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An ethnopharmacological survey and anti-urolithiatic activities of some traditional medicine plants against calcium oxalate stones

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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Dedications

First of all, praise be to God who has granted us the grace of reaching this great day that embraces one of my dreams.

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To my family; they gave me a noble upbringing, and their love made me who I am today.

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Rim

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Abstract

Algerian herbalists use a very important range of medicinal plants to cure urolithiasis (kidney stones). These traditional healers rely mainly on their ancestral knowledge to prescribe recipes for the reduction or to heal the urolithiasis. This study describes the traditional practices for the use of medicinal plants against kidney stones in Mila region for future pharmacological validations. The survey was carried out among residents without specifying age or gender or educational level. Twenty-five plants were reported for use in the traditional treatment of kidney stones. It was found that *Herniaria glabra*. L and *Paronychia Capitata*. L are the two plants most recommended by interviewers. This study also aimed to investigate medicinal uses of the different plant parts of the two plants and to examine their anti- urolithiatic activities. The data of an ethnopharmacological survey showed that 29% of local habitants used these plants in folk medicine. *P.capitata* and *H.glabra* contain various compounds such as polyphenols and flavonoids in both leaves and flowers extracts. Furthermore, results showed that the decoction leaf extract had the larger percentage of inhibition against calcium oxalate nucleation compared to other extracts, with value of 98.65% and the decoction flower extract, had the best potency on inhibitory activity with percentage of 99.65% against crystals aggregation compared to other extracts. From this investigation, we suggest that leaves extracts of *H. glabra*. L and flowers extracts of *P.capitata*.L could be used as good sources of natural anti-urolithiatic. In addition, the results support the use of *Herniaria glabra*. L and *Paronychia capitata*. L in folk medicine.

Keywords: phytotherapy, ethnopharmacological study, polyphenols, anti-urolithiatic activity.

Résumé

Les herboristes algériens utilisent une gamme très importante de plantes médicinales pour soigner l'urolithiase. Ces traditionnels praticiens s'appuient principalement sur leurs connaissances ancestrales pour prescrire des recettes pour la réduction ou la guérison de l'urolithiase. Cette étude décrit les pratiques traditionnelles d'utilisation des plantes médicinales contre les calculs rénaux dans la région de Mila en vue de futures validations pharmacologiques. L'enquête a été menée auprès des habitants sans préciser l'âge, le sexe ou le niveau d'éducation. 25 plantes ont été signalées comme étant utilisées dans le traitement traditionnel des calculs rénaux. Il a été constaté que *Herniaria glabra*. L et *Paronychia capitata*. L sont les deux plantes les plus recommandées par les informateurs. Cette étude visait également à étudier les utilisations médicinales des différentes parties de ces deux plantes et à examiner leurs activités anti-urolithiaques. Les données d'une enquête ethnopharmacologique ont montré que 29% des habitants locaux utilisaient ces plantes en médecine populaire. *P.capitata* et *H.glabra* contiennent divers composés tels que des polyphénols et des flavonoïdes dans les extraits de feuilles et de fleurs. En outre, les résultats ont montré que l'extrait de feuilles en décoction avait le plus grand pourcentage d'inhibition contre la nucléation de l'oxalate de calcium par rapport aux autres extraits, avec une valeur de 98,65% et que l'extrait de fleurs en décoction avait la meilleure activité inhibitrice avec un pourcentage de 99,65% contre l'agrégation des cristaux par rapport aux autres extraits. Cette étude suggère que les extraits de feuilles de *H. glabra*. L et les extraits de fleurs de *P.capitata*.L pourraient être utilisés comme de bonnes sources d'anti-urolithiaques naturels. En outre, les résultats soutiennent l'utilisation d'*Herniaria glabra*. L et de *Paronychia capitata*. L dans la médecine populaire.

Mots clés : phytothérapie, étude ethnopharmacologique, polyphénols, activité anti-urolithiatique

الملخص

يستخدم المعالجون بالأعشاب الجزائريون مجموعة واسعة من النباتات الطبية لعلاج حصى الكلى. ويعتمد هؤلاء الممارسون التقليديون بشكل أساسي على معارف أجدادهم لوصف وصفات طبية لعلاج حصى الكلى أو الحد منه. تصف هذه الدراسة الممارسات التقليدية لاستخدام النباتات الطبية لعلاج حصى الكلى في منطقة ميلة، بهدف التحقق من صحة الأدوية في المستقبل. تم إجراء الدراسة بين السكان دون تحديد العمر أو الجنس أو مستوى التعليم. تم ايجاد 25 نبات مستخدم في العلاج التقليدي لحصى الكلى. وكان فتات الحجر وبساط الملوك هما أكثر نباتين أوصى بهما المعالجون. هدفت هذه الدراسة أيضاً إلى معرفة الاستخدامات الطبية للأجزاء المختلفة من هذين النباتين وفحص أنشطتهما المضادة للحصى. أظهرت نتائج الدراسة أن 29% من السكان المحليين استخدموا هذه النباتات في الطب الشعبي. تحتوي نباتات *P.capitata* و *H.glabra* على مركبات مختلفة مثل البوليفينول والفلافونويد في مستخلصات الأوراق والزهور. بالإضافة إلى ذلك، أظهرت النتائج أن مستخلص الأوراق في عن طريق الغلي كان له أعلى نسبة تثبيط ضد تنوي أكسالات الكالسيوم مقارنة بالمستخلصات الأخرى، حيث بلغت98.65%، وأن مستخلص مغلي الزهرة كان له أفضل نشاط مثبط بنسبة 99.65% ضد التراكم البلوري مقارنة بالمستخلصات الأخرى. تشير هذه الدراسة إلى أن مستخلصات أوراق نبات *H. glabra*.L ومستخلصات زهرة *P.capitata*. L يمكن استخدامها كمصادر جيدة لمضادات حصى الكلى الطبيعية. بالإضافة إلى ذلك، تدعم النتائج استخدام مستخلصات هذه النباتات في الطب الشعبي.

الكلمات المفتاحية: العلاج بالنباتات، دراسة عرقية صيدلانية، البوليفينول، نشاط مضاد لحصى الكلى.

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$Alcl_3$: Aluminum chloride

$CaCl_2$: Calcium chloride

CaOx: Calcium oxalate

DecE: Decoction extract

DPPH: 1, 1-diphenyl-2-picrylhydrazyl

FE: Flowers extract

GAE: Gallic acid equivalents

***H.glabra*:** *Herniaria glabra*

Hcl: Acide chlorhydrique

InfE: Infusion extract

LE: Leaves extract

MacE: Maceration extract

$Na_2C_2O_4$: Sodium oxalate

NaOH: Sodium hydroxide

***P.capitata*:** *Paronychia Capitata*

QE: Quercetin equivalents

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Introduction

Introduction

Antibiotics and chemical synthetic drugs cause various disadvantages and side effects (Pham *et al*., 2020). However resistance of bacteria to the available antibiotic is growing rapidly and the available antibacterial agent also cause diverse adverse reactions such as hypersensitivity and immunosuppression (Amari, 2023). The synthetic antioxidants can present unwanted side effect and some have been suspected of being responsible for liver damage and carcinogenesis (Pharm *et al*., 2020). Also, the prolonged utilization of non- steroidal anti-inflammatory drugs (NSAIDs) can produce deterious effects on the gastrointestinal tract (Karbab *et al*., 2021).

Thereby, patients turn to search for alternative or complementary medicine to cure their diseases (Bharthi *et al*., 2017). The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history. It is known that even today over 80% of the world population depends on herbal medicines. The quality of herbal medicines is believed to be directly related to its active principles, but herbal medicines can be variable in their composition (Souilah, 2018).

Ethnopharmacological studies exploring traditional and herbal medicines may represent a valuable alternative approach for drug discovery and replacement therapy (Taïbi *et al*., 2020).The variability of ecological and climatic conditions in Algeria has favored the rise of an important plant biodiversity represented by 3183 species that constitutes a considerable opportunity for the exploration of various biological active molecules of interests (Boussaid *et al*., 2018). Consequently, traditional herbal remedies have gained more popularity to treat and manage urinary diseases as they are thought to be in general effective, safe and with less side effects (Bharthi *et al*., 2017).

Both *Herniaria glabra* and *paronychia Capitata* were used traditionally in the treatment of kidney stones, *Herniaria glabra*, the whole plant, was used as astringent, diuretic and expectorant. It appeared to have an antispasmodic effect upon the bladder and was used in the treatment of dropsy, catarrh of the bladder, cystitis and kidney stones. Externally, it was used as a poultice to speed the healing of ulcers. *Herniaria glabra* possessed hypotensive, diuretic, antiurolithiasis, antimicrobial, insecticidal and antioxidant effects (Al-Snafi, 2018). *Paronychia Capitata*, plant of the genus *Paronychia* are also known for their therapeutic and biological properties including antioxidant and hypoglycemic activity

(Elshamy *et al*, 2021). Although the use of *Paronychia capitata* in traditional medicine is not well known, information on its medicinal properties is limited. Recent studies have focused on the chemical constituents of *Paronychia capitata* (Allaoua *et al*, 2022). Similarly, the evaluation of biological activities has been the subject of a limited number of recent studies (Bouzidi, 2018).

In this context, our study focuses on the evaluation of the anti-urolithiatic activity of *Herniaria glabra*. L *and Paronychia capita*. L based on their use in traditional pharmacopeia in the Mila region of Algeria. Our study is structured in a classical manner, divided into three parts.

- The first part is a literature review, providing a synthesis of data related to our research topic.
- The second part is the experimental section, which describes the methodological approaches, including quantitative analysis of the extracts from our selected plant, as well as the evaluation of their antiurolithic activities.
- The final part consists of the discussion of the obtained results. Finally, a conclusion summarizing the main findings, along with future perspectives, concludes our manuscript

Chapter I

Phytotherapy and medicinal plants

1. Phytotherapy

1.1. Definition

The term "phytotherapy" is derived from two Greek words: "therapeia" meaning "treatment" and "phyton" meaning "plant." It is one of the oldest curative methods used by people (Leite *et al*., 2021). Phytotherapy is can be defined as an allopathic discipline that utilizes medicinal plants and herbal products for the prevention of diseases, often drawing upon traditional knowledge (Bellamin, 2017; Souilah, 2018).Phytotherapy generally uses the chemical composition and pharmacological activity of the source plant (entire herb, buds, leaves, sprouts, flowers, fruits, seeds, bark, and/or roots) for therapeutic purposes (Azmi & Shukla, 2021).

1.2. Types and complementary practice

The phytotherapy may be used as an alternative treatment when classical therapy is not giving satisfactory results (Azmi & Shukla, 2021) and it is increasingly seen as an essential complement to them. Two distinct approaches can be identified, the traditional and modern phytotherapy. The traditional phytotherapy draws upon empirical knowledge accumulated over generations, passed down through oral tradition and experiential practice (Jorite, 2015).Then, the advent of modern chemistry marked a turning point in the development of phytotherapy. However, the scientific advancements facilitated researchers to research the mechanisms of action and the healing properties of traditionally used plants for the development of standardized extracts and synthetic derivatives **)**Merad &Mahiout, 2019). The following (Table I) demonstrates some others complementary practice can be existed using plants.

Table I: Some complementary practice, definitions and applications (Rák &Csutak, 2024)

Type	**Definitions**	**Applications**
Aromatherapy	Utilization of naturally extracted lipophilic essential and vegetal oils such as hydrophilic herbal essences	Massage, medical, psycho-, cosmetical, and olfactory aromatherapy
Gemmotherapy	Application of extracts obtained by the ethanolic or glycerolic maceration of fresh meristematic plant tissues, such as buds	Herbal and cosmetic industries

Bach flower therapy	Utilization of alcoholic fresh flower tinctures	Tinctures, skin creams, pastilles, chewing gums, and liquid sprays

1.3. Route of use and herbal preparation

Herbal plants exist in diverse forms, including fresh, dried, and extracts, with occasional use of entire dried plants (Balkrishna *et al*., 2024).). The following (Figure 1) demonstrates the major type of herbal preparations.

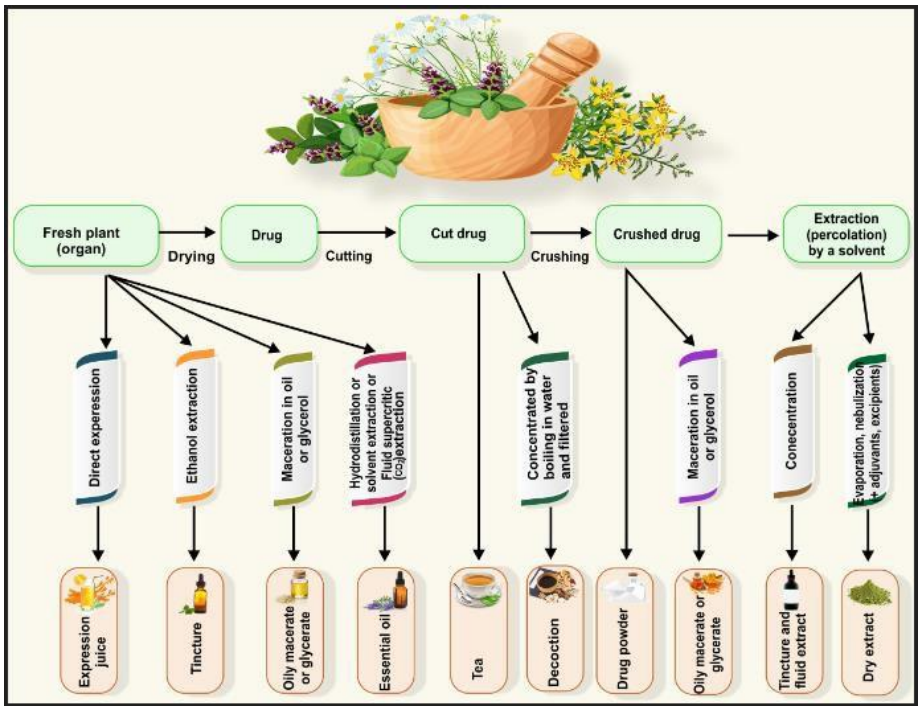


Figure 1: The major types of herbal preparations (Balkrishna *et al*., 2024).

1.3.1. Internal routes

1.3.1.1. Herbal Tea

Herbal tea is a beverage made by infusing, decocting, or macerating plant materials such as flowers, leaves, stems, or roots in hot or cold water. This process extracts various bioactive compounds from the plant materials, which can provide health benefits (Ghenabzia *et al*., 2023).

1.3.1.1.1. Infusion

The infusion is the most well-known preparation method. This process is suitable for leaves, flowers and plants rich in essential oils. It allows for a good extraction of water-

soluble active principles. It involves pouring boiling water over the previously fragmented drug, then covering the container and letting it steep for five to ten minutes (5-10 minutes) (Terniche & Tahanout, 2018).

1.3.1.1.2. Decoction

Decoction involves putting the plant in cold water and boiling the water for 15 to 30 minutes (Figure 2). A "decoction" can be kept in the refrigerator for three days. This procedure is suitable for plant parts with a hard or very hard consistency (wood, roots, bark, stems, rhizomes, seeds, or berries) that release their active principles less easily during an infusion (Huang *et al*., 2024).

Figure 2 : The pictorial representation of decoction (Alara*et al*., 2021).

1.3.1.1.3. Maceration

Maceration is a process that involves soaking a certain quantity of dry or fresh plants in a liquid (water, alcohol, oil, etc.) for 12 to 18 hours for the most delicate parts (flowers and leaves) and 18 to 24 hours for harder parts, then leaving it at room temperature. Before drinking, it should be well filtered (Lazli *et al*., 2019).

1.3.1.2. Inhalations

Inhalations have the effect of decongesting the nasal passages and disinfecting the respiratory tract. They are useful against colds, bronchitis, and sometimes to relieve asthma attacks. The simplest method is to pour boiling water into a large container containing finely chopped aromatic plants, or, in the case of essential oils, to add a few drops (Bouziane, 2017). Essential oils are mixtures containing multiple volatile compounds isolated from plant sources (He *et al*., 2024).

1.3.1.3. Powders

Dried plant material is finely ground into a powder, preserving the plant's natural constituents. Powders can be consumed directly, encapsulated, or added to other preparations (Bone &Mills, 2017).

1.3.1.4. Syrups and juice

Honey and unrefined sugar are effective preservatives that can be mixed with infusions and decoctions to make syrups, making them excellent remedies for relieving sore throats and more palatable for children to consume voluntarily (Meddour *et al*., 2010).

1.3.2. External routes

1.3.2.1. Ointments

Ointments are creamy preparations made from oils or other fatty substances in which the active principles of plants are dissolved. They are applied to wounds to prevent inflammation. Ointments are effective against hemorrhoids or yeast cracks (Bouziane, 2017).

1.3.2.2. Creams

Creams are emulsions prepared using substances such as oils and plant preparations (infusion, decoction, tincture, essences, and powders). Unlike ointments, creams penetrate the epidermis. However, they degrade very quickly and must be stored away from light, in airtight containers placed in the refrigerator (Bouziane 2017).

1.3.2.3. Lotions

Lotions are preparations made from water and plants (infusions, decoctions, or diluted tinctures) that are dabbed onto irritated or inflamed skin (Ghedabnia *et al*., 2008; EL Alami *etal*., 2010).

1.3.2.4. Poultices and compress

Poultices is the application of a fairly pasty preparation of a plant to the skin (Ghedabnia *et al*., 2008) to calm muscle pains, relieve fractures, and help draw pus from infected wounds and ulcers (Meddour *et al*., 2010). The plant can be crushed, chopped hot or cold, or mixed with linseed flour to obtain the right consistency. (Meddour *et al*., 2010).Compress involves applying gauze soaked in decoction, infusion, or macerate to the areas to be treated (Ghedabnia *et al*., 2008).

1.3.2.5. Essential oil

Essential oils are volatile, aromatic compounds derived from various parts of plants, including leaves, flowers, stems, and roots. These oils are extracted using techniques such as steam distillation, hydrodistillation, solvent extraction, and cold pressing. The chosen extraction method can significantly impact the oil's chemical composition and therapeutic effectiveness (Thangaleela *et al*., 2022).

1.3.2.6. Tincture

A tincture is a concentrated liquid herbal extract made by soaking herbs in a solvent, typically alcohol or vinegar, to draw out the active ingredients of the plant. This process results in a potent and easily absorbable liquid form of the Herbal tinctures may be available to purchase but they are not regulated in the United States (Debra *et al*.,2023),

1.3.2.7. Gargle and eye bath

Gargle consisting of an infusion or decoction as hot as possible is used to rinse the back of the mouth, throat, pharynx, tonsils, and mucous membranes. It is used to disinfect or soothe but should never be swallowed. The eye bath is done using an eyecup filled with an infusion or decoction, and it is essential to filter the solution before use (Ghedabnia *et al*., 2008).

1.4. Research methods

Herbal pharmacology utilizes various research methods to understand how herbal medicines work including *in vitro*, *in vitro*, *in silico* studies and clinical trials (Ramzan,2015).

Figure 3 : Different screening approaches utilized in herbal pharmacology (Couto *et al*., 2019).

