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Title

Biosynthesis, Characterization, and Cosmetic Applications of Metallic Nanoparticles in Hair Dye and Skin Care

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Abstract

In the context of sustainable development and green chemistry, this study explores the eco-friendly synthesis of silver (Ag) and zinc oxide (ZnO) nanoparticles using aqueous extracts from Curcuma longa (turmeric), Lawsonia inermis (henna), Hibiscus sabdariffa (hibiscus), and Lavandula stoechas (tasalgha). The biosynthesized nanoparticles were thoroughly characterized using UV-Vis spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD), confirming their nanoscale dimensions, crystalline nature, and morphologies. These nanoparticles were subsequently incorporated into cosmetic formulations, namely skin care creams and herbal-based hair dyes.

The creams were evaluated for physicochemical properties including pH, stability, and type of emulsion, as well as for pharmacological activities such as antioxidant and antibacterial effectiveness. The antioxidant activity was quantified using the DPPH assay, with IC_{50} values indicating significant radical scavenging properties, especially in creams containing AgNPs. Antibacterial tests showed selective activity against Staphylococcus aureus (Gram-positive), with no effect on Escherichia coli (Gram-negative). Additionally, dyeing trials on white and brown hair demonstrated good adherence and color stability, particularly in nanoparticle-based formulations .

This study confirms the potential of plant-mediated nanoparticles as multifunctional ingredients in natural and sustainable cosmetic products, combining therapeutic and aesthetic benefits.

Keywords: ZnO nanoparticles, AgNPs, biosynthesized, characterization, cosmetics, dye cream, skincare

Résumé

Dans le contexte du développement durable et de la chimie verte, cette étude explore la synthèse écologique de nanoparticules d'argent (Ag) et d'oxyde de zinc (ZnO) à partir d'extraits aqueux de Curcuma longa (curcuma), Lawsonia inermis (henné), Hibiscus sabdariffa (hibiscus) et Lavandula stoechas (tasalgha). Les nanoparticules biosynthétisées ont été caractérisées par spectroscopie UV-Visible, microscopie électronique à balayage (MEB) et diffraction des rayons X (DRX), confirmant leur taille nanométrique, leur nature cristalline et leur morphologie.

Ces nanoparticules ont ensuite été intégrées dans des formulations cosmétiques, notamment des crèmes de soin pour la peau et des colorations capillaires à base de plantes. Les crèmes ont été évaluées pour leurs propriétés physico-chimiques (pH, stabilité, type d'émulsion) ainsi que pour leurs activités pharmacologiques, notamment l'efficacité antioxydante et antibactérienne. L'activité antioxydante a été mesurée par le test DPPH, avec des valeurs de CI_{50} indiquant un fort pouvoir piégeur de radicaux libres, en particulier pour les crèmes contenant des nanoparticules d'argent. Les tests antibactériens ont révélé une activité sélective contre Staphylococcus aureus (Gram positif), sans effet sur Escherichia coli (Gram négatif).

Les essais de teinture sur cheveux blancs et bruns ont montré une bonne adhérence et une stabilité de la couleur, notamment dans les formulations à base de nanoparticules. Cette étude confirme ainsi le potentiel des nanoparticules d'origine végétale en tant qu'ingrédients multifonctionnels dans les produits cosmétiques naturels et durables, alliant bienfaits thérapeutiques et esthétiques.

Mots-clés : Nanoparticules de ZnO, nanoparticules d'Ag, biosynthétisées, caractérisation, cosmétiques, crème colorante, soin de la peau.

الملخص

في سياق التنمية المستدامة والكيمياء الخضراء، تستكشف هذه الدراسة التحضير الصديق للبيئة لجسيمات نانوية من الفضة (Ag) وأكسيد الزنك (ZnO)باستخدام المستخلصات المائية من *الكركم* (*Curcuma longa*)، *الