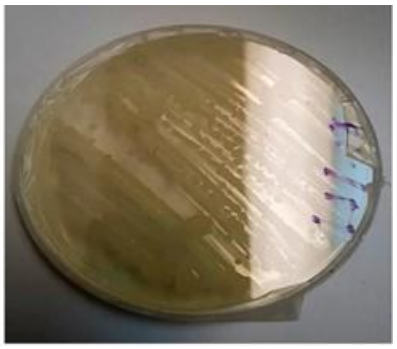
AE: ethyl acetate fraction



CH: chloroform fraction



BU: Butanolic fraction



CN: negative control

Figure 29. The results of the antibacterial evaluation of the crude extract and fractions against *Staphylococcus aureus* strain

The Gram- (*P. aeruginosa* and *E. coli*) strains are sensitive against the chloroform fraction (18.33-18.66mm), resistant against both butanolic and ethyl acetate fractions (6 mm), not very sensitive against the crude extract (11-11.66). The Gram+ (*S. aureus*) strain is of a nature: Resistant against the butanolic and ethyl acetate fractions (6mm), Not very sensitive against the chloroformic fraction and crude extract (11-14.33mm).

According to **Sqalli et *al*., (2007),** the crude extract of the leaves of *R. montana* shows a total inhibition of the growth of the five-mycobacterium tested (*Mycobacterium aurum, smegmatis, kansasii, bovis and vaccae*). The evaluation of the antibacterial activity by the method of the discs described by **Allouni, (2018),** reveals that all the ethanolic (leaves and seeds) and alkaloid extracts have a strong inhibitory capacity against the four strains tested (*B. cereus, S. typhimurium, E. coli, S. aureus*) which increases proportionally with concentration. The minimum inhibitory concentration (MIC)was determined to be 3.124 mg / ml. On the other hand, **Daoudi et *al*. (2016),** shows that neither the infused nor the decocted have an

antibacterial effect against the pathogenic strains tested (*E. coli, S. aureus, Klebsiella pneumoniae, Proteus mirabilis*), this is due to the multi-resistance of these vis-à-vis the crude extracts of this plant. These results suggest that the antibacterial activity of the extracts depends on their chemical composition. Plants synthesize several types of active compounds to protect themselves against attacks from pathogens. These molecules have always been considered a potent source of new compounds with biological activities such as antimicrobial activities (**Christova-Bagdassarian et *al*., 2013**). According to **Lindelöf et *al*. (1991)** and **Gonzalez *et al.* (1977)**, the two coumarins Umbelliferone (7-hydroxycoumarin) and Scopoletin identified by LC-MS possess antibacterial activity. Also, giving to several authors, Gram- bacteria are generally more resistant than Gram+, this is due to structural differences in their outer membranes, in Gram- bacteria, the outer membrane constitutes a very permeable barrier, rich in negatively charged lipopolysaccharides preventing the diffusion of hydrophobic molecules such as some low molecular weight phenolic compounds. These compounds are able to disrupt the membrane plasma and the outer membrane of Gram-bacteria by causing its permeability and cell death (**Houria et *al*., 2014**).

2.3.3. Cytotoxic Activity

In order to assess the cytotoxic potential of our plant crude extract and fractions, we used the "Brine shrimp" test. This test is considered to be a useful tool for the preliminary assessment of the toxicity of a product. It has been used for the discovery of active cytotoxic and antitumor agents (**Ajoy and Padma, 2013; Hamidi et *al*., 2014).** The results of the cytotoxic activity and the mortality rates (M%) of the crude extract were determined after 24 hours exposure to the different extracts and are summarized in **tables 8** and **9.**

Table 8. Mortality rate (M%) of the crude extract and the different fractions tested at different doses.

[C] µg /ml	**Crudeextract**	**Butanolic fraction**	**Chloroform fraction**	**Ethylacetate fraction**

Control	0±0	0±0	0±0	0±0
250	0 ±0	0±0.7	15±0.4	6.66±0.33
500	0±0	6.66±0.33	23.33±0.33	6.66±1.20
1000	10±0	6.66±0.66	33.33±0.66	7.33±0.33
2000	66.66±0.88	15±0.15	45±2.04	10±0
4000	100±0	15±0	45.33±0.88	10±0

Table 9. Correspondence between LC50 and toxicity.

LC_{50}	**Toxicity**
0 > LC_{50}>100µg/ml	Very toxic
100µg/ml > LC_{50}> 500µg/ml	Moderately toxic
500µg/ml > LC_{50}> 1000µg/ml	Slightly toxic
LC_{50}> 1000µg/ml	Nontoxic

Our results revealed that no mortality was observed in the control solutions. Our results demonstrated that the tested extract and fractions did not have a cytotoxic effect because the mortality rate was too low depending on the different doses, which varied between 0% to 45%. While the crude extract revealed a high and considerable mortality rate varying between 66.66% and 100%.

In order to determine the value of the lethal concentration LC_{50} of the crude extract, we plotted the regression curve, which expresses the rate of dead larvae as a function of the logarithm of the concentration of the crude extract, which has a mortality rate greater than 50% (**Figure30**).

According to **Hamidi** (**2014**), the crude extract of *R. montana* has no cytotoxic activity with an LC50> 1000 µg/ml (LC50 = 5623 µg/ml). Indeed, there is a positive correlation between toxicity against Artemia salina larvae and cytotoxicity against human nasopharyngeal carcinoma cells.

This test was used as a pre-screening for several human tumor cell lines. It is an internationally accepted bioassay for screening for anti-tumor compounds (**Ajoy and Padma, 2013**). The results showed that *R. montana* had no cytotoxic effect. For some authors, there is a correlation between this test and the toxicological effects on a whole animal; nevertheless, in a study on 20 extracts of plants that were tested using in vivo and in vitro methods (**Parra et *al.*, 2001**), the results showed a good correlation, suggesting that the Artemia test is a relatively useful alternative toxicity model.

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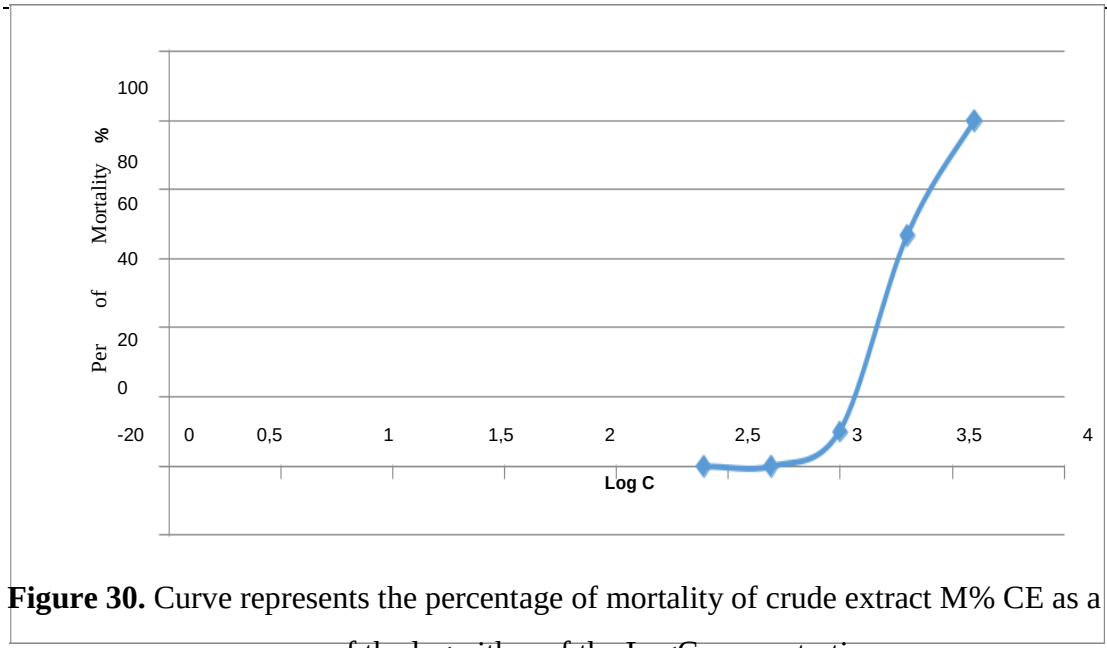

Figure 30. Curve represents the percentage of mortality of crude extract M% CE as a function of the logarithm of the LogC concentrations

CONCLUSION AND PERSPECTIVE

Conclusion and perspectives

Boussaâda stands as a significant region for medicinal plants and the associated

indigenous knowledge. The surveys conducted allowed for the compilation of medicinal species and the collection of extensive information on traditional therapeutic applications. A total of 193 species, spanning 69 families, were recognized for their use in traditional healing practices. The most frequently cited plant families include Lamiaceae (85 species), Fabaceae (37 species), Asteraceae (71 species), Apiaceae (38 species), Rosaceae (23 species), and Rutaceae (18 species). Among the local population, the primary plants used in herbal medicine are *Artemisia herba-alba, Juniperus oxycedrus, Mentha viridis, Thymus vulgaris,* and *Artemisia vulgaris*. Notably, over half of the inventoried plants are available during the spring season.

Based on habitat, native plants are the most prominent for traditional medical applications, constituting 53% of the total. Cultivated plants account for 45%, while exotic species are the least utilized, making up just 2% of the plants used. The ethnobotanical study conducted in this region highlights the significant role of traditional herbal medicine in the lives of Boussaâda's residents. To analyze the data, various quantitative indices were applied to socio-demographic information, including species use value (UV), fidelity level (FL), and informant consensus factor (ICF). The highest UV values were attributed to *Citrus lemon* L.*Burm*, *Ficus carica* L., *Moringa oleifera* Lam., and *Olea europaea* L. (UV=5). Furthermore, 73 species achieved the highest FL values. Regarding disease categories, the ICF results indicated that gastrointestinal disorders and diseases of the digestive system's associated glands have the most significant value.

The data collected from 534 survey responses show that older women, particularly those over 60 years of age (36%) and with a moderate level of education, are the primary users of traditional medicine. The findings also indicate that leaves are the most commonly utilized plant parts for medicinal purposes (33%). This survey has provided valuable insights into the traditional practices employed by the residents of Boussaâda.

While this knowledge appears to be rich based on the results obtained, it would be highly beneficial to extend such research to other regions of the country to preserve this invaluable cultural heritage. Consequently, the results obtained can potentially serve as a foundation for phytochemical investigations aimed at identifying the active compounds within the studied plants.

The phytochemical analysis of *R. montana* revealed that all the tested extracts exhibit significant anti-radical and antioxidant properties. However, when it comes to antibacterial activity, only the chloroform extract demonstrated effectiveness against the growth of the tested bacterial strains, with inhibition zone diameters ranging from 14 to 18 mm. On the

other hand, the ethyl acetate and butanol extracts showed limited or no activity against the tested bacteria. The crude extract produced similar results, with inhibition zone diameters between 11 and 11.66 mm.

To assess cytotoxicity, the "Brine shrimp" test was conducted, and it was found that none of the tested extracts exhibited cytotoxic effects within the concentration limit of 4000 µg/mL.

Furthermore, the extracts were subjected to qualitative analysis using modern liquid chromatography coupled with mass spectrometry (LC-MS). This analytical method identified 16 phenolic compounds, including various types of phenolic acids, coumarins, and flavonoids. Consequently, this study suggests that *R. montana* is a promising plant with a rich content of secondary metabolites, encompassing a variety of biologically active molecules responsible for antioxidant and antibacterial activities.

All these results obtained represent just a preliminary step in the search for biologically active natural compounds. Additional work is required to isolate and identify the bioactive substances responsible for the observed activities. Medicinal plants often contain multiple active compounds with different modes of action. Therefore, it would be of interest to conduct a detailed in vivo study on Rutaceae plants to explore their other activities. Additionally, highlighting their potential mechanisms of action at various levels would be valuable.

Further research related to this study should aim to deepen our understanding of the biological and pharmacological activities of these plants. This can pave the way for the development of modern medicines based on the findings of these explorations.

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Annexe **01**: Questionnaire used in the ethnobotanical study (French language)

Université Ibn Khaldoun- Tiaret
Facultés des Sciences de la Nature et de la Vie
Département des Sciences de la Nature et de la Vie

Fiche d'enquête ethnobotanique (01)

Nom et prénom :

Age :<2 [2-30] [30-40] [40-50] [50-60] >60

Sexe : Masculin Féminin

Niveau académique : Néant Primaire Secondaire Universitaire

Espèces médicinales	*Micromeriainodora*(DESF.) Benth.	*CalaminthaCandidissima*(Munby.) Benth.
Nom vernaculaire		
Habitat et répartition		
Récoltée ou Achetée		
Usage thérapeutique		
Partie utilisée		
Mode de préparation		
Mode d'administration		
Posologie		

Fiche d'enquête ethnobotanique (02)

Age :<2 [2-30] [30-40] [40-50] [50-60] >60

Sexe : Masculin Féminin

Niveau académique : Néant Primaire Secondaire Universitaire

Plante médicinale :

Nom vernaculaire :

Usage de la plante : Thérapeutique Cosmétique Autres

Plante seule En association

Partie utilisée : Tige Feuilles Fleurs Plante entière

Forme d'emplois : Tisane Poudre Huile essentielle

Mode de préparation : Infusion Décoction Cataplasme Cru Cuit Autre

Mode d'administration : Oral Massage Rinçage Badigeonage Autres

Posologie :

Pour les enfants : 1 fois / jour 2 fois / jour 3 fois / jour Autres

Pour les personnes âgées : 1 fois / jour 2 fois / jour 3 fois / jour Autres

Pour les adultes : 1 fois / jour 2 fois / jour 3 fois / jour Autres

Durée d'utilisation (durée de traitement) :

Journalière Hebdomadaire Mensuelle Jusqu'à la guérison

Utilisation :

Type de maladies :

Affections dermatologique
Affection respiratoire
Affections cardio-vasculaire
Affections génito-urinaires
Affections ostéo-articulaire
Affections métaboliques
Affections du tube digestif
Affections des glandes annexes du tube digestif
Affections neurologiques

Annexe 02: List of medicinal plants from Bousâada and its environs traditionally used by the local population.

N	Species	Family	Common name in French	Vernacular name arabic	TP	NDC	N	UV	Np
1	*Acanthus mollis* L.	Acanthaceae	Acanthe molle	اقنة رهلية	Herbs	4	1	4	1
2	*Actinidia deliciosa* (A. Chev.) C.F. Liang&A.R. Ferguson	Actinidiaceae	Kiwi	الكيوي	Liana	2	1	2	1
3	*Ajuga iva*L.	Lamiaceae	Ivory	شندقورة	Herbs	2	2	1	1
4	*Alchemilla vulgaris* L.	Rosaceae	Alchémille	عباءة السيدة	Herbs	2	1	2	1
5	*Alchemilla vulgaris* L.	Rosaceae	Alchémille commune	رجل الأسد	Herbs	1	1	1	1
6	*Allium ampeloprasum* L.	Liliaceae	Poireausauvage	الكراث/ ثوم الشرق	Herbs	2	1	2	1
7	*Allium cepa* L.	Liliaceae	Oignon	البصل	Herbs	1	1	1	1
8	*Allium sativum* L.	Amaryllidaceae	Garlic	الثوم	Bulbs	10	7	1.42	6
9	*Anacyclus pyrethrum* (L.) Link	Asteraceae	Pyréthred'Afrique	قنطس	Perennial	2	1	2	1
10	*Anastatica hierochuntica*L.	Brassicaceae	Rose de Jéricho	كف مريم	Herbs	4	2	2	1
11	*Anemone coronaria* L.	Ranunculaceae	Anémonecouronaire	عكر فاسي	Herbs	2	1	2	1
12	*Angelica archangelica* L.	Apiaceae	Angélique vraie	حشيشة الملائكة	Herbs	1	1	1	1
13	*Anthyllis vulneraria* L.	Fabaceae	Violette des haies	حشيشة الجرح	Herbs	4	3	1.33	3
14	*Anvilleagarcinii*subsp.*radiata*(Coss. &Durieu) Anderb.	Asteraceae	*Anvillea*	النقد	Bushy undergrowth	8	6	1.33	5
15	*Apium graveolens* L.	Apiaceae	Céleri	الكرافس	Herbs	4	3	1.33	3
16	*Aquilaria malaccensis* Lam.	Thymelaeaceae	Garou de malacca	عود غريس	Tree	3	3	1	1
17	*Arachis hypogaea* L.	Fabaceae	Cacahuète	الفول السوداني	Herbs	6	4	1.5	3
18	*Artemisia dracunculus* L.	Asteraceae	Estargon	الطرخون/ الحوذان	Perennial herb	3	1	3	1

19	*Artemisia herba-alba* Asso**.**	Asteraceae	Sagebrush	الشيح	Perennial.	45	22	2.04	15
20	*Artemisia vulgaris* L.	Asteraceae	Armoise commune / Armoise citronelle	التفقد	Perania l	23	14	1.64	9
21	*Artiplexhalimus* L.	Amarantaceae	Atriplex	القطف	Shrub	2	1	2	1
22	*Arum creticum*Boiss. et Heldr.	Araceae	Arum	اللوف	Tuberous plant	3	1	3	1
23	*Astragalus gummifer* Labill.	Fabaceae	Tragacanthe	الكثيراء	Shrub	1	1	1	1
24	*Avena sativa* L.	Poaceae	Avoine	الشوفان	Herbs	3	1	3	1
25	*Beta vulgaris* L.	Amaranthaceae	Épinard	السلق	Herbs	4	2	2	1
26	*Beta vulgaris*Subsp. *vulgaris*(autonyme).	Amaranthaceae	Betterave	الشمندر	Herbs	2	1	2	1
27	*Borrago officinalis* L.	Boraginaceae	Bourrache	لسان الثور/ حمحم	Herbs	4	1	4	1
28	*Boswella sacra* Flueck.	Burseraceae	Encens / résine oliban	اللبان الدكر	Tree	2	1	2	1
29	*Brassica oleracea* var.*asparagoides*DC.	Brassicaceae	Brocli	البروكلي	Herbs	2	1	2	1
30	*Brassica oleracea* var.*capitata*	Brassicaceae	Chou pommé	الملفوف/ الكرنب	Herbs	4	1	4	1
31	*Buniumpachypodum*P.W.Ball	Apiaceae	Bunium	التلغودة	Tree	2	1	2	1
32	*Buxus sempervirens* L.	Buxceae	Buis commun	بقس/ شمشير	Shrub	4	1	4	1
33	*Calendula officinalis* L.	Asteraceae	Souci officinal	أذريون الحدائق	Herbs	7	5	1.4	5
34	*Calluna vulgaris* (L.) Hull	Ericaceae	Bruyère	خلنج	Shrub	3	1	3	1

35	*Capparis spinosa* L.	Capparaceae	Câpriercommun	الكبار	Shrub	2	1	2	1
36	*Capsicum annuum* L.	Solanaceae	Piment	الفلفل الحار	Sub shrub	6	4	1.5	2
37	*Ceratonia siliqua* L.	Fabaceae	Caroubier	الخروب	Tree	2	2	1	1
38	*Chrysanthemum pacificum*Nakai.	Asteraceae	Chrysanthème Ajania	الاقحوان	Perennial	1	1	1	1
39	*Cichorium intybus* L.	Asteraceae	Cichoréaam ère	الهندباء	Herbs	12	6	2	5
40	*Cinnamomum verum* J.Presl.	Lauraceae	Cannelle	القرفة	Tree	1	1	1	1
41	*Citrus lemon* (L.) Burm. f.	Rutaceae	Citron	الليمون	Tree	5	1	5	1
42	*Citrus sinesis*(L.) Osbeck	Rutaceae	Orange	قشور البرتقال	Shrub	13	7	1.857	5
43	*Cocos nucifera* L.	Arecaceae	Cocotier	جوزة رقيقة	Tree	2	1	2	1
44	*Coffea arabica* L.	Rubiaceae	Cafier	القهوة	Shrub	5	3	1.66	3
45	*Commiphoramyrrha*(Nees) Engl.	Burseraceae	Arbe a myrrhe	مر الصبر	Tree	4	4	1	2
46	*Corchorus olitorius*L.	Malvaceae	Corètepotagère	الملوخية	Shrub	4	1	4	1
47	*Coriandrum sativum* L.	Apiaceae	Coriandreculti vé	الكسبر	Herbs	5	4	1.25	3
48	*Crataegus azarolus*L.	Rosaceae	Aubépine	ورق الزعرور	Tree	3	1	3	1
49	*Crocus sativus* L.	Iridaceae	Safran	الزعفران	Herbs	6	4	1.5	3
50	*Cucumis sativus* L.	Cucurbitaceae	Concombre	الخيار	Vegetable plant	5	2	2.5	3
51	*Cucurbita maxima* L.	Cucurbitaceae	Citrouille	اليقطين	Herbs	8	3	2.66	3
52	*Cucurbita pepo* L.	Cucurbitaceae	Courged'été	الكوسة	Herbs	4	3	1.33	1
53	*Cuminum cyminum* L.	Apiaceae	Cumin	الكمون	Herbs	6	4	1.5	3
54	*Curcuma longa* L.	Zingiberaceae	Curcuma	الكركم	Herbs	5	3	1.66	2
55	*Cutrulluscolocynth*	Cucurbitaceae	Coloquintev	الحنظل	Peranni	3	2	1.5	2

	is(L.) Schrad.		raie		al				
56	*Cynaracarduncululs*var.*scolymus*L.	Asteraceae	Artichautcultivé	الخرشف	Herbs	3	1	3	1
57	*Cynodondactylon*(L.)Pers.	Poaceae	Chiendent	النجيل	Herbs	2	1	2	1
58	*Cyperus diffusus* L.	Cyperaceae	Suchet galingale	السعد/ تارة	Herbs	1	1	1	1
59	*Cyperus esculentus* L.	Cupressaceae	Hab el-aziz	حب العزيز	Herbs	4	4	1	4
60	*Daucus carota L.*	Apiaceae	Carotte	الجزر	Herbs	4	2	2	2
61	*Diospyros kaki* Thunb.	Ebenaceae	Plaqueminier du japon	التين الكاكي	Tree	4	1	4	1
62	*Dipsacusfullonum* L.	Dipsacaceae	Cardère	مشط الراعي / مشيطة	Herbs	3	1	3	1
63	*Dittrichiaviscosa* (L.) Greuter	Asteraceae	Inulevisqueuse	مقرمان	Perennial	2	1	2	1
64	*Dorema ammoniacum*D. Don	Apiaceae	Doréma	فاسوخباطول	Tree	2	1	2	1
65	*Dorseraspatulata*Labill.	Droseraceae	Droséra	الدورسيرة	Carnivorous plant	2	1	2	1
66	*Dracaena cinnabari*Balf.f.	Asparagaceae	Dragonnier de socotra	دم الاخوة	Tree	2	1	2	1
67	*Dryas integrifolia* Vall.	Rosaceae	Dryade	الدرياس/ بونافع	Shrub	7	6	1.16	4
68	*Ecballium elaterium*(L.) A. Rich.	Cucurbitaceae	Concombred'ane	فقوس الحمير	Herbs	3	1	3	1
69	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Cardamome	الحبهان	Perennial	1	1	1	1
70	*Equisetum arvense* L.	Equisetaceae	Prêle des champs	ذنب الخيل	Herbs	3	1	3	1
71	*Eruca sativa* Mill.	Brassicaceae	Roquette	الجرجير	Herbs	4	3	1.33	1
72	*Erygiumcampestre* L.	Apiaceae	Chardon rolandpanicaut	شنداب/ فقاع الجمال	Perennial	2	1	2	1

