Mémoire

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Therapeutic innovation in the treatment of skin diseases: from phytotherapy to nanotechnology

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ABSTRACT:

This study presents a novel microwave-assisted method for the synthesis of silver nanoparticles (AgNPs) using an endemic plant species from the northern Sahara of Algeria: *Euphorbia guyoniana*. The plant, known for its high content of bioactive compounds, was used as both reducing agent and stabilizing agent in the synthesis process. The effects of microwave power, irradiation time, extract concentration and silver nitrate concentration on the formation and properties of AgNPs were investigated and optimized with the use of response surface methodology (RSM). The synthesized AgNPs were characterized using UV-Vis spectroscopy, zeta potential (ZP) and dynamic light scattering (DLS). The microwave-assisted synthesis method demonstrated several advantages, including rapid synthesis, uniform particle size distribution, and enhanced yield compared to conventional methods. The results of the statistical analyses carried out attest to the accuracy of the mathematical models proposed by the RSM and allow us to affirm that they describe well the behavior of the studied system.

RESUME :

Ce travail a pour objectif la mise au point d'une nouvelle méthode de synthèse de nanoparticules d'argent (AgNPs) assistée par micro-ondes en utilisant une espèce endémique du Sahara septentrional d'Algérie : *Euphorbia guyoniana*. La plante, connu pour sa forte teneur en composés bioactifs, a été utilisé à la fois comme agent réducteur et agent stabilisant dans le processus de synthèse. Les effets de la puissance du micro-ondes, le temps d'irradiation, la concentration de l'extrait et la concentration du nitrate d'argent sur la formation et les propriétés des AgNPs ont été étudiés et optimisés en utilisant la méthodologie de surface de réponse (RSM). Les AgNPs synthétisées ont été caractérisées en utilisant la spectroscopie UV-Vis, le potentiel zeta (ZP) et la diffusion dynamique de la lumière (DLS). La méthode de synthèse assistée par micro-ondes a présenté plusieurs avantages, notamment une synthèse rapide, une distribution uniforme de la taille des particules et un rendement amélioré par rapport aux méthodes conventionnelles. Les résultats des analyses statistiques réalisées attestent de la justesse des modèles mathématiques proposés par la RSM et permettent d'affirmer que ces derniers décrivent bien le comportement du système étudié.

يهدف هذا العمل إلى تطوير طريقة جديدة لتركيب الجسيمات النانوية الفضية (AgNPs) بمساعدة الموجات الدقيقة التي تستخدم نوعًا مستوطنًا في شمال الصحراء الجزائرية: Euphorbia guyoniana. تم استخدام النبات ، المعروف بمحتواه العالي من المركبات النشطة بيولوجيًا ، كعامل اختزال وعامل استقرار في عملية التوليف. تم دراسة تأثير قوة الميكروويف ووقت التشعيع وتركيز المستخلص وتركيز نترات الفضة على تكوين وخصائص AgNPs باستخدام منهجية السطح للاستجابة (RSM). تم تمييز AgNPs المُصنَّع باستخدام التحليل الطيفي للأشعة المرئية وفوق البنفسجية ، وإمكانات زيتا (ZP) ، وتشتت الضوء الديناميكي (DLS). أظهرت طريقة التوليف بمساعدة الميكروويف العديد من المزايا ، بما في ذلك التركيب السريع ، وتوزيع حجم الجسيمات المنتظم ، وتحسين العائد مقارنة بالطرق التقليدية. نتائج التحليلات الإحصائية التي تم إجراؤها تشهد على دقة النماذج الرياضية المقترحة من قبل RSM وتجعل من الممكن التأكيد على أن هذا الأخير يصف سلوك النظام المدروس.

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Lastly, but certainly, not least, we would like to take this opportunity to extend our gratitude to all the teachers and everyone who has contributed to our university education, especially those from the Department of Process Engineering.

Dedication

I wholeheartedly dedicate this thesis to my dear parents, Mom and Dad, you have been the foundation upon which my aspirations have been built. Your unconditional love, sacrifices, and tireless efforts have shaped me into the person I am today. Your unwavering encouragement and endless support have been the driving force behind my accomplishments. I am forever grateful for your guidance and the values you instilled in me.

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Lastly, I dedicate this thesis to myself, as a testament to my perseverance, dedication, and self-belief. This work represents the countless hours of research, analysis, and hard work that I have poured into it.

May this dedication serve as an expression of my heartfelt gratitude, love, and appreciation for all those who have played a significant role in shaping my academic and personal life.

RIHAB

Dedication

I dedicate this thesis to my incredible parents, whose love, support, and sacrifices have shaped me into the person I am today. Your belief in me and encouragement have fueled my academic pursuits. This achievement is a reflection of your unwavering dedication and values.

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I am grateful to my friends who have accompanied me on this academic journey, enriching my research and providing a sense of community.

Lastly, I dedicate this thesis to all individuals who thirst for knowledge and have a passion for discovery.
May this work inspire meaningful contributions to our respective fields.

MALLAK

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List of Abbreviations

%	Percent
Adj-R^2	Adjusted correlation coefficient
API	Active Pharmaceutical Ingredient
DNA	Deoxyribonucleic acid
DLS	Dynamic light scattering
FCCD	Face-centered composite experimental design
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
LC/MS	Liquid chromatography/mass spectroscopy
mM	Mili-molar
Q^2	Prediction coefficient
R^2	Correlation coefficient
ROS	Reactive oxygen species
RSM	Response surface methodology
RSD	Relative standard deviation
s	seconds
SEM	Scanning electron microscopy
SNEDDS	Self-nanoemulsifying drug delivery system
TEM	Transmission electron microscopy
UV	Ultraviolet
UVA	Ultraviolet Radiation A
UVB	Ultraviolet Radiation B
UV-Vis	Ultraviolet-visible
W	Watt
W/W	Weight/Weight
WHO	World Health Organization
XRD	X-ray diffraction
ZP	Zeta potential

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References

General Introduction

According to the World Health Organization (WHO), 80% of the world's population is primarily dependent on indigenous medicine and about 33% of all traditional therapies are prescribed for the treatment of dermatological disorders (Robinson & Zhang, 2011). The search for natural remedies for skin disorders has received a great deal of interest from the scientific community in recent years. Such infatuation lead to the marketing of several modern drugs and medicines originated from ethnic herbal medicine for the treatment of skin diseases.

Nanoparticles hold a significant place in scientific research due to their unique and diverse properties i.e. optical, magnetic, electronic, and catalytic properties and thus have the potential to be harnessed for practical applications. Nanoparticles are commonly used in development of pharmaceutical formulations for topical, ocular, and intravenous administrations; moreover, they have been proved as excellent drug vehicle systems for drug local targeting through skin becoming of vital importance in skin disorders and skin cancer (Mazayen et al., 2022).

In the framework of the present study, we focused on the utilization of *Euphorbia Guynoniana* plant species, which belongs the Euphorbiaceae family and is acknowledged for its endemic nature, being primarily found in Algeria. The unique ecological characteristics of this plant prompted our exploration into its properties and potential benefits. Also, *Euphorbia Guynoniana* has a rich history of use in the traditional medicine of the Maghreb countries. Indeed, throughout the years, local communities in Algeria have harnessed the versatile properties of this plant to address various health conditions including digestive disorders, skin diseases, and respiratory ailments, among others. However, despite its use in traditional medicine, to the best of our knowledge, there is a very limited amount of scientific research regarding the medicinal potential of *Euphorbia Guynoniana*. This scarcity of information further piqued our interest and motivated us to delve deeper into the plant's properties.

The presented research work is divided into three sections:

The first section is devoted to a bibliographical study that brings together essential preliminary notions concerning skin diseases, phytotherapy and the antioxidant, anti-inflammatory and antibacterial properties of plants' secondary metabolites that indicate that they deserve recognition as potent effective compounds in treatment of various skin problems. A general introduction to pharmaceutical nanoparticles is presented at the end of this chapter.

The second section presents the material and methods used for the extraction of bioactive compounds from *Euphorbia Guynoniana*, the synthesis of silver nanoparticles and their analysis. We first briefly present the method used for the extraction assisted by microwave from *Euphorbia Guynoniana*. Secondly, we describe the synthesis of silver nanoparticles with the application of experimental designs, followed by their quantification and characterization. Finally, we present an LC-MS analysis of the extracted secondary metabolites of *Euphorbia Guyoniana* for the identification of phenolic compounds present in the plant.

The third and final part presents and discusses the results obtained in the different experiments.

After summarizing the main results obtained, our general conclusion aims to open up new research perspectives.

Chapter 1 – Skin diseases

The skin is the largest organ in our body and comprises about 15% of the body weight. It's the primary barrier against the external environment and is continuously subject to a constant battle against mechanical impacts, dehydration, and infections (Walker, 2022). As a vital organ, skin disorders are a major health problem affecting people of all ages over the world (Karimkhani et al., 2017). Although most of the dermatological conditions do not result in death, they lead to incapacitations with quality of life patients compromised in different ways including agony from social stigma and low self-esteem.

I.Anatomy and physiology of the skin

I.1 Structure of the skin

The skin is composed of three layers: epidermis, dermis, and subcutaneous tissue (Kolarsick et al., 2011).

The epidermis, the outermost layer is directly contiguous with the environment and has a protective function(Program, 1937),the dermis is the middle layer, composed of collagen, it is the tough and resilient part of the skin and finally the subcutaneous layer is the innermost which serves primarily as a reserve energy supply (fat).

The detailed structure of the skin is presented in **Figure 1**.
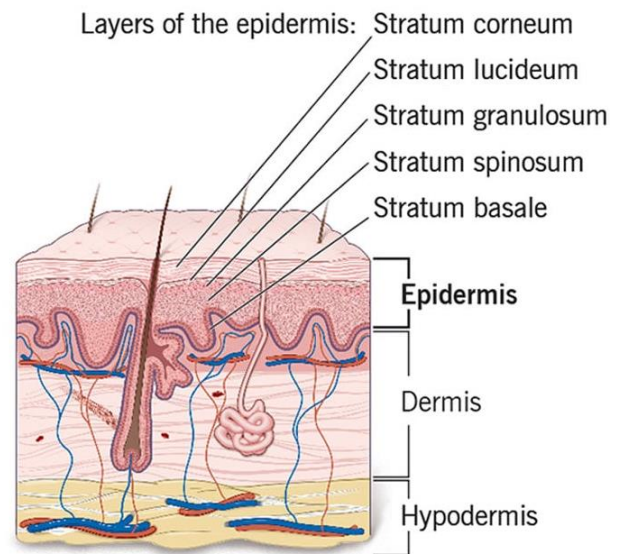
Figure 1 : Cross section of the epidermis showing all five layers together with the cells

❖ **Epidermis** (Kolarsick et al., 2011): The epidermis is a stratified, squamous epithelium layer that is composed mainly of two types of cells, keratinocytes and dendritic cells. The epidermis is commonly divided into five layers according to keratinocyte morphology and position including the stratum basale (the deepest portion of the epidermis), the stratum spinosum, the stratum granulosum, the stratum lucidum, and the stratum corneum (the most superficial portion of the epidermis). The epidermis is a continually renewing layer and gives rise to derivative structures, such as pilo-sebaceous apparatuses, nails, and sweat glands.

- **The stratum basale** contains column-shaped keratinocytes (skin cells) that attach to the basement membrane zone with their long axis perpendicular to the dermis. Keratinocyte stem cells produce the protein keratin. Also, the stratum basale contains melanocytes which are responsible for producing melanin, which provides the pigment of the epidermis. The basal layer is the primary location of mitotically active cells in the epidermis that give rise to cells of the outer epidermal layers (Bergfelt, 2009).
- **The stratum spinosum** is overlying the basal cell layer and is composed of a variety of cells that differ in shape, structure, and subcellular properties. The most abundant

cells are keratinocytes which held together by sticky proteins called desmosomes. The stratum spinosum helps make the skin flexible and strong.

- **The stratum granulosum** contains keratinocytes which are flatter and more irregular in shape and have granules within them. These granules contain lipids, which along with the desmosomal connections form lipid-rich waterproof lamellar bodies that will help to prevent fluid loss from the body. This layer is the one visible under a microscope.

- **The stratum lucidum** is the thinnest layer of the epidermis and is translucent. It is composed of three to five layers of dead and flattened keratinocytes filled with eleidin, an intermediate form of keratin. It has a protective role by reducing frictions between the outer layers stratum corneum and stratum granulosum, which helps to protect the skin in areas most common to damage, such as the palms of the hands and the side of the fingers.

- **The stratum corneum** is the most superficial layer with mainly corneocytes (dead keratinocyte cells) that shed periodically and are progressively replaced by cells formed from the basal layer. The stratum corneum is very thick with fifteen to thirty layers of cells and serves as the primary barrier against environment i.e. abrasions, light, heat and pathogens. This layer entails also fats that keep water from entering or leaving the body.

❖ **Dermis** (Gaboriau & Murakami, 2001): The dermis represents the inner layer of skin between the epidermis and subcutaneous fat. It consists of two layers, a thin superficial papillary dermis and a thicker reticular dermis that lies deeper. The dermis houses the sweat glands, hair follicles, muscles, sensory neurons, and blood vessels. Collagen, elastic tissue, supports the dermis and are greatly responsible of the mechanical strength and extensibility of the skin. However, the skin collagen decreases by 1 % per year throughout adulthood.

- **The papillary dermis** is the top layer of the dermis and is mainly composed of collagen fibers, fibroblast cells, fat cells, blood vessels that give nutrients to the epidermis to produce skin cells i.e. keratinocytes, touch receptors and phagocytes. The papillary layer forms irregular projections called **dermal papillae, they are** extensions of the dermis into the epidermis which form people's fingerprints.

- **The reticular dermis** is the bottom layer of the dermis and it contains blood vessels, sweat and sebaceous glands, hair follicles, lymphatics, nerves and fat cells. A net-like structure of elastin fibers and collagen fibers surrounds the reticular dermis. These fibers support the skin's overall structure, strengthen the skin and also helps to preserve the tensile elasticity of the skin.

❖ **Hypodermis** (Gilaberte et al., 2016, Kolarsick et al., 2011): The subcutaneous layer is the deepest layer of the skin and serves to connect the skin to the underlying fascia of the bones and muscles. The hypodermis serves as a reserve energy supply, protects the skin, and allows mobility by sliding over underlying structures. The hypodermis is mostly formed by adipocytes, indeed, most of the body's fat is stored in this layer.

I.2 Functions of the skin

The skin covers the body's entire external surface, has many vital functions in the human body and protective functions, including (Kolarsick et al., 2011) :

- Protecting the body from heat, sunlight, injuries due to mechanical or chemical damage, bacterial and viral infections.
- Regulating the body's temperature thanks to the blood flowing to the skin's surface allowing the heat to escape to the outside.
- Sweating which allows the body to regulate its temperature to 37°C and to get rid of waste substances.
- Preventing the body from losing water and electrolytes crucial to its well hydration.
- Sensation of the touch and monitoring the environment by sensing cold, heat, pain and pressure through dermis nerve receptors.
- Synthesizing vitamin D and defensive proteins.

II.Significance and brief description of common skin diseases

II.1 Inflammatory skin diseases

II.1.1 Dyshidrotic eczema

Dyshidrotic eczema also known as pompholyx, is a skin condition that affects the fingers, palms, and soles (**Figure 2**). It is characterized by the dry, scaly patches of skin with blisters, which can be intensely itchy and painful. The condition can be triggered by minor irritants or allergens, allergies, and frequently sweaty or wet hands or feet (Abreu-Velez et al., 2009).

Figure 2 : Dyshidrotic eczema on a woman's finger

Dyshidrotic eczema is more common in warm weather, some patients are attacked annually in summer and these patients have intensely pruritic or burning, vesicular eruption on their palms, and soles.

❖ **Medical treatment**: Successful treatment for dyshidrotic eczema consist of a low-strength topical steroid such as hydrocortisone or topical calcineurin inhibitors like tacrolimus (Protopic 0.1%, cream). However, alternative such dupilumab (dupixent 300mg/2ml, injection) were reported to be efficient (Leung et al., 2014; US FDA, 2000).

❖ **Alternative treatment :** A topical herbal formulation, Natural Armor Skin Care®, containing extracts from various plants, such as *Juglans Nigra*, *Artemisia Absinthium*, *Curcuma Longa*, *Allium sativum*, *Matricaria Chamomile*, etc. in combination with oral niacin supplementation was developed for the treatment of dyshidrosis and related skin diseases (Mazzio & Karam, 2008). Also, recently, a pilot study to evaluate the efficacy of a moisturizer

cream containing dimethicone and *Vitellaria paradoxa* showed significant improvement in patient with eczema by reducing itching, inflammation, dryness and erythema (Draelos, 2016).

II.1.2 Contact Dermatitis

Contact dermatitis is a type of eczema condition that occurs when the skin comes into contact with a substance that causes an allergic or irritant reaction. The word "dermatitis" comes from the Greek word "derma," meaning skin, and "-itis," meaning inflammation. Contact dermatitis is characterized by erythematous and pruritic skin lesions (**Figure 3**). The condition causes also the skin to become itchy, blistered and dry (Usatine & Riojas, 2016)

Figure 3 : Contact Dermatitis on a man's arm

❖ **Medical treatment:** Dermatitis lesions are successfully treated with topical steroids, such as (triamcinolone 0.1%, cream) or (clobetasol 0.05%, cream). On areas with thinner skin, lower-potency steroids such as desonide ointment (Desowen® 0.05%, cream) can be helpful and minimize the risk of skin atrophy. If allergic contact dermatitis involves extensive areas of the skin, systemic steroid (dupixent 300mg/2ml, injection) therapy is often required and offers relief within 12 to 24 hours (Usatine & Riojas, 2016).

❖ **Alternative treatment:** LMNOOP® Cream includes essence extracted from *Dictamnus dasycarpus*, *bassia scoparia*, *sophora flavescens*, etc. intensely moisturizes to help strengthen skin's natural moisture barrier and restore skin's protective function and thus helps heal eczema, rosacea and dermatitis. *Achillea millefolium* and *Curcuma longa* possess various bioactive compounds that exhibit anti-inflammatory, antioxidant, and antimicrobial properties, have been confirmed for their potential in treating contact dermatitis (Calapai et al., 2014).

II.1.3 Seborrheic dermatitis

Seborrheic dermatitis is a common inflammation of the skin, occurring most often on the face, scalp and chest (**Figure 4**). It is closely related to infantile seborrheic dermatitis, or diaper rash. The name 'seborrheic dermatitis' implies an oily inflammation of the skin. It can cause itchy red patches and greasy scales on the skin, along with white or yellow crusty or powdery flakes on the scalp (Gupta & Bluhm, 2004).

Figure 4: Seborrheic dermatitis on a man's face

❖ **Medical treatment:** Treatment for seborrheic dermatitis depends on the cases. It can be medicated with shampoos containing 2.5% selenium sulfide or 1 to 2% pyrithione zinc or ketoconazole shampoo (Loprox®). Treatments of the face and body include topical antifungals (ketoconazole 1%, cream or terbinafine 1%, cream), corticosteroids (betamethasone valerate 0.1%, cream) and calcineurin inhibitors such pimecrolimus cream (Elidel®) or tacrolimus ointment (Protopic®) (Gupta & Bluhm, 2004).

❖ **Alternative treatment:** Roycederm Seborrheic Dermatitis & Psoriasis® is a **s**calp treatment cream designed to help relieve the itching, burning, scaling and pain experienced. It is exclusively composed of plant extracts such as *Borneolum syntheticum*, *Sophora Flavescens*, *Kochia scoparia*, *Dictamni Cortex*, etc. Apple cider vinegar is one of the most *well-known natural remedies used* to treat seborrheic dermatitis by diminishing inflammation in the area of the flare-up. In a double blind, placebo controlled clinical trial, the efficacy of an emulsion derived from *Aloe Vera* was assessed showing a significant reduction of pruritus and scaling in 58% of patients (Gupta & Versteeg, 2017).

II.1.4 Rosacea

Rosacea is a chronic facial **inflammatory** dermatosis characterized by background facial erythema and flushing. This particular pathology may be accompanied by inflammatory papules and pustules, cutaneous fibrosis and hyperplasia known as phyma, and ocular involvement which can lead to visual dysfunction (**Figure 5**) (Asai et al., 2016).

Figure 5: Rosacea on a woman's face

The diagnosis of rosacea is exclusively a clinical one and according to the classification of the National Rosacea Society Expert Committee (Wilkin et al., 2002), the presence of at least one of the following primary features is diagnostic of rosacea: flushing (transient erythema), non-transient erythema, papules and pustules, and telangiectasia.

❖ **Medical treatment:** The past decade has witnessed the introduction of novel treatment called Topical ACU-D1 which demonstrates its efficacy in reducing inflammatory lesions and erythema in patients with rosacea (Picardo et al., 2017). Other rosacea medications have been developed and prescribed in recent years including topical drugs that reduce flushing for mild to moderate rosacea such as brimonidine (Mirvaso 3 mg/g, gel) or oxymetazoline (Rhofade 1%, cream). Also, Azelaic acid (Azelex 20%, cream), metronidazole (Metrogel 1%, gel) and ivermectin (Soolantra 10 mg/g, cream) assess their efficacy for the treatment of patients with inflammatory lesions. Oral antibiotics especially tetracycline derivatives are the most widely systemic agents used (Del Rosso 2017).

❖ **Alternative treatments:** Grahams Rosacea Cream® is a natural formula containing *Simmondsia chinensis* (Jojoba) seed oil and coconut oil to reduce inflammation and soothe sensitive skin. Green tea derived from *Camellia sinensis* proved to exhibit antioxidant and anti-inflammatory properties useful in the treatment of rosacea. In addition, topical application of green tea extracts has been shown to decrease UV-induced redness and to reduce DNA damage suggesting it's a potent treatment for UV-induced rosacea (Emer et al., 2011).

II.1.5 Acne vulgaris

Acne vulgaris is a common inflammatory disorder caused by blockage and/or inflammation of the pilosebaceous unit. (Juhl et al., 2018). It is characterized by non-inflammatory follicular papules or comedones and by inflammatory papules, pustules, and nodules in its more sever forms. Acne vulgaris affects the areas of skin with the densest population of sebaceous follicles such as the face (**Figure 6**), the upper part of the chest, and the back. Symptoms of acne vulgaris include blackheads, whiteheads, pimples, nodules, and cysts (Juhl et al., 2018).

Figure 6: Acne vulgaris face infection

This skin disease affects 85-100% of people at some time during their lives and is triggered by *Cutibacterium acnes* in adolescence, under the influence of hormonal changes and severe anxiety and anger (Juhl et al., 2018).

❖ **Medical treatment:** Treatments are directed toward follicular hyper-proliferation, *Cutibacterium acnes* and excess sebum and inflammation. Combination of benzoyl peroxide (2.5-10%) and topical antibiotics like erythromycin or clindamycin are mainly used for their role against *Cutibacterium acnes* and to reduce oil production. Topical retinoids (adapalene 0.1%, gel or cream) or (tazarotene 0.1%, cream) are comedolytic and normalize follicular hyper-proliferation and hyper-keratinization and anti-inflammatory. As oral antibiotic medications, doxycycline in combination with isotretinoin (Roaccutane 50 mg, gel) have been shown to be effective for severe acne vulgaris (Haider, 2004).

❖ **Alternative treatment:** Tea Tree Medicated Gel For Acne® is a gel that contains 100% pure Australian Tea tree (*Melaleuca alternifolia*) oil that helps to dissolve blackheads and whiteheads, dry out pimples to help control acne. Leaves of *Amaranthus hypochondriacus Linn* and *Amaranthus cruentus Linn* have been used effectively as an astringent and also make a good wash for skin problems like acne vulgaris (Reddy & Jain, 2019). In a preliminary clinical investigation, lotion products formulated with the undiluted or 50% *Aloe vera* in *Ocimum gratissimum* oil were most active and resolved inflammatory lesions faster than 1% clindamycin in the treatment of Acne vulgaris (Orafidiya et al., 2004).

II.2 Infectious skin

II.2.1 Dermatophytosis

Dermatophytosis, also known as ringworm, is a chronic, superficial, fungal infection of the skin that results in a red, itchy, scaly, circular rash (**Figure 7**). There are different Different fungal tinea capitis (scalp), tinea corporis (body), tinea unguium (nails), tinea pedis (feet), or tinea cruris (groin). The most common pathogens in skin infections are *Microsporum*, *Trichophyton*, and *Epidermophyton* species (AL-Khikani & Ayit, 2021).

Figure 7: Dermatophytosis skin infection

Individuals aged between 21 and 40 years old are most commonly affected and prevalence of dermatophytosis varies depending on the area, clinical subtype, and dermatophyte.

❖ **Medical treatment:** In general terms, Systemic antifungal therapies (Griseofulvin® 500 mg, tablets) as well as imidazole family (ketoconazole, itraconazole, and fluconazole) and terbinafine (Lamisil® 250 mg) are effective. Also, topical cream therapies including imidazole derivatives (Daktarin® 2%), allylamines derivatives (Lamisil® 1%), benzylamine derivatives (Butenafine® 1%) and hydroxypyridones derivatives (Ciclopirox olamine® 1%) are effective short-duration therapies by preventing the growth of fungus (AL-Khikani & Ayit, 2021).

❖ **Alternative treatment:** Forces of Nature-Natural, Organic Ringworm Treatment® is a homeopathic medicine composed of *Natrum Muriaticum* and *Silicea* species used to relieve itching and clears ringworm. Also, in a study on ringworm in animals, it has been concluded that *Aloe vera* and *Allium sativum* can be used for treating ringworm infection as they have soothing and healing properties (Meena & Ramaswamy, 2014). In a randomized, double-blind study, a cream containing an extract of *Solanum chrysotrichum* proved to be as effective as a 2 % ketoconazole preparation in the treatment of one of the fungal causing dermatophytosis i.e. tinea pedis (Reuter et al., 2010).

II.2.2 Molluscum contagiosum

Molluscum contagiosum is a viral infection that is usually a benign, mild skin disease characterized by lesions that may appear anywhere on the body including the face, neck, arms, legs, abdomen and genital area. The lesions, known as Mollusca, are small, and usually white or pink with a dimple or pit in the center (**Figure 8**). They often have a pearly appearance considered a sexually transmitted infection (Brown et al., 2006).

Figure 8: Molluscum contagiosum skin infection

❖ **Medical treatment:** Topical medications are often used to treat molluscum contagiosum (Tretinoin® 0.05% or 0.1%, cream) and keratolytic agents such as salicylic acid, potassium hydroxide and benzoyl peroxide as they produce desquamation and inflammation (Meza-Romero et al., 2019). Physical removal of mollusca contagiosum can be done through cryotherapy with liquid nitrogen, curettage, or laser treatment.

❖ **Alternative treatment:** Podophyllotoxine (Podofilox® 0.5%, topical solution) is s a non-alkaloid extract of the *Podophyllum* species used to treat molluscum contagiosum by inducing an inflammatory reaction, thus accelerating recovery. In a study where thirty-one children diagnosed with molluscum contagiosum were treated with a 10% solution of essential oil of *Backhousia citriodora*, more than 90% in the number of lesions were reduced, indicating that the plant is a moderately effective and safe as a treatment (Meza-Romero et al., 2019).

II.2.3 Herpes labialis

Herpes labialis, or cold sores, is caused by herpes simplex virus type 1 and can result in significant irritation, pain, discomfort, and worry (Opstelten et al., 2008). The infection is a rash of the skin and mucous membranes mostly of the lips and is characterized by erythema and blisters that are preceded and accompanied by burning pain (**Figure 9**).

Figure 9: Herpes labialis on a woman's lips

Up to 90% of persons over the age of 50 would test seropositive for simplex virus type 1, and an estimated 20 to 40% of adults experience cold sore outbreaks. The infection is most often acquired in childhood, but the incidence increases with age (Opstelten et al., 2008).

❖ **Medical treatment:** Antiviral drugs in both cream and ointment forms are the main mode of treatment prescribed such acyclovir, valacyclovir or famciclovir. Also, the classic clinical manifestation of herpes simplex virus type 1 is gingivostomatitis that often requires either topical or oral administration of analgesics like viscous lidocaine, topical benzocaine or over-the-counter medications based on docosanol (Abreva® 10%, cream) (Raborn et al., 2004).

❖ **Alternative treatment:** Urban ReLeaf Lemon Balm Salve® is a natural lip balm used to soothe symptoms of cold sores. It is made of *Vitellaria paradoxa* Butter, lemon (*Citrus limon*), Tea tree (*Melaleuca alternifolia*) oil and Peppermint (*Mentha piperita*) Oil. Under double-blind conditions, a study was conducted to compare the efficacy of a lip cream containing propolis extract concentrated at 0.5% to aciclovir 5% confirmed that the application of propolis extract lip balm was clinically effective and safe showing for both early and late start of treatment during an episode of herpes labialis (Jautová et al., 2019).

II.2.4 Impetigo

Impetigo is a primary superficial **bacterial skin infection**, initially vesicular or bullous, and later crusted. It is caused by staphylococcus aureus, Streptococcus pyogenes or both. It is highly contagious and most commonly affects infants and young children. The symptoms of impetigo include red sores that quickly burst and ooze prior to the formation of a crust accompanied with itching and soreness (**Figure 10**) (Cole & Gazewood, 2007).

Figure 10: Impetigo on a woman's face

❖ **Medical treatment:** The aims of treatment include relieving the discomfort and improving cosmetic appearance of the lesions. Several different oral antibiotic treatment e.g. penicillins (Methicillin®), amoxicillin/clavulanate (Augmentin®) or cephalosporins (Cephalexin®) are considered for patients with impetigo with extensive spread and associated with systemic symptoms. In case of limited body surface area, topical antibiotics such as mupirocin (Bactroban® 2%, cream) is the preferred therapy (Cole & Gazewood, 2007).

❖ **Alternative treatment:** ImpetigoCream® is an over-the-counter ointment composed of 16 essential oils (*Azadirachta indica*, *Melaleuca alternifolia*, *Salvia rosmarinus,* etc.) is commercialized to handle acute and chronic impetigo and all types of impetigo problems. The extract of dried leaves of *Azadirachta indica* leaves and *Aloe vera* gel and creams formulated with these extracts exhibited antimicrobial activity that can be utilized in the treatment of skin disorders caused by susceptible organisms such as impetigo (Azubuike et al., 2015).

II.2.5 Warts

Warts are small, noncancerous growths that appear on the skin when it is infected with one of the viruses of the human papillomavirus family. They are more frequently seen on the hands of children and young adults (**Figure 11**); they may be located on any cutaneous or mucosal surface are highly contagious and can be passed by direct skin contact (Nofal & Nofal, 2010).

Figure 11: Wart on a man's finger

❖ **Medical treatment:** Although there are many destructive and immunotherapeutic options available for the treatment of common warts, no single treatment has yet proven 100% effective. Cryotherapy is mainly used and causes cryocytolysis, resulting in tissue sloughing and wart destruction. Also the immune response modifiers (Imiquimod® 5%, cream) is used to treat warts on the skin of the genital and anal areas (Perry et al., 1999).

❖ **Alternative treatment:** Podowart Podophyllin Red Cream®, a resin extracted from the roots of *Podophyllum peltatum* and *Podophyllum emodi*, has been evaluated extensively in randomized treatment-comparison studies in human populations. It is used topically as a treatment for genital warts.

II.3 Auto-immune and oxidative stress diseases

II.3.1 Alopecia Areata

Oxidative stress has been found to be a common feature in alopecia areata, which is an autoimmune disorder characterized by transient, non-scarring hair loss and preservation of the hair follicle (**Figure 12**). Hair loss can take different forms ranging from loss in well-defined patches to total hair loss affecting all hair-bearing sites of the body. This particular pathology affects nearly 2% of the general population at some point during their lifetime (Pratt et al., 2017).

Figure 12: Alopecia areata on a man's head

❖ **Medical treatment**: The main goal of alopecia areata therapy is to stop the immune system attack on hair follicles and to stimulate the regrowth of hair. Topical corticosteroids have been reported to have some effect in the treatment of alopecia areata, (triamcinolone 0.1%, cream) is the preferred intralesional product administered with a 0.5 inch long needle as multiple intradermal injections of 0.1 mL per site (Madani & Shapiro, 2000).

❖ **Alternative treatment:** Capsules of *Ginkgo biloba* oil are commercialized under the name Ginkgo Biloba B+ ® for the treatment of alopecia as it improves cerebral microcirculation, and hence increases oxygen supply. A randomized clinical trial of the efficacy of 5% garlic (*Allium sativum*) gel in combination with betamethasone cream showed that the use of garlic gel significantly increased the therapeutic efficacy of topical betamethasone as compared with in the control group (Ezekwe et al., 2020).

II.3.2 Systemic sclerosis (scleroderma)

Systemic sclerosis is a connective tissue disease, which affects skin, blood vessels, heart, lungs, kidneys, gastrointestinal tract and musculoskeletal system. Lower-limb swelling and muscle weakness or fatigue might be reported in early-stage cutaneous systemic sclerosis (**Figure 13**). Involvement of internal organs results in significant morbidity and mortality of patients (Denton & Khanna, 2017).

Figure 13: Systemic sclerosis in a woman's fingers

Weight loss, often associated with reduced appetite or food intake, and exertional breath troubles are common symptoms along with puffy fingers, ulcers on the fingertips and the extensor aspect of the digits (Denton & Khanna, 2017).

❖ **Medical treatment:** Management of skin-associated complications consists of immune-modulating therapies. Clinical trial have supported clinical benefits for oral methotrexate or immunosuppressive agents such as mycophenolate mofetil (Phenocept® 500 mg) (Denton & Khanna, 2017).

❖ **Alternative treatment:** In a study investigating the potential benefits of *Hypericum perforatum* and *Azadirachta indica*, patients with difficult-to-heal scleroderma were enrolled, yielded promising results, indicating that these plants could serve as valuable tools for managing scleroderma (Giuggioli et al., 2019).

II.3.3 Psoriasis

Psoriasis is a persistent, non-infectious inflammatory skin condition that affects 1 to 2 % of the population. It is characterized by the emergence of distinct pink or red lesions topped with silvery scales on the extensor surfaces of the body and the scalp (**Figure 14**). Although certain systemic and environmental factors are recognized to impact the disease, its progression is unpredictable, marked by periods of spontaneous improvement and flare-ups of lesions ((Medovic et al., 2022).

Figure 14: Psoriasis on a woman's arm

The underlying cause of the condition is attributed to immune system dysfunction in conjunction with a genetic predisposition. It was also established that the skin disorder is related to patients that show signs of increased oxidative damage (Medovic et al., 2022).

❖ **Medical treatment:** When psoriasis affects less than 20% of the body surface, topical therapy is generally recommended such as Vaseline® ointment and exposing the affected areas to sunlight. Vitamin D3 analogues: (Calcitriol® 3 µg/g, ointment) and (Calcipotriol® 50 µg/g, ointment) and third-generation retinoid (Tazarotene 0.05% or 0.1%, cream) is used for the treatment of psoriasis by regulating keratinocyte proliferation and maturation.

❖ **Alternative treatment:** Comizla Psoriasis Cream® is a natural cream on the market that contains plant-based (*Mahonia* aquifolium, Prunus armeniaca, etc.) stem cells to block inflammation and rebuild damaged skin for psoriasis condition. The clinical effects of topical *Hypericum perforatum* in plaque-type psoriasis were studied and showed that erythema, scaling, and thickness, were substantially reduced in places where the formulated ointment was applied (Mansouri et al., 2017).

Chapter 2 – Phytotherapy and its application in the treatment of skin diseases

Phytotherapy is the science that deals with the treatment and prevention of diseases through the use of medicinal plants and herbal products. Phytotherapy has witnessed significant advancements over the years as a result of several factors. Firstly, the availability of raw materials for herbal remedies makes it easier for manufacturers to produce herbal products. Additionally, the price of these raw materials is low compared to the cost of manufacturing commercial chemical drugs. Furthermore, the few side effects associated with herbal remedies has contributed to their increasing popularity unlike many commercial chemical drugs. All the above mention factors led to striking statistics, according to the World Health Organization, claiming that about 60% of the world's population relies on herbal medicine and about 80% of people in developing countries employ traditional herbal medicine (Khan & Iqbal, 2019).

As the demand for natural and sustainable healthcare options grows, phytotherapy has gained prominence as a valuable adjunct or alternative therapy for various health conditions, including skin diseases. Skin diseases impact millions of people worldwide, leading to discomfort and diminished quality of life. Phytotherapy presents a promising avenue for addressing these conditions (Albahri et al., 2023).

I.Plant families with dermatological benefits

Plant-based therapeutic preparations offer a safer alternative or, in some cases, the only effective treatment for skin disorders. In this realm, to the best of our knowledge, the most used plant families in Asia, Europe, Africa, and the Middle East are Asteraceae, Fabaceae, Lamiaceae and Euphorbiaceae families (Tsioutsiou, et al., 2022; Almoshari et al., 2022). However, it is important to highlight the presence of plant species belonging to almost all plant families presenting activity for dermatological diseases. For example, coconut oil obtained from *Cocos nucifera* (Arecaceae) is used as an ingredient in dermal and skin formulations whereas mango butter from *Mangifera indica L.* (Anacardiaceae) is valued for its significant potential as a cosmetic ingredient (Poljšak et al., 2020). The trendy *Aloe Vera* herbal medicine, extensively presented in media as a miraculous remedy, has been found to reduce the healing time of burn wounds and to enhance the production of collagen (Eshghi et al., 2010).

I.1 The Asteraceae family

The Asteraceae family, also known as the sunflower family, is one of the largest groups of flowering plants with a diverse range of appearances, there are 23,000 species spread across 1600 genera (**Figure 15**). They can take various forms such as herbs, shrubs, vines, and trees, showcasing their remarkable adaptability. This family has a widespread distribution, from subpolar to tropical regions (Mandel et al., 2019).

Figure 15: Examples of the Asteraceae family plants

The Asteraceae family has long been recognized for its medicinal properties worldwide due to its bioavailability (Mandel et al., 2019). The aerial parts of Asteraceae plants possess antibacterial, antiviral, antioxidant, anti-inflammatory, hepatoprotective, anthelminthic, antifungal, anti-diarrheal and antituberculosis properties (Nguyen et al., 2021).

Although, many Asteraceae plants are recognized as allergenic plants causing eczema, hay fever, asthma, or even anaphylaxis, several plants from the family are known as wound-healing agents such as *Ageratina pichinchensis* which showed decreases healing time and lesion size in a human clinical study to treat foot ulcer (Carvalho et al., 2018). Also, a cream containing *Calendula officinalis* flowers extract was recently found to enhance collagen organization in the early phase of the healing process (Nicolaus et al., 2017). Plant species of the genus *Artemisia* are well-known to play an important role in the skin treatments in traditional medicine. Their pharmacological potentials have been confirmed in *in vivo* healing models where for example *Artemisia montana* promoted the healing of rats with dorsal wounds and *Artemisia campestris L.* presented a positive effect in the progress of wound healing (Carvalho et al., 2018). Finally, it's important to highlight the fact that Asteraceae species are commonly used for fabrication of nanoparticles where innovative strategies where made for treating skin infections and cutaneous wounds (Ouyang et al., CN 102552414-A, 2012).

I.2 The Fabaceae family

The Fabaceae family or Leguminosae family is is a large and agriculturally important family of flowering plants that includes trees, shrubs, vines, and herbaceous plants, both perennials and annuals (Rahman & Parvin, 2014). This plant family has a wide global distribution and ranks as the third-largest family of land plants based on species count. It comprises 730 genera and over 19,400 (**Figure 16**).

Figure 16: Examples of plant genera of the Fabaceae family

Some of the healthiest legumes from the Fabaceae family include chickpeas, lentils, black beans, kidney beans and navy beans. They are fundamentals in health prevention such as cardiovascular and digestive health prevention thanks to their high concentration in fibers that helps to lower blood cholesterol and promotes regular bowel movements respectively, diabetes prevention thanks to their low glycemic index, vegetarian protein source because of their "meaty" texture, etc.

Being such a large family, the enormous diversity of secondary metabolites does not surprise. The Fabaceae Family is usually utilized to treat various ailments such as abscesses, asthma, coughs, colds, dysentery, skin diseases, ulcers, and leprosy (Rahman & Parvin, 2014). *Senna alexandrina* Mill. is used topically in traditional medicine to treat injuries and skin diseases as a leaf paste (Almoshari et al., 2022). Several plants of the *Copaifera* genus has a

very long history of medicinal use for their oleoresin healing properties. Thus, *Copaifera guyanansis* oleoresin is today used in modern medicine in anti-acne creams, formulations for treating stretch marks and scars, as well as shampoos, capillary lotions, soaps, and bathing foams (Da Silva et al., 2012).

I.3 The Lamiaceae family

The Lamiaceae family is a highly diverse and widely distributed plant family renowned for its significant contributions to ethno-medicine primarily attributed to its high concentration in volatile oils. The Lamiaceae family presents 236 genera and more than 7,000 species of which the most known members are aromatic spices like thyme, mint, oregano, basil, sage, rosemary, lemon balm, etc,. (Uritu et al., 2018). **Figure 17** show some plants of different genera of the Lamiaceae.

Figure 17: Examples of plant genera of the Lamiacea family

A number of species from this family has been widely employed as ethnomedicine against allergic inflammatory skin diseases. As a reference examples, topical treatment of *Scutellaria baicalensis* aqueous extract showed to be effective against immunoglobulin E and induced suppression of dermatitis and *Prunella vulgaris* exhibited significant inhibitory effects on histamine release and thus proved to have high potential to be developed into anti-allergic agent (Choia et al., 2016 ; Kim et al., 2016). Also, the Lamiaceae family is widely used in cosmetic; a very well-known plant in cosmetics industry namely *Satureja hortensis* is a precious beauty ingredient for skin conditioning and soothing (essential oil Winter Mountain Savory®), *Salvia officinalis* is used as clarifying anti-scar serum (Ningen Salvia Skin Serum®) and *Siegesbeckia Orientalis* is sold as a support to fragile skin around the eyes by reducing the visible effects of dark circle pigments (Beautifeye Anti-Wrinkle®).

I.4 The Euphorbiaceae family

The selected plant in this Master research project is *Euphorbia Guyoniana* which belongs to the Euphorbiaceae family. Thereby, the presentation of this particular family in this section will be more detailed.

I.4.1 Presentation of the Euphorbiaceae family

The Euphorbiaceae family is the fifth most diverse and expansive group of flowering plants in the world, with around 300 genera and 5000 à 100000 species. The family is found mainly in the South of North America, in the Mediterranean region, Middle East and South Africa. It is also found in all dry, semi-arid regions and tropics (Ramalho et al., 2018).

The Euphorbiaceae family is commonly known as the spurge family and is remarkable for its variety of plant shapes and sizes including herbs, trees and shrubs. The Euphorbiaceae family exhibits nearly every growth form used by plants, including annuals, perennials, trees, succulents species, etc (**Figure 18**). The greatest range is seen in the genera *Phyllanthus* and *Euphorbia* which have adapted to both wet and dry living conditions (Webster, 1994).

Figure 18: Examples of plant genera of the Euphorbiaceae family

❖ **The genus *Euphorbia*** (Frodin, 2004) is the second major genus of the Euphorbiaceae family with over 2100 species occurring in all temperate and tropical regions. This genus has a worldwide distribution and is characterized by a large variety of herbaceous annuals or perennials, big shrubs, small trees, subshrubs, and cactus-like plants. Their main common points are the production of a viscous milky latex present in the stem implicated in the defense against herbivorous insects and used to produce rubber and the presence of a characteristic cyathium that resemble a dicotyledonous flower. Among the countries of south-west Asia, Turkey and Iran are the most species-rich places with about 8650 and 7500 species respectively. Likewise, some endemic species are rare and restricted from only a single locality like the *Euphorbia Guyoniana* species which primarily grows in the desert shrubland biome of the northwest Sahara and is thus a species of plant that is endemic to Algeria (**Figure 19**).
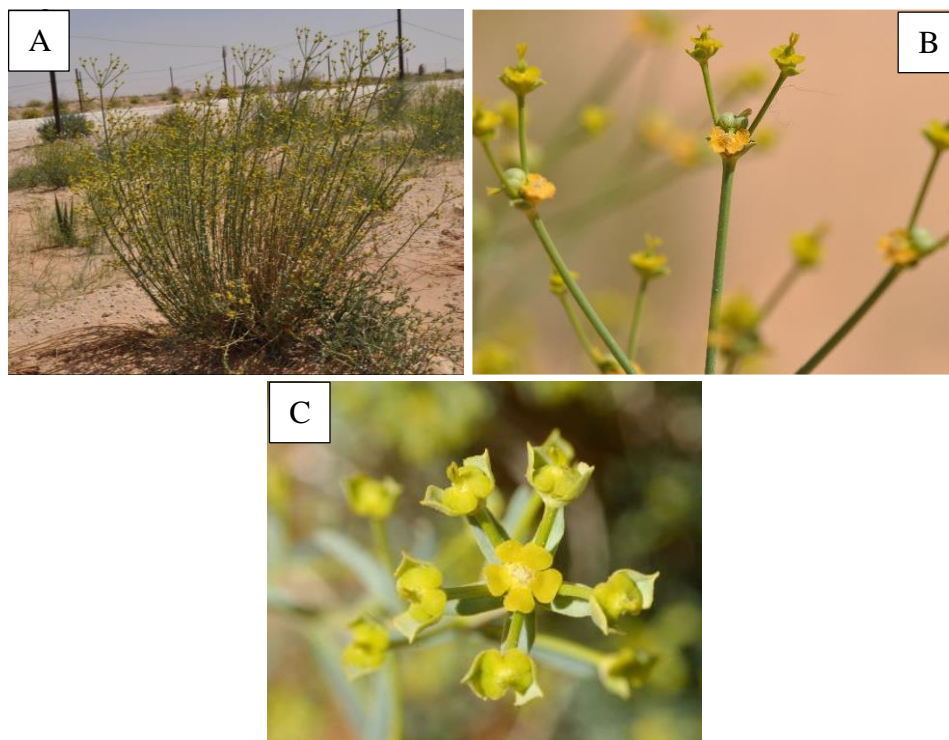
Figure 19: *Euphorbia guyoniana*: Whole plant (A), Flowery steam (B), Detail of a cyathium (C)

I.4.2 Therapeutic interest of the Euphorbiaceae family (Mwine & van Damme, 2011)

In Chinese medicine, Euphorbiaceae family has been reported to have 33 species used in herbal medicine, 17 of these species were used to treat snakebites. The family is known for its rich variety of secondary metabolites, such as alkaloids, terpenoids, flavonoids and tannins. This makes it one of the biggest plant families with probably the highest species richness in many habitats. The implication is that in absolute terms, there is a higher probability of having more species that are medicinal in that one family as compared to other families. A numerous plants belonging to the Euphorbiaceae family are daily used and present an industrial and therapeutic interest. We can cite:

- Roots of *Bridelia retusa (L.)* are used in the treatment of rheumatism and obtained methanolic extract showed great antifongic properties.
- Oils of *Ricinus communis* and *Sapium sebiferum* are used in the fabrication of soaps, paints and varnishes.
- The species *Hevea brasiliensis* provides the large majority of natural rubbers.
- *Phyllanthus emblica* provides comestible fruits whereas *Sapium sebiferum*, *Chozophora tinctoria* and *Mallotus philippinensis* are used as dyes.

Also, the effectiveness of many plants of the Euphorbiaceae family were evaluate against many skin diseases and infections even if the milky latex present in the genus *Euphorbia* plants is known to be irritating to skin. It was suggested that *Euphorbia* species have skin curative properties due to the presence of various phytochemicals exclusively found in this genus. For instance, the active ingredient (ingenol mebutate) of Picato® medicine used in topical therapy against the precancerous skin condition actinic keratosis was identified in *Euphorbia peplus* L., *lathyris* L., *nivulia*, *esula* L., *antiquorum* L., *serpens* and *fischeriana* (Salehi et al., 2019).

In the table below, few examples of *Euphorbia* plants studied or used traditionally in the management of skin or oral infections are presented.

Table 1: *Euphorbia* plants studied/used in the management of skin or oral infections

Plant species	Treatment	Reference
Euphorbia hirta	Treatment of warts and fungal infections in 35 students' toes	Kripa et al., 2022
	Improvement of wound healing after topical and oral administration	Tuhin et al., 2017
Euphorbia abyssinica	Treatment against ringworm and demodex infections	Mengiste et al., 2014
Euphorbia peplus	treatment of skin cancers	Ramsay et al., 2011
Euphorbia thymifolia	Age-sustaining properties	Mali et al., 2013
Euphorbia antiquorum	Treatment of burns	Dilara & Subhan., 2000
Euphorbia neriifolia	Treatment of sores, pimples and skin eruptions	Mali et al., 2017
Euphorbia heterophylla	Treatment of skin infections	Falodun et al., 2006
Euphorbia lateriflora	Treatment of dermatitis	Oyedemi et al., 2018

II. Active Compounds with dermatological benefits

Plant constituents, or phytochemicals, are divided into primary and secondary metabolites based on their activity in the plant metabolic process. Secondary metabolites protect plants from diseases, damage, various environmental hazards i.e. pollution, stress, drought, UV exposure, pathogenic attack and contribute to their color, aroma, and flavor (Roelandts, 2002). Secondary metabolite classes include phenolic compounds, lipids, saponins, carbohydrates, alkaloids, and terpenes (Hussein & El-Anssary, 2019). All above cited classes of secondary metabolites found in plants have particular compounds that exhibit therapeutic effects on the skin (Farmacognosia, 2005). In our research project, our interest was mainly turned toward phenolic compounds, so only compounds of that specific secondary metabolite class that exhibit therapeutic effects on the skin are presented hereafter.

II.1 What are phenolic compounds?

Phenolic compounds are commonly known as the largest phytochemical class of molecules in the plant reign and play a key role in the growth, the reproduction and the protection of plants against ubiquitous pathogens and predators (Kumar et al., 2020). Based on the chemical structure of their nucleus, phenolic compounds are divided into simple phenols (phenolic acids and coumarins) and polyphenols i.e. flavonoids and non-flavonoids (tannins, lignans and stilbenes). Our interest was mainly turned toward simple phenols, flavonoids and tannins, so only those particular secondary metabolite are presented hereafter.

II.2 Chemical structure of phenolic compounds

II.2.1 Phenolic acids

Phenolic acids present at least one aromatic nucleus characterized with the presence of one hydrogen substituted with hydroxyl. There are two main classes of phenolic acids: hydroxybenzoic acid derivatives and cinnamic acid derivatives.

❖ **Hydroxybenzoic acids**

The hydroxybenzoic acid derivatives present are phenolic compounds with a general structure C6-C1. They usually occur as esters or attached to a glycosyl moiety. Variations in the structure of hydroxybenzoic acid derivatives include also hydroxylation and methoxylation of the aromatic ring. The most well-known hydroxybenzoic acids are: vanillic acid, salicylic acid, para-hydroxybenzoic acid, gallic acid and catechic acid (**Table 2** and **Figure 20**).

Table 2: Radical substitutions of Hydroxybenzoic acids
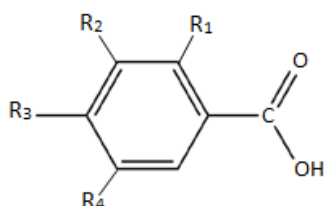
Figure 20: General chemical structure of hydroxybenzoic acids

Compounds	R_1	R_2	R_3	R_4
Vanillic acid	H	H	OH	OCH_3
Salicylic acid	OH	H	H	H
Para-hydroxybenzoic acid	H	H	OH	H
Gallic acid	H	OH	OH	OH
Catechic acid	H	OH	OH	H

❖ **Hydroxycinnamic acids**

The hydroxycinnamic acid derivatives are phenolic compounds with a characteristic side chain where the double bond can be *cis* or *trans*. They are usually combined to other organic structures. The most well-known hydroxycinnamic acids are: cinnamic acid, coumaric acids, caffeic acid and ferulic acid (**Table 3** and **Figure 21**).

Table 3: Radical substitutions of hydroxycinnamic acids
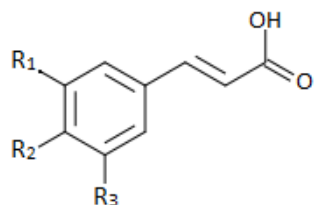
Figure 21: General chemical structure of hydroxycinnamic acids

Compounds	R_1	R_2	R_3
Cinnamic acid	H	H	H
Ortho-coumaric acid	OH	H	H
Meta-coumaric acid	H	H	OH
Para-coumaric acid	H	OH	H
Caffeic acid	H	OH	OH
Ferulic acid	H	OH	OCH_3

II.2.2 Flavonoids

Flavonoids are the largest family of over 6500 hydroxylated phenolic compounds in the vegetal reign. Flavonoids are found in all parts of plants and serve as significant contributors to the colors and enticing fragrances exhibited by fruits and flowers. They also play a crucial role in safeguarding plants against damage caused by sunlight (Havsteen, 2002). All flavonoids present the same basic chemical structure characterized by a C6-C3-C6 structure containing two benzene rings, A and B, connected by a heterocycle pyrene ring C (**Figure 22**).
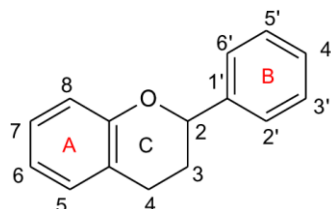
Figure 22: Chemical structure of flavonoids nucleus

On the basis of the configuration and the number of substitutions of the aromatic rings A and B on one hand, and of the oxidation degree of the position 3 of the heterocycle C on the other hand, flavonoids can be divided into seven sub-groups:

❖ **Flavones:** simplest structure, they represent 80% of all flavonoids and are responsible of the yellow color in flowers and leaves: apigenin, luteolin, diosmetin, etc.

❖ **Flavanols or flavan-3-ols:** presence of an -OH group in position 3 of the heterocycle; they are the most abundant flavonoids in tea, apples and cocoa: catechin, etc.

❖ **Flavonols:** presence of an -OH group in position 3 and a carbonyl in position 4 of the central heterocycle C, they are usually occur attached to a glycosyl moiety; widly distributed, they are one of the major pigments in higher plants: quercetin, kampferol, myricetin, etc.

❖ **Flavanones:** absence of an -OH group in position 3, generally attached to a diglycosyl moiety in position 7: naringenin, eriodictyol, etc.

❖ **Isoflavones:** occurrence of the cycle B in position 3, they are the less widespread taxonomically: genistein, daidezin, etc.

❖ **Anthocyanins:** pigments responsible of the colors blue, red, purple or pink in plant's flowers and leaves: peonnidin, malvidin, cyanidin, delphinidin, etc.

❖ **Chalcones:** C3-α,β-unsaturated carbonyl group attachs the two aromatic rings A and B, they occur as *Z* or *E* stereo-molecules : naringenin chalcone, butein, sappanchalone, etc.

II.2.3 Tannins

Tannins are highly hydroxylated polar phenolic compounds which have the ability to precipitate proteins, alkaloids and polysaccharides in aqueous solutions. They are present in all parts of the plant: bark, wood, leaves, fruits and roots, they are soluble in water and their molecular weights range from 500 to 3000 Daltons. (Ky et al., 2016) Tannins are divided into two sub-groups:

❖ **Hydrolysable tannins** are monomeric or oligomeric polyesters of a sugar moiety (generally glucose) or a related sugar or polyol (fructose, sucrose, etc.) and organic acids (gallic for gallotannins and ellagic acids for ellagitannins). The designation hydrolyzable tannins is due to the fact that gallotannins can be hydrolyzed releasing gallic acid, whereas ellagitannins give hexahydroxydiphenic acid which spontaneously dehydrates to ellagic acid.

❖ **Condensed tannins** or non-hydrolyzable tannins are particular polymers formed from two or more flavan-3-ol molecules or flavan-3,4-diols units linked by carbon–carbon bonds that are not susceptible to cleavage by hydrolysis. The total number of monomeric flavanol units are generally ranged from 2 to 17. They don't bear any sugar moiety in their structure.

II.3 Importance of phenolic compounds in skin diseases

Plant secondary metabolites can be effective for humans in treating a variety of disorders (Działo et al., 2016). The most common properties of polyphenols i.e. antioxidant, anti-inflammatory and antimicrobial properties indicate that they deserve recognition as potent highly effective compounds in treatment of various skin problems. Those three mentioned properties constitute the main mechanisms of action against various skin disorders.

II.3.1 Antioxidant Activity (Valko et al., 2007)

While oxygen is vital for organisms, reactive oxygen species (ROS) can have detrimental effects and cause mutations. ROS induce oxidative stress and damage cellular components such as lipids, proteins, and DNA. Such damages negatively impact immunological processes, aging, and the pathophysiological mechanisms involved in skin disorders. The skin possesses two essential defense mechanisms against oxidative stress: antioxidant enzymes and non-enzymatic molecules. However, in many cases, the endogenous defense system against ROS is insufficient.

❖ **Phenolic compounds** are excellent candidates to enhance the level of natural antioxidants through dietary intake or external application. Natural exogenous antioxidants, such as vitamins C and E, lipoic acid, coenzyme Q, melatonin, resveratrol, curcumin, and

almost all simple phenolic compounds, serve as examples. These compounds are safe and exhibit higher biological activity compared to synthetic antioxidants (Sadowska-Bartosz & Bartosz, 2014). Ferulic acid contributes to the inhibition of the skin photo-aging process and the regeneration and absorption of UVB and UVA radiation. Thus, it is used for the production of sunscreens (Sytar Oksana, 2012).

❖ **Flavonoids** are known for their crucial role in safeguarding plants against damage caused by sunlight. Similarly, when extracted and used in humans, they can effectively protect against oxidative damage. Indeed, the positive effects on human skin and anti-glycation of proteins that degrades collagen activity of taxifolin was proven (Heim et al., 2002). Also, the white pulp of citrus fruits which is today recognized as an affordable source of flavonoids, contains ascorbic acid, which is a powerful antioxidant agent against dermatologic conditions. Quercetin and rutin are known antioxidant agent that can scavenge free radicals, inhibit lipid peroxidation, and protect cells and tissues from oxidative damage and thus maintain cellular health (Russo et al., 2012, Enogieru et al., 2018).

❖ **Tannins** are more and more being recognized as the new natural class of antioxidants. However, the exact mechanism of tannin antioxidant action is still unclear. Ellagitannins can scavenge free radicals, inhibit lipid peroxidation, and protect cells from oxidative damage (González-Barrio et al., 2011). Standardized *Pinus maritima* bark extract commercialized under the name Pycnogenol® is mainly composed of oligomeric procyanidins and phenolic monomers present strong antioxidant properties thanks to its ability to regenerate ascorbyl radical and protect endogenous vitamin E and glutathion against oxidative damage (Packer et al., 1999). Another example concerns punicalagin present in pomegranate which participates in its antioxidant potential by inhibiting lipid peroxidation (Seeram et al., 2005).

II.3.2 Anti-Inflammatory activity

On a daily basis, our bodies encounter external factors (pollution, junk food, make-up, etc.) that cause damage, irritation, or skin allergies. During the complex process of inflammation, an excess of free radicals and pro-inflammatory cytokines and interleukins are produced, which lead to skin inflammation. The crucial functions of polyphenols are inhibition of pro-inflammatory mediators, neutralization of free radicals and thus inhibition of lipid peroxidation.

❖ **Phenolic compounds** like curcumin is well-known for its potent anti-inflammatory properties. It inhibits various inflammatory mediators and enzymes, such as COX-2 and NF-kB, which play crucial roles in the inflammatory process (Gupta et al., 2013). Resveratrol has been shown to inhibit the production of inflammatory mediators, such as cytokines and prostaglandins, and modulates inflammatory signaling pathways (Shakibaei et al., 2009). Various derivatives of p-coumaric acid revealed exhibited similar activity to the one showed by dexamethasone used as anti-inflammatory standard (Taofiq et al., 2015).

❖ **Flavonoids** such as Kaempferol exhibits anti-inflammatory by modulating multiple signaling pathways involved in inflammation, leading to a reduction in inflammatory responses (Calderon-Montano et al., 2011). Luteolin showed anti-inflammatory effect by inhibiting the production and release of pro-inflammatory mediators leading to a reduction in inflammation

(Xia et al., 2016). In a very interesting study conducted by Trinh et al., in 2010, <u>baicalin</u> isolated from rhizome of *S. baicalensis* ant its metabolites <u>baicalein</u> and <u>oroxylin A</u> possessed inhibitory activity against histamine-induced scratching behavior.

❖ **Tannins** have been extensively used for hundreds of years for medical and mainly dermatological purposes and are currently indispensable in in the therapy of inflamed superficial skin diseases. <u>Tannic acid</u> was recently assessed as a suppressor of the 12-O-tetradecanoylphorbol 13-acetate induced skin inflammation in an *in vivo* study by using a mouse model (Nakamura et al., 2018). <u>Punicalagins</u> and <u>Epigallocatechin gallate</u> exhibited anti-inflammatory properties by suppressing inflammatory signaling pathways and reducing the production of pro-inflammatory cytokines (Islam et al., 2021 ; Khan & Mukhtar, 2007).

II.3.3 Antimicrobial Activity

Various infections, including those affecting the skin, are often treated with broad-spectrum antibiotics. However, the use of antibiotics can have adverse effects on the natural microflora of the skin and contribute to the development of antibiotic resistance. Thus the activity of polyphenols has special significance in the case of strains resistant to antibiotics, e.g., *Staphylococcus*, *Enterococci*, *Pneumococci* and *Pseudomonas* which are the most frequent causes of skin hospital infections i.e. ulcers, bedsores or burns (Parisi et al., 2013).

❖ **Phenolic acids** demonstrate antimicrobial properties against a large spectrum of microorganisms and sensitize multi-drug resistance strains. The mechanism of action of phenolic acids seems to be related to the ability to inhibit bacterial virulence factors such as enzymes and toxins, to interact with cytoplasmic membrane, to suppress biofilm formation and to exert a synergistic effect with antibiotics (Miklasińska-Majdanik et al., 2018). <u>Gallic acid</u> and <u>catechin</u> has been found to possess antimicrobial properties against a wide range of microorganisms, including bacteria, fungi, and viruses (Basrani & Haapasalo, 2014). <u>Protocatechuic acid ethyl ester</u> displayed diverse activity against *Staphylococcus aureus* with an augmented antibacterial effect in the presence of clindamycin (Miklasińska et al., 2015).

❖ **Flavonoids:** Flavonoids exhibit a wide range of antimicrobial activities against various microorganisms, including bacteria, fungi, and viruses. <u>Myricetin</u> has been shown to possess antimicrobial effects against by inhibiting the growth and replication of pathogens by disrupting their cellular processes (Cushnie & Lamb, 2011). <u>Apigenin</u> has been studied for its antimicrobial activity against a range of bacteria, including antibiotic-resistant strains. It can inhibit the growth of pathogens and interfere with their virulence factors (Nayaka et al., 2014).

❖ Recently, it was reported that methanol extracts from *C. mucronatum* leaves rich in **tannins** exhibited antibacterial properties against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bacillus subtilis* (Kisseih et al., 2015). Also, <u>hydrolyzable tannins</u> are known to exhibit antimicrobial activity, for instance, *Mango kernel* extracts contain hydrolyzable tannins and have been demonstrated to inhibit the growth of various bacterial species including foodborne pathogens (Kabuki, 2000). <u>Epigallocatechin gallate</u> ability to augment the antistaphyloccocal activity of antibiotics has been widely tested and it was indicated that the compound shows synergistic effect with including *β*-lactams against methicillin-resistant Staphylococcus aureus strains (Zhao et al., 2002).

Chapter 3 – Pharmaceutical nanotechnology

Nanotechnology is a branch of science and engineering devoted to manipulate atoms and <u>molecules</u> at <u>nanoscale</u> i.e. 100 millionth of a millimeter or less (Sharma, 2012). Nanotechnology represents the convergence of the understanding of fundamental physics, chemistry, biology and technology of nanometer-scale objects. This modern tool creates intelligent materials, structures, devices and systems with entirely new properties with significantly improved physical, chemical, and biological properties. These advancements unlock new phenomena and processes in a wild range of scientific explorations including pharmaceutical research and industry. Thus, over 50 nano-pharmaceuticals are currently available in the market with a wide range of different nano-formulations (Germain et al., 2020).

I. Pharmaceutical nanotechnology – Non-metallic types of nanoparticles

In pharmaceutical nanotechnology research, the active pharmaceutical ingredient (API) can be entrapped, encapsulated, dissolved, or linked to the nanoparticle matrix resulting in so-called API nanoparticles. These latter have been proven to be useful as drug delivery vehicles with a major purpose to control particle size, surface properties and API release at site-targeted drug activity at an appropriate therapeutic rate. Accordingly arose application of nanoparticles drug delivery in gene therapy, cancer therapy, AIDS therapy, and radiation with reduced toxic side effects, controlling biodistribution and accelerating effects or reactions (Mazayen et al., 2022). Correspondingly, the research in pharmaceutical nanotechnology has nowadays a growing focus on expanding the range of medicines currently under clinical studies.

Over the last three decades, nanotechnology has been considered one of the most important and emerging fields of modern science resulting in the fabrication of many different types of nano systems. These latter differ from each other based on their different physiochemical properties, chemical structures, the starting materials and the selected method of preparation. A few examples of non-metallic nanostructures are summarized below.

I.1 Nanoemulsions (Nazzal et al., 2002)

Nanoemulsions (**Figure 23**) are non-homogenous emulsions with droplet sizes between 20 and 500 nm systems made up of immiscible liquids. Nanoemulsions and self-nanoemulsified drug delivery systems (SNEDDS) increase the bioavailability of medicines of low aqueous solubility. When integrated into aqueous phases under mild mixing, SNEDDS are isotropic mixes of oil, surfactant, co-surfactant and drug that produce nanoemulsions.
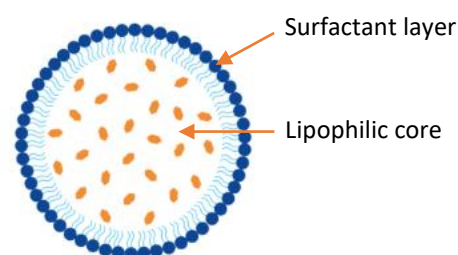
Figure 23: General structure of a nanoemulsion

Furthermore, the small size of the droplets reduces the surface tension between the oil droplets and the aqueous medium of the gastrointestinal tract, allowing for more uniform and widespread drug distribution in the gut. Nanoemulsions are commonly used in development of pharmaceutical formulations for topical, ocular, and intravenous administrations; moreover, they have been proved as excellent drug vehicle systems for drug local targeting through skin becoming of vital importance in skin infections, skin cancer, and psoriasis.

I.2 Liposomes

Liposomes are one of the nanosystems that have been the most extensively explored due to their biocompatibility, stability, biodegradability, ease to synthesize and high drug loading efficacy (Nsairat et al., 2022). They are spherical or multilayered spherical phospholipid vesicles and range in size from 50 nm to 500 nm as presented in **Figure 24**.
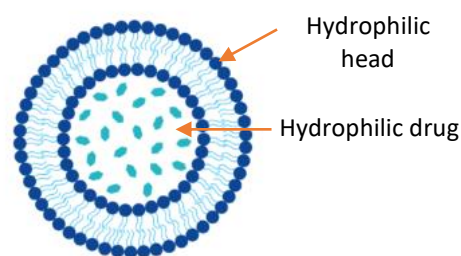
Figure 24: General structure of a liposome

Liposomes can be made from both natural and synthetic phospholipids and are the most often utilized as drug vehicles in clinical trials to treat cancer, fungal and viral infections thanks to their good pharmacokinetic characteristics. Recently, COVID-19 mRNA based-vaccines using liposomes protection to increase their *in vivo* stability have been designed by Pfizer/BioNTech and Moderna, and were administered worldwide (Gregoriadis, 2021).

I.3 Polymeric micelles (Mazayen et al., 2022)

Polymeric micelles are core–shell structures made up of a center of lipophilic blocks that is stabilized by a corona of lipophilic polymeric chains and range in size from 10 nm to 1000 nm. This particular structure make them able to hold hydrophobic drugs inside the core and hydrophilic bioactive molecules outside shell of polymeric micelles (**Figure 25**).
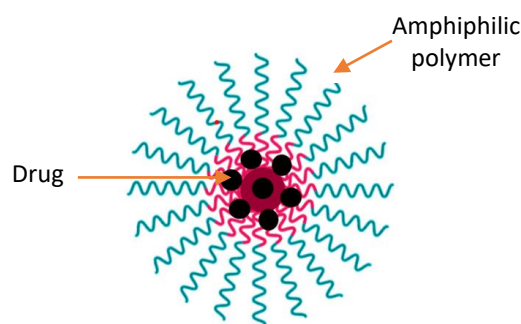
Figure 25: General structure of a micelle

As a medication carrier, a micellar system has a major advantage over conventional systems in promoting poorly water-soluble drug solubility and increase their bioavailability that resulted in approval of the first micellar paclitaxel preparation for the treatment of cancer (Socinski et al., 2012).

I.4 Nanocapsules

Nanospheres are typically solid polymers with drugs embedded in the polymer matrix whereas nanocapsules consist of a core and a protective shell with an inner space loaded with the drug of interest (**Figure 26**) (Mora-Huertas et al., 2010).
They range from 10 nm to 1000 nm and the drug might be dissolved, entrapped, or encapsulated.
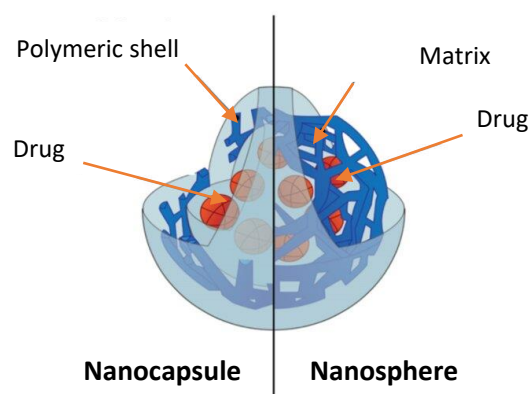
Figure 26: General structure of a nanocapsule and nanosphere

The substantial interest in the nanocapsules comes from their capacity to be used, for the controlled release and targeting of drugs against the protection of enzymes, proteins, and foreign cells. Thereby, this nanocapsules with enhanced drug selectivity, improved drug bioavailability and alleviates drug toxicity, made them an excellent alternative for cancer therapy and other applications (Kayser et al., 2005).

I.5 Dendrimers (Huang & Wu, 2018)

Dendrimers are a particular category of polymers, tree-like structures (**Figure 27**) with a controllable size (less than 10 nm) and shape. They contain three different regions: core moiety, branching units and surface and their spherical branching creates voids that can be benefited for drug entrapment and deliver. The free ends of dendrimers can be conjugated to other molecules.
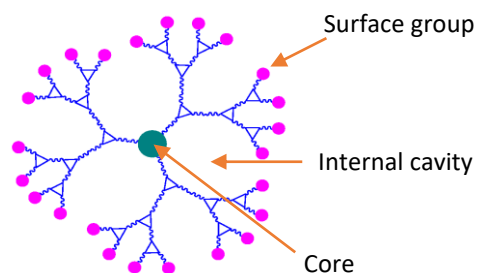
Figure 27: General structure of a dendrimer

When comparing dendrimers with other nanosystems, these latter have a limited structural diversity, thus dendrimers are advanced in terms of surface functionalization and stability. As such, it was shown that they enhance the delivery of medicines and vaccinations to specific tissues (Pedziwiatr-Werbicka et al., 2018).

I.6 Polymeric nanoparticles (Bhatia, 2016)

Polymeric nanoparticles are synthetic solid and colloidal polymers with sizes ranging from 10 to100 nm and are generally spontaneous self-assembly structures in which drugs are entrapped within the main core. They are formed of an API that is within/adsorbed on macromolecular substances (polymer) as shown in **Figure 28**.
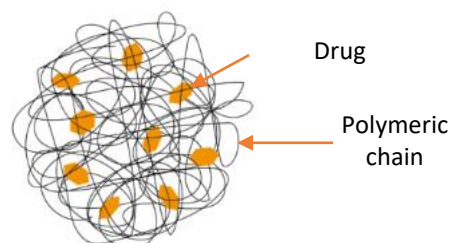
Figure 28: General structure of a Polymeric nanoparticle

Polymeric nanoparticles are manufactured through both natural (like albumin, collagen, gelatin, etc.) and synthetic polymers (such as polyglycolic copolymers, etc.). Polymeric nanoparticles are able to deliver drugs to the site of action with minimum toxic levels, they hydrolyze inside the body to biodegradable lactic acid and glycolic acid. These particularities lead polymeric nanoparticles to be ideal candidates to control many cancer therapies that suffer from cytotoxicity effects and allow sustained release of small doses (Danhier et al., 2012).

II.Pharmaceutical nanotechnology – Organometallic types of nanoparticles

There was a huge growing fascination and development of inorganic nanoparticles during the past 20 years. These nanoparticles, made of metals, metal oxides or metal-based compounds are formed based on the use of an organometallic precursor which is capable to decompose naturally or in the presence of a reducing agent.

Organometallic chemistry offers significant advantages in the realm of nanoparticle synthesis and characterization. By allowing precise control over reaction conditions, organometallic chemistry enables researchers to tailor the surface characteristics of particles with great accuracy. Through this approach, oxidation can be minimized, ensuring the stability and integrity of the nanoparticles (Campeau & Fogg, 2019). Another notable advantage of organometallic chemistry lies in its ability to regulate surface reactivity and facilitate the gradual growth of clusters (Kahn et al., 2009). This controlled growth mechanism ensures the formation of well-defined and homogeneous nanoparticles. Finally, all the above cited advantages of organometallic nanoparticles have shown great potential in overcoming the limitations of conventional drug delivery systems such as poor bioavailability, low solubility, and nonspecific targeting (Patra et al., 2018).

II.1 Traditional methods for the synthesis of organometallic nanoparticles

II.1.1 Chemical reduction method (Jamkhande et al., 2019)

The chemical reduction is a low cost method and is typically used for the preparation of magnetic metal nanoparticles (iron, cobalt, nickel nanoparticles, etc.). In this method, the organometallic precursor salt is reduced to its elemental state in the presence of a reducing agent. Some of the reducing agents used are: sodium borohydride, sodium citrate, dextrose, ascorbic acid, plant extracts, etc. Also, the stabilizing agents are indispensable in the fabrication of nanoparticles in order to increase their biomedical functionality by reducing their toxicity and enhancing their biocompatibility and bioavailability. This method present some restrictions concerning the scaling-up for industrial applications, the use of toxic reducing agents harmful to the environment and human health and the limited control over particle size and shape.

II.1.2 Thermal decomposition method (Jamkhande et al., 2019)

The thermal decomposition method produces nanoparticles with a narrow size distribution and good crystal quality when compared with other methods. The method is also clearer and more economical with very simple synthetic protocol and no additives thus involving minimize costs. The thermal decomposition method involves the heating of the precursor until the decomposition of its chemical bonds allowing the nanoparticles to form. However, this method present some limitations such as the difficult to control the properties of the final product (lack of reproducibility) and the required high temperatures, which can lead to the formation of impurities and defects in the produced nanoparticles.

II.1.3 Electrochemical method (Jamkhande et al., 2019)

The electrochemical method is an efficient and versatile way to synthesize nanoparticles with different shapes and sizes. Electrochemical methods utilize an electrode to reduce the organometallic precursor, leading to the formation of nanoparticles. Electrochemical deposition occurs at the interface of an electrolyte solution containing the metal to be deposited and an electrically conductive metal substrate. The most popular solutions used as electrolytes are phosphoric acid and a combination of citric acid and alkali hydroxide in water. Various nanostructures can be easily fabricated with advantages of low synthetic temperature, high purity, simplicity, and environmental friendliness.

II.2 Modern methods for the synthesis of organometallic nanoparticles

While traditional methods have been widely used, they do have limitations. These include low yield, poor size control, and difficulties in scaling up the synthesis process. To address these limitations, new methods have been developed to enhance the preparation of organometallic nanoparticles. Some of these methods are presented hereafter.

II.2.1 Microwave-assisted method (Thamima & Karuppuchamy, 2015)

The microwave irradiation is a green and effective method used to accelerate the nanoparticle synthesis process with exact control over particle size and morphology. This method is used for the preparation of metallic, zinc oxide, silver, lead sulfite etc. nanoparticles. The microwave irradiation relies on its penetration characteristic which depends on the absolute permittivity (ε) of the selected solvent that makes possible to homogeneously heat up the reaction solution. This controlled heating result is uniform nucleation and rapid crystal growth and thus to the formation of nanoparticles that have a narrow size distribution and an improved yield. Moreover, the microwave-assisted method can reduce the agglomeration of nanoparticles thanks to the faster reaction kinetics. Finally, microwave-assisted synthesis is more environmentally friendly method compared to other synthesis methods as it can reduce the use of toxic chemicals, produce less waste and diminish the electricity use.

II.2.2 Ultrasound-assisted method (Thamima & Karuppuchamy, 2015)

Ultrasound-assisted method or sono-chemistry involves the use of ultrasound waves to induce chemical reactions. Ultrasonic waves permit the formation, growth, and implosive breakdown of bubbles in a liquid. This creates localized high-pressure zones with increased temperature which promote the formation of nanoparticles. Ultrasound-assisted method is appropriate for the synthesis of inorganic nanoparticles including gold, iron oxide and zinc oxide nanoparticles. This method is eco-friendly and cost-effective because it does not require the use of any chemical or sophisticated equipment. Also, it can be used to synthesize nanoparticles with specific shapes and sizes, good morphology and high-quality crystal structures.

II.2.3 Green synthesis (Ying et al., 2022)

Green synthesis focuses on environmentally friendly and cost-effective approach to nanoparticle preparation. This method emphasizes the use of sustainable and non-toxic materials, solvents and reducing agents. At present, green synthesis mainly uses microorganisms (fungal, bacteria, and algae) or plant extracts. The phenolic compounds and proteins present in the green materials replace usual chemical reagents as reducing agents to reduce metal ions into lower valence state. Such method permits the production of biocompatible nanoparticles that are suitable for biomedical applications. However green synthesis method, for now, does not assure the production of nanoparticles with consistent particle size, morphology and reproducibility. Overall, green synthesis of nanoparticles is still at its early stages but represents a promising approach for the production of eco-friendly biocompatible nanoparticles.

II.3 Silver nanoparticles

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care and industrial purposes, due to their unique physical and chemical properties. AgNPs have been proved to exert several biological properties that include antibacterial in industrial and healthcare-related products, anticancer agents with improved tumor-killing effects of anticancer drugs, burn treatment, wound dressings, medical device coatings, optical sensors, orthopedics as bone implants, drug delivery, etc. (Chernousova & Epple, 2013).

In order to synthetize AgNPs, several methods have been adopted. The most popular method being the chemical approaches by means of chemical reduction using a variety of organic and inorganic reducing agents in the presence of a stabilizing agent. However, many of them may be associated with potential environmental toxicity as well as biological risks (Zaheer et al., 2011). Interestingly, biologically-prepared AgNPs with green approaches show high yield, solubility, and high stability with well-defined size and morphology under optimized conditions. Therefore, the synthesis of silver nanomaterials using biological methods is a reliable approach, more environmentally friendly than chemical expensive and hazardous methods. However, it is important to highlight the main disadvantage of the synthesis of silver nanomaterials using biological methods which is the very long time consumption. This limitation was circumvented by introducing microwave chemistry for the synthesis of AgNPs. This last decade, microwave-assisted method was used for the green synthesis of AgNPs using crude plant extracts, viruses, fungi, etc. Some of these studies are summarized in **Table 4**.

Table 4. The use of microwaves in the preparation of silver nanoparticles

Method	Description	Size range	Reference
Microwave-assisted synthesis	Synthesis of silver nanoparticles using sodium alginate as stabilizer and reducer	5-50 nm	(Zhao et al., 2014)
Microwave-assisted polyol method	Synthesis of silver nanoparticles based on a polyol process and variable frequencies	10-50 nm	(Jiang et al., 2006)
Microwave-assisted polyol reduction method	Synthesis of silver nanoparticles in an aqueous medium using bamboo hemicelluloses as stabilizer and glucose as reducer	8.3-14.8 nm	(Peng et al., 2013)
Microwave-assisted method	Synthesis of silver nanoparticles using aqueous peel extracts of *Citrus paradisi* as a reducing, stabilizing and capping agent	14.84 ± 5 nm	(Ayinde et al., 2019)

Based on all the above cited advantages of the microwave chemistry combined with the unique remarkable properties of silver nanomaterials, the microwave-assisted irradiation for the synthesis of silver nanoparticles was selected for our research work.

III. Characterization of the nanoparticles

After synthesis, nanosystems' precise particle characterization is compulsory, because the physicochemical properties of a particle has a significant impact on its physico-chemical properties and biological activities. The characteristic feature of nanomaterials, such as size,

shape, size distribution, surface area, shape, solubility, aggregation, etc. are thus evaluated. Additionally, the surface atoms of nanoparticles play a significant role in determining their chemical and physical properties, making it necessary to control the surface species. To do so, a diverse measurement techniques exist to assign precise numerical values to those parameters.

III.1 Ultraviolet-visible spectroscopy (Hassellöv et al., 2008)

Ultraviolet-visible spectroscopy, commonly known as UV-Vis spectroscopy, is a non-destructive indispensable analytical technique utilized across various scientific disciplines, including chemistry, biochemistry, and materials science. By examining the absorption and transmission of light within the ultraviolet and visible regions of the electromagnetic spectrum, this powerful method enables scientists to glean crucial insights into molecular structure, concentration, and purity.

Nanoparticles have optical properties related to their size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV/Vis spectroscopy a valuable tool for identifying, characterizing, and studying these materials.

III.2 Fourier Transform Infrared (FTIR) spectroscopy (Adebayo et al., 2020)

Fourier Transform Infrared (FTIR) spectroscopy, a renowned analytical technique, plays a crucial role in studying molecular vibrations, thereby aiding in the identification of functional groups in various compounds. The vibration and rotation of molecules influenced by infrared radiation at a particular wavelength is measured using FTIR. By measuring the absorption of infrared radiation, FTIR spectroscopy effectively enables the *in-situ* analysis of interfaces and the composition of a given sample.

This technique finds significance in the analysis of nanoparticles and the study of organometallic compounds, making it an indispensable tool in modern scientific research. Thus, the molecular data obtained allows to establish structural and conformational changes of the self-assembled functional groups on the surface of synthesized nanoparticles.

III.3 Transmission electron microscopy and scanning electron microscopy

Electron microscopy has been a revolutionary imaging technology for scientists and engineers over the past 80 years, opening up the world of nanoscale materials and enabling characterization of their unique properties (Inkson, 2016).

❖ **Transmission electron microscopy (TEM)** is a quantitative imaging technique that uses a beam of electrons to create high-resolution images of thin samples, such as biological tissues, cells, and nanoparticles. In TEM, the electrons pass through the sample and interact with the atoms, which scatter the electrons in different directions. The scattered electrons are then detected and used to create an image of the sample. TEM is capable of producing 2D images with a resolution of a few picometers and thus to directly measure nanoparticle size, grain size, size distribution, and morphology. This makes it an essential tool for studying the distribution, the morphology and dispersion of nanoparticles in polymer matrices.

❖ **Scanning electron microscopy (SEM)** is another powerful imaging technique that uses a beam of electrons to create high-resolution images of surfaces. In SEM, the electron

beam is focused onto the sample and scans across it, creating a three-dimensional image of the surface. SEM is capable of producing 3D images with a resolution of a few nanometers, this makes it an essential tool for studying the surface morphology of materials, such as metals, ceramics, and polymers. In comparing both methods, TEM is used to study the internal structure of a sample, while SEM is used to study the surface of a sample and TEM provides higher magnification and resolution than SEM, but requires thin samples.

III.4 Dynamic light scattering (Jana et al., 2007)

Dynamic light scattering (DLS) instruments utilize a highly efficient analytical technique to precisely measure the particle size distribution of various substances. By illuminating a sample with a laser beam, these advanced instruments allow scientists and researchers to analyze the scattered light and determine the particle size range and distribution.

This invaluable technique finds extensive application in nanotechnology. Polymer coating layers on metallic cores can be observed and measured by microscopy techniques; however, therefore, the sizes measured correspond only to the metallic cores, whereas DLS provide the total size of the metallic core and the coating layer.

III.5 X-ray diffraction (XRD) (Epp, 2016)

X-ray diffraction (XRD) is an analytical technique widely used in materials science, geology, and solid-state physics for studying the composition, phase transitions, and crystallography of various materials. By analyzing the resulting diffraction pattern, XRD can determine critical information about the crystal lattice arrangement and other properties of the material under investigation. Its ability to provide detailed information about the atomic and molecular structure of materials makes it particularly useful in the field of nanotechnology.

XRD finds utility in the field of organometallic nanochemistry where it helps in determining the crystal structures facilitating the understanding of their fundamental properties and aiding in the design of new organometallic catalysts.

III.6 Zeta potential (ZP) (Kaszuba et al., 2010)

The Zeta potential (ZP) refers to the potential observed at the slipping/shear plane when a colloid particle is in motion under an electric field. The electric potential of a surface is a fundamental concept in electrochemistry that refers to the amount of work that is required to transport a unit positive charge from an infinite distance to the surface without any acceleration. This concept is critical in understanding the behavior of charged particles in various systems. The ZP is a related concept that reflects the potential difference between the EDL of electrophoretically mobile particles and the layer of dispersant surrounding them at the slipping plane. This difference in electric potential is a critical factor in determining the stability and behavior of colloidal systems, which are ubiquitous in many natural and industrial processes.

Zeta potential is an important parameter for nanoparticles as it determines their colloidal stability. The zeta potential can be used to optimize nanoparticle formulation, resulting in more effective formulation development for suspensions, emulsions, or nanoparticle dispersions.

Chapter 4 – Material and Methods

I. Material

I.1 Plant Material

The plant material used in our study consisted of a single sample of the plant *Euphorbia Guyoniana*. The mature aerial part of the plant, including the stems, buds, leaves, and flowers, was collected in Touggourt during the period from February 20, 2022, to March 10, 2022. The botanical identification of the species was carried out at the Botany Laboratory of the National Higher School of Agronomy of El-Harrach (Algeria).

The plant material was first cleaned of dust and roots, and then air-dried until a constant mass of the test portion was obtained. The dried plant was ground into a fine powder using an industrial rotor grinder (**Figure 29**). This powder was stored in light- and moisture-proof containers that were tightly sealed prior to the start of extractions.

Figure 29: Grinding of the dried plant material

I.2 Chemical products

Silver nitrate (AgNO$_3$) was purchased from Sigma Aldrich (Saint Louis, USA).

II. Extract preparation

The extraction purpose was to recover the maximum amount of phenolic compounds present in the aerial part of *Euphorbia Guyoniana* plant.

The extraction method used in this study was a Microwave-Assisted Extraction (MAE) method. It is an extractive technique that uses microwave energy to help transfer plant metabolites from the plant matrix into a solvent. It is a non-conventional extraction method that has been introduced in the last decade and is an efficient and modern tool with multiple benefits compared to traditional methods of extraction. The benefits of MAE include a reduction in cost, time of extraction, amount of solvent used and energy consumption. This technique enables the rapid heating of the extractive solvent, in our case water, which, through conduction, will heat the plant material, facilitating the penetration of the solvent into the plant matrix and allowing a more efficient extraction.

The extraction was performed using the dried aerial part of *Euphorbia Guyoniana* plant material, employing a microwave oven operating at a frequency of 2450 kHz and an output power set to 600W (**Figure 30**).

Extractions were carried out using a setup consisting of a flat-bottom flask loaded with 5g of plant material and supplemented with a volume of 100 ml of water (a plant matrix to solvent ratio of 1:20). The flask was connected to a round-ball condenser.

After 20 minutes of irradiation, the mixture was vacuum filtered using a Büchner funnel with a 4µm diameter, and then the solvent was evaporated by drying in an oven at a temperature of 40°C for 24 hours.

The extract was stored at 4°C for subsequent use.
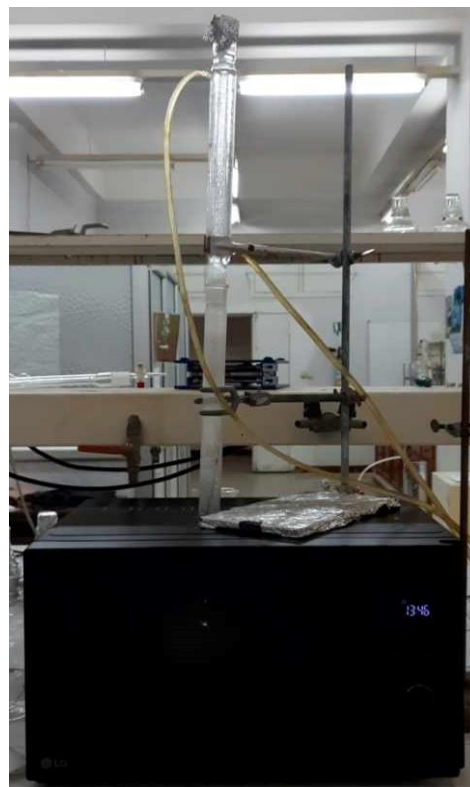
Figure 30: Setup of Microwave-Assisted Extraction (MAE)

The extraction yield is defined as the ratio between the mass of the dried extract obtained after evaporation and the initial mass of the plant material used. The recovery yield of *Euphorbia Guyoniana* was calculated using the following equation

$$\% \, Yield \, of \, extract = \frac{Mass \, of \, dried \, extract \, (g)}{Mass \, of \, plant \, powder \, (g)} * 100$$

III. Analysis of the extract by LC/MS

The analysis of the extract using liquid chromatography coupled with mass spectrometry (LC/MS) was conducted at the laboratory of Associate professor Attila Hunyadi, Institute of Pharmacognosy, University of Szeged, H-6720 Szeged, Hungary.

Phenolic compounds profile was realized by the sample that was analyzed using a Waters 2695 (Milford, MA) HPLC system composed of LC30AD pump, DGU-20AR 5R vacuum degasser, SIL-30AC auto sampler, CTO-20AC column oven, and SPD-M20A diode array detector coupled to a Waters ACQUITY QDA equipped with an electrospray ionization source (ESI). Separation of phenolic compounds was carried out on Phenomenex (Torrance, CA, USA) Kinetex 5µm XB-C18A 100 Å 250X4,6mm, both set at 30 °C, using a binary mobile phase composed of acidified water (1% v/v acetic acid) and methanol (B). The following gradient program was applied at a flow rate of 1 mL.min−1: 0–1 min 95% A; 1–55 min 95% B; 55–57 min 95% A; 57–65 min 95% A. UV-detection was performed from 200 to 400 nm and MS analysis was carried out using the following settings: at positive ion mode voltage of

0.8kV, nebulizer gas (N$_2$) flow: 3.0L min^{-1}, drying gas flow: 15L min^{-1}, desolvation line temperature 250 °C and heat block temperature 400°C. Full Scan mode (m/z 100–2000) was used for qualitative analysis.

Figure 31: A photograph of the LC/MS instrument used (Hungary)

IV. Synthesis of nanoparticles

IV.1 General procedure

The silver nanoparticles were synthesized using an environmentally friendly green process involving a natural aqueous extract and silver nitrates. The system was heated using microwaves (**Figure 32**).

In the first step, different solutions of extract and silver nitrates were prepared in distilled water. The solutions were mixed in equal proportions, with 25ml of extract solution combined with 25ml of silver nitrate solution.

The mixtures were heated in a microwave reactor, and for each test, the heating power and reaction time were controlled.
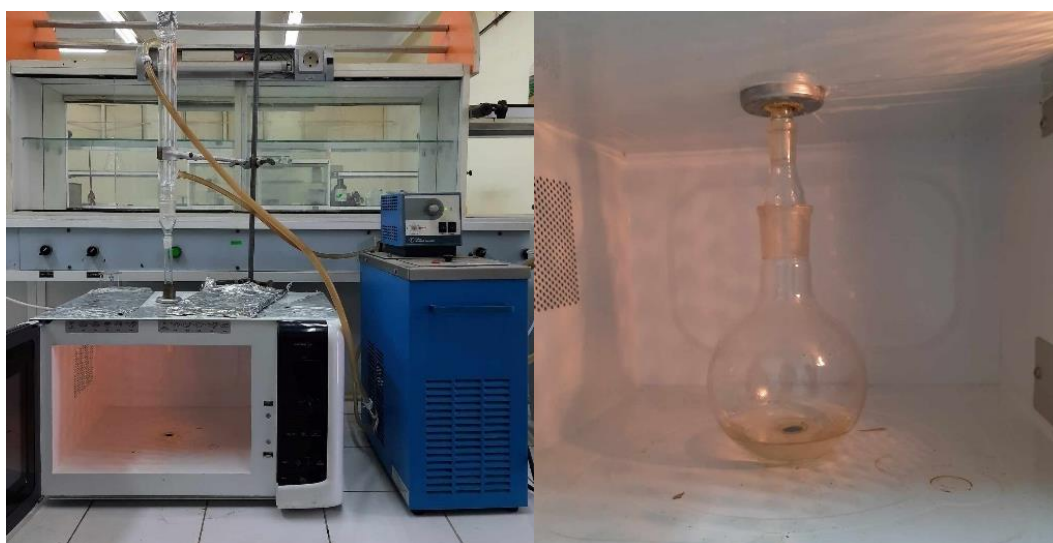
Figure 32: Setup for microwave-assisted synthesis (left) and synthesis reactor inside the microwave (right)

IV.2 Process optimization

The nanoparticle preparation process was optimized using response surface methodology (RSM) of experimental design plans. The response surface methodology is a mathematical and statistical technique that uses quantitative data from experimental design to demonstrate the correlation between extraction parameters and their responses at different optimal levels. It also evaluates multivariate equations by fitting functional relationships between factors and response values. RSM is a powerful tool for designing experiments, analyzing experimental data, and optimizing the effect process variables. It reduces the number of trials and recognizes the influence of process parameters on the removal process.

For this purpose, a face-centered composite experimental design (FCCD) with four factors, two levels and a central level, and three center points was employed, resulting in a total of 27 experimental tests.

The central composite design is a widely used statistical approach for optimization. It allows for the exploration of the factors' effects and interactions while minimizing the number of experiments required. By varying the levels of the four factors in the design, the response of the system can be evaluated, and the optimal conditions can be determined.

IV.2.1 Factors studied and levels

In order to explore the effect of independent variables in the research margin, a central composite design with uniform precision with four independent variables was executed. The four factors investigated during the optimization of this process were:

- X_1: Microwave heating power (W)
- X_2: Synthesis time (s)
- X_3: Concentration of the extract (%)
- X_4: Concentration of silver nitrate (mM)

The levels for each factor are provided in the table below.

Table 5: Description of the studied factors and their levels

Factors		Levels		
Code		-1	0	+1
X_1	Microwave heating power (W)	350	500	650
X_2	Synthesis time (s)	30	120	210
X_3	Concentration of the extract (%)	0.5	1	1.5
X_4	Concentration of silver nitrate (mM)	0.5	1	1.5

IV.2.2 Experimental matrix

A central composite face-centered experimental design was chosen in the present study to model and optimize and to analyze the effect of each variable, their interactions and second-order terms. It is generated by combining a two-level full factorial design with axial experiments requiring a number of experiments equal to $N = L^k + 2*k + Nc$ where L represents the number of levels for the investigation (two in our case), k represents the number of process variables or factors (four in our case) and N_c is the number of central experiments

leading to $N = 2^4 + 2*4 + 3 = 27$ experiments. The operational conditions for each experiment are provided in the table below:

Table 6: Experimental matrix

N°	Coded values				Experimental values			
	X_1	X_2	X_3	X_4	X_1	X_2	X_3 [Extract]	X_4 [AgNO$_3$]
1	+1	+1	+1	+1	650	210	1.5	1.5
2	-1	+1	+1	+1	350	210	1.5	1.5
3	+1	-1	+1	+1	650	30	1.5	1.5
4	-1	-1	+1	+1	350	30	1.5	1.5
5	+1	+1	-1	+1	650	210	0.5	1.5
6	-1	+1	-1	+1	350	210	0.5	1.5
7	+1	-1	-1	+1	650	30	0.5	1.5
8	-1	-1	-1	+1	350	30	0.5	1.5
9	+1	+1	+1	-1	650	210	1.5	0.5
10	-1	+1	+1	-1	350	210	1.5	0.5
11	+1	-1	+1	-1	650	30	1.5	0.5
12	-1	-1	+1	-1	350	30	1.5	0.5
13	+1	+1	-1	-1	650	210	0.5	0.5
14	-1	+1	-1	-1	350	210	0.5	0.5
15	+1	-1	-1	-1	650	30	0.5	0.5
16	-1	-1	-1	-1	350	30	0.5	0.5
17	+1	0	0	0	650	120	1	1
18	-1	0	0	0	350	120	1	1
19	0	+1	0	0	500	210	1	1
20	0	-1	0	0	500	30	1	1
21	0	0	+1	0	500	120	1.5	1
22	0	0	-1	0	500	120	0.5	1
23	0	0	0	+1	500	120	1	1.5
24	0	0	0	-1	500	120	1	0.5
25	0	0	0	0	500	120	1	1
26	0	0	0	0	500	120	1	1
27	0	0	0	0	500	120	1	1

IV.2.3 Studied responses and data analysis

In the present study, two different responses were optimized:

R_1: Average diameter (nm).

R_2: Polydispersity index.

The experimental results obtained for these responses are input into the MODDE6 software for further analysis. This software allows for studying the effects of each factor and their

interactions on the different responses. By utilizing this computational tool, the present process can be modeled, and predictions can be made for the studied responses.

The statistical analysis will provide valuable information, such as the coefficient of determination (R^2), prediction capability (Q^2), and reproducibility of the obtained results.

V. Characterization of Nanoparticles

V.1 Particle Size Analysis by DLS (Dynamic Light Scattering)

The particle size analysis for all obtained nanoparticles was performed using a laser particle size analyzer HORIBA Nanopartica SZ-100 type. The analysis allowed us to determine the particle size distribution for each sample and derive two very important parameters, namely the mean diameter and polydispersity index.

Before the analysis, each sample was diluted (1/100) in distilled water. The analysis was performed in a glass cuvette (1x1x5 cm) at 25°C.

V.2 Zeta Potential Measurement

The electrophoretic mobility for each sample was measured using the Zeta potential function of HORIBA Nanopartica SZ-100 equipment, and the value of zeta potential was automatically determined by the device's software. Before the analysis, each sample was diluted (1/100) in distilled water. The analysis was performed in a cell with gold electrodes at 25°C.

VI. Biological Activity (Theoretical Approach)

This part of the study is considered the main perspective of the current experimental work. Due to time constraints, we were unable to conduct this part, but we have decided to discuss it as it represents the final application for our new active principle.

The chosen cutaneous pathology after our extensive and comprehensive literature review on dermatological diseases is dermatophytosis. This pathology is caused by an infection from fungi belonging to the family of dermatophytes.

The anti-dermatophytic activity can be studied *in vitro* through microbial culture on Sabouraud dextrose agar, or *in vivo* by inoculating the fungus on the skin surface of mice. Among the most studied dermatophytes is *Microsporum canis*.

The anti-dermatophytic activity of volatile and non-volatile extracts of plants has been extensively studied, but so far, very few studies have been conducted on nanoparticles. A thorough literature search has been carried out on the treatment of dermatophytosis using nanoparticles, and a brief summary of this theoretical work will be presented in results and discussion section.

Chapter 5 – Results and discussion

I. Preparation of the extract

 The extract prepared using the MAE method exhibited a light brown color after filtration, which darkened further upon drying (**Figure 33**).

The extract also possessed a characteristic odor of the studied plant *Euphorbia Guyoniana*. After drying, the extract yielded a solid, friable, non-oily mass that readily dissolved in water at percentages of 1%, 2%, and 3% (W/W).

The application of the equation presented in section (Chapter 4 – Material and methods, II. Extract preparation) for the calculation of the extracted yield resulted in an extraction yield of approximately 19%.

Figure 33: Extract obtained after filtration (right) and after drying (left)

II. Chemical composition of the extract

 Determining the composition of the obtained extract is a crucial step for the continuation of the study, as the extracted organic molecules will serve as a system for the fixation of silver atoms present in silver nitrates. For this purpose, we employed a highly effective analysis method involving liquid phase chromatography for the separation of different molecules, along with mass spectrometry for the identification of the separated molecules. The chromatogram and mass spectra of the conducted analysis is provided in **Figure 34**.

 Analysis enabled to determine the number of compounds present in the extract, which amounted to approximately 18 compounds. Among these compounds, only 11 could be identified by comparing the results of mass spectrometry with the database of used device. The 11 identified compounds are listed in **Table 7**.
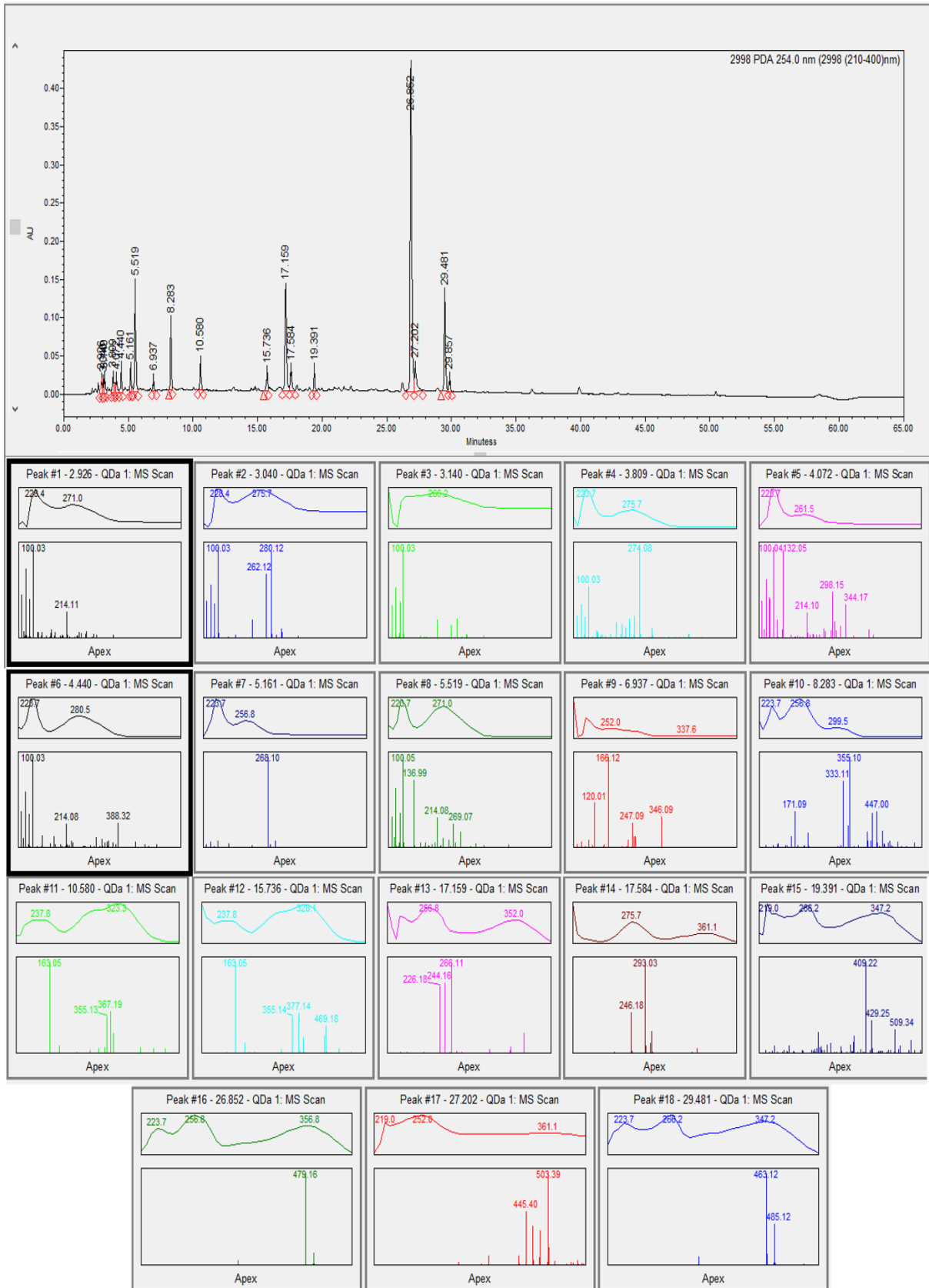
Figure 34: Chromatogram of LC/MS analysis for obtained extract and the different mass spectra for the 18 extract compounds

Table 7: Chemical composition of the extract

No.	Rt (min)	Compound formula	Tentative identification
1	2.926	-	N.I
2	3.040	$C_{18}H_{33}NO$	Linoleic acid amide
3	3.809	-	N.I
4	4.072	-	N.I
5	4.440	-	N.I
6	5.161	$C_{10}H_{13}N_5O_4$	Adenosine
7	5.519	-	N.I
8	6.937	-	N.I
9	8.283	$C_{20}H_{30}O_{11}$	Gallic acid derivative
10	10.580	$C_{13}H_{16}O_{10}$	Caffeoylquinic acid
11	15.736	$C_{20}H_{30}O_{11}$	2-phenylethyl glucopyranosy glucopyranoside
12	17.159	$C_{21}H_{20}O_{12}$	Quercetin glucoside
13	17.584	$C_{13}H_8O_8$	Brevifolin carboxylic acid
14	19.391	-	N.I
15	26.852	$C_{21}H_{18}O_{13}$	Quercetin glucuronide
16	27.202	$C_{21}H_{18}O_{12}$	Ellagic acid derivative
17	29.481	$C_{21}H_{18}O_{12}$	Kaempferol glucuronide
18	29.857	$C_{22}H_{22}O_{11}$	Kaempferol methyl ether glucopyranoside

Rt: retention time; NI: non-identified.

III. Preparation and characterization of nanoparticles

III.1. Results of the experimental plan

The preparation of nanoparticles was conducted using the methodology of experimental designs. Composite-centered designs were employed, which include a factorial design with central points, along with a group of star points that allow for estimating curvature. Their main advantage, compared to other existing designs, is the presence of star points that ensure an equal variance of prediction at equidistant points from the central point, thus expanding the application range of the study. In this regard, 27 experiments were performed, and the response matrix is provided in **Table 8**. Photographs of the 27 formulations are presented in **Figure 35**.
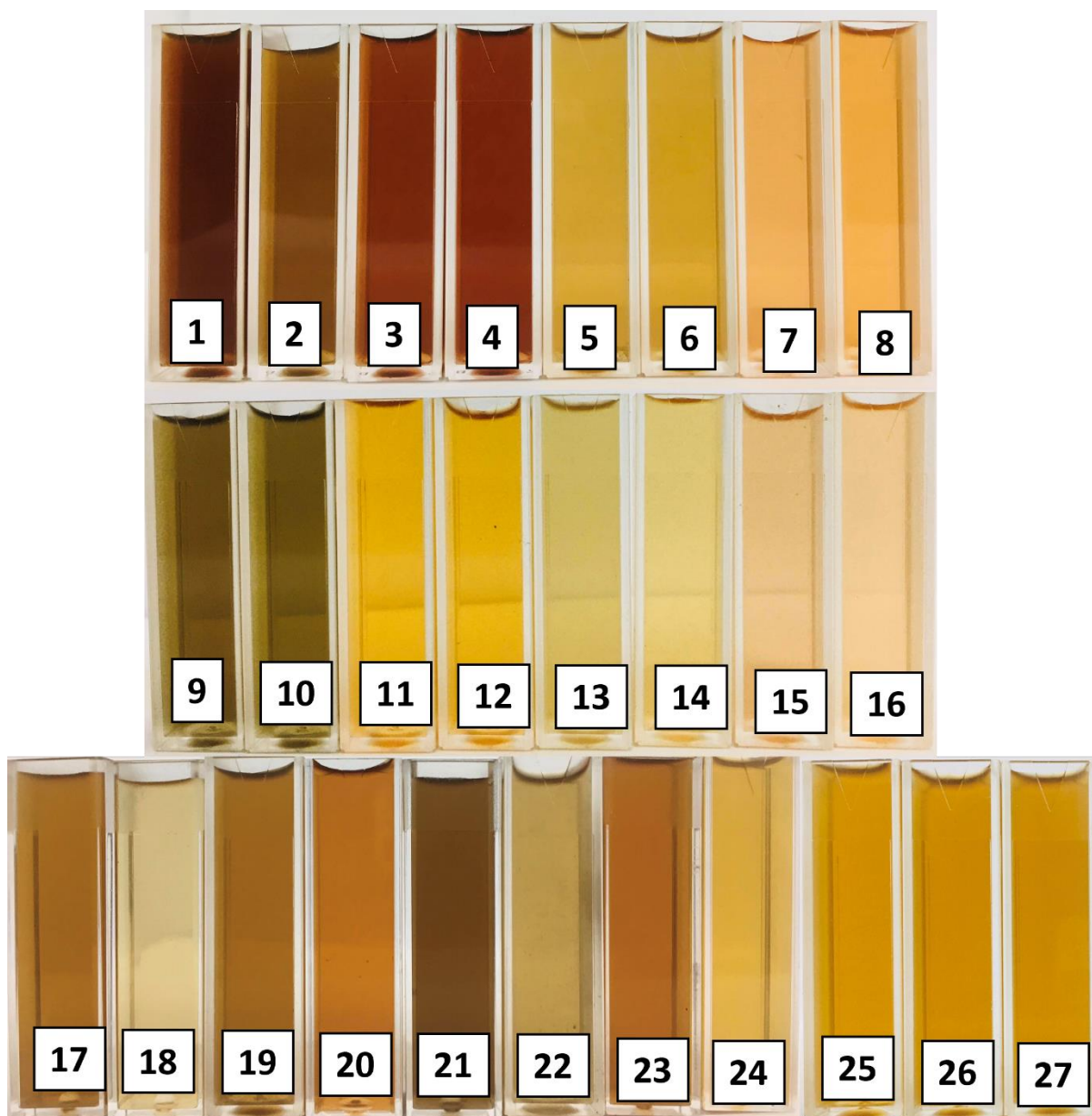
Figure 35: Photograph of the 27 synthesized nanoparticles

Based on the obtained results, the experiment randomly conducted at number 5 exhibited the highest polydispersity index. The optimal operating conditions are as follows:

- Microwave heating power = 650 W (niveau +1).
- Synthesis time = 210 s (niveau +1).
- Concentration of extract = 1.5 % (level 0).
- Concentration of silver nitrates = 1.5 mM (level 0).

Table 8: Response matrix

Exp No.	Exp Name	Run Order	Microwave heating power	Synthesis time	[Extract]	[AgNO₃]	Polydispersity index	Mean diameter (nm)
1	N1	12	350	30	0.5	0.5	0.946	58.5
2	N2	22	650	30	0.5	0.5	0.906	50.4
3	N3	17	350	210	0.5	0.5	0.716	82.5
4	N4	7	650	210	0.5	0.5	0.721	80.9
5	N5	23	350	30	1.5	0.5	0.845	71.6
6	N6	1	650	30	1.5	0.5	0.774	79.4
7	N7	20	350	210	1.5	0.5	0.788	85.8
8	N8	26	650	210	1.5	0.5	0.638	85.5
9	N9	19	350	30	0.5	1.5	0.448	109
10	N10	25	650	30	0.5	1.5	0.431	116
11	N11	21	350	210	0.5	1.5	0.363	217
12	N12	6	650	210	0.5	1.5	0.461	211
13	N13	8	350	30	1.5	1.5	0.642	150
14	N14	2	650	30	1.5	1.5	0.564	169
15	N15	14	350	210	1.5	1.5	0.375	268
16	N16	5	650	210	1.5	1.5	0.298	237
17	N17	18	350	120	1	1	0.648	133
18	N18	9	650	120	1	1	0.645	110
19	N19	27	500	30	1	1	0.85	71.9
20	N20	24	500	210	1	1	0.599	145.1
21	N21	16	500	120	0.5	1	0.47	74.7
22	N22	15	500	120	1.5	1	0.753	145.9
23	N23	10	500	120	1	0.5	0.973	56.6
24	N24	13	500	120	1	1.5	0.488	129.4
25	N25	3	500	120	1	1	0.659	127.7
26	N26	11	500	120	1	1	0.777	106.1
27	N27	4	500	120	1	1	0.86	144.8

The choice of the optimum was based on the polydispersity index rather than the mean diameter for two reasons:

- All the obtained nanoparticles exhibited a very small size, not exceeding 268 nm (with diameters ranging from 50.4 to 268 nm). Nanoparticles within this size range generally possess similar biological properties, such as their ability to penetrate living organisms or distribute within them.

- The polydispersity index exhibited a significant variation ranging from 0.298 to 0.973. This parameter holds great importance in the pharmaceutical field, both from a pharmacological perspective, particularly regarding distribution, and from a technological standpoint for the incorporation of the active ingredient into pharmaceutical formulations.

Indeed, having a homogeneous polydispersity index in the particle size distribution of pharmaceutical products is of great importance. It ensures a uniform particle size, optimal formulation efficacy, precise dosing, dissolution control, and reduction of adverse effects. These factors contribute to the quality, safety, and efficacy of medications for patients.

III.2 Effect of factors on the responses

The effect of studied factors and their interactions are shown in **Figure 36** for the polydispersity index and in **Figure 37** for the mean diameter.

III.2.1. Effect of factors on the polydispersity index

Based on the information provided, the factors of synthesis time (X_2), microwave heating power (X_1), and concentration of silver nitrates (X_4) have a negative influence on the polydispersity index, while the concentration of the extract (X_3) has a slight positive influence. Among these factors, the concentration of silver nitrates is the most influential, unlike the other factors.

The interactions between the factors also have a significant influence on the operation of the studied process. The interactions between extract/extract, power/power, and power/extract all have a negative influence. These three interactions exhibit the largest effects compared to the other interactions, which have smaller effects.
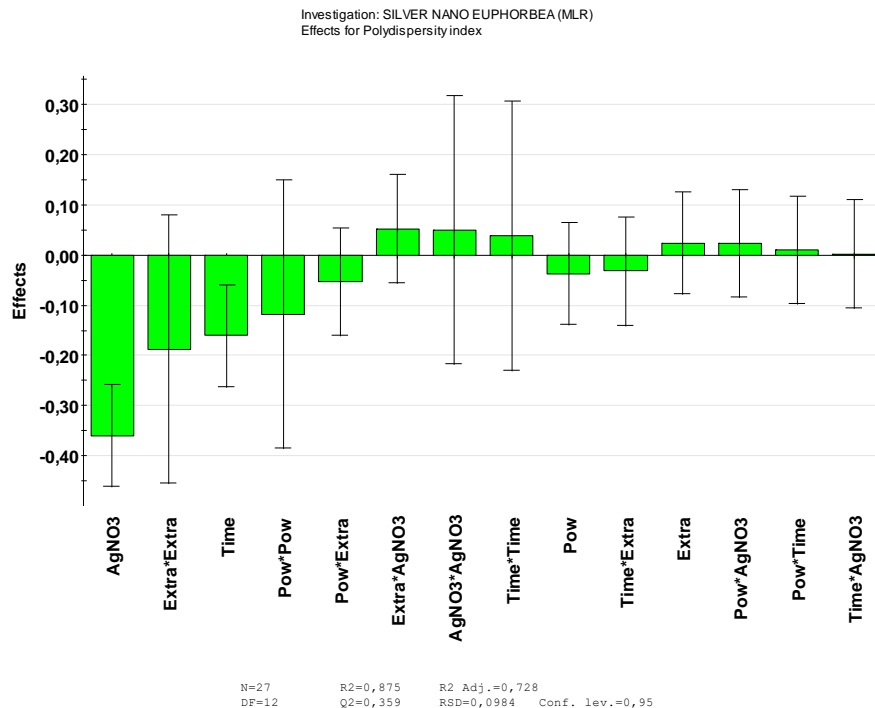
Figure 36: Effects of factors and their interactions on the

III.2.2 Effect of factors on the mean diameter

The factors of synthesis time (X_2), concentration of the extract (X_3), and concentration of silver nitrates (X_4) have a positive influence on the mean diameter, while the microwave heating power (X_1) has a slight negative influence. Among these factors, the concentration of silver nitrates is the most influential, unlike the other factors.

The interactions between the factors also have a significant influence on the operation of the studied process. The interactions between time/concentration of silver nitrates (positive influence), power/power (positive influence), concentration of silver nitrates/concentration of silver nitrates (negative influence), concentration of silver nitrates/concentration of the extract (positive influence), and concentration of the extract/concentration of the extract (positive influence) are the most influential, unlike the other interactions between the factors.
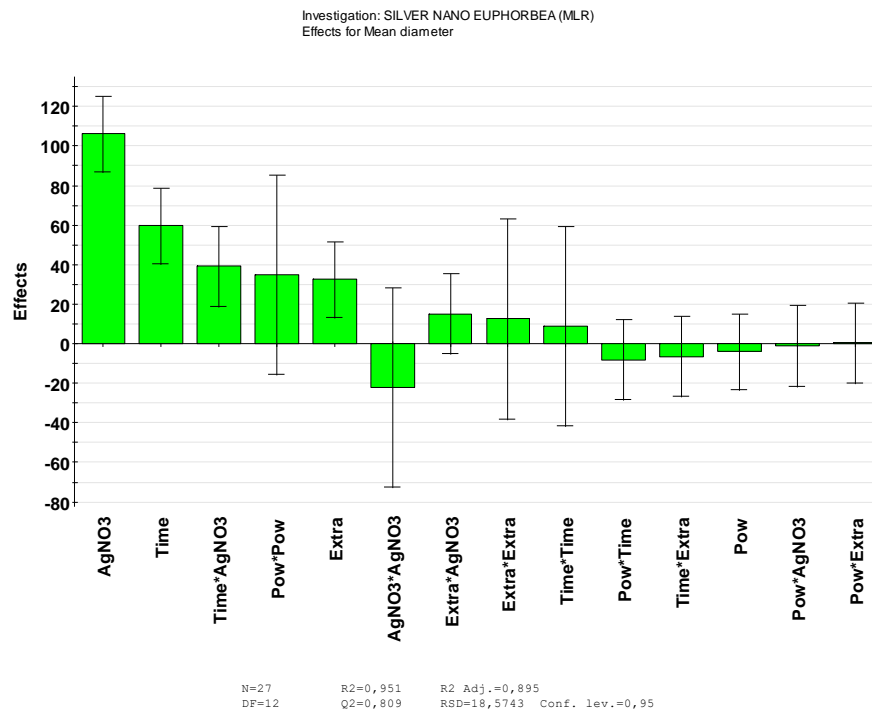
Figure 37: Effects of factors and their interactions on the mean diameter

III.2.3 Mathematical modeling of the process and statistical analysis

An analysis of variance (ANOVA) is a statistical analysis that aims to interpret the coefficients associated with each action of the model by testing the effect of the studied parameters: linear, quadratic, and interactions between factors. It also involves graphically representing the importance of each factor on the studied response.

For each parameter of the studied process, the significance of the linear, quadratic, and possible interaction effects is tested using software that associates the calculated F-value with a probability known as the p-value at a confidence level of 95% by referring to the statistical table.

- If the p-value < 5 % (0.05), the hypothesis (H_0) is rejected, and the factor will be declared significant.

- If the p-value > 5% (0.05), the hypothesis (H_0) is retained, and the factor will be declared non-significant.

The ANOVA analysis provided by the software includes the decisions made regarding the significance of the parameters based on the comparison of the calculated F-value (F_{exp}) with the critical F-value ($F_{critical}$) and the probability value (p-value). The software will indicate whether each parameter is deemed significant or not based on these comparisons.

Table 9 and **Table 10** present the ANOVA analysis for polydispersity index and mean diameter, respectively.

Table 9: Analysis of polydispersity index variance

Polydispersity index	Coeff. SC	Std. Err.	P-value	Conf. int
Constant	0.725284	0.0362459	1.3866e-010	0.0789727
Microwave heating power	-0.0185	0.023183	0.440377	0.0505111
Synthesis time	-0.0803889	0.023183	0.00465165	0.0505111
[Extract]	0.0119445	0.023183	0.615754	0.0505111
[AgNO$_3$]	-0.179833	0.023183	5.14785e-006	0.0505111
Power*Power	-0.0587592	0.0613364	0.356975	0.13364
Time*Time	0.0192407	0.0613364	0.759141	0.13364
[Extract]*[Extract]	-0.0937593	0.0613364	0.152284	0.13364
[AgNO$_3$]*AgNO$_3$]	0.0252407	0.0613364	0.687952	0.13364
Power*Time	0.00512498	0.0245892	0.838394	0.0535751
Power*Extra	-0.026375	0.0245893	0.304538	0.0535751
Power*[AgNO$_3$]	0.011375	0.0245893	0.651926	0.0535751
Time*[Extract]	-0.016	0.0245893	0.527509	0.0535751
Time*[AgNO$_3$]	0.00125001	0.0245893	0.960294	0.0535751
[Extract]*[AgNO$_3$]	0.02625	0.0245893	0.306735	0.0535751

For the mathematical model of the polydispersity index, the significant factors and interactions are the constant, the synthesis time (X_2) and the concentration of silver nitrates (X_4). Thus, the mathematical model of the polydispersity index as a function of the different studied parameters is represented by the following equation:

$$\textbf{Polydispersity index} = 0.725284 - 0.0803889X_2 - 0.179833X_4$$

Table 10: Analysis of mean diameter variance

Mean diameter	Coeff. SC	Std. Err.	P-value	Conf. int
Constant	111.43	6.8449	1.51922e-009	14.9137
Microwave heating power	-2.01111	4.37802	0.654182	9.53883
Synthesis time	29.8333	4.37802	1.86439e-005	9.53883
[Extract]	16.2334	4.37802	0.00299213	9.53883
[AgNO$_3$]	53.0667	4.37802	4.32342e-008	9.53883
Power*Power	17.4556	11.5831	0.157681	25.2374
Time*Time	4.45555	11.5831	0.70722	25.2374
[Extract]*[Extract]	6.25555	11.5831	0.599038	25.2374
[AgNO$_3$]*AgNO$_3$]	-11.0444	11.5831	0.359153	25.2374
Power*Time	-4.03749	4.64358	0.401643	10.1175
Power*Extra	0.262504	4.64359	0.955848	10.1175
Power*[AgNO$_3$]	-0.55	4.64359	0.907676	10.1175
Time*[Extract]	-3.2	4.64359	0.503853	10.1175
Time*[AgNO$_3$]	19.6375	4.64359	0.00117017	10.1175
[Extract]*[AgNO$_3$]	7.5625	4.64359	0.129352	10.1175

For the mathematical model of mean diameter, the significant factors and interactions are: the constant, the synthesis time (X_2), the concentraction of the extract (X_3), the concentration of silver nitrates (X_4) and interaction between the synthesis time and the concentration of silver nitrate (X_2X_4). Thus, the mathematical model of the mean diameter as a function of the different studied parameters is represented by the following equation:

$$Mean\ diameter = 111.43 + 29.8333X_2 + 16.2334X_3 + 53.0667X_4 + 19.6375X_2X_4$$

III.4 Prediction of the responses

The previously established mathematical models, as described in the previous section of this document, allow the generation of response surfaces for predicting the studied responses.

III.4.1 Prediction of polydispersity index

The response surface showing the effect of microwave heating power and reaction time on the polydispersity index, for fixed values of extract concentration at level 0 (1.5%) and silver nitrate concentration at level 0 (1.5 mM), is presented in **Figure 38**. It is observed that as the reaction time increases, the polydispersity index decreases. The effect of power is very weak and negligible, making it challenging to predict accurately.

Figure 38: Response surface showing the effect of microwave heating power and reaction time on the polydispersity index for fixed values of extract concentration at level 0 (1.5%) and silver nitrate concentration at level 0 (1.5 mM)

The response surface showing the effect of extract concentration and silver nitrate concentration on the polydispersity index, for fixed values of microwave heating power at level 0 (500 W) and reaction time at level 0 (120 s), is provided in **Figure 39**. It can be observed that as the silver nitrate concentration increases, the polydispersity index decreases. On the other hand, the extract concentration has a very weak and negligible effect, making it difficult to predict accurately.

Figure 39: Response surface showing the effect of extract concentration and silver nitrate concentration on the polydispersity index for fixed values of microwave heating power at level 0 (500 W) and reaction time at level 0 (120)

III.4.2 Prediction of mean diameter

The response surface showing the effect of microwave heating power and reaction time on the mean diameter, for fixed values of extract concentration at level 0 (1.5%) and silver

nitrate concentration at level 0 (1.5 mM), is given in **Figure 40**. We observe that as the reaction time increases, the mean diameter becomes larger. The microwave heating power, which has a very weak and negligible effect, is very difficult to predict because the mean diameters are larger at the extreme values of the range (650 and 350 W) than at the center value (500 W).
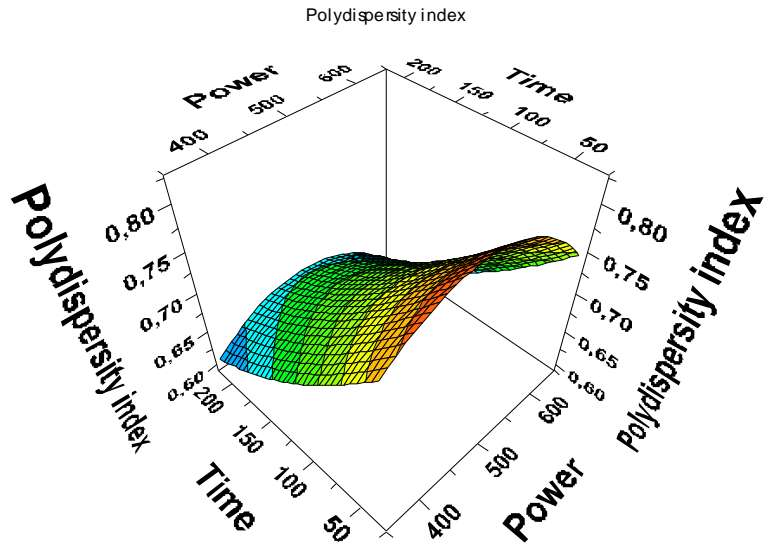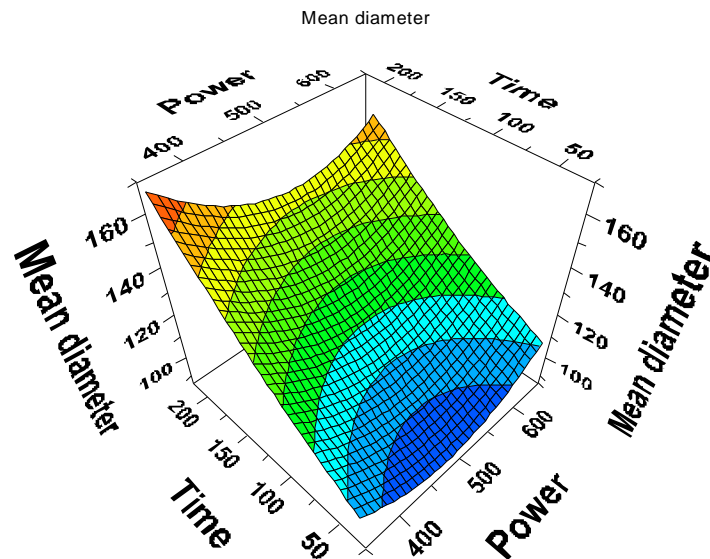

Figure 40: Response surface showing the effect of microwave heating power and reaction time on the mean diameter, for fixed values of extract concentration at level 0 (1.5%) and silver nitrate concentration at level 0 (1.5 mM)

Response surface showing the effect of extract concentration and silver nitrate concentration on the mean diameter, for fixed values of microwave heating power at level 0 (500 W) and reaction time at level 0 (120 s) is presented in **Figure 41**. We observe that as the silver nitrate concentration increases and the extract concentration increases, the mean diameter of the nanoparticles also increases.
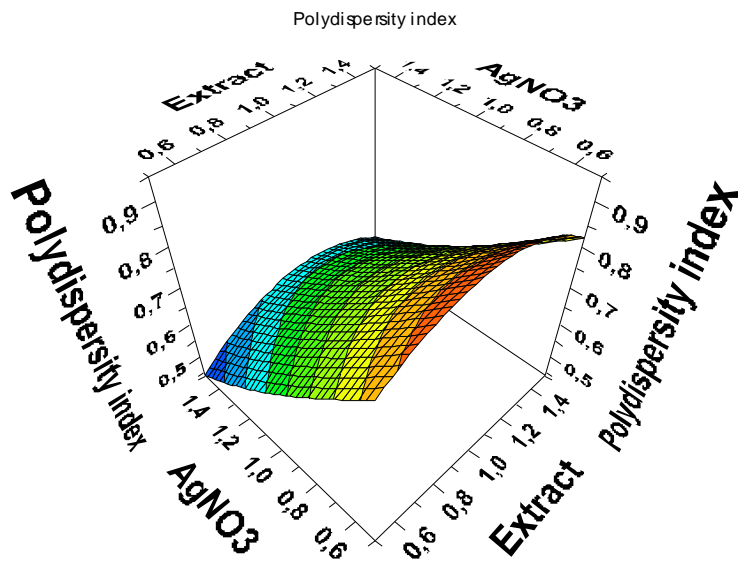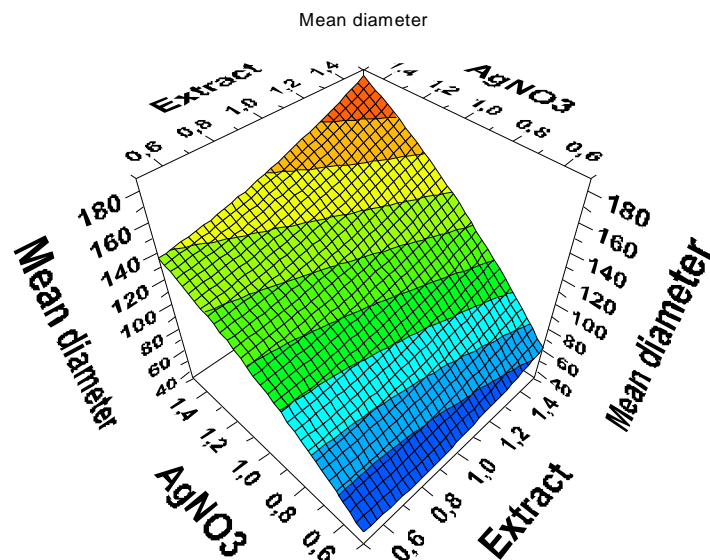

Figure 41: Response surface showing the effect of extract concentration and silver nitrate concentration on the mean diameter for fixed values of microwave heating power at level 0 (500 W) and reaction time at level 0 (120s)

III.5 Study of R^2 and Q^2 coefficients, model validity, and process reproducibility

The results of the correlation coefficients R^2, prediction coefficients Q^2, model validity, and process reproducibility are presented in **Table 11** and **Figure 42** for the polydispersity index and mean diameter.

The polydispersity index showed correlation coefficient (R^2), prediction coefficient (Q^2), model validity, and process reproducibility values of approximately 0.87, 0.36, 0.88, and 0.71, respectively. The correlation between the obtained results is significant; however, the prediction of responses remains challenging within the studied factor intervals. The model validity is high, and the reproducibility of the results remains satisfactory.

The mean diameter showed correlation coefficient (R^2), prediction coefficient (Q^2), model validity, and process reproducibility values of approximately 0.95, 0.8, 0.89, and 0.89, respectively. The correlation between obtained results is significant, and prediction of responses within studied factor intervals is possible. The model validity is high, and the reproducibility of results is very satisfactory.

Table 11: Coefficients R^2, Q^2, model validity, and process reproducibility

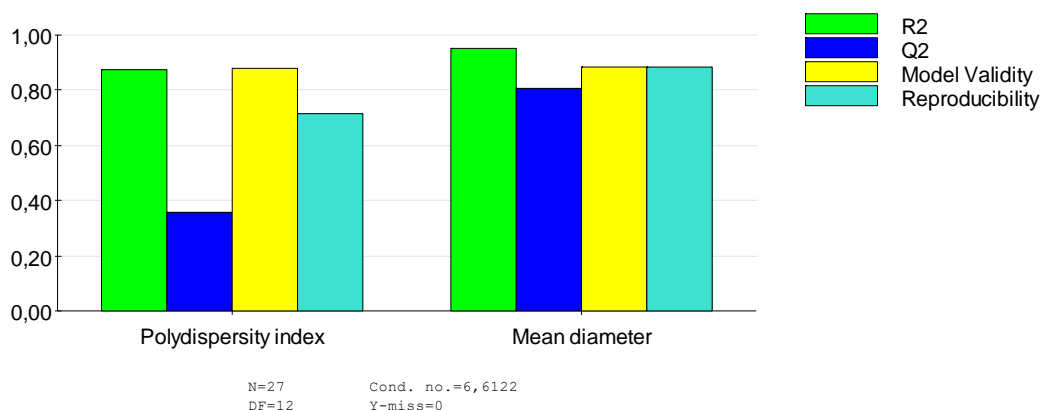|	R^2	R^2 Adj.	Q^2	RSD	N	Model Validity	Reproducibility
Polydispersity index	0.874528	0.728143	0.358601	0.098357	27	0.880155	0.713298
Mean diameter	0.951479	0.894872	0.808636	18.5743	27	0.885543	0.885394

Figure 42: Coefficients R^2, Q^2, model validity, and process reproducibility

IV. Biological Activity (Theoretical Approach)

IV.1 Antidermatophytic activity of silver metal

Silver metal has demonstrated significant antidermatophytic activity against various dermatophytes, which are fungi that cause common skin infections. The antimicrobial

properties of silver have been extensively studied and applied in various medical and healthcare settings.

Several studies have investigated the antidermatophytic activity of silver metal or silver-based formulations. For example, a study by Samuels et al. (2017) evaluated the antifungal effects of silver nanoparticles against dermatophytes, including Trichophyton rubrum and Microsporum gypseum. The results showed potent antifungal activity, inhibiting the growth of dermatophytes and reducing their viability.

Similarly, Gupta et al. (2013) examined the antifungal activity of silver nanoparticles against dermatophytes isolated from patients with skin infections. The study demonstrated the inhibitory effects of silver nanoparticles on dermatophyte growth, highlighting their potential as an alternative antifungal treatment.

Furthermore, a study by Ahmad et al. (2018) investigated the antifungal activity of silver nanoparticles synthesized using plant extracts against various dermatophytes. The findings revealed significant antidermatophytic activity, suggesting the potential use of silver nanoparticles in the management of dermatophyte infections.

Another research study by Subbaiya et al. (2015) evaluated the antifungal activity of silver nanoparticles against clinical isolates of dermatophytes, including Trichophyton mentagrophytes and Epidermophyton floccosum. The results demonstrated the effective inhibitory activity of silver nanoparticles against these dermatophytes.

These studies collectively suggest that silver metal or silver nanoparticles possess considerable antidermatophytic activity against dermatophytes. However, it is important to note that further research is needed to fully understand the mechanism of action, optimize dosages, and evaluate the safety and efficacy of silver-based treatments for dermatophyte infections.

IV.2 Antidermatophytic activity of plant extracts

The use of plant extracts for their antidermatophytic activity has gained considerable attention in recent years. Various plant species have been explored for their potential in combating dermatophytic infections, which are caused by fungi that invade the skin, hair, and nails. The antimicrobial properties of these plant extracts make them promising candidates for the development of alternative antifungal therapies.

One widely studied plant extract is tea tree oil derived from the *Melaleuca alternifolia* tree. Tea tree oil has been shown to possess potent antifungal activity against dermatophytes such as Trichophyton spp. and Microsporum spp. Mondello et al. (2006) demonstrated the efficacy of tea tree oil in inhibiting the growth of azole-susceptible and -resistant human pathogenic yeasts, including dermatophytes.

Garlic (*Allium sativum*) is another plant extract that has exhibited significant antidermatophytic potential, it was investigated the antifungal activity of garlic extract against Trichophyton rubrum and Trichophyton mentagrophytes, two common dermatophyte species. The study reported the inhibitory effects of garlic extract on fungal growth, further supporting its antidermatophytic properties.

Neem (*Azadirachta indica*) is a well-known medicinal plant with broad-spectrum antimicrobial activity. Sharma et al. (2018) evaluated the antifungal potential of neem leaf extract against dermatophytes, including Trichophyton rubrum and Trichophyton mentagrophytes. The study demonstrated the inhibitory effects of neem extract on the growth of these dermatophytes, indicating its potential as an antidermatophytic agent.

Turmeric (*Curcuma longa*) has also shown promise as an antidermatophytic agent. Swamy et al. (2016) investigated the antifungal effects of turmeric extract against various dermatophyte species, including Trichophyton spp., Epidermophyton floccosum, and Microsporum gypseum. The study reported significant antifungal activity of turmeric extract against these dermatophytes.

Aloe Vera (*Aloe barbadensis*) gel extract has been traditionally used for its medicinal properties, including its antimicrobial activity. Savithramma et al. (2011) evaluated the antifungal potential of Aloe Vera gel extract against various dermatophyte species. The study demonstrated the inhibitory effects of aloe vera gel extract on the growth of Trichophyton spp., Epidermophyton floccosum, and Microsporum spp.

The diverse antimicrobial compounds present in these plant extracts provide an alternative approach for the treatment of dermatophytic infections. However, further research is needed to explore their mechanisms of action, optimize extraction methods, and evaluate their efficacy and safety in clinical settings.

IV.3 Antidermatophytic activity of silver nanoparticles

Silver nanoparticles have gained significant attention for their potential as antidermatophytic agents due to their unique physicochemical properties and antimicrobial activity. Dermatophytes are fungi that cause common skin infections, and the use of silver nanoparticles has shown promising results in inhibiting their growth and activity.

Several studies have investigated the antidermatophytic activity of silver nanoparticles. For example, a study by Pal et al. (2015) evaluated the efficacy of silver nanoparticles against dermatophytes such as Trichophyton rubrum and Microsporum gypseum. The results showed that silver nanoparticles effectively inhibited the growth of dermatophytes and reduced their viability.

In another study, Jadhav et al. (2019) investigated the antifungal activity of silver nanoparticles against clinical isolates of dermatophytes, including Trichophyton mentagrophytes and Epidermophyton floccosum. The study demonstrated the potent antifungal effects of silver nanoparticles, suggesting their potential as a therapeutic option for dermatophyte infections.

Furthermore, a study by Sharma et al. (2018) examined the antidermatophytic activity of silver nanoparticles synthesized using a plant extract against various dermatophytes. The results revealed significant inhibitory effects of silver nanoparticles on dermatophyte growth, indicating their potential use as an alternative antifungal treatment.

These studies collectively suggest that silver nanoparticles possess strong antidermatophytic activity against dermatophytes. The mechanisms underlying this activity include the disruption of cell membranes, inhibition of enzyme activity, and generation of reactive oxygen species, leading to the inhibition of fungal growth.

It is important to note that further research is needed to fully understand the mechanisms of action of silver nanoparticles, optimize their formulation and dosage, and evaluate their safety and efficacy in clinical settings. Nonetheless, the antimicrobial properties of silver nanoparticles make them a promising candidate for the development of antifungal therapies against dermatophytic infections.

IV.4 Mechanisms underlying silver nanoparticles antidermatophytic activity

The antidermatophytic activity of silver nanoparticles is attributed to their unique physicochemical properties and their interactions with fungal cells. The mechanisms underlying the antifungal activity of silver nanoparticles against dermatophytes involve several key processes:

1. Disruption of Cell Membranes: Silver nanoparticles can interact with the cell membranes of dermatophytes, leading to membrane damage and permeabilization. The nanoparticles can penetrate the fungal cell wall and disrupt the integrity of the cell membrane, causing leakage of cellular contents and ultimately leading to cell death.

2. Inhibition of Enzyme Activity: Silver nanoparticles have been shown to inhibit the activity of key enzymes involved in fungal metabolism and growth. These nanoparticles can interact with enzymes such as cytochrome P450, which are essential for the synthesis of important cellular components in dermatophytes. Inhibition of enzyme activity disrupts vital metabolic pathways and hinders fungal growth.

3. Generation of Reactive Oxygen Species (ROS): Silver nanoparticles have the ability to generate reactive oxygen species, such as superoxide radicals and hydrogen peroxide, upon contact with fungal cells. These ROS can induce oxidative stress in dermatophytes, leading to damage to cellular components, including proteins, lipids, and DNA. The accumulation of ROS disrupts cellular homeostasis and can ultimately lead to fungal cell death.

4. Interaction with Fungal DNA: Silver nanoparticles can interact with the genetic material of dermatophytes, specifically DNA. The nanoparticles can bind to the DNA molecules, interfering with replication and transcription processes. This disruption of DNA integrity and function impairs fungal growth and survival.

It is important to note that the exact mechanisms of antidermatophytic activity may vary depending on factors such as nanoparticle size, shape, surface charge, and concentration, as well as the specific characteristics of the targeted dermatophyte species.

Overall, the multifaceted antifungal mechanisms of silver nanoparticles, including disruption of cell membranes, inhibition of enzyme activity, generation of reactive oxygen species, and interaction with fungal DNA, contribute to their potent antidermatophytic activity.

Please keep in mind that while silver nanoparticles have shown promising antifungal properties, further research is necessary to fully understand their mechanisms of action, optimize their formulation, and evaluate their safety and efficacy in clinical settings.

General conclusion

In this study, we investigated the preparation, composition, and characterization of an extract obtained from a plant using the MAE method. We also examined the synthesis and optimization of nanoparticles using the prepared extract and silver nitrate.

The extract obtained through the MAE method exhibited a light brown color after filtration, which became darker upon drying. It also had a characteristic odor associated with the studied plant. The dried extract resulted in a solid, friable, and easily soluble mass in water at various concentrations.

Analyzing the composition of the extract was crucial for understanding its potential in serving as a system for silver atom fixation. We employed advanced techniques such as liquid chromatography and mass spectrometry to identify the compounds present in the extract. The analysis revealed the presence of 18 compounds, out of which 11 were successfully identified. These identified compounds included linoleic acid amide, adenosine, gallic acid derivative, caffeoylquinic acid, and various other derivatives and glucosides.

To synthesize the nanoparticles, we adopted a methodology based on experimental designs. A composite centered design comprising a factorial plan with central points and additional star points was used. This design allowed for the estimation of curvature and ensured a consistent variance of prediction across equidistant points from the central point, thereby expanding the applicability of the study domain. A total of 27 experiments were conducted, and the response matrix was presented.

Based on the obtained results, it was determined that the experiment randomly assigned as number 5 exhibited the highest polydispersity index. The optimized operating conditions for the synthesis of nanoparticles were determined as follows: power of 650 W, reaction time of 210 s, extract concentration of 1.5%, and silver nitrate concentration of 1.5 mM. It is important to note that the choice of optimization was based on the polydispersity index rather than the mean diameter for two reasons. Firstly, all the obtained nanoparticles exhibited a very small size, not exceeding 268 nm. Nanoparticles within this size range generally possess similar biological properties in terms of penetration into living organisms and their distribution within them. Secondly, the polydispersity index demonstrated a wide variation ranging from 0.298 to 0.973. In the pharmaceutical field, an evenly distributed polydispersity index is of utmost importance for pharmacological and technological reasons. It ensures uniform particle size, optimal formulation efficiency, precise dosing, dissolution control, and reduction of adverse effects, thereby contributing to the quality, safety, and efficacy of medications for patients.

In conclusion, this study successfully prepared an extract using the MAE method and characterized its physical properties. The extract exhibited a unique composition with multiple identified compounds. The synthesized nanoparticles demonstrated a range of sizes with varying polydispersity indices. The optimized conditions for nanoparticle synthesis were determined based on the polydispersity index, ensuring a homogeneous particle size distribution. The findings of this study contribute to the understanding of extract preparation, nanoparticle synthesis, and optimization processes, thereby providing valuable insights for further applications in the pharmaceutical and biomedical fields.

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