1.4.1. *In vitro* studies

These lab-based experiments investigate the effects of herbal extracts or isolated compounds on cells or tissues. This helps identify potential mechanisms of action at the molecular level, for example, the studying of cytotoxicity in cancer cells (Ramzan, 2015).

1.4.2. *In vivo* studies

Preclinical studies using whole animals like mice or rats assess the safety and efficacy of herbal medicines. These studies also help determine how the body absorbs and processes herbal compounds (pharmacokinetics), for example, the testing of diabetes medication in diabetic rats (Ramzan, 2015).

1.4.3. Clinical trials

Human studies are essential to confirm the safety and effectiveness of herbal medicines in people (Ramzan, 2015).In fact, in order for a new biomedical product to enter human clinical trials and be approved for commercialization, the treatment must first be tested for efficacy and safety in at least two different animal models (Couto *et al*., 2019).

1.4.4. *In silico* studies

Computational biology is the use of computer models and simulations to study biological systems. These studies enable researchers to analyze complex systems, design experiments, and predict possible results without relying uniquely on traditional laboratory experiments. It is a valuable tool in various fields, especially drug discovery and toxicology (Luconi *et al*., 2022).

1.5. Advantages and disadvantages

Phytotherapy nowadays occupies a prominent place in both traditional and modern medicine. It can be dangerous at the same time when misused (Ekor, 2014).

1.5.1. Benefits

1.5.1.1. Medical applications and pharmacology importance

Phytotherapy hold significant global importance in international trade, featuring noteworthy clinical, economic, health, and pharmaceutical value. The escalating recognition of their worth, whether justified or not, is contributing to a steady expansion of their market(Balkrishna *et al*., 2024).The applications of phytotherapy can address various health concerns, from skin issues like acne and aging to chronic conditions like diabetes, high blood

pressure, and even cancer (Parfait & Lawrence, 2023).Phytotherapy can offer satisfactory results in relieving rheumatic pain, managing osteoarthritis, and treating urinary tract infections. Many studies also suggest its potential as a supportive therapy to boost the immune system during serious illnesses like cancer (Vogel *et al*., 2021).

1.5.1.2. Tolerance and safety

Herbal remedies are often considered gentler on the body compared to some pharmaceutical drugs, offering a favorable benefit-to-risk ratio (Parfait &Lawrence, 2023). On other hand, the major perception for the use of herbal drugs is that "they are safe because they are natural and have fewer side effects than prescription drugs" (Abdel-Aziz &Kahil., 2016).

1.5.1.3. Economic and nutritive value

Phytomedicines can be a more inexpensive option compared to conventional medications (Parfait &Lawrence, 2023). Herbs and spices are vital ingredients in many dishes. They add flavor, aroma, color, texture, and even nutrients (Abdel-Aziz &Kahil., 2016).

1.5.1.4. New drugs source

Many active ingredients in modern medicine are derived from plants, and research continues to explore this vast potential (Parfait & Lawrence, 2023).For new drugs to be available for commercialization, they must first go through the necessary steps such as the preclinical trials and clinical trials (Couto *et al*., 2019).

1.5.2. Risks

1.5.2.1. Potentiel side effects

Various studies have highlighted possible side effects of herbal medicine, if taken irregularly, in excessive amounts or in combination with some medicines (Abdel-Aziz &Kahil., 2016). Prolonged use of some herbal remedies can also lead to adverse effects due to their active ingredients (Parfait & Lawrence, 2023). These serious adverse events, often linked to self-prescription and non-professional sources (Abdel-Aziz &Kahil., 2016).

1.5.2.2. Pregnancy and allergy risk

Phytomedicines are not always recommended during pregnancy and pregnant women should avoid herbal products and should consult a doctor or pharmacist before taking any

herbal remedy (Abdel-Aziz &Kahil., 2016; Parfait & Lawrence, 2023). As phytomedicines contain various chemical compounds, there's a potential for allergic reactions (Parfait & Lawrence, 2023).

1.5.2.3. Serious limitations

Phytotherapy is not a substitute for conventional treatment for severe illnesses like AIDS, cancer, or severe migraines (Parfait & Lawrence, 2023).

1.5.2.4. Processing and potency

Drying methods can affect the potency of herbal remedies. Loss of essential oils and changes in structure can negatively impact the content of active ingredients and their stability (Parfait& Lawrence, 2023).

1.5.2.5. Toxicity

Scientific evidence on toxicity of herbal medicines is gathered from various sources such as systematic reviews, clinical trials, animal studies, cellular studies, and chemical analyses. Highly toxic herbs should be prohibited for medicinal use, while others should be restricted to qualified practitioners (Ramzan& Li, 2015).

1.6. Balancing benefits and risks

While phytotherapy offers many benefits, it is essential to be aware of the potential downsides and limitations. Unfortunately, the lack of qualified practitioners in traditional and herbal medicine can increase the risk of receiving inappropriate treatment. Consulting a qualified healthcare professional before using herbal remedies can help and understanding both the advantages and disadvantages associated with herbal remedies, individuals can make informed decisions about them (Vogel *et al*., 2021)**.**The advantages and disadvantages of some well-known herbs are mentioned in Table II.

Table II : Benefits and possible side effects of some important herbs

Herb	Benefits	Side effects
Peppermint	Cold, cough, sinus infections, and respiratory infections, digestive problems. Peppermint oil is applied for headache, muscle and nerve pain, toothache, mouth inflammation, allergic rash, bacterial and viral infections.	Allergic reactions, including flushing, headache and mouth sores. Large quantities of peppermint oil could damage the kidneys
Parsley	Urinary tract infections, kidney stones ,gastrointestinal disorders, constipation, jaundice, intestinal gas, indigestion, colic, diabetes, cough, asthma, edema, osteoarthritis, anemia, high blood pressure, prostate conditions, rheumatoid arthritis.	Allergic skin reactions, it is very high in oxalic acid may result in gouty arthritis, kidney stones, and mineral nutrient deficiencies.
Aloe vera	Anti-inflammatory, antiproliferative, antiaging, wound healing, recovery from burn injury, cell growth, and immune modulation.	Hepatotoxicity, abdominal spasms, pain, allergic reactions, cramps, and kidney damage.
Rosemary	Powerful antioxidant, anti-inflammatory, antiviral, and antibacterial. Carnosic acid offers protection against harmful carcinogens and Alzheimer's disease	Taking large amounts of undiluted rosemary oil by mouth can cause vomiting, uterine bleeding, kidney irritation, increased sun sensitivity, kinredness, and allergic reactions

2. Medicinal plants

2.1. Secondary metabolites

Plants act as living chemical factories, synthesizing a diverse array of secondary metabolites (Figure 4). These metabolites are not directly involved in primary processes such as growth and reproduction but play important ecological roles (Morreel *et al*. 2014). These chemical constituents in medicinal plants provide biological activities beneficial to human health, with applications in the pharmaceutical and food industries, as well as significant value in the perfume, pesticides, disinfectants, cosmetic industries, and as important raw materials for chemical industries (Hassan, 2013).

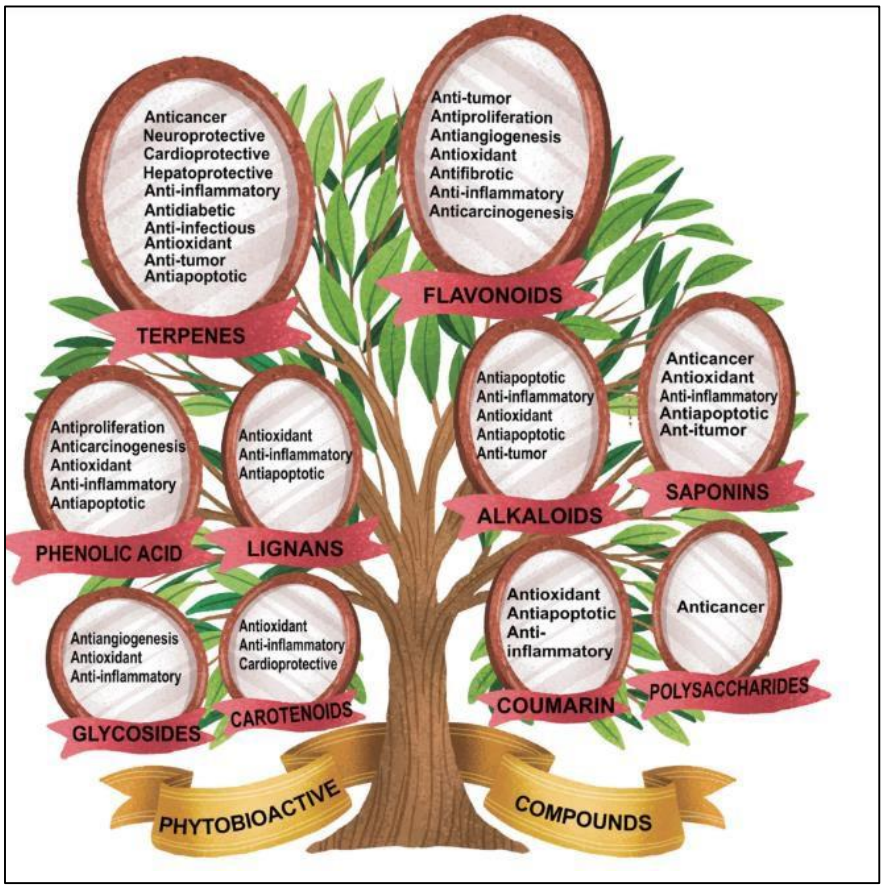


Figure 4 : Therapeutic potentiel of bioactive compounds (Balkrishna *et al*., 2024).

2.1.1. Terpenoids

Terpenoids, are known as isoprenoids, are a vast group of plant secondary metabolites. However, Due to their diverse biological activities and unique physical and chemical properties, terpenoid plant compounds have been utilized by humans since ancient times, either as complex mixtures or in purified forms recent, understanding has highlighted their

crucial ecological functions in plant biology (Mohiuddin, 2019).The following (Figure 5)illustrates examples of different classes of terpenoids by chain length and biosynthetic origin.

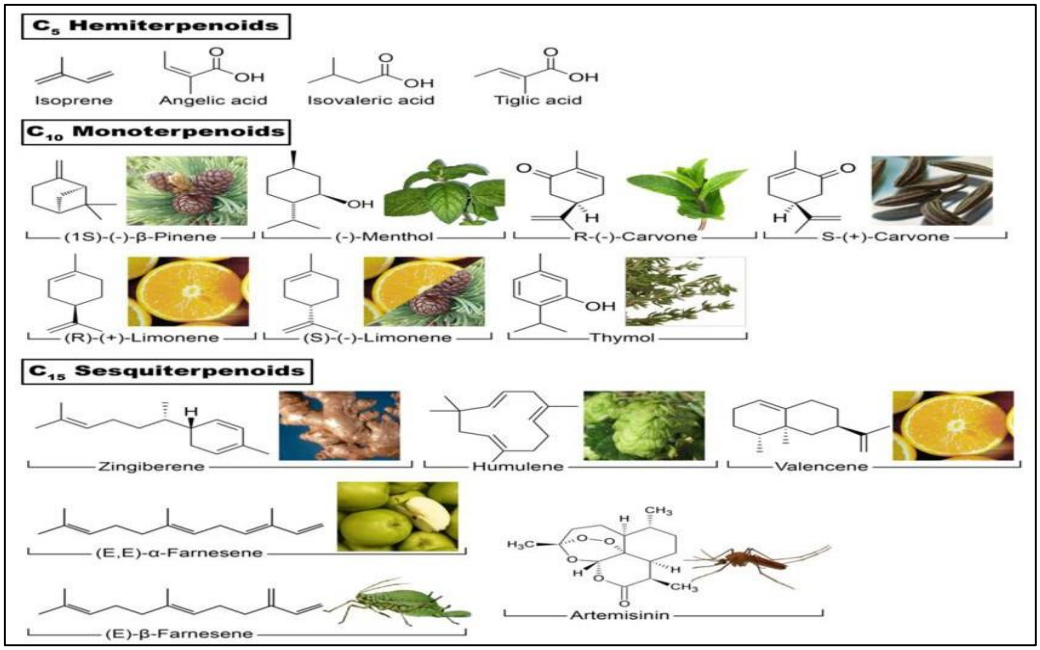


Figure 5 : Examples of different classes of terpenoids by chain length and biosynthetic origin (Polito, 2023).

2.1.2. Polyphenols

Phenolics are aromatic compounds with benzene rings and hydroxyl groups, and secondary metabolites synthesized through the shikimic acid pathway and pentose phosphate pathway via phenylpropanoid metabolism. They vary from simple phenolic molecules to complex polymerized forms (Figure 6). Phenolics include simple flavonoids, phenolic acids, complex flavonoids, and colored anthocyanins, playing roles in defense, fertilization, coloration, and antibacterial/antifungal activities in plants (Mohiuddin, 2019).

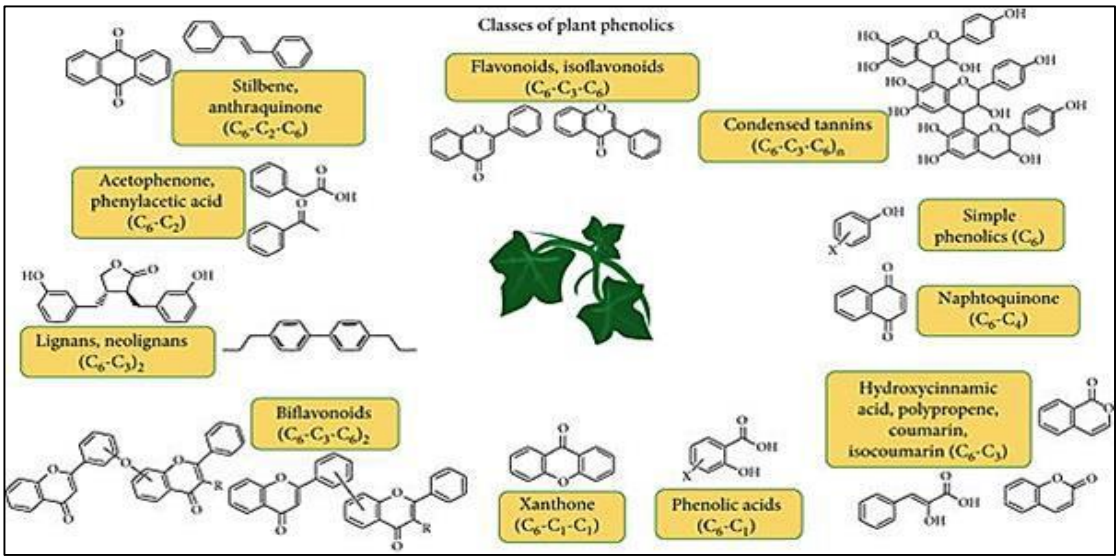


Figure 6 : Classes of plants phenolic (Chandran *et al*., 2020).

2.1.3. Alkaloids

Alkaloids or glucosinolates are characterized as essential compounds orchestrated by living creatures containing at least one heterocyclic nitrogen particles, got from amino acids (with certain exemptions) and pharmacologically dynamic (Mohiuddin, 2019).

2.2. Medicinal plants used

2.2.1. *Paronychia capitata* L.

2.2.1.1. Botanical description

Paronychia capitata L is perennial, polymorphic and ranges in height from 5 to 15 centimeters (Figure 7). It is widely distributed in the Mediterranean region and the Auras in mountainous of Algeria (Alluola, 2016). It thrives on rocky soils on sunny slopes at altitudes up to 300 meters. Its stems covered with dense, spreading hairs that give it a velvety appearance. The flowers are white in color, clustered in terminal heads with a calyx 3.5-5 mm long. The unequal sepals are much longer than the capsule and curved at the apex. The leaves are always pointed, mostly very small, sometimes lanceolate, all covered with diffuse hairs. The flowering time occurs from April to June (Alluola, 2016).

Figure 7 : *Paronychia capitata* L species (Tison *et al*., 2014).

2.2.1.2. Taxonomical classification and nomenclature

The plant is classified according to the following taxonomy mentioned in Table III (Bouzidi,2018).

Table III : Taxonomical classification and nomenclature of *Paronychia capitata*. L

Order	Caryophyllales
Family	Caryophyllaceae
Subfamily	Paronychioideae
Genus	Paronychia
species	*Paronychia capitata* Lamarck
French	Paronyque en tête
English	Algerian tea
Arabic (Algeria)	بساط الملوك

2.2.1.3. Chemical composition

Paronychia species have received little attention apart from the phytochemical investigations and the majority of the studies carried out on *Paronychia* plants showed that

oleanane-type triterpene glycosides were the main phytochemicals of these species. The aglyconesaponins of *Paronychia* were identified as gypsogenic acid, polygalacic, zahnic acid and medicagenic acid. In addition, many flavonoids and some other phenolic compound have been previously documented in this genus (Allaoua, 2022).Allaouaand his colleagues (2022) focused the phytochemical analysis of n-butanol extract, identifying two new saponins (capitatosides A and B) and characterizing seven known compound.

2.2.1.4. Traditional use and biological activities

P. capitata represents one of the most interesting species, after *Paronychia argenteana*, for the treatment of renal diseases. In addition, the infusion of the aerial parts of this species is used to regulate blood circulation and for treating gout diseases (Allaoua, 2022). The extracts did not inhibit the tested microbial strains. In addition, acute oral toxicity evaluations in rats confirmed the safety of butanol extracts at a dose of 2000 mg/kg (Bouzidi, 2018).

2.2.2. *Heniaria glabra*. L

2.2.2.1. Botanical description

H. glabra. L is perennial plant (Figure 8), 5 to 20 cm tall, glabrous, light green, with or slightly thick roots. The stems are usually smooth or slightly hairy. The leaves are opposite, small, narrow, and linear. They are sessile and are often arranged in pairs along the stems. The flowers are tiny, greenish-white, and clustered in axillary or terminal cymes. They lack petals but have five sepals that are fused at the base. The fruit is a small, bladder-like capsule, containing one to three seeds. The roots are fibrous helping the plant to anchor firmly in the substrate (Ghédira, 2017).

Figure 8: *Heniaria glabra.* L species.

2.2.2.2. Taxonomical classification and nomenclature

The plant is classified according to the following taxonomy mentioned in Table IV

Table IV: Taxonomical classification and nomenclature of *Heniaria glabra*.

Order	Caryophyllales
Family	Caryophyllaceae
Sub-family	Caryophylloideae
Genus	*Herniaria*
species	*Herniaria glabra* L
French	Herniaire glabre
English	Rupture wort
Arabic	فتات حجر

2.2.2.3. Chemical composition

H.glabra contained triterpene glycosides, saponins, flavonoids, hydroxycoumarins, phenolic acids, tannins and essential oil. Three flavonoids (isoquercitrin, luteolin and rutin), two phenolic acids (caffeic and chlorogenic acid) and nine amino acids were identified. The quantitative analysis of flavonoids and phenolic acids showed that *H. glabra* contained 0.29 % flavonoids and 0.34 % phenolic acids (Al Snafi, 2018).

2.2.2.4. Traditional use and biological activities

Infusions and decoctions of the aerial parts have been used in traditional medicine to treat various diseases. Thus, *H.glabra* is widely used traditionally to evacuate kidney stones. It is also utilized as diuretic for the treatment of edema, water retention, catarrh of the bladder and urinary diseases. The plant is also used in the treatment of hypertension in south-east region (Hakim *et al*., 2021). Hypertensive rats were treated with *H. glabra* saponins at a dosage of 200mg/Kg. Treatment led to progressive decline in both systolic and diastolic blood

pressures. The extracts of *H. glabra* showed the highest growth inhibitory effects against bacteria (Al Snafi, 2018).

Chapter II

Urinary system and urolithiasis

1. Urinary system

1.1. Definition

The urinary system, also known as the renal system, comprises organs that regulate blood's chemical composition and volume while excreting metabolic waste. It is divided into the upper urinary tract, which includes two kidneys and two ureters, and the lower urinary tract, which consists of the bladder and urethra (Figure 9) (Tortora *et al*., 2003).

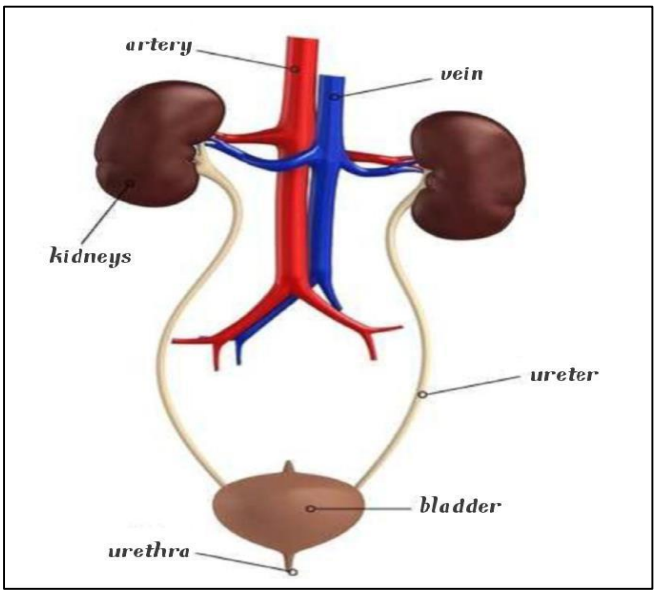


Figure 9: Anatomy of the urinary system (Ellatifi, 2011).

1.2. Urinary tract

1.2.1. Kidneys

The kidneys are two reddish-brown, bean-shaped organs located in the retroperitoneal space on the left and right sides (Figure 10). Blood enters the kidneys through the paired renal arteries and exits through the paired renal veins. The kidneys regulate the volume of body fluids, fluid osmolality, acid-base balance, electrolyte concentrations, and toxins (Mescher, 2016). Each kidney contains approximately one million nephrons, the functional units that produce urine. Each nephron consists of a glomerulus in the cortex and a tubule in the medulla (Briggs, 2014). The renal parenchyma is made up of these nephrons (Yacouba, 2012).

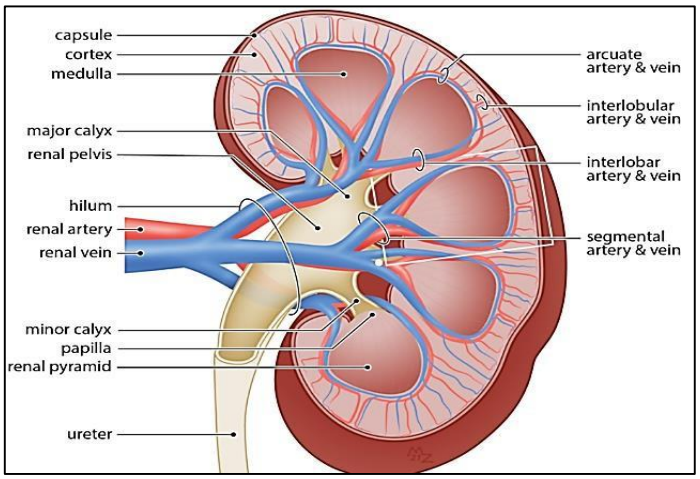


Figure 10: Anatomy of kidneys system (Apelt *et al*., 2021)

1.2.2. Ureter

The ureter is a musculo-membranous canal extending from the renal pelvis to the bladder. The ureter, by its vesical submucous path, prevents the reflux of urine during micturition towards the upper urinary tract and thus protects it: this is the anti-reflux valve. The bladder serves as a reservoir for urine and for evacuation through the urethra. Three layers can be identified in the structure of the ureter: adventitia, muscularis, and mucosa (yacouba, 2012). The ureter is a tube connecting each kidney to the bladder (Glenn, 2022).

1.2.3. Bladder

The bladder is a pouch like organ with walls made of smooth muscle and epithelial tissue the lower part of the bladder connects to the urethra at the vesico-urethral junction the bladder collects urine that comes to it from the ureters (Ellatifi, 2011).

1.2.4. Urethra

The urethra is a fibro muscular tube that allows urine to be transported from the bladder to the outside of the body. The base of the urethra is located at the anter-inferior surface of the bladder (Ducrocq, 2019).

1.3. Urinary system function

The main function of the urinary system is to produce and eliminate urine. This helps remove waste products from the body, such as urea and creatinine, and maintain the body's fluid, electrolyte, and acid-base balance. It also has endocrine functions that help regulate blood pressure by secreting a hormone called renin-angiotensin. Another function is to ensure

bone metabolism by activating vitamin D, which plays a role inregulating calcium and phosphorus metabolism by promoting their intestinal absorption (Kouta, 2009).

2. Urolithiasis

2.1. Generality

Urinary stone disease continues to occupy an important place in every day urological practice (Atmani, 2003) and considered one of the most common medical problems in the present society as it affects a high percentage of people but this illness exists since antique societies (Wein *et al*, 2007). The formation of urinary tract stones is worldwide, sparing no geographical, cultural or racial groups (Atmani, 2003) and derived from the Greek words ouron (urine) and lithos (stone) (Agarwal *et al*., 2007). It can be also defined as the clinical condition where the formation of crystal aggregates in the urinary tract results in kidney stones(Wein *et al* ., 2007).

2.2. Epidemiology

The lifetime incident rate of urolithiasis is approximately 13% in men and 7% in women. Moreover, this problem is most common in middle-age people as the most common age Apart from the gender and age, there are some other aspects which also affect the presence of kidney stones, for instance race, weight and body mass index. Moreover, whites have highest incidence of stones compared with Asians and African. Men and women who are obese have a greater risk to suffer urolithiasis. Finally, the risk of stone is directly related with the geographic variability with the highest prevalence of stone disease in the Southeast (Wein, 2007).

2.3. Signs and symptoms

The most prominent signs and symptoms include haematuria, back pain, nausea, dizziness, and vomiting. The systemic symptoms including restless, writhing, nausea, vomiting, fever, and foul-smelling urine. Asymptomatic symptoms including incidental stones, fluctuations in ache depth, with durations of pain lasting 20 to 60 minutes and chronic urge to urinate (Samantha *et al.*, 2021). The most prominent signs and symptoms of urinary tract stones are mentioned in Figure 11.

Figure 11: Clinical signs and symptoms of nephrolithiasis (Malhotra *et al*., 2022).

2.4. Risk factors

Various factors can produce calcium stones such as diet, low fluid intake and family history (Figure 12).The impact of diet on the prevalence of stone disease may be significant. Thus, dietary components that have been linked to stone disease include animal protein, extra calcium, sodium, oxalate, and fruit juices. According to an epidemiological study, the frequency of stone disease increase with supersaturation of urine solutes, the protease inhibitor indinavir sulphate taking, the presence of metabolic risk factors for kidney stones include obesity, hypercalciuria, hyperuricosuria, hyperoxaluria, and hypocitraturia (Malhotra *et al*., 2022) .

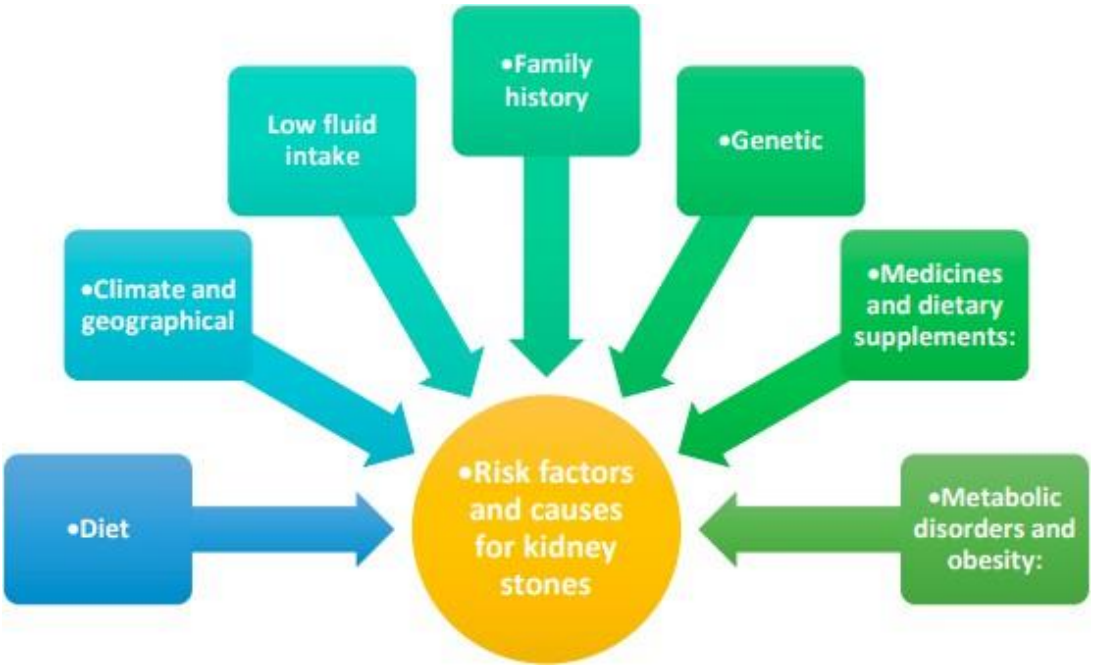


Figure 12: Various causes and risk factors of kidney stones (Khan. 2015).

2.5. Types

There are several types of calculi that differ in their chemical composition (Table V). The most common are calcium stones, which include calcium oxalate stones (75%) and calcium phosphate stones. Uric acid stones are also common, while struvite (magnesium) stones are less frequent. Cystine stones are rare composed of apatite and brushite crystals, appear beige or white (Battu, 2016).

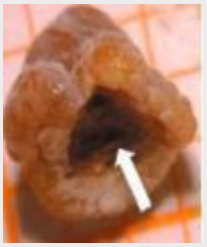
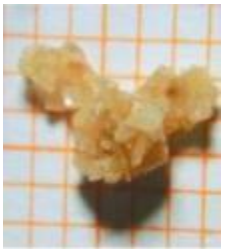
Table V: Some exemples of Stone chemical composition (Skolarikos *et al*., 2023)

Chemical name	**Mineral name**	**Chemical formula**
Calcium oxalate monohydrate	Whewellit	$CaC_2O_4.H_2O$
Calcium oxalate hydrate	Weddelite	$CaC_2O_4.2H_2O$
Basic calcium hydroxyl phosphate	Apatite	$Ca_{10}(PO_4)_6$-$(OH)_2$
Calcium hydroxyl phosphate	Carbonate	$Ca_5(PO_4)_3(OH)$
Carbonate apatite phosphate	Dahllite	$Ca_5(PO4)_3OH$
Calcium hydrogen phosphate dihydrate	Brushite	$CaHPO_4.2H_2O$
Calcium carbonate	Aragonite	$CaCO_3$
Uric acid	Uricite	$C_5H_4N_4O_3$
Uric acid dehydrate	Uricite	$CH_4O_3.2H_20$

2.5.1. Calcium oxalate stones

Calcium oxalate crystals in the urine are the most common constituent of human kidney stones represent about 80% of all cases in the United State (Reilly, 2007). Calcium oxalate crystal formation is also one of the toxic effects of ethylene glycol poisoning (Vijaya *et al* .,2013). Factors that promote the precipitation of oxalate crystals in the urine, such as primary hyperoxaluria, are associated with the development of calcium oxalate stones (Hoppe *et al*., 2003).The constituent of calcium stone is mentioned in Table VI.

Table VI: Constituents of calcium stones (Denu *et al*., 2011).

Type	Sub-type	Chemical name	Crystal name	Morphology	Characteristics
Calcium lithiasis	Oxalate calcium	Oxalate of calcium monohydrate	Whewellite		Brown or pale yellow, small, and well defined on the radiographs. They may appear as dumbbells orpyramids.
		Oxalate of calcium dihydrate	Whewellite		
	Phosphate calcium		Carbatite Oubrushite		Beige color in blanche

2.5.2. Struvite stones

About 10–15% of urinary calculi are composed of struvite (ammonium magnesium phosphate) (Vijaya *et al*., 2013).Struvite stones also known as "infection stones", form most often in the presence of infection by urea-splitting bacteria (Figure 13). Using the enzyme urease, these organisms metabolize urea into ammonia and carbon dioxide. This alkalinizes the urine, resulting in favorable conditions for the formation of struvite stones. These infection stones are commonly observed in people who have factors that predispose them to urinary tract infections. They are also commonly seen in people with metabolic disorders, such as hypercalciuria, hyperparathyroidism, and gout (Weiss *et al*., 2007).

Figure 13: Struvite calculi (Sekkoume, 2011)

2.5.3. Uric acid stones

Uric acid stones (Figure 14) appear as pleomorphic crystals, usually diamond-shaped. They may also look like squares or rods which are polarizable (Mais , 2009). About 5–10% of all stones are formed from uric acid (Moe, 2006). People with certain metabolic abnormalities; including obesity may produce uric acid stones(Johri, 2010).They also may form in association with hyperuricosuria, disorders of acid/base metabolism where the urine is excessively acidic (Reilly, 2005). Thus, patients with hyperuricosuria can be treated with allopurinol which will reduce urate formation (Fjellstedt, 2003). These calculi represent 10 to 15% of all stones and are the main cause of staghorn calculi; they are more common in women. They are light brown in color (Cynthia *et al*., 2011).

Figure 14: Uric acid calculi (Dominique, 2014).

2.5.4. Cystine stones

Cystinuria is the most common defect in the transport of an amino acid. Cystine kidney stones (Figure 15) are due to cystinuria, an inherited (genetic) disorder of the transport of an amino acid (a building block of protein).Thus, the excess of cystine in the urine (cystinuria) led to the formation of cystine stones (Fjellstedt , 2003).

Figure 15 : Cystine calculi (Cochat *et al*., 2012).

2.6. Mechanism of urolithiasis

Calcium oxalate kidney stones for example is characterized by mineral deposition in urinary system and the calcium oxalate monohydrate are most preponderant stones deposited, which formed generally by binding free oxalate to calcium (Amari, 2023).Supersaturation of urine, crystallization and retention of stone within urinary system are the prominent steps involves in the formation of urinary stones. The most prominent steps of kidneys stones formation are illustrated in Figure 16.

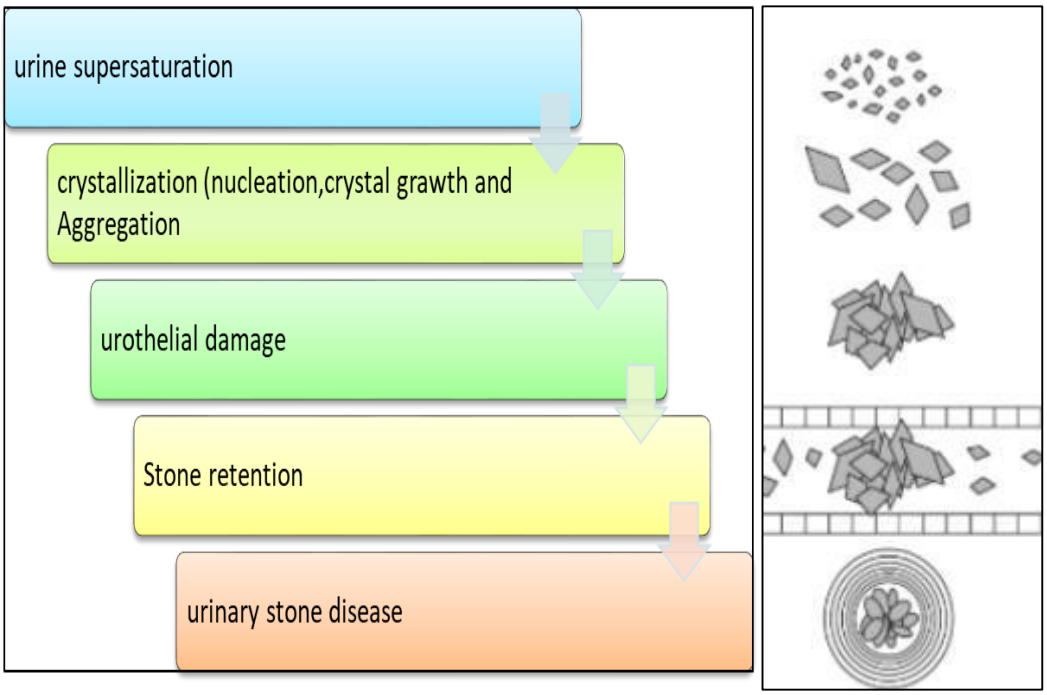


Figure 16: Stone formation mechanism (Daudon *et al*., 2008).

2.6.1. Crystallization

The Crystallisation represents the first phase of urinary stone formation and further includes three steps: crystal nucleation, growth and aggregation (Daudon *et al*., 2008).

2.6.1.1. Nucleation

The nucleation is a process of formation of a solid crystal in solution (Basavaraj *et al*., 2007). Generation of a crystal can occur by homogeneous nucleation when local supersaturation allows spontaneous organization of the atoms into the appropriate lattice. However, heterogeneous nucleation is more likely to occur within complex mixtures in which peculiar proteins provide patterns on their surface to organic molecules for the formation of the initial crystal lattice (Cerini *et al*., 1999).

The organic matrix of the stone compositions constitute around 2.5% of total weight, which mainly include small proteins such as albumin, (Abdel *et al*., 2009). Generally, the kidney walls are protected by an anti-adherent glycosaminoglycans layer and because of this, nucleation of stone can only occur at damaged areas or perhaps just at reduced protective layer sites. Nucleation may occur in renal tubules, on bladder walls, on normal or damaged cells, on areas denuded of cells by certain forms of injury, or at interstitial sites (Grases & Sohnel, 1993; Evan *et al*., 2003; Ratkalkar &Kleinman, 2011).

2.6.1.2. Crystal growth

After nucleation, crystal growth is the next major step of stone formation. In this process, several atoms or molecules in supersaturated liquid starts to form clusters. Crystal growth is determined by the molecular size and shape of the molecule, the physical properties of the material, supersaturation levels, pH, and defects that may form in the crystal's structure (Basavaraj *et al*., 2007).

2.6.1.3. Aggregation

Aggregation an important step of stone development, and is commonly defined as a process in which crystals agglomerate and form larger multicomponent particles in free solution Ratkalkar & Kleinman, 2011). Aggregation of particles in solution is determined by a balance of forces, including both aggregating and disaggregating effects (Basavaraj *et al*., 2007).

Trace elements in the urine, such as fluoride, iron, iodine, manganese, molybdenum, nickel, selenium, silicone, germanium, vanadium, copper, zinc, chromium, and lithium are found to initiate the process of crystallization (Słojewski, 2011). These elements act as a nucleus for the formation of the stone, or influence the external morphology of growing

crystals. They may also increase or decrease the speed of the crystallization process (Scott *et al*., 1980; Słojewski, 2011).

2.6.2. Stone retention

After the crystallization process is complete, the retention of urinary stones within the urinary system is an important step in the development of the disease. However, chemical or mechanical urothelial damage may promote crystal binding and aggregation (Gnessin *et al*., 2010).

So far, two hypotheses have been put forwarded for retention of urinary stones: free particle and fixed particle hypothesis. According to the free particle hypothesis¸ the process of nucleation occurs entirely in the tubular lumen. As crystals move through the renal tubules, rapidly aggregate and grow large enough to get stuck within the tubular lumen (Gnessin *et al*., 2010). Whereas, in fixed particle hypothesis it has been proposed that crystals gets adhered to a fixed point, such as renal epithelial cells or Randall's plaque (Evan *et al*., 2006).

Four different possible modes of stone retention have been identified in the fixed particle hypothesis (Evan *et al*., 2015) namely:

- Growth over white (Randall's) interstitial hydroxyapatite plaque;
- Growth over Bellini duct plugs;
- Formation of micro-liths within inner medullary collecting ducts;
- Formation in free solution within the calyces or renal collecting system

2.7. Prevention and Treatment

2.7.1. Preventive measurements

The best strategy to reduce urine supersaturation and stop stones from returning is to increased fluid intake, balance diet, and citrate supplement (Fontenelle & Sarti, 2019).

2.7.2. Treatment approaches

The symptoms, size, and placement of the stone inside the kidneys all affect how kidney stones are treated. Stones small in size should be eliminated using pharmacological therapies and prophylactic measures. A surgical method should be used to remove big stones (Malhotra *et al*., 2022).

A variety of surgical techniques techniques, including ureteroscopy, extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy (PCNL).There are numerous commercially available herbal preparations for treating kidney stones such as Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India) and Uriflush (Inti Sumatera Global, Indonesia), are marketed as composite herbal and formulations that have been successfully used in clinical trials to dissolve urinary stones in the kidney and bladder (Malhotra *et al*., 2022).

Renal colic is treated with steroids and painkillers. Diuretic are used to reduce kidney stones and produce more urine. The choice of drug is depending upon the type and cause of the kidney stones. In Table VII, various classes of drugs used in the treatment of kidney stones are mentioned (Malhotra *et al*., 2022).

Table VII: Various drugs used with their doses and side effects (Malhotra *et al*., 2022).

kidney stone type	**Aetiology**	**Pharmacological Treatment**	**Dose (mg/dose)**	**Side effects**
Calcium disease	Hypercalciuria	Hydrochlorothiazide	50	Hypokalemia
		Chlorthalidone	25-50	Glucose tolerance
		Amiloride	5	Hypomagnesemia
		Indapamide	1.2-2.5	Hypertriglyceridemia
	Hyperuricosuria	Allopurinol	100-300	Rare or severe hypersensitivity
	Hypocitraturia	Alkali treatment	30-60	Safer
	Hyperoxaluria	Pyridoxine	25-50	Safer
Non-	Uric acid stones	Alkali treatment	30-60	Safer

Calcium disease	Cystine stone	d-Penicillamine	1000-2000	d-penicillamine can result in arthralgia, leukopenia, proteinuria nausea, vomiting, diarrhoea, fever, skin rashes, and lupus-like syndrome.
	Infectious stones	Acetohydroxamic acid	10-15	Intractable headache, hemolytic anemia, and thrombophlebitis.

ChapterIII
Materials and methods

1. Materials

1.1. Plant material

The plants were purchased from an herbal shop called Baghdadi Natural Herbs from the region of Ferdjioua Mila in eastern Algeria (April , 2024). represented by the leaves of (*Herniaria glabra. L*). and the flowers of (*Paronychia capitata.* L) The harvested material was cleaned and then dried in the shade at room temperature, then crushed and sieved through manual sieve and separately powdered using an electric grinder. The resulting fine powders (leaves, flowers) were separately stored in tightly closed glass containers in the dark at room temperature (Figure 17).

Figure 17: Store of medicinal plants used (Original, 2024).

1.2. Chemicals and reagents

The chemicals and reagents used for extraction and the determination of total polyphenols and flavonoids, as well as those used for the evaluation of antiurolithiatic activity as follows:Tris Hcl, aluminum chloride($Alcl_3$), concentrated HCl, Folin-ciocalteu reagent, sodium oxalate($Na_2C_2O_4$), calcium chloride($CaCl_2$), sodium hydroxide (NaOH).

2. Methods

2.1. Ethnopharmacological study

2.1.1. Study area

The region of Mila covers a total area of 3,480 square kilometers. Approximately 85% of this area (294,300 square kilometers) is dedicated to agricultural activities (Figure 18). The remaining 15% (53,700 square kilometers) is located primarily on the northern border of the Wilaya. Mila is comprised of 13 administrative divisions called "daïras" and 32 communes, with a population of approximately 780,000 inhabitants.Mila city itself is located in eastern Algeria, roughly 391 kilometers northeast of Algiers, 80 kilometers south of Jijel, and 40 kilometers northwest of Constantine(Mouloud *et al*., 2024)

There are 32 municipalities and 13 districts(Mila;Sidi Merouane,El graram, Goug,Ferjaoua,Ain Beida Ahrich,Tsedat Hadada,Bouhatem,Oued N'ja,El Rouached,Terrai Bainan,Chelghoum Laid,Tadjenanet,Telaghma(.

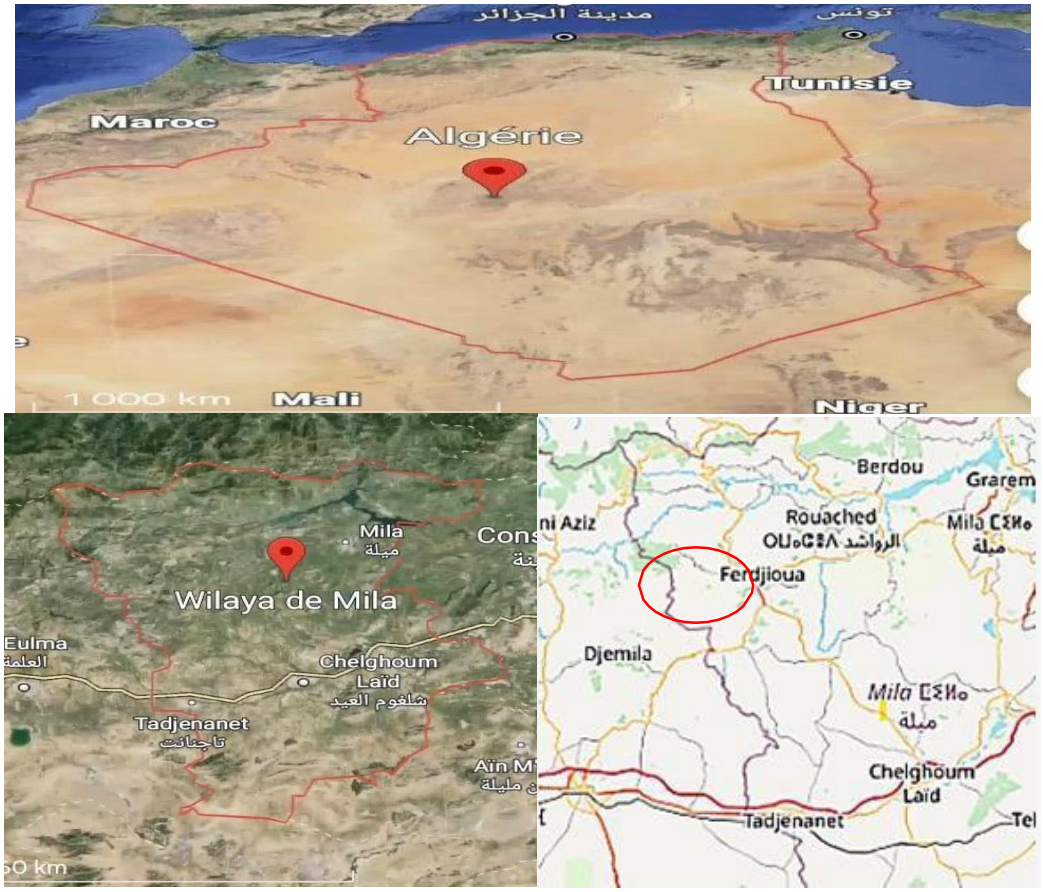


Figure 18: Geographical location of study area (Original, 2024).

2.1.2. Survey

The ethnobotanical study was conducted using a pre-designed survey. The survey was completed orally and through an electronic questionnaire. The survey used (Annex 01) addresses important aspects of people's treatment habits in combating urinary tract stones. Respondents were asked to identify specific factors that can be categorized into two main sections. The first section concerns informant identification (age, gender, education level, place of residence, etc.), and the second section concerns plant information (common name, ecological distribution, part used, harvesting season, method of use, preparation method, duration of use, dosage amount, therapeutic indications and side effects...).

2.2. Extraction

2.2.1. Preparation of the aqueous extract by decoction

The preparation of plant extracts was carried out according to the method of (Martins *et al*., 2015). Decoction extract was prepared by boiling 20 g of each leaves and flowers in 200 mL of distilled water at heating plate, for 5min. Then the mixture was filtered through filter paper and dried to obtain a dry extract (Figure 19).

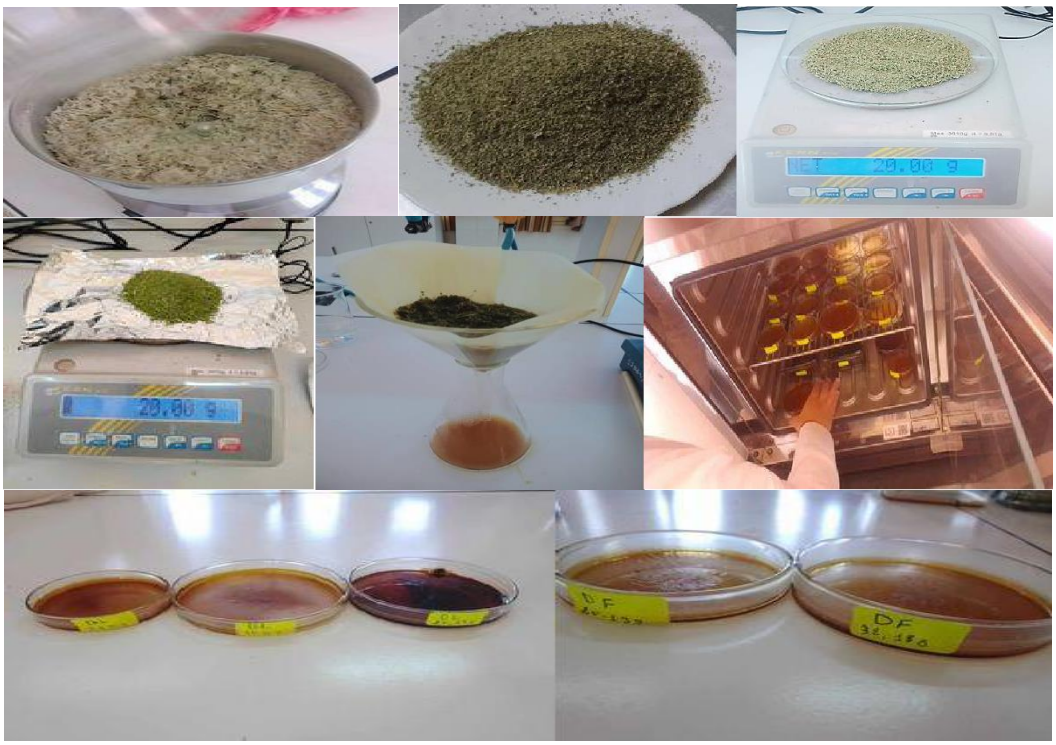


Figure 19: Stages of the plant extraction process by decoction (Original, 2024).

2.2.2. Preparation of the aqueous extract by infusion

The preparation of the infusion extract was carried out according to the method of (Martins *et al*, 2015). Infusion extract was prepared by mixing20 g of dry sample in 200mLof boiled distilled water and left to stand at room temperature during 5 min. Then the mixture was filtered and dried to obtain a dry extract (Figure 20).

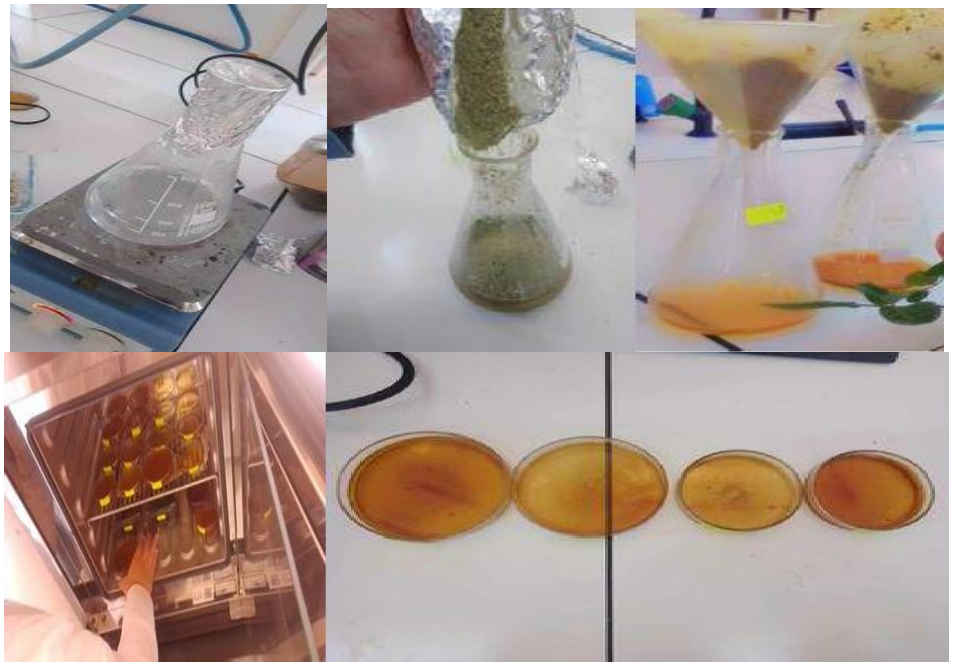


Figure 20: Stages of the plant extraction process by infusion (Original, 2024).

2.2.3. Preparation of the ethanolic extract by maceration

Hydroethanolic extracts (EAME) from different parts of plant were obtained using the maceration technique (Karbab *et al*., 2020). According to this method, 20 g of each ground part (leaves and flowers) were extracted in 200 mL of the ethanol and left in stirrer, at room temperature for 24 h. then the mixture was filtred and dried to obtain a dry extract (Figure 21).

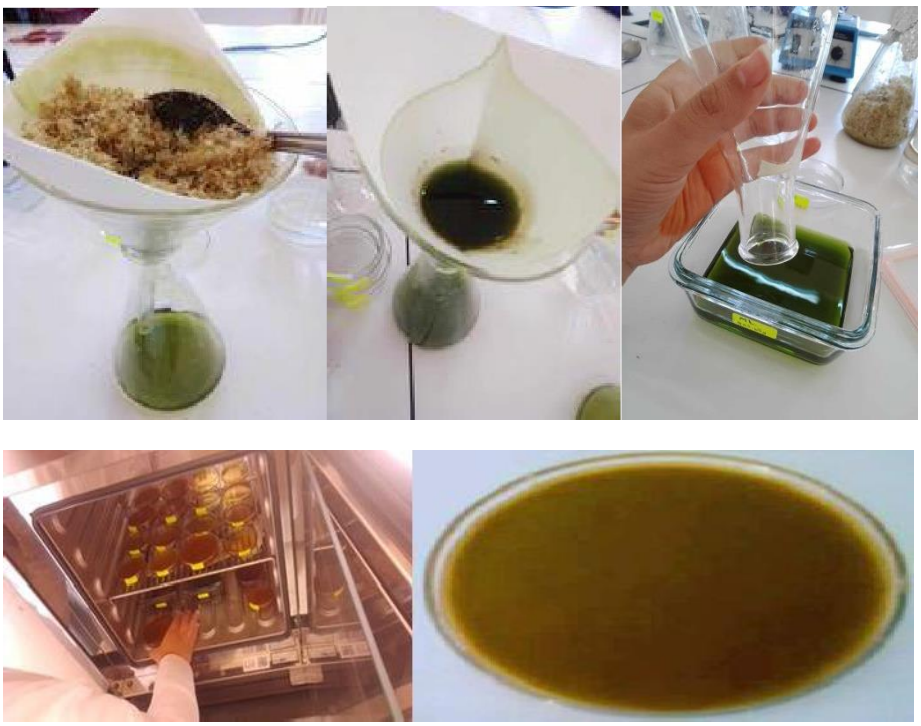


Figure 21: Stages of the plant extraction process by maceration (Original, 2024).

2.3. Determination of extraction yield

The percentage of extraction yield for each extract was calculated by the following formula as the ratio of the mass of the dried extract to the mass of the ground plant sample.

%yield= weight of extract obtained / total weight of the sample*100

2.4. Phytochemical evaluation

2.4.1. Quantitative phytochemical analysis

The extracts of two plants leaves of *H. glabra* and flowers of P.*capitata*. were subjected to detect the presence of potential phytochemical constituents such as polyphenols and flavonoids.

2.4.1.1. Determination of phenolic compounds

The total phenolic in extracts is estimated using the method of Folin-Ciocalteu method (Karbab *et al*., 2020). In tubes, a volume of 200 µL of each extract was added to the1000 µL of Folin-Ciocalteu reagent for 4min, 800 µL of a

7.5 % sodium carbonate solution was added. Then, the tubes are shaked and incubated for 2h. The absorbance is determined at 765 nm (Figure 22).

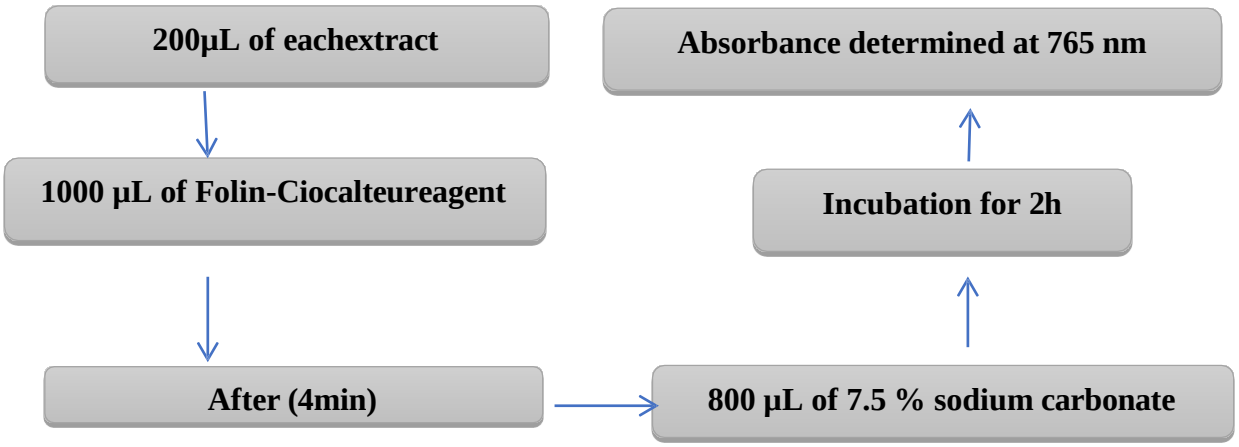


Figure 22: Tolal polyphenolic dosage protocol.

2.4.1.2. Determination of flavonoids

The total flavonoids content was evaluated by the method of aluminum chloride ($Alcl_3$) (Karbab *et al*., 2020). A 1000 µL of each extract was added to 1000 µL of the solution of $Alcl_3$ (2% in ethanol). After 15 min of incubation of darkness room temperature, the absorbances are measured at 430 nm (Figure 23).

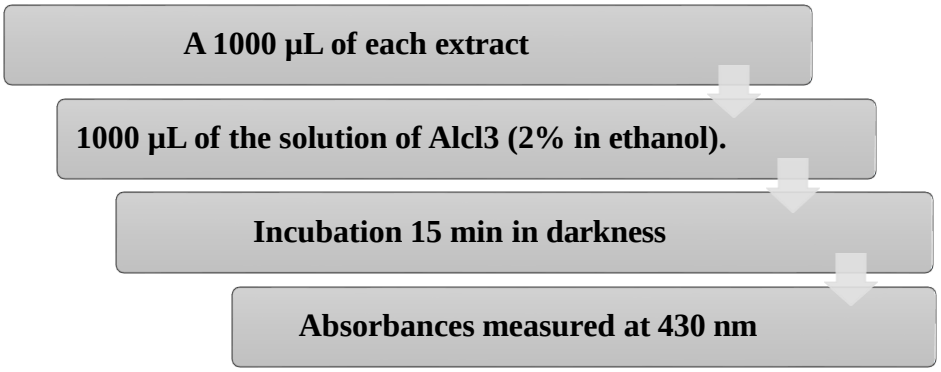


Figure 23 : Total flavonoids dosage protocol.

2.5. Anti-urolithic activity evaluation

2.5.1. Nucleation assay

Effect of extracts on calcium oxalate (CaOx) crystal formation was carried out by nucleation assay according to a previously described method of (Amari *et al*., 2023). Calcium chloride ($CaCl_2$) (0.5 g/L) and sodium oxalate ($Na_2C_2O_4$) solution (0.75 g/L) were prepared in Tris-HCl (5g/L) buffer (pH 6.5). Dilutions of

extract ranging from (62.5-500) µg/mL were prepared in buffer. 100 µL of each extract concentration was mixed with 950 µL $CaCl_2$ solution followed by the addition of950 µL NaC_2O_4 solutions. Final mixtures were incubated for 30 min at37ºC (Figure 24).

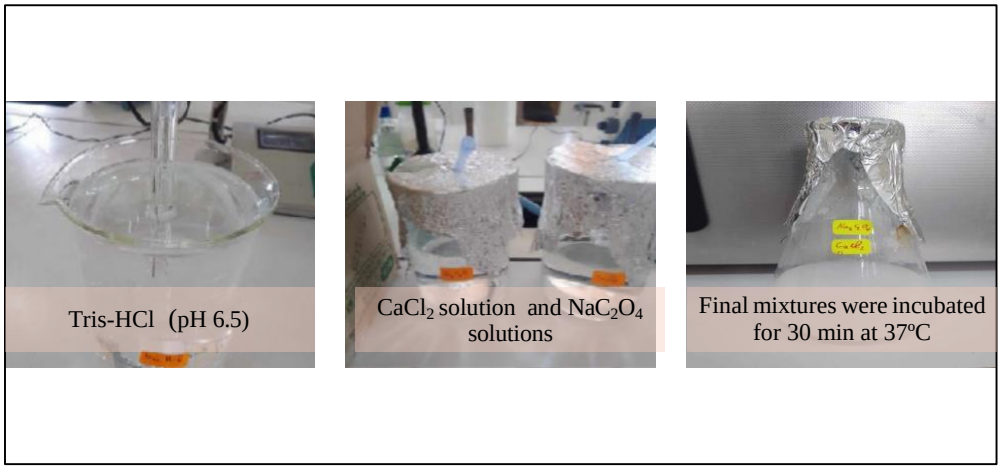


Figure 24: Effect of extracts on CaOx by nucleation assay (Original, 2024).

The absorbance of the mixtures was then measured at 620 nm wavelength. Percent inhibition of nucleation by extracts was calculated using the under mentioned formula and compared to that calculated for the standard allopurinol .

$$\mathbf{I\ (\%) = 100 \times (1- A\ test/A\ control)}$$

Where A control = absorbance of the negative control, and A test = absorbance of the hydroethanolic extracts and allopurinol.

2.5.2. Aggregation assay

The CaOx crystal aggregation was determined by aggregation assay described by Amari *et al*. (2023). The CaOx crystals were prepared by mixing together calcium chloride $CaCl_2$ and sodium oxalate NaC_2O_4 (50 mmoL/ each). The mixed solution were heated in a water bathfor 1 h to 60 ºC and then incubated overnight at 37ºC. After drying, CaOx crystals were used at a final concentration of 0.8 mg/mL, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L atpH 6.5. 200 µl

of extract (62.5-500) µg/mL of extract were added to 600 µL CaOx solution vortexed and then incubated at 37ºC for 30 min (Figure 25).

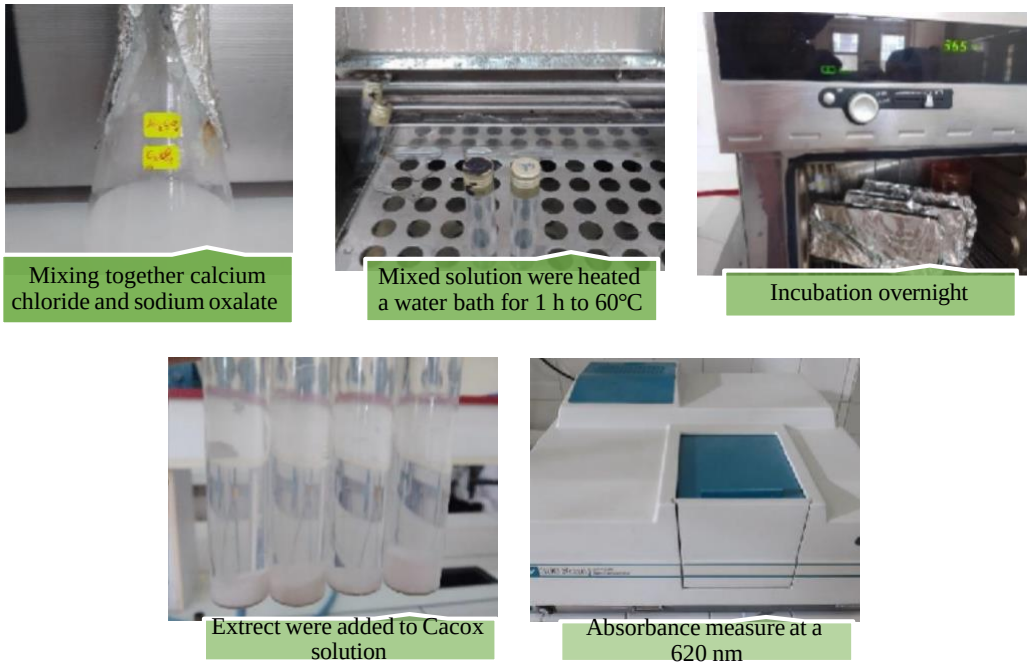


Figure 25: Effect of extracts on CaOx by aggregation assay(Original, 2024).

The absorbance of the mixtures was then measured at 620 nm wavelength. Percent inhibition of nucleation by extracts was calculated using the under mentioned formula and compared to that calculated for the standard allopurinol .

$$I\ (\%) = 100 \times (1 - A\ test / A\ control)$$

Where A control = absorbance of the negative control, and A test = absorbance of the hydroethanolic extracts and allopurinol.

Chapter IV
Results and discussion

1. Ethnopharmacological survey

1.1. Description of the population surveyed

During our ethnobotanical survey in the Mila region, which is carried out at using a questionnaire intended for the population which represents 213 people.

1.1.1. Gender

The percentage of using medicinal plants for females is larger than males, as orange color represents 75% for females and the pink color represents 35% for males (Figure 26).

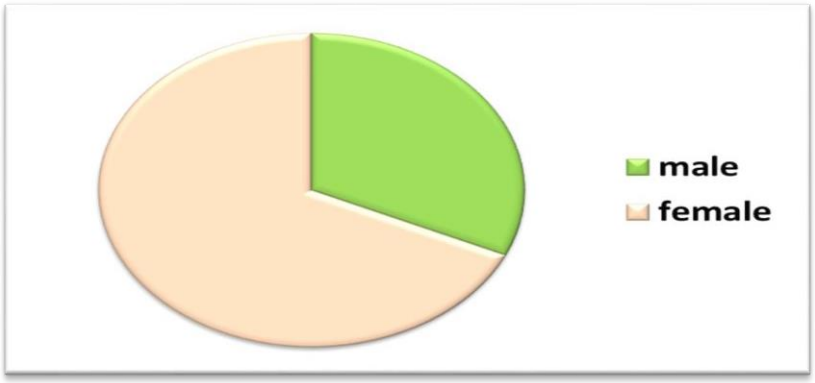


Figure 26: Distribution of interviewers by gender.

This predominance of females can be explained by the vigilance of women for the balance of the disease, and their attachment to all that is traditional. Indeed, women give sustenance and healthcare to their families in case of an illness. (Morocco, 2020; Chaachouay *et al.*, 2023). This must be due to that women are in charge of drying, stocking of medicinal plants, and preparing recipes for the care of family members. (Bouyahya *et al*., 2017).

1.1.2. Age

The following diagram (Figure 27) clearly shows the distribution of the respondents' age group using medicinal plants, with the majority in the 20-30 age group (59%), followed by the 30-40 age group (17%), then by the 18-20 age group (12%), 40-60 years (10%), and finally 60-80 years (2%).

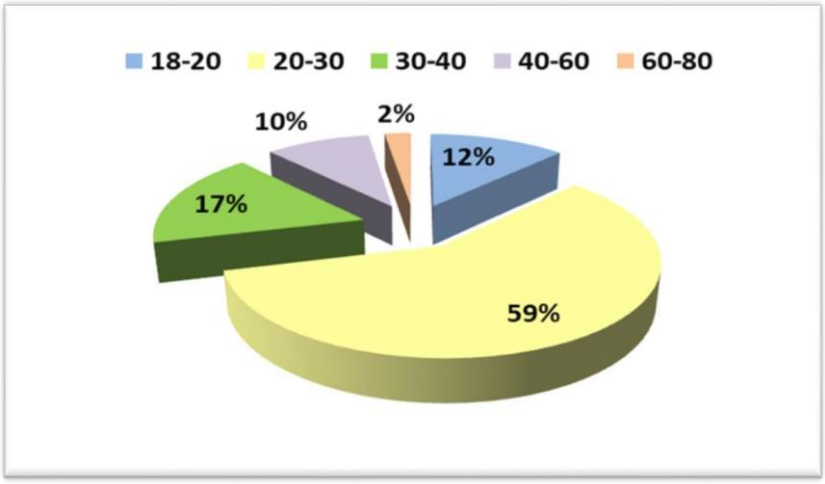


Figure 27: Distribution of interviewers by age.

1.1.3. Intellectual level

The following diagram shows the distribution of informants by intellectual level (Figure 28). The largest percentage, with value equal to (36%), is registered by the "no level" category. The second largest category is marked by "university level" with (26%). The Distribution of the remaining categories is as follow: Primary level (8%) and secondary level (12%).

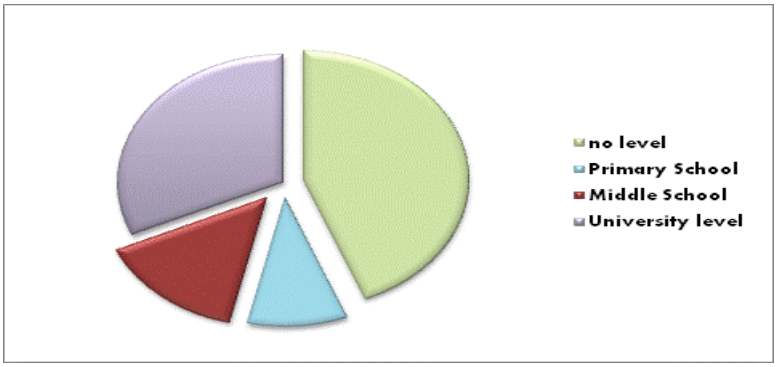


Figure 28: Distribution of interviewers by intellectual level.

1.1.4. Habitat

During our ethnobotanical survey we managed to complete 213 questionnaires which were spread over the thirteen regions (Figure 29).Mila has the highest percentage of the population using medicinal plants with (50%) in Ferdjioua, followed by Sidi Merouane (30%), Mila (27%), El Rouached (24%), Terrai Bainan (14%), Ain Beida Ahrich (11%), Oued El N'ja (11%), Tadjenanet (12%), G r a r e m Gouga (10%), Tesdat Hadada (8%), Bouhatem (5%), Chelghoum Laid (4%) and Telaghma (5%).

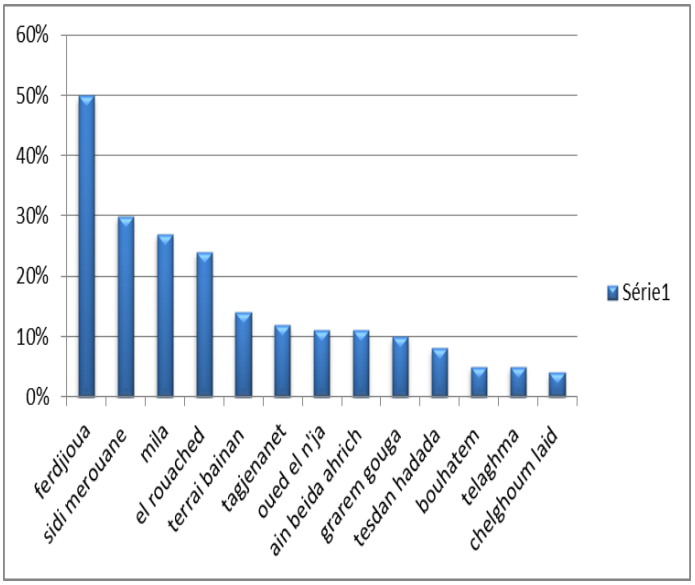


Figure 29: Percentages of subjects interviewed by region.

1.1.5. Exposure to urolithiasis diseases

Among the 213 questionnaire studied. The diagram revealed 140 people (66%) affected by urolithiasis and the rest 73 people (34 %) not affected (Figure 30).

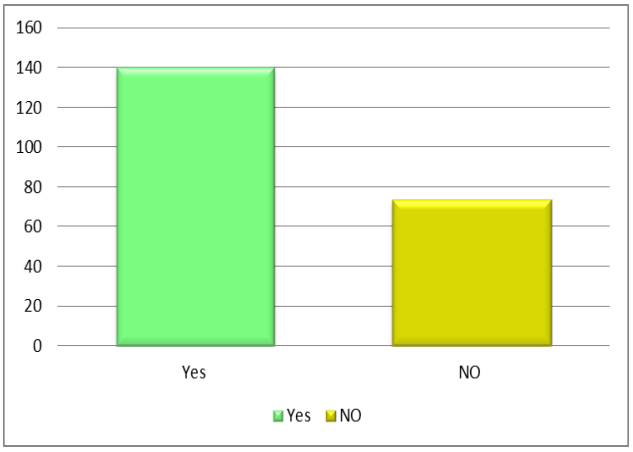


Figure 30: Number of people with or without kidney stone diseases.

1.1.6. Conventional treatment taken for urolithiasis

Among the 213 people interviewed, 132 people (62%) treat with medicinal plants and know them, while 81 people (38%) don't know them (Figure 31).

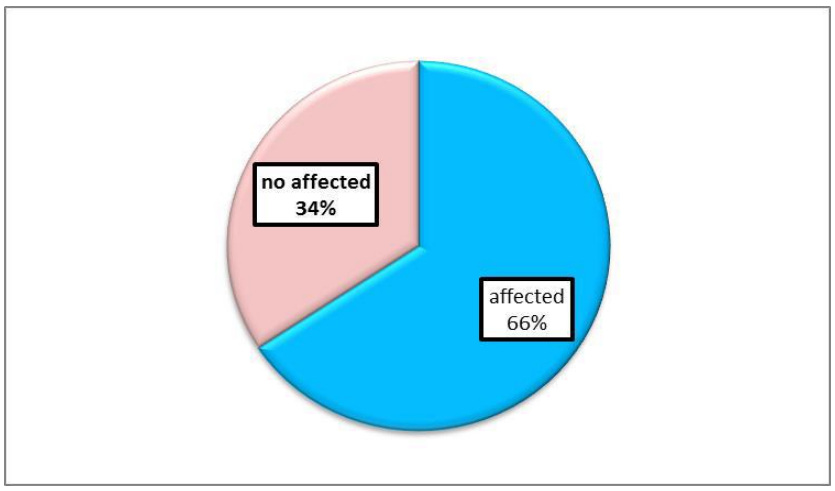


Figure 31: Treatment taken using medicinals plants by people interviewed.

1.2. Analysis of floristic data and phytotherapeutic uses

1.2.1. List of medicinal plants used

We have grouped together a number of 36 medicinal plants, cited by 213 people. The most commonly used plant species in the Mila region is *Herneria glabra* and *Paronychia capitata*, mentioned by people with a respective percentages of 16.27 and 13.37 %, and followed by (12.80 %) *of Petroselinum crispum* and *Thymus vulgaris* (8.13%). As for the rest of the herbs mentioned, their percentages ranged are between 0.58 and 6.97 %.

The following Table VIII highlights medicinal plants used to treat lithiasis cited by the population during our survey in Mila, mentioning: the vernacular name, scientific name, family, part used, mode of use.

Table VIII: Medicinal plants used for the treatment of kidney stones in Mila region.

Family	**Scientific name**	**Vernaular name /photo**	**Part used**	**Mode of use**	**Citation**
Aloeaceae	*Aloe barbadensis* L	Sabra/sebar 	AP	R	1
Apiaceae	*Foeniculum vulgare* L	Besbase	ER	D/R	4

					
	Apium graveolens L	Krafes 	AP	D/IN	5
	Daucus carota L	Argal 	FR	D/IN	2
	Pimpinella anisum L	Habaat hlawa 	FR	D/IF	1
	Petroselinum crispum L	Bakdouns 	AP	D/IN	22
Astraceae	*Anthemis nobilisL* L	Babounj 	AP/FL/LE	D	1
	Artemisia herba-alba Asso L	Chih 	SD	D	4

	Taraxacum officinalis L	Hindibaa 	FL	D	1
Brassicaceae	*Raphanus sativus* L	Fjel 	SD	IN/D	1
	Lepidium sativum L	Hebbrchad 	SD	D/IN	1
Caryophyllaceae	*Herniaria glabra* L	Herraslhejr 	LE/AP	D/IN	28
	paronychia capitata L	Bisatal molok 	FL	D/IN	23
Chenopodiaceae	*Atriplex halimus* L	Alkhate 	AP	D	3
Cucrurbitaceae	*Ecballium elaterium* L	Fakus alhamier	FR	D	4

					
Ericaceae	*Erica arborea* L	Khalndj 	LE/FL	D/IN	4
Fabaceae	*Trigonella foenumgraecum* L	Halba 	SD	M	2
Lamiaceae	*Mentha spp* L	Naanaa 	AP/LE	D/IN	2
	Thymus vulgaris L	Zaater 	LE	D/IF	14
	Lavandula angustifolia Mill	Al khozama 	AP	D	4
	Ocimum basilicum L	Rayhan	FL	D	6

					
	Rosmarinus officinalis L	Iklil al djabal 	LE	D	4
	Melissa officinalis L	Tarnijan 	LE	D	3
Linaceae	*Linum usitatissimumL* L	Zeriatlketan 	SD	D	12
Malvaceae	*Hibiscus sabdariffa* L	Korkodiya 	LE	D	2
Myrtaceae	*Syzygium aromaticum* L	Qronfel	SD	D	10

					
	Eucalyptus globulus L	Eukaliptus 	LE	D	2
Poaceae	*Zea mays* L	Dra 	ST	IN	2
	Hordeum vulgare L	Chaar 	SD	M/D/IN	4
Punicaceae	*Punica granatum* L	Raman 	PE	IN	9
Rosaceae	*Malus domestica* L	Khaltofah 	FR	D/IN	1
	Rubus fruticosus L	Tut	FR/LE/RO	D	2

					
Rutaceae	*Betula pendula* L	Satov 	FR	D	2
	Citrus limon L	Karas 	FR	D	1
Solanaceae	*Lycium shawii* L	Alousaj 	LE	D/IF	2
Verbenaceae	*Aloysia citodora* L	Tizana 	LE/AP	D/IN	1

AP: Aerial part ; **FR**: Fruits ;**RZ**: Rhizome ;**ST**: Stigmata ; **FL** :Flower; **EN**:Wholeplant; **SD**:Seeds;**RO**:Root; **PE**: Pericarp; **LE**: Leaves; B:Bulb. **D**: Decoction; **IN**: Infusion; **M**: Maceration; **PL**: Poultice; **R**: Raw, **PW**: Powder.

1.2.2. Distribution of medicinal plants according to botanical families

The analysis of the survey allowed us to distinguish 34 species belonging to 19 families (Figure 32). The prominent families include Caryophyllaceae with 24%, Apiaceae with 16%,

Lamiaceae with 15%, Linaceae with 13%, Astraceae and Myrtaceae. The other families appearing in small percentages ranging from 1 to 3%.

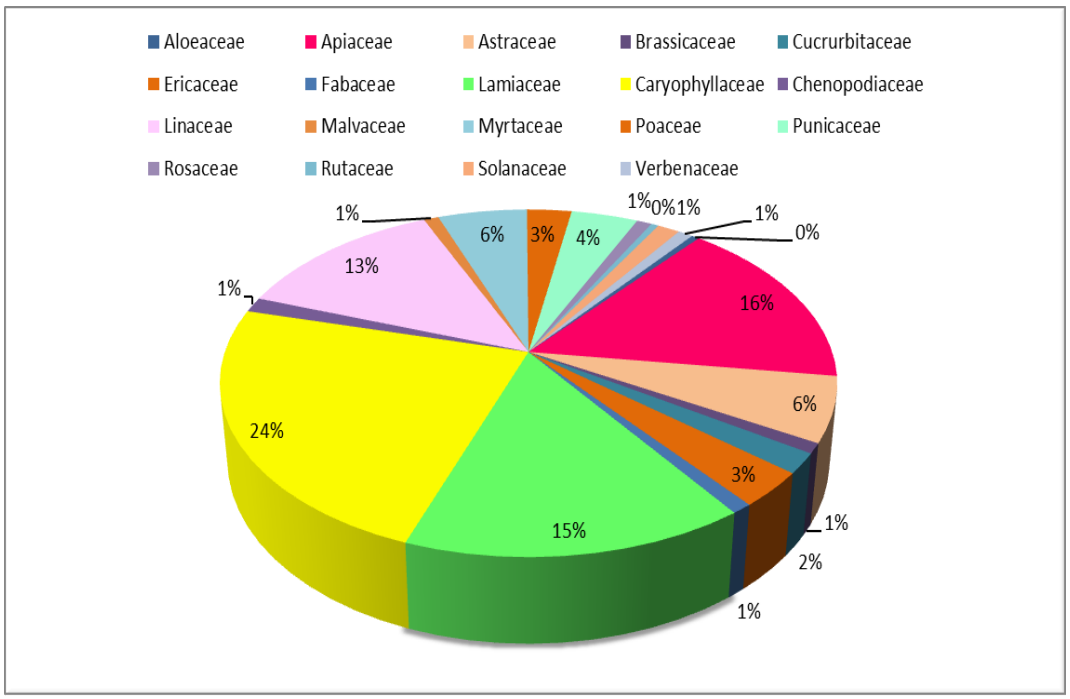


Figure 32: Distribution of medicinal plants according to botanical families.

1.2.3. Environmental distribution

The graph shows the distribution of plants according to environmental (spontaneous, cultivated, native, imported). Spontaneous plants showed the largest percentage of the total plants, accounting for 56%, followed by cultivated plants and native plants accounting for 21% and 18% cultivated. Thus, imported plants showed the lowest percentage of total plants, accounting for 5% (Figure 33).

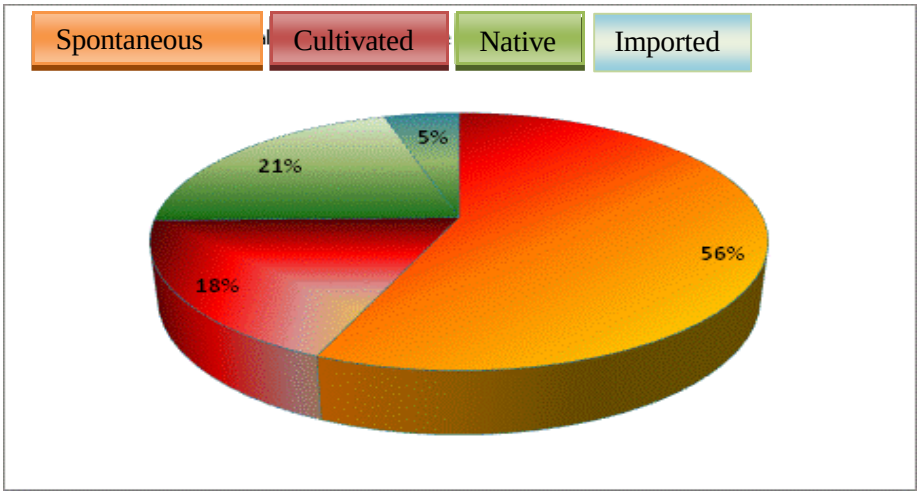


Figure 33: Environmental distribution of plants medicinal.

1.2.4. Parts used

The following graph (Figure 34) shows the distribution of parts used by informants. The preferred part used by the 80 person is leaves, corresponding to the percentage of (37%), followed by flowers by 40 person (19%), and fruits by 26 person (12 %). The utilization of other parts by informants appearing in small percentages ranging from 1 to 7 %.

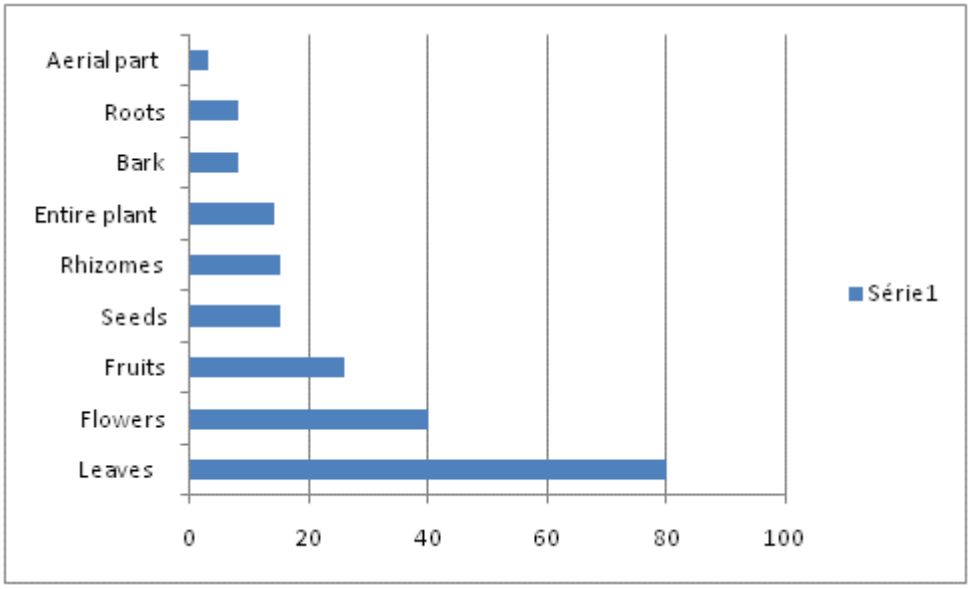


Figure 34: Diagram representing plants parts used for kidney stone disease.

Leaves are the most frequently utilized part of the plant. The choice of leaves is due to their natural availability, ease of collection, and simplicity in the preparation of herbal remedies (Yemele *et al*., 2015).

Similar results have indicated that leaves are used as a major part of plants in phytotherapy in different studies (Benlamdini *et al*., 2014; Aulas et al. 2024). Many communities in other regions of Morocco and other countries use leaves to prepare herbal medicines (Yemele, 2015; Mrabti *et al*., 2019; Yebouk *et al*., 2020).

The frequent use of one part over another in herbal medicine depends on its content of active substances. Leaves are the site of photosynthesis and sometimes the storehouse of active secondary metabolites of the pharmaceutical properties of medicinal plants. Besides, harvesting these organs is a relatively sustainable practice compared to other plant parts, such as roots and stem. Harvesting roots can contribute to plant extinction and disappearance (Yemele *et al*., 2015).

1.2.5. Source of information

The graph (Figure 35) shows the source information of plants medicinal. The major source information employed by 127 informants is family heritage, corresponding to percentage of (60%), followed by 30 people (14%) that who choose herbalist, then internet by 22 people (10%), scientific documents by 21 people (9.85 %) and social environment by 13 people (6%).

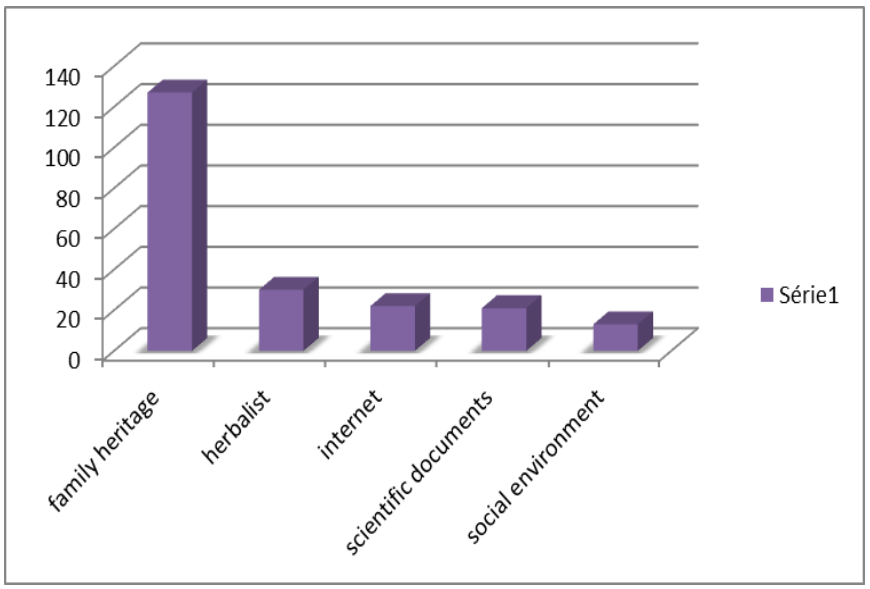


Figure 35: Information source on plant usage.

1.2.6. Reason of plant utilization

The diagram shows the reason for the use of medicinal plants (Figure 36).The plants were favored by the majority of 100 people because it effective for a percentage of 47 % (100 person), inexpensive by 72 people (34%0 and because it signify the most commonly used by 42 people (19%).

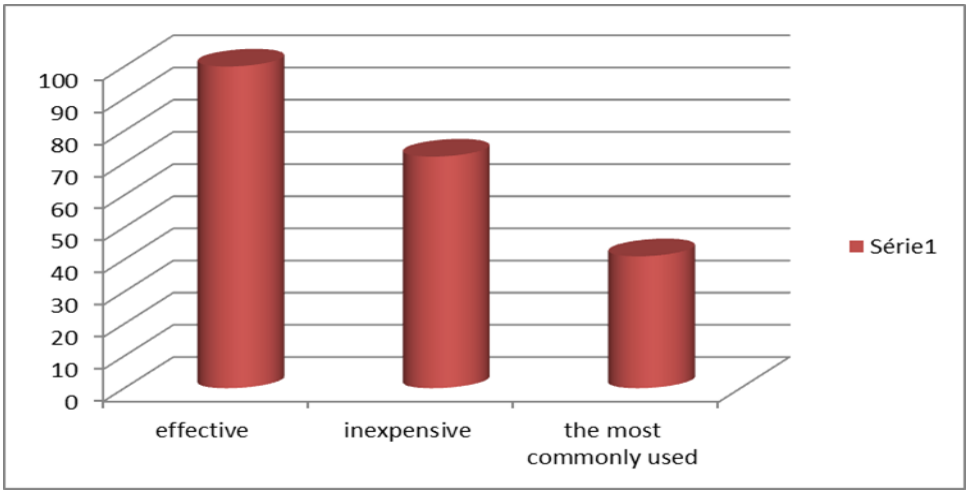


Figure 36: Reason of plant utilization.

1.2.7. Harvest season

The diagram shows the distribution of medicinal plant harvesting seasons (Figure 37). Summer season is the longest season for harvesting medicinal plants, accounting for (31%), followed by spring and length of the year, accounting for (26%) and finely winter with (6%).

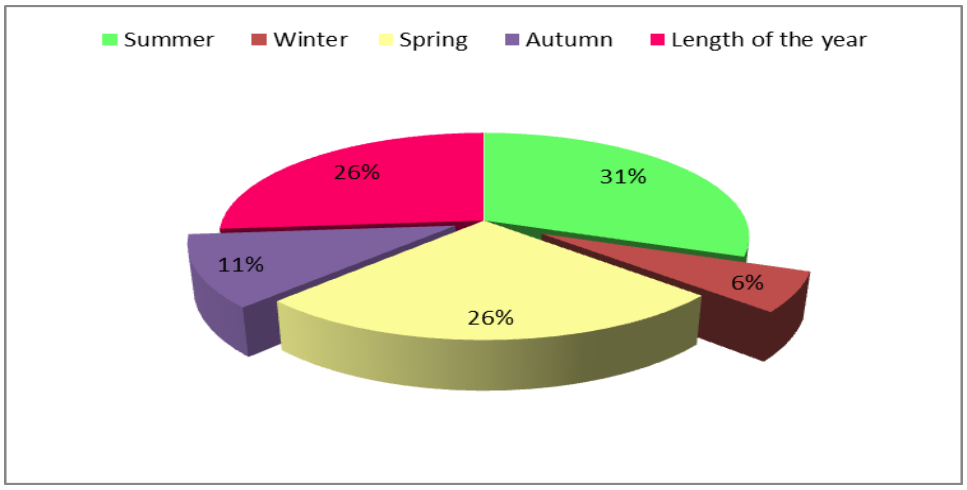


Figure 37: Percentages of harvest seasons of medicinals plants.

1.2.8. Application method

The tisane or dried plants application are the most ways to use medicinal plants, accounting for respective percentages 61 and 21 %. The fresh plants application is the second way to use medicinal plants for treatment, accounting for 11%. After treatment of medicinal plants and oil topical application showed respective percentages, 5 and 2% of use (Figure 38).

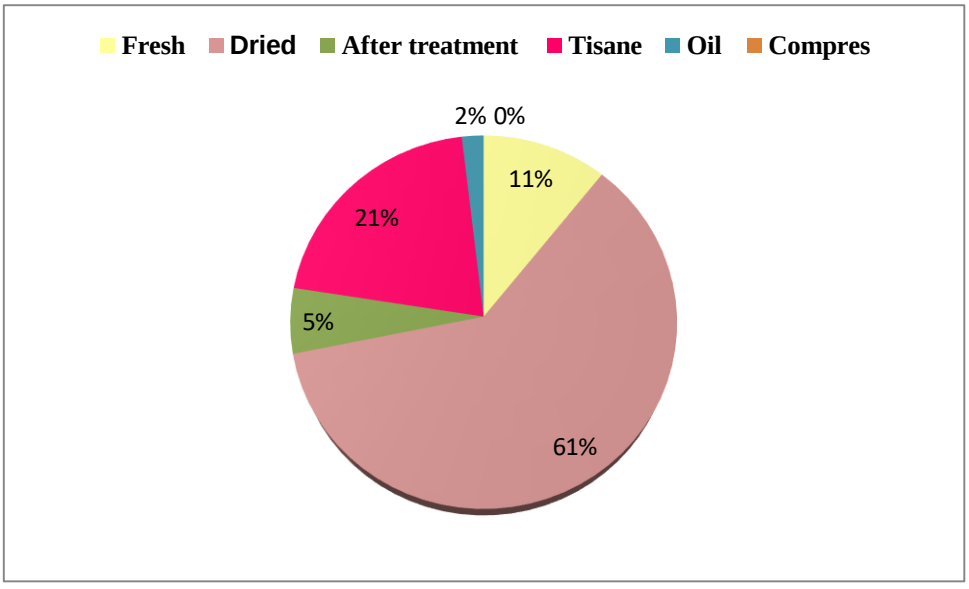


Figure 38: Percentages of application methods of plants medicinal.

1.2.9. Mode of preparation

In order to facilitate the administration of the drug, several methods of preparation are used, namely infusion, decoction, crude, glue, syrop, and oil. Decoction is the most widely method of preparation (46%) used par 97 person, followed by infusion (36%) used by 76 person, glue (16%) used by 34 person, then syrup, oil, crude and paste displayed the very low percentages and applied by 1 to 2 persons (Figure 39).