73	*Eucalyptus globulus* Labill.	Myrtaceae	Eucalyptus	الكاليتوس	Tree	5	3	1.66	3
74	*Ferulaassa-foetida*L.	Apiaceae	Asefétide	الحنتيت	Herbs	4	3	1.33	2
75	*Ficus carica*L.	Moraceae	Figuier	التين	Tree	5	1	5	1
76	*Ficus religiosa* L.	Moraceae	Figuier des pagodes	لسان العصفور	Tree	2	1	2	1
77	*Foeniculum vulgare* var. *dulce* (Mill.) Batt.	Apiaceae	Fenouil doux	الشمر/ الشمار	Perennial	1	1	1	1
78	*Foeniculum vulgare* Mill.	Apiaceae	Fenouilsauvage	البسباس	Shrub	2	2	1	1
79	*Fraxinus excelsior* L.	Oleaceae	Frêne	الدردار	Tree	3	1	3	1
80	*Fumaria officinalis* L.	Papaveraceae	Fumeterre officinale	بقلة الملوك	Herbs	4	2	2	2
81	*Globularia alypum*L.	Globulariaceae	Globulaire	التسلقة	Sub-shrub	3	1	3	1
82	*Glycine max* (L.) Merr.	Fabaceae	Soja	فول الصويا	Herbs	7	4	1.75	3
83	*Glycyrrhiza glabra L.*	Fabaceae	Réglisse	عرق السوس	Perennial	9	4	2.25	2
84	*Hammda scoparia***(**Pomel) Iljin	Amaranthaceae	Remth	الرمث	Schrub	4	4	1	1
85	*Harpagophytumprocumben* L.	Pedaleaceae	Harpagophytn	مخلب الشيطان	Herbs	2	1	2	1
86	*Helianthus annuus* L.	Asteraceae	Tournesol	عباد الشمس	Herbs	9	6	1.5	3
87	*Hibiscus sabdariffa* L.	Malvaceae	Oseille de guinée	الكركدية	Herbs	2	2	1	1
88	*Hordeum vulgare* L.	Poaceae	Orge	الشعير	Herbs	4	2	2	2
89	*Humulus lupulus*	Cannabaceae	Houblon	حشيشة الدينار	Herbs	2	1	2	1

	L.								
90	*Hyssopus officinalis* L.	Lamiaceae	Hyssopus	الزوفا	Shrub	3	1	3	1
91	*Iris germanica* L.	Iridaceae	Iris	السوسن	Perennial	4	1	4	1
92	*Jasminum polyanthum*Franch.	Oleaceae	Jasmin	الياسمين	Shrub	3	1	3	1
93	*Juglans regia* L.	Juglandaceae	Noix	ورق الجوز	Tree	1	1	1	1
94	*Juniperus communis* L.	Cupressaceae	Genévrier	العرعار	Tree	36	20	1.8	20
95	*Juniperus phoenicia*L.	Cupressaceae	Genévrier de phénicie	الطاقة / العرعارالفينيقي	Shrub	1	1	1	1
96	*Laurus nobilis* L.	Lauraceae	Laurier	الرند	Shrub	5	4	1.25	3
97	*Lavandula angustifolia* Mill.	Lamiaceae	Lavande	اللافندر	Sub-Shrub	2	1	2	1
98	*Lavandula officinalis* L.	Lamiaceae	Lavande officinale	الخزامى	Sub-Shrub	4	2	2	1
99	*Lawsoniainermis*L.	Lythraceae	Henné	الحناء	Shrub	2	1	2	1
100	*Lellium temulentum* L.	Poaceae	Ivraieenivrante	الجليف	Herbs	2	1	2	1
101	*Lens culinaris* Medick.	Fabaceae	Lentille	العدس	Herbs	6	3	2	3
102	*Lepidium sativum* L.	Brassicaceae	Cresson alénois	حب الرشاد	Herbs	8	6	1.33	5
103	*Linum usitatissimum*L.	Linaceae	Lin cultivé	زريعة الكتان	Herbs	1	1	1	1
104	*Lisimachiaarvensis* (L.) U.Manns&Anderb.	Primulaceae	Mouron	حشيشة العلق / زغليلة	Herbs	3	1	3	1
105	*Lupinus luteus* L.	Fabaceae	Lupin	الترمس	Herbs	1	1	1	1
106	*Lyciumafrum* L.	Solanaceae	Lyciet	العوسج	Shrub	2	1	2	1
107	*Lytrumsalicaria* L.	Lythraceae	Salicaire commune	صابون العرائس	Herbs	5	3	1.66	3

108	*Malus domestica* Borkh.	Rosaceae	Pommier	التفاح	Tree	2	2	1	2
109	*Malva parviflora* L.	Malvaceae	Mauve	الخبيز	Herbs	12	10	1.2	1
110	*Marrubium vulgar* L.	Lamiaceae	Marrubeblanc	تمرويت	Herbs	15	7	2.14	5
111	*Matricariadiscoidea*DC.	Asteraceae	Matricaire	البابونج	Herbs	2	2	1	1
112	*Melissa officinalis* L.	Lamiaceae	Verveine	اللويزة	Herbs	14	11	1.27	6
113	*Mentha viridis* L.	Lamiaceae	Green mint	النعناع	Perenial	48	18	2.66	13
114	*Moringa oleifera* Lam.	Moringaceae	Moringa	المورينجا	Shrub	5	1	5	1
115	*Morusalba* L.	Moraceae	Murier	ورق التوت	Shrub	2	1	2	1
116	*Musa paradiciaca*L.	Musaceae	Banane	الموز	Shrub	4	2	2	1
117	*Mycotaalexop*L.	Pleurotaceae	Champignon	الفطر	Mushroom	1	1	1	1
118	*Narcissus tazetta* L.	Amaryllidaceae	Narcisse à bouquet	النرجس	Herbs	4	1	4	1
119	*Nerium oleander* L.	Apocynaceae	Laurier rose	الدفلى	Schrub	1	1	1	1
120	*Nigella sativa* L.	Ranunculaceae	Nigelle	الحبة السوداء	Herbs	7	6	1.16	4
121	*Ocimumbasilicum* L.	Lamiaceae	Basilic	الريحان / الحبق	Herbs	5	3	1.66	3
122	*Olea europaea* L.	Oleaceae	Olivier	الزيتون	Tree	5	1	5	1
123	*Opuntia ficus-indica* (L.) Mill.	Cactaceae	*Cactus raquettes*	الصبار	Shrub	4	4	1	3
124	*Origanum majorana*L.	Lamiaceae	Origan marjolaine	البردقوش / مردقوش	Perennial	2	2	1	1
125	*Panax ginseng*C.A. Mey.	Araliaceae	Panax	الجينسينغ	Perennial	1	1	1	1
126	*Panicum virgatum*	Poaceae	Millet	الدرع	Perenni	2	1	2	1

	L.		vivace		al				
127	*Peganum harmala* L.	Nitrariaceae	Harmel	الحرمل	Perania l	8	6	1.33	6
128	*Petroselinum crispum*(Mill.) Fuss	Apiaceae	Persil	البقدونس	Herbs	5	4	1.25	2
129	*Phoenix dactylifera* L.	Arecaceae	Palm	طلع النخيل	Tree	3	1	3	1
128	*Phylanthusemblica* L.	Phyllanthacea e	Amla	الاملج	Tree	1	1	1	1
129	*Pimpinella anisum*L.	Apiaceae	Anis	اليانسون / زريعة البسباس	Herbs	7	6	1.16	5
131	*Pinus halpensis* Mill.	Pinaceae	Pin d'Alep	الصنوبر	Tree	7	6	1.16	6
130	*Pinus krempfii*Lecomte.	Pinaceae	Tannage	الدباغة	Tree	2	1	2	1
132	*Pistacia lentiscus* L.	Anacardiacea e	Lentisque	الضرو	Schrub	3	2	1.5	1
133	*Pistacia lentiscus* L.	Anacardiacea e	Arbre de mastic/ Pistachier lentisque	المستكة	Shrub	1	1	1	2
134	*Plantago ovata* Forssk.	Plantaginacea e	Psyllium blond	القاطونة	Herbs	5	2	2.5	1
135	*Plantago ovata* Forssk.	Plantaginacea e	Psyllium blond	قشور السيليوم / القطونة	Herbs	2	1	2	1
136	*Portulaca oleracea* L.	Portulaceae	Pourpiermar aîcher	بذورالرجلة	Herbs	2	1	2	1
137	*Prunus armeniaca* L.	Rosaceae	Abricotier	المشمش	Tree	2	1	2	1
138	*Prunus cerasus* L.	Rosaceae	Cerise	أذناب الكرز	Tree	2	1	2	1
139	*Prunus dulcis*(Mill.) D.A.Webb.	Rosaceae	Amandier	اللوز	Tree	2	1	2	1
142	*Prunus persica*(L.) Batsch	Rosaceae	Pêcher	ورق الخوخ	Tree	1	1	1	1
140	*Psidium guajava* L.	Mytraceae	Goyavier	الجوافة	Schrub	2	1	2	1
141	*Punica granatum* L.	Lythraceae	Grenadier	الرمان	Tree	14	6	2.33	5
143	*Quercus ilex* L.	Fagaceae	Chêne vert	البلوط	Tree	1	1	1	1

144	*Retama raetam* (Forssk.) Webb &Berthel.	Fabaceae	Retam	الرتم	Shrub	1	1	1	1
145	*Rhamusalternus*L.	Rhamnaceae	Nerprunalaterne	المليلس	Shrub	1	1	1	1
146	*Rhus typhina*L.	Anacardiaceae	Sumac vinaigrier	السماق/ زوان	Tree	2	1	2	1
147	*Ricinus communis* L.	Euphorbiaceae	Ricin	الخروع	Shrub	1	1	1	1
148	*Rosa canina* L.	Rosaceae	Eglantier	نسرين	Herbs	2	1	2	1
149	*Rosa damascena* Mill.	Rosaceae	Rose	الورد	Shrub	4	2	2	1
150	*Rosmarinus officinalis* L.	Lamiaceae	Romarin	اكليل الجبل	Subshrub	13	8	1.62	3
151	*Rubia tinctorum* L.	Rubiaceae	Rubia	الفوة	Shrub	2	1	2	1
152	*Ruta montana*L.	Rutaceae	Rue de Chalep	الفجل	Herbs	12	6	2	6
153	*Saguisorba officinalis* L.	Rosaceae	Sanguisorbe officinale	توت الثعلب / عشبة كل بلية	Perennial	9	5	1.8	3
154	*Salvadora persica* L.	Salvadoraceae	Souek / Bois d'Araq	عود الاراك	Schrub	1	1	1	1
155	*Salvia hispanica*L.	Lamiaceae	Graine de chia	بذور الشيا	Herbs	2	1	2	1
156	*Salvia officinalis* L.	Lamiaceae	Sauge	الميرمية / سواك النبي	Sub-Schrub	3	1	3	1
157	*Salvia rosmarinusL.*	Lamiaceae	Romarin	اكليل الجبل	Schrub	13	8	1.62	3
158	*Saussureacostus*(Falc.) Lipsch.	Asteraceae	Costus	قسط بحري / القسط الهندي	Herbs	3	1	3	1
159	*Senegaliasenegal*(L.) Britton	Fabaceae	Gomme arabique	صمغ العربي	Tree	4	1	4	1
160	*Senna alexandrina*Mill.	Fabaceae	Sénéalexandrin	السنمكي/ عشرق	Small shrub	6	3	2	3
161	*Sesamum indicum* L.	Pedaliaceae	Sésame	السمسم	Herbs	5	3	1.66	1

162	*Silbym marianum* (L.) Gaertn.	Asteraceae	Chardon-Marie	شوكة الجمل/ العكوب	Herbs	2	1	2	1
163	*Sinapis arvensis* L.	Brassicaceae	Moutarde	الخردل	Shrub	1	1	1	1
164	*Solanum lycopersicum*L.	Solanaceae	Tomate	الطماطم	Herbs	7	5	1.4	4
165	*Solanum melongena* L.	Solanaceae	Aubergine	الباذنجان	Herbs	3	1	3	1
166	*Spergularia rubra* J.Presl&C.Presl	Caryophylacées	Sabline rouge	فتاتالحجر	Herbs	2	1	2	1
167	*Stipa tenacissima* L.	Poaceae	Stipe	الحلفة	Peranni al	1	1	1	1
168	*Syzygium aromaticum*(L.) Merr.&L.M.Perr	Myrtaceae	Girofle	القرنفل	Herbs	5	2	2.5	2
169	*Tamarindus indica* L.	Fabaceae	Tamarinier	التمر الهندي	Tree	3	1	3	1
170	*Tebebuiaavellened a*Gomes ex DC.	Bignoniaceae	Lapacho	اللاباشو	Tree	2	1	2	1
171	*Terfezia arenaria*(Moris) Trappe	Pezizaceae	Truffes	الترفاس	Mushro om	1	1	1	1
172	*Tetraclinis articulata*(Vahl) Mast	Cupressaceae	Thuya de Berbérie	سندروس	Tree	2	1	2	1
173	*Teucrium polium*L.	Lamiaceae	La germandréet omenteuse	الجعيدة	Herbs	14	8	1.75	5
174	*Theobroma cacao* L.	Malvaceae	Cacao	الكاكاو	Small tree	2	1	2	1
175	*Thuja occidentalis* L.	Cupressaceae	Thuya	العفصة	Tree	2	1	2	1
176	*Thymelaeahirsuta*(L.) Endl.	Thymelaeacea e	Passerine hérissée	المثنان	Shrub	6	4	1.5	4
177	*Thymus serpyllum*L.	Lamiaceae	Thyme	الجرتيل	Sub-shrub	15	9	1.66	8
178	*Thymus vulgaris* L.	Lamiaceae	Thyme	الزعتر	Sub-shrub	28	15	1.86	12
179	*Tirmania nivea* (Desf.) Trappe	Pezizaceae	Terfesse	الكماة	Mushro om	4	2	2	1
180	*Trigonella foenum-graecum* L.	Fabaceae	Fenugrec	الحلبة	Herbs	11	8	1.37 5	5

181	*Triticum aestivum* L.	Poaceae	Son de blé	النخالة	Herbs	2	2	1	1
182	*Triticum durum* Desf.	Poaceae	Blé	جنين القمح	Herbs	2	1	2	1
183	*Tritium vulgare* L.	Poaceae	Blé	القمح	Herbs	12	4	3	3
184	*Urtica dioica* L.	Urticaceae	Ortie	القراص/ الحريقة	Perennial	2	1	2	1
185	*Vachellianilotica*(L.)	Fabaceae	Gommier rouge	القرض	Shrub	2	1	2	1
186	*Valeriana officinalis* L.	Valerianaceae	Valériane officinale	جذور الناردين	Herbs	1	1	1	1
187	*Vinca minor* L.	Apocynaceae	Pervenche	قضاب	Perennial	5	3	1.66	2
188	*Viola odorata* L.	Violaceae	Violettes	البنفسج	Pernnial	2	1	2	1
189	*Vitex agnus-castus* L.	Verbenaceae	Gattilier	أم الجلاجل	Shrub	1	1	1	1
190	*Vitis vinifera* L.	Vitaceae	Raisin	العنب	Herbs	3	3	1	1
191	*Zea mays* L.	Poaceae	Maïs	شعر الكبال	Herbs	6	5	1.2	3
192	*Zingiber officinale* Roscoe	Zingiberaceae	Gingembre	الزنجبيل	Herbs	4	2	2	2
193	*Ziziphus lotus* L.	Rhamnaceae	Jujubier	السدرة	Tree	6	2	3	2

ABSTRACT

The purpose of our thesis is to know the different uses of medicinal plants collected for medicinal purposes and identify the important medicinal plants used for medicinal therapy by the local people of Bousaâda (M'sila province, South Est Algeria) then, to study the phytochemical composition and to evaluate the biological activities of *Ruta montana* L. (Rutacea) extracts which is a medicinal plant with a long history of traditional use to treat ailments. **Methods**: 534 Semi-structured interview questionnaires were used to collect and provide significant ethnobotanical information on the plants used. Bioactive compounds of *R. Montana* were obtained using solid-liquid extraction using solvents of increasing polarity. The obtained extracts were qualitatively analyzed by liquid chromatography coupled with mass spectrometry (LC-MS). The pharmacological properties of *R. montana* were also investigated. Antioxidant activity was achieved "in vitro" using two methods: scavenging of the free radical DPPH and total antioxidant capacity. Antimicrobial activity was evaluated using disc diffusion method on 3 pathogenic bacterial strains (*Escherichia Coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*). In addition, cytotoxic activity was determined by the "Brine shrimp" test. **Results**: most users' plants were women over 60 years old, with a middle level of education, the most frequently used parts of plants for the treatment, was leaves (33%) followed by seeds and fruits, the infusion (23%), followed by decoction (20%), were represent to be used more, while, the most treated disease was gastrointestinal disorders with a rate of 31.2%. A total of 193 species, grouped within 69 families were identified. Lamiaceae and Asteraceae were the most commonly reported medicinal plants with 85 and 71 species. *Artemisia herba-alba*, and *Juniperus oxycedrus* were the most widely used plants in the traditional medicine by the local population. The highest use value (UV) was observed for Citrus lemon (L.) Burm., *Ficus carica* L., *Moringa oleifera* Lam. and *Olea europaea* L. (UV=5). The highest fidelity level (FL) value was for 73 species. The calculated informant consensus factor (ICF) showed that diseases related to gastrointestinal disorders and diseases of the glands attached to the digestive system diseases present the highest values. Chemical investigation allowed the identification of 14 phenolic compounds. The identified compounds were mainly phenolic acids, coumarins and flavonoids. The crude extract and the different tested fractions exhibited an interesting antioxidant activity. Chloroform extract was effective against the growth of the tested bacterial strains with zones of inhibition varying between 14 and 18 mm. In contrast, ethyl acetate and butanolic extracts were almost inactive on all of the tested bacterial strains. Furthermore, the crude extract was found to exhibit antibacterial activity with 11 to 11.66 mm of inhibitions zone. No cytotoxic effect was recorded for all the tested extracts up to a concentration of 4000 µg/mL. **Conclusion**: Our study provided an opportunity to access and knows about the traditional uses of the inhabitants of Boussaâda, and showed that in the Bousaâda the folk use of plants still derives from daily practice. So, evaluation of pharmacological activity for the important medicinal plants is suggested. Our thesis highlights the potent bioactivity and acceptable drug-likeness of this plant, which supports its further uses.

Keywords: Ethnobotanical study, medicinal plants, Bousaada, M'sila, Biological activities, LC-MS, Phytochemical study, *Ruta montana*

الملخص

ان ال غرض من هذه الدراسة كان معرفة مختلف استخدامات النباتات الطبية التي تم احصاءها للأغراض الطبية وتحديد النباتات الطبية الهامة المستخدمة في العلاج الطبي من قبل السكان المحليين لمناطق مختلفة من بوسعادة (ولاية المسيلة، جنوب شرق الجزائر) ثم دراسة الكيمياء النباتية ، من خلال التركيب الكيميائ ي وتقييم الأنشطة البيولوجية لمستخلصات *Ruta montana L. (Rutacea)* وهي نبات طبي معروف جدا من حيث الاستخدام التقليدي للعلاج. الطرق والوسائل : تم استخدام 534 استبيانًا للمقابلة المباشرة مع الاشخاص لجمع اكبر ال معلومات المهمة عن النباتات المستخدمة. اما الجزء العملي فقد تم الحصول على المركبات النشطة بيولوجيا من *R. montana* باستخدام الاستخلاص الكيميائي باستخدام المذيبات ذات القطبية المتزايدة. تم تحليل المستخلصات التي تم الحصول عليها نوعيا بواسطة التحليل اللوني السائل إلى جانب قياس الطيف الكتلي *(LC-MS)* كما تم دراسة الخصائص الدوائية لنبات *R.montana.* وتم تقييم النشاط المضاد للأكسدة "في المختبر" باستخدام طريقتين: *DPPH*والقدرة الكلية لمضادات الأكسدة. تم تقييم النشاط المضاد للميكروبات باستخدام طريقة الانتشار القرصي على ثلاث سلالات بكتيرية ممرضة *Escherichia Coli*، *Pseudomonas aeruginosa*و *Staphylococcus aureus*وبالإضافة إلى ذلك، تم تحديد النشاط السام للخلايا. النتائج: معظم الافراد المستخدمين كان من النساء فوق 60 سنة، مع مستوى تعليمي متوسط، وكانت أكثر أجزاء النباتات استخداماً في المعالجة هي الأوراق (33%) تليها البذور والثمار، اما طريقة ا لنقيع (23%)، يليه المغلي (20%)، كانت اكثر طرق الاستخدام ، في حين أن أكثر الأمراض علاجاً هي اضطرابات الجهاز الهضمي بنسبة 31.2%. تم تحديد إجمالي 193 نوعًا، مجمعة ضمن 69 عائلة. كانت الفصيلة الشفوية والفصيلة النجمية من أكثر النباتات الطبية التي تم الإشارة اليها حيث تضم 85 و 71 نوعًا. كانت نباتات *Juniperusoxycedrus* من أكثر النباتات استخدامًا على نطاق واسع في الطب التقليدي من قبل السكان المحليين. أعلى قيمة استخدام *(UV)* لوحظت في الحمضيات الليمونية *.(L.) Burm*، *Ficus carica L.* ، *Moringa oleifera Lam.*و *Olea europaea L* أظهر عامل *ICF*أن الأمراض المرتبطة باضطرابات الجهاز الهضمي وأمراض الغدد المرتبطة بأمراض الجهاز الهضمي تمثل أعلى القيم. وقد سمح الفحص الكيميائي بتحديد 14 مركبًا فينوليًا. وكانت المركبات التي تم تحديدها بشكل رئيسي هي الأحماض الفينولية والكومارينوالفلافونويدات. أظهر المستخلص الخام والأجزاء المختلفة التي تم اختبارها نشاطًا مضادًا للأكسدة مثيرًا للاهتمام. كان مستخلص الكلوروفورم فعالاً ضد نمو السلالات البكتيرية المختبرة بمناطق تثبيط تتراوح بين 14 و 18 ملم. في المقابل، كانت مستخلصات أسيتات الإيثيل والبيوتانوليك غير فعالة تقريبًا على جميع السلالات البكتيرية التي تم اختبارها. علاوة على ذلك، وجد أن المستخلص الخام يظهر نشاطًا مضادًا للبكتيريا مع منطقة تثبيط تتراوح من 11 إلى 11.66 ملم. لم يتم تسجيل أي تأثير سام للخلايا لجميع المستخلصات المختبرة حتى تركيز 4000 ميكروجرام/مل. الخلاصة : أتاحت دراستنا فرصة الوصول ومعرفة الاستخدامات التقليدية للنباتات الطبية لسكان بوسعادة، وأظهرت أن الاستخدام الشعبي للنباتات في بوسعادة لا يزال مستمدا من الممارسة اليومية. لذا، يُقترح تقييم النشاط الدوائي للنباتات الطبية المهمة. تسلط أطروحتنا الضوء على النشاط الحيوي القوي والتشابه الدوائي المقبول لنبات الفيجل ، مما يدعم استخداماته الإضافية .

الكلمات المفتاحية: دراسة نباتية عرقية، نباتات طبية، بوسعادة، المسيلة، أنشطة بيولوجية، *LC-MS*، دراسة كيميائية نباتية، *Ruta montana*