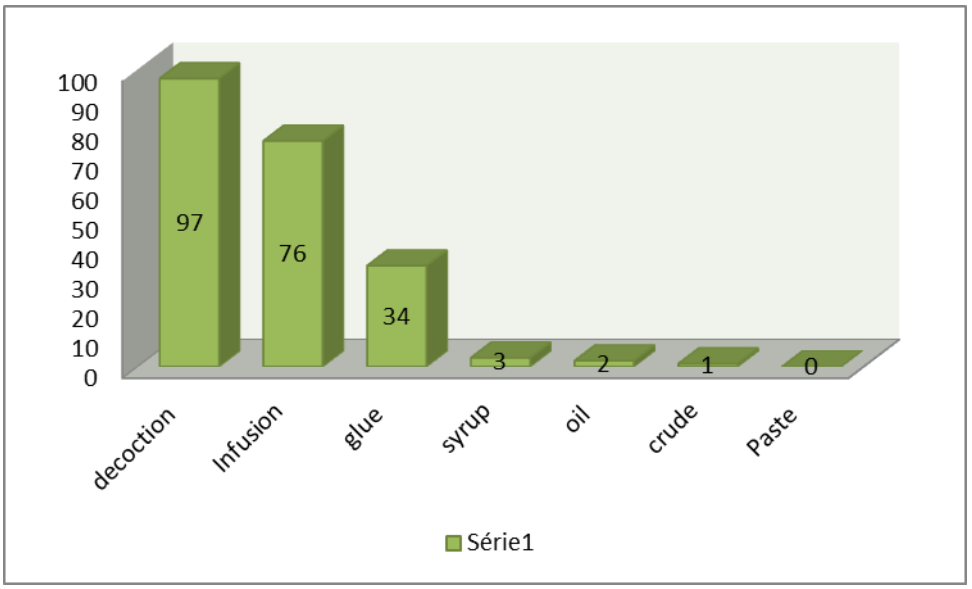


Figure 39: Percentages of preparation mode of plants medicinal.

The ethnobotanical research conducted in many regions of the world has discovered that the majority of informants prepare herbal remedies by boiling and infusing (Bencheikh *et al*., 2021; Chaachouay *et al*., 2023).

This high percentage of decoction indicates that the local population accepts this style of preparation and finds it suitable for warming the body and cleansing the plant. On the other hand, boiling makes it possible to collect the most active ingredients and dilutes or eliminates the toxic effects of certain recipes (Bencheikh *at al*., 2021),

1.2.10. Duration of use

The histogram analysis (Figure 40) on the duration of use of herbal medicines for kidney stones is represented in descending order as follow: More than Weekly by 197 people (66%), until healing by 58 people (19%), Weekly 39 people (13%) and finely daily by 6 people (2%).

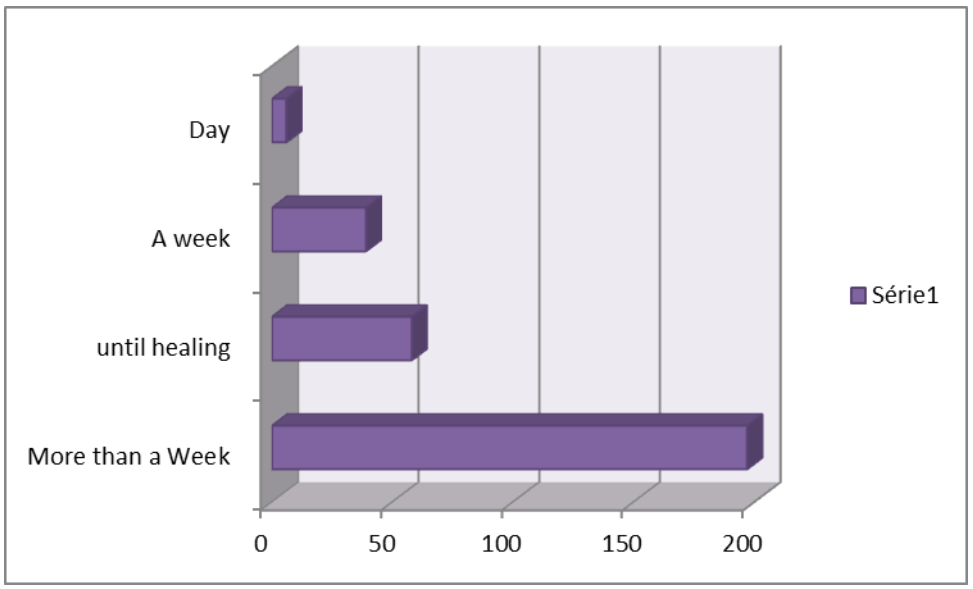


Figure 40: Percentages of duration use of medicinal plants.

1.2.11. Time of use

The diagram (Figure 41) shows that the most common time to use herbal medicine is in the evening (34%), followed by the morning (28%) and all times (20%). There are who say the plant can be used before sleep (18%).

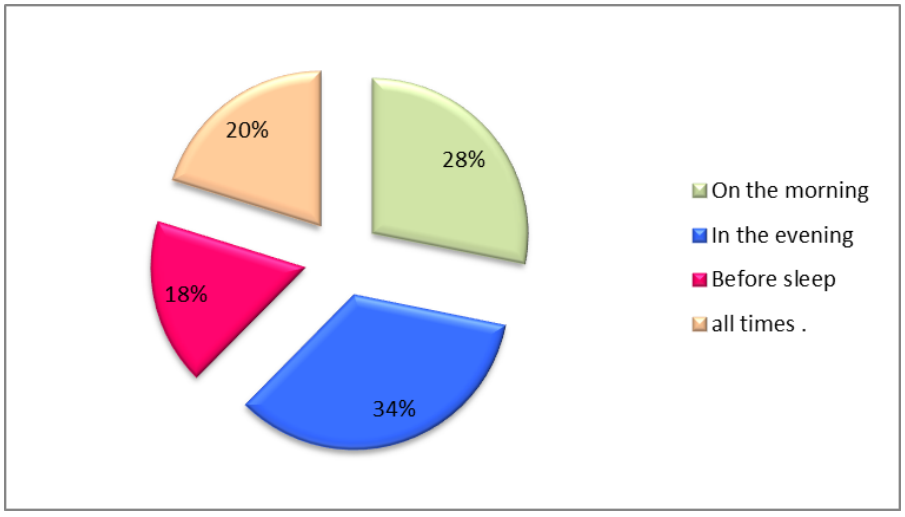


Figure 41: Percentages of use time of medicinal plants.

1.2.12. Dose used

The graph (Figure 42) represent the doses used in treatment by informants. The accurate doses is applied by 73 people (34%) that who presented the lower percentage of dose than inaccurate doses applied by 140 people (66%).

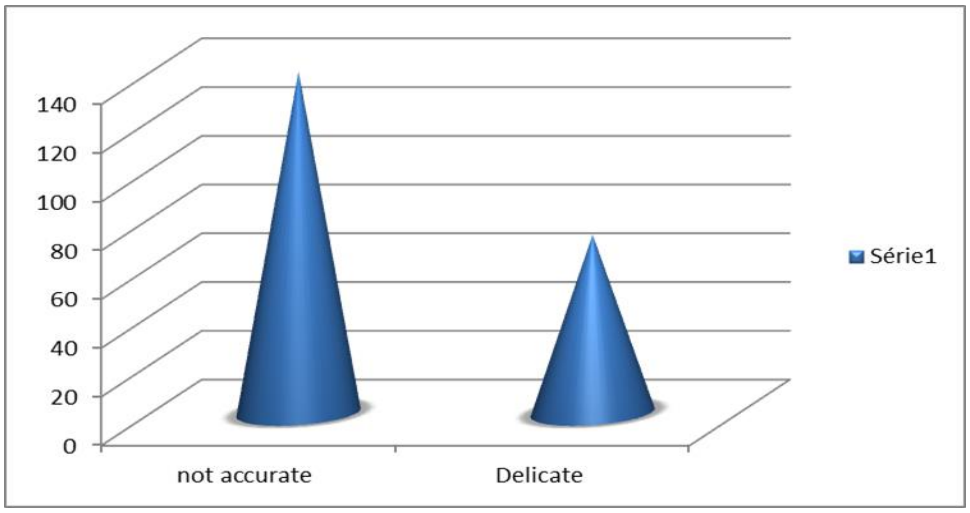


Figure 42: Percentages of dose used of medicinal plants.

1.2.12.1. Accurate dose

The medicinal plants reported are used with precise doses, mentioned by 73 users, including 34% of plants cited used per one cup, 22% per several cups and 19% per one to two cups. The following Table IX represents the percentages of accurate dose applied by some users.

Table IX: Percentages of accurate dose applied by some users.

Accurate dose	Citation number	Percentage (%)
Spatula	6	8%
half a cup	2	3%
One cup	25	34%
One to two cups	14	19%
Two to three cups	10	14%
Several cups	16	22%
Total	73	100%

1.2.13. Toxicity

The effect of the herb is represented in the graph (Figure 43) 142 people (90%) as non-toxic, followed by 12 people (8%) as toxic and three people (2%) as lethal dose.

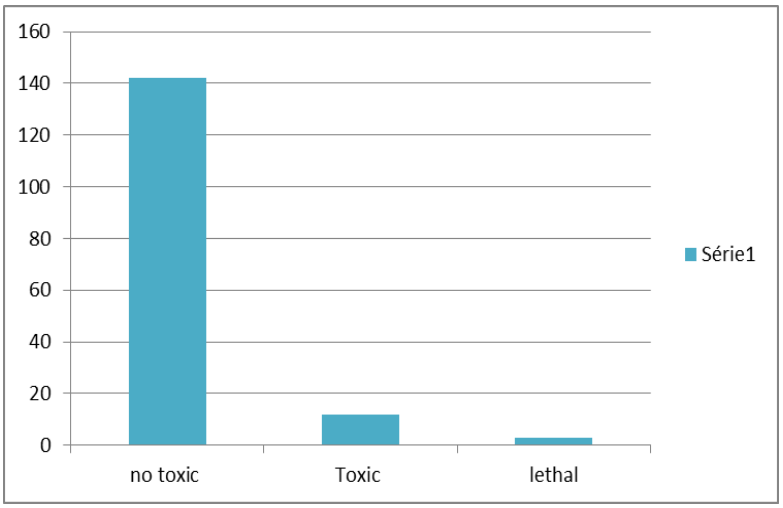


Figure 43: Percentages of toxicity of medicinal plants cited

1.2.14. Therapeutic aim

The relative diagram (Figure 44) represents the largest proportion of people who use medicinal plants for treatment (50%), followed by the proportion of people who use plants for disease prevention (30%), and a small proportion of people who use plants for both treatment and prevention (20%).

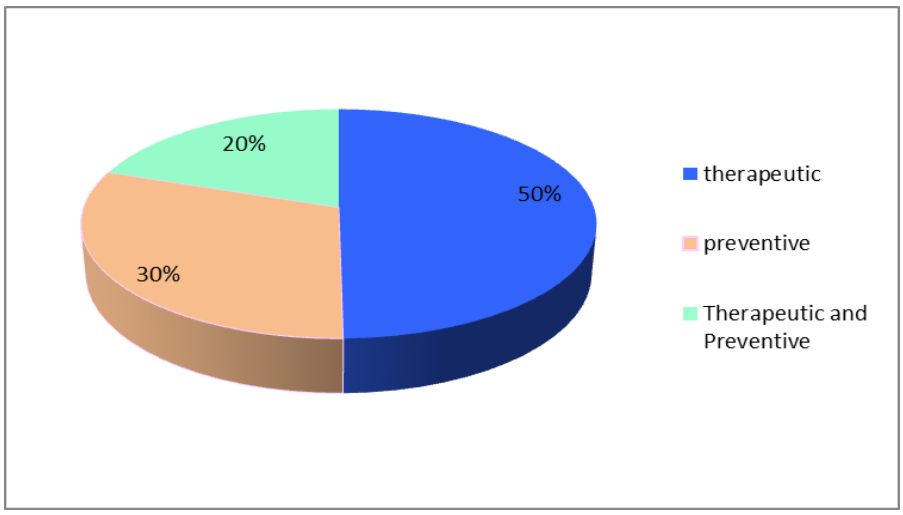


Figure 44: Percentages of therapeutic aim of users.

1.2.15. Treatment results

The highest percentage of complete healing was about 38%, then followed by marked improvement for 31% and slight improved for 29%. The lowest percentage of treatment results was marked by not effective for 2% (Figure 45).

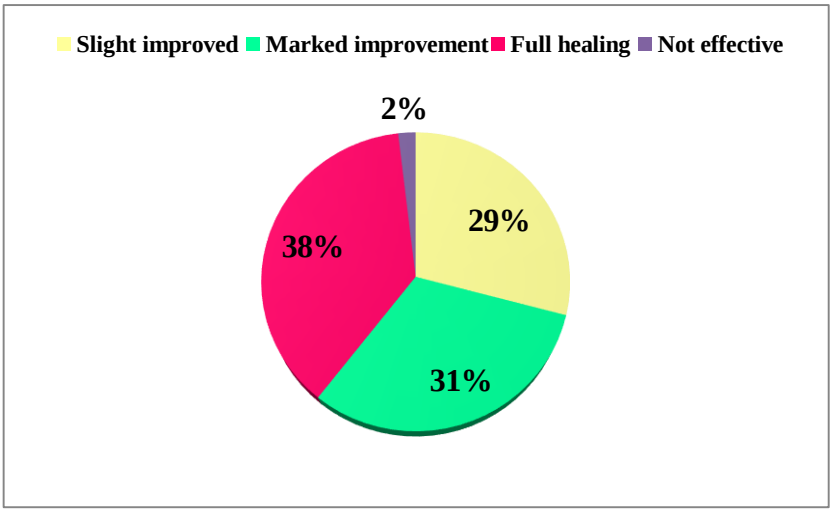


Figure 45: Percentages of different effects of the plants mentioned after their uses.

1.2.16. Additifs

The diagram with three sections labeled "water," "oil," and "honey" is shown in Figure 46, and the respective percentages in descending order are as follow: Water who added by 186 people (58%), honey by 21 people 10% and oil by 6 people (3%).

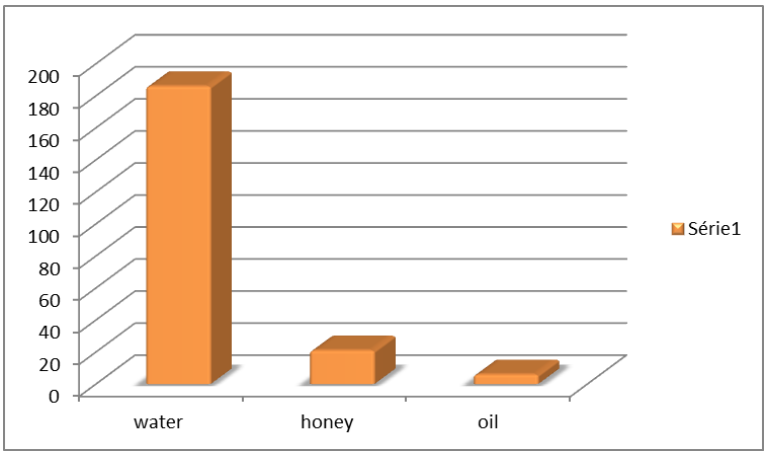


Figure 46: Percentages of additifs added to preparations based on medicinal plants.

1.2.17. Association with others plants

The pie chart (Figure 47) shows the percentage of people who use the plant alone (81%) and the percentage of people who use other plants with it (19%).

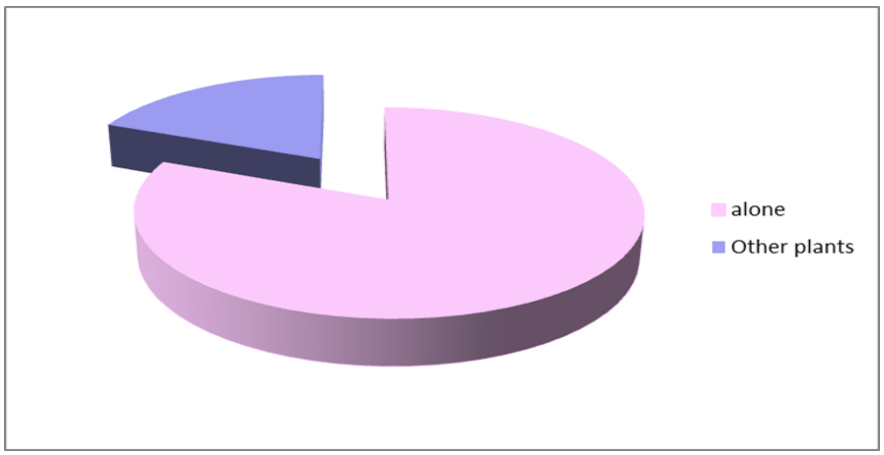


Figure 47: Percentages of the use of the plant alone or in combination.

1.2.18. Precautions for use

The pie chart (Figure 48) shows the presence or absence of precautions for plants use. Thus, the percentage of precautions of use applied by 79% users include:

- The storage the plant in a dry place with low humidity
- Not associated the medicinal plant with other plants whose their effect is not well-known.

Other informants represented by (18%), which says there are no precautions of use and (3%) about the ignorant informants of informations regarding this matter.

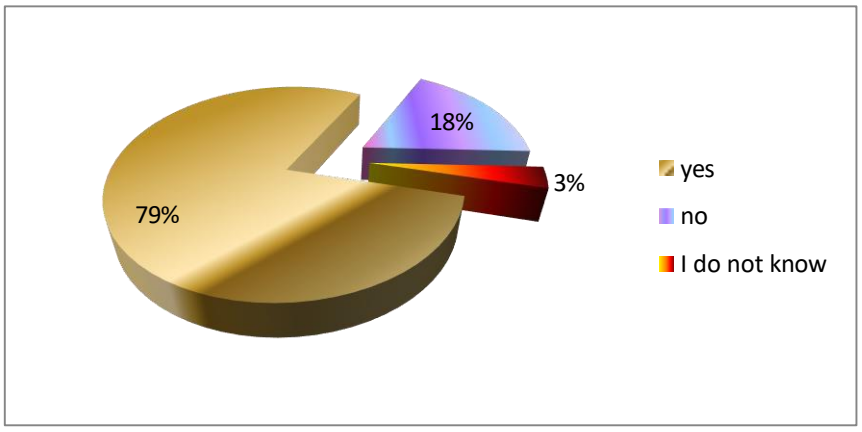


Figure 48: Percentages of presence or absence of precautions use of medicinal plants.

1.2.19. Secondary effects

The chart pie (Figure 49) represents the side effects when using different plants, where the some people response may cause possible allergies for 59 people, with the highest percentage of 28%, followed by diarrhea for 29 people (14%), vomiting for 23 people (11%),

no side effects for 20 people (8%), hypotension 20 people with 8%, blood clots for 18 people (7%), hypoglycemia for 16 people (8%), Sore throat for 11 people (8%), constipation for 9 people (4%), low blood sugar 16 people (5%), excessive urination for 4 people (2%). Finely, the percentages of people who answered (kidney failure, epilepsy, stomach pain, increased liver enzymes) are equal to 1%.

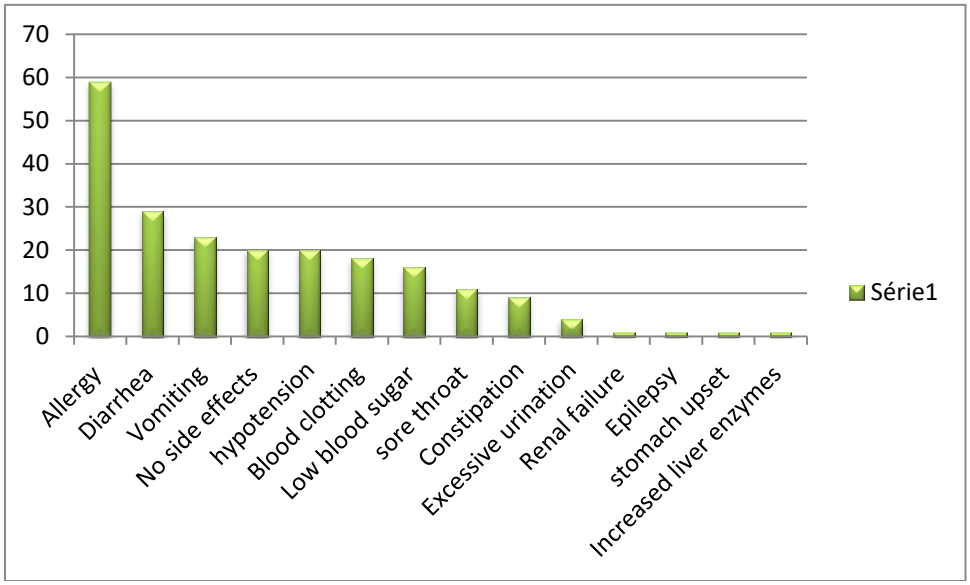


Figure 49: Diagram represents the side effects.

The people interviewed mentioned also certain plants requiring special precautions use to follow given the undesirable effects are summarized in the Table X.

Table X: Precautions that some people use when using medicinal plants.

plants	***Interactions Drugs***	***Interaction plants***	***Side effects***
Artemisia herba alba Asso.	Primidone	*-Solanum lycopersicum* *-Mentha spp*	Epilepsy Pregnancy Breastfeeding
Trigonella foenum-graecum	Warfarin	*Rubus fruticosus*	Blood clotting diseases
Petroselinum crispum	Thiazides	*Hibiscus sabdariffa L*	Hypglycemia

Cichorium intybus	Ciprofloxacin	*Anthemis nobilisL.*	-Pregnancy BreastfeedingChronic liver disease

2. Extraction yield

There are many steps to obtain phytochemicals from plant such as milling, grinding, homogenization, and extraction. Due to the structural diversity and complexity of phenolic compound in plants. Today, a number of different extraction techniques are used (Amari, 2023). In this study, solvent extraction procedures applied to *Herniaria glabra* (leaves) and *Paronychia capitata* (flowers) include maceration, decoction and infusion. In the first one, an extraction with ethanol to obtain ethanolic extract. In decoction and infusion the water is used as an extractant. The yield of each extract is shown in Table XI.

Table XI: Extraction yield of *H.glabra*.L and *P.capitata*.L extracts.

Plants	**Extracts**	**Extraction yield (%)**
P.capitata	DecE	5.60
	InfE	3.87
	MacE	3.25
H.glabra	DecE	15.72
	InfE	9
	MacE	4.30

DecE: Decoction extracts; **InfE**: Infusion extract; **MacE**, Maceration extract.

According to the results of Table XI, the variability of extraction yield depends on the plant part used and extraction methods. The highest yield was noted with leaves part of *H. glabra.* L with range from 4.3% to 15.72%. Although, the flower extract of *P. capitata.* L displayed a lower yield with range from 3.25% to 5.6%. Auxiliary, it was observed that the decoction extract of *P.capitata* and *H.glabra* produced an almost maximum yield of phytochemicals about 15.72% and 5.6%, respectively. Their yields decreased in the following

order decoction>infusion> maceration extract which ranged about 3.87% and 9% for infusion. The maceration extract of leaves and flowers displayed the lower yields which range about 3.25%.

The extraction yield of ethanolic of leaves (*H. glabra*), 4.3% is much less to those obtained by Kenza *et al*. (2018) with percentage of 31.2% of *Herniaria hirsuta* extract. In addition, the extraction yield of decoction of leaves of 15.72% is less than those reported by Kenza *et al*. (2018) with a percentage of 28.73% of *Herniaria hirsuta*. Furthermore the extraction yield of ethanolic flowers of *P.capitata* (3.25%) is lower than those reported by Omar. (2021) with a percentage of 13.83% who work on ethanolic extract of flowers of *Parenchia argentea*. The variability of extraction yield depends on the plant species, parts used as well as, extraction processes and solvent used. Do *et al*. (2014).

2. Determination of total polyphenols and flavonoids

Quantitative tests were performed to determine the concentration of the phytochemicals in the extracts. Polyphenols and flavonoids were quantified using spectrometric methods. The total phenolic contents (TPC) and total flavonoids contents (TFC) of decoction infusion and maceration extracts of leaves of *H.glabra.* L and flowers of *P.capitata.*L were evaluated employing the Folin-Ciocalteu and aluminum chloride reagents respectively. Results of TPC and TFC are shown in Figure 50.

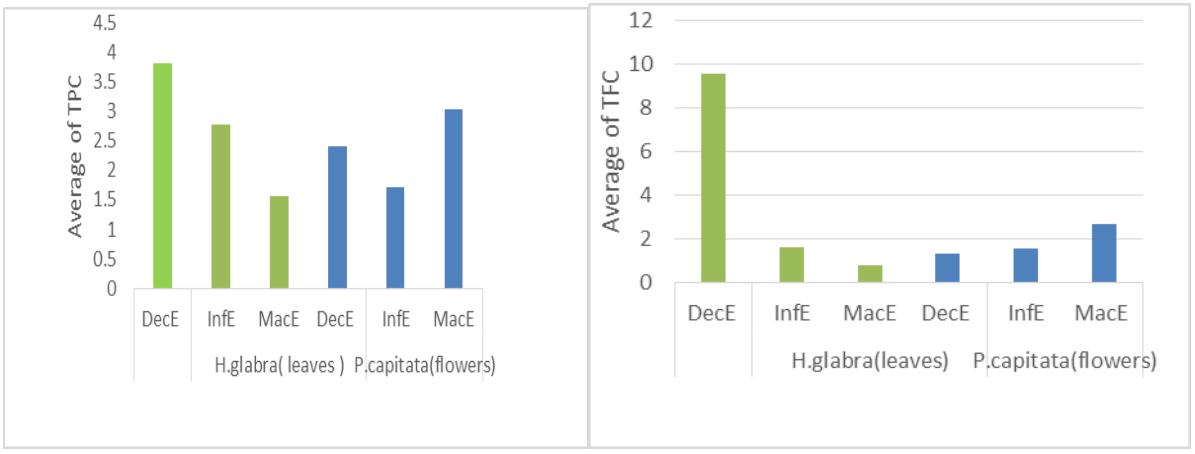


Figure 50: TPC and TFC of *H.glabra*. L and *P.capitata.* L.

DecE: Decoction extracts; **InfE**: Infusion extract; **MacE**, Maceration extract. Each value is represented by the average of three repetitions ± SD.

The largest amount of phenolic was obtained via leaves extracts, followed by flowers extracts (FE), with the exception in Mac E. Firstly, in DecE and InfE extracts the highest level

of polyphenols compounds were recorded in LE followed by FE. Secondly for the MacE, great values were shown in FE then LE. The TPC ranged from 1.57 mg GAE/gE for Mac extract of leaves to 3.82mg GAE/gE for DecE extract of leaves. The TPC decrease in the following order: DecE>InfE>MacE for LE and MacE>DecE>InfE for FE.

Furthermore, the content of flavonoids varying from 0.7 for MacE of leaves to 3.21 for DecE of leaves. The largest amount of flavonoids was obtained via LE followed by FE with the exception in MacE. In DecE and InfE extracts the highest level of flavonoids were recorded in LE followed by FE. Secondly for the MacE, great values were shown in FE then LE. The TFC decrease in the following order: DecE>InfE>MacE for LE and MacE>InfE>DecE for FE.

Furthermore, the decoction *P.capitata* flowers extract, which present the concentration of total phenolic compounds, amounting to 2.42 ± 0.29 (mg GAE/g dry extract), is lower than that previously reported in Allaoua. (2016) with value of 10.19 ± 2.24 mg GAE/g) in decoction extract. But our result is highest than those reported by Bouzidi. (2018) which is of the order of 1.0 µg. Also the comparaison of our results of TPC of ethanolic flowers extract with those of literature shows that our TFC amounting 3.03± 0.13 mg GAE/g are near similar to that obtained by Omar *et al*.(2021) Who work on *Paronychia argentea* flowers extract amount 5.92 ± 0.14 mg GAE/g. Several factors could influence the content of phenolic compounds, recent studies have shown extrinsic factors (such as geographic and climatic factors) genetic factors but also the degrees of maturation of the plant and the duration of storage have a strong influence on the polyphenols content (Omar *at al*., 2021).

The flavonoid content of *P.capitata* flowers extract is 1.3 ± 0.3 mg QE/g, these results are lower than 6.07 ± 0.44 mg QE/g those reported by Allaoua. (2016). In addition; these results are similar to those reported by Bouzidi. (2018). Which is 1,000 mg QE/g the flavonids content of ethanolic flowers extract (2.67 ± 1.91 mg GAE/g. are highest than those reported by Omar *et al*.(2021) Who work with *Paronychia argentea* flowers extract wiith value of 0.47± 0.0035 mg GAE/g. The differences in flavonoid content may be due to the growing conditions of the plant such as soil, geographic location, and environmental conditions during organ development, degree of maturity, harvest and genetic differences (Pawlowska *et al.*, 2010).

3. Antiurolithic activities

This present study involved both CaOx crystal nucleation and agglomeration, as two important processes in the urinary tract for crystal retention (Ly *et al*., 2021). The results shown in Tables XII, XIII and XIV. Given indication for different methods extracts from *H.glabra* and *P.capitata* at doses of 62.5–1000 µg/mL, which have significant tremendous anti-urolithiasis effects for CaOx formation in vitro tests. In which, at highest concentration of 1000 µg/mL, the decoction leaf extract had the larger percentage of inhibition against calcium oxalate nucleation compared to other extracts, with value of 97.81% ,which was higher than allopurinol at value of 93.56 %.Thus, the decoction flower extract, had the best potency on inhibitory activity with value of 99.65 against crystals aggregation compared to other extracts, while positive control, allopurinol was nearly similar to decoction flower extract presented percentage of inhibition at 96.43%. The results of ethanolic extract of flowers (*Paronychia Capita*) are superior to those obtained by Omar *et al*. (2021), who work on the ethanolic extract of flowers of *Paronychia argentea*, the concentration of 0.5 mg/mL gave 52.78% of inhibition.

Table XII: Antiurolithiatic effect of *H.glabra.L* leaves extracts evaluated by nucleation and aggregation assays.

Extracts	Dose (µg/mL)	Nucleation	Aggregation
	1000	97.81 ± 0.22	95.23 ± 1.6
	500	97.5 ± 0.87	96.26 ± 2.3
DecE	250	98.65 ± 0.35	86.09 ± 0.9
	125	95.4 ± 3.25	72.87 ± 3.4
	62.5	75.8 ± 6.25	76.26 ± 1.2
	1000	92.98 ± 0.16	98.16 ± 0.16
	500	89.65 ± 2.11	95.86 ± 5.2
InfE	250	92.98 ± 0.97	92.18 ± 1.7

	125	91.83 ± 6.5	87.47 ± 9.5
	62.5	92.75 ± 3.75	90.97 ± 2.1
	1000	93.5 ± 3.33	97.98 ± 0.4
	500	93.18 ± 3.03	95.63 ± 3.5
MacE	250	90.11 ± 2.9	97.64 ± 3.1
	125	87.35 ± 2.27	89.77 ± 0.6
	62.5	82.41 ± 0.98	84.48 ± 0.8

DecE: Decoction extracts; **InfE**: Infusion extract; **MacE**, Maceration extract. Each value is represented by the average of three repetitions ± SD.

Table XIII : Antiurolithiatic effect of *P.capitata* leaves extracts evaluated by nucleation and aggregation assays

Extracts	**Dose (µg/mL)**	**Nucleation**	**Aggregation**
	1000	95 ± 1.21	99.65 ± 0.32
	500	92.26 ± 1.99	74.13 ± 13
DecE	250	81.43 ± 0.4	92.81 ± 0.4
	125	85.91± 6.42	93.79 ±1.46
	62.5	66.49 ± 3.65	95.36 ±1.83
	1000	88.1 ± 3.33	98.79 ±1.21
	500	90.51 ± 5.6	94.94 ± 6.98
InfE	250	91.32 ± 1.38	94.94 ± 6.33
	125	87.47 ± 0.52	95.86 ±1.46
	62.5	80.17 ± 1.38	89.82 ± 8.69

	1000	71.55 ± 4.3	99.13 ± 0.4
	500	84.77 ± 0.24	93.62 ± 3.5
MacE	250	87.35 ± 2.27	91.2 ± 7.07
	125	88.85 ± 0.97	88.16±13.49
	62.5	72.18 ± 6.82	85.34±17.96

Table XIV: Antiurolithiatic effect evaluated by nucleation and aggregation assays of Allopurinol. Each value is represented by the average of three repetitions ± SD.

Dose (µg/mL)	**Nucleation**	**Aggregation**
1000	93.56±1.78	96.43±1.3
500	92.29±1	89.94±3.16
250	87.87±0.89	92.98±2.6
125	88.44±3.49	92.75±9.1
62.5	83.90±1.3	94.31±0.4

Conclusion

Conclusion

Medicinal plants are inseparable from local livelihoods because they have long been collected, consumed, and managed through local customs and knowledge. Management of traditional therapies is urged, because the therapies are empirically and knowledge based, often culturally inherited and important to pharmacology and local livelihoods. *Herniaria glabra*. L and *Paronychia capitata*. L are two plants used in the traditional therapies and they are widely distributed in Algeria. The local Algerian population for their kidney stone diseases mainly uses their aerial parts.

In the first point, an ethno medicinal data revealed that both *H. glabra*. L and *P. capitata.* L plant was the major plants used in the ethnobotanical practice in the treatment of urolithiasis. Additionally, the extraction of phenolic compounds was carried out by different methods and their yield of extraction could be influenced by several factors include the method of extraction, the part used and the nature of extracted phytochemical compounds.

H.glabra leaves and *P.capitata* flowers contains various compounds such as polyphenols and flavonoids. All the tested extracts of leaves and flowers showed an appreciable total content of phenolic compounds. The highest total phenolic contents was present in the decoction extract of leaves with an average of 3.82 µg GAE/mg of E. While, the highest flavonoids content was present in the decoction leaves with an average of 9.56 µg QE/mg of E.

Finally, finding from antiurolithiatic activity nucleation and aggregation assays showed that the decoction leaves extract had the larger percentage of inhibition against calcium oxalate nucleation compared to other extracts, with value of 98.65% and the decoction flowers extract, had the best potency on inhibitory activity with percentage of 99.65% against crystals aggregation compared to other extracts.

The findings from this investigation suggest that leaves and flowers extracts of the two plants could be used as prominent sources of natural anti-urolithiatic agents. In addition, our findings provided the utilization of *Herniaria glabra*. L and *paronychia capitata*. L (leaves, flowers) in traditional medicine as a potential source of beneficial bioactive compounds for the development of new drugs against kidney stones. However, further studies are required to explore the mechanisms of action.

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Annexes

استبيان حول التداوي بالأعشاب لمرض حصى الكلى

1) الجنس

☐ ذكر ☐ انثى

2) العمر

☐ 18_20 ☐ 20_30 ☐ 30_40 ☐ 40_60 ☐ 60_80

3) المسكن (البلدية)

.....

4) مستوى التعليمي

☐ بدون مستوى ☐ ابتدائي ☐ متوسط ☐ ثانوي
☐ جامعي

5) النشاط المهني

.....

6) هل أصبت من قبل بمرض حصى الكلى؟

☐ نعم ☐ لا

إذا نعم ما هو العلاج الذي أخذته؟

.....

7) هل سبق لك وأن أخذت علاج تقليدي ضد مرض حصى الكلى؟

☐ نعم ☐ لا

8) هل تعرف النباتات الطبية المستعملة في علاج حصى الكلى؟

☐ نعم ☐ لا

9) الاسم العامي للعشبة

.....

10) التوزيع البيئي

☐ تلقائية ☐ مزروعة ☐ محلية ☐ مستوردة

11) الجزء المستعمل

☐ الأوراق ☐ الساق ☐ الجذور ☐ الازهار ☐ بذور ☐ ثمار ☐ الجزء الهوائي ☐ النبتة بأكملها ☐ الجذمور ☐ اللحاء

أخرى: .....

12) مصدر المعلومات

☐ وثائق علمية ☐ المحيط الاجتماعي ☐ العطار ☐ انترنت ☐ ارث عائلي

أخرى: .....

13) سبب استعمال العشبة

☐ رخيصة الثمن ☐ فعالة ☐ الاكثر استعمالا

أسباب أخرى: .....

14) موسم الحصاد

☐ الصيف ☐ الخريف ☐ الشتاء ☐ الربيع ☐ طول السنة

15) طريقة الاستعمال

☐ طازجة ☐ جافة ☐ بعد المعالجة ☐ تيزانة ☐ زيت ☐ كمادة ☐ بخار

طريقة أخرى: .....

16) طريقة التحضير

☐ مغلى ☐ النقع ☐ شراب ☐ زيت ☐ صمغ ☐ نيء ☐ عجينة

طريقة أخرى: .....

17) مدة الاستعمال

☐ يوم ☐ أسبوع ☐ أكثر من أسبوع ☐ حتى الشفاء

إجابة أخرى: .....

18) كمية الجرعات

☐ دقيق ☐ غير دقيق

إذا كانت الجرعة دقيقة او غير دقيقة حددها

.....

19) تأثير العشبة

☐ سمية ☐ غير سمية ☐ مميتة

20) هدف العلاج

☐ علاجية ☐ وقائية ☐ علاجية ووقائية

21) هل استعمالك لهذه النبتة ادى الى

☐ تحسن خفيف ☐ تحسن ملحوظ ☐ شفاء تام ☐ غير فعالة

إجابة أخرى: .....

22) هل استعملت إضافات أو مواد اخرى ممزوجة مع النبتة

☐ ماء ☐ زيت ☐ عسل

مواد أخرى: .....

23) هل يستخدم النبات بمفرده أم مع نباتات أخرى؟

☐ لوحده ☐ نباتات اخرى

24) هل هناك احتياطات استعمال النبتة

☐ نعم ☐ لا

إذا كانت نعم حددها:

.....

25) أوقات الاستعمال

☐ عند الصباح ☐ عند المساء ☐ قبل النوم

أوقات أخرى: .....

26) الاثار الجانبية

☐ حساسية ☐ الاسهال ☐ الإمساك ☐ انخفاض ضغط الدم ☐ القيء ☐ التهاب الحلق

آثار جانبية أخرى: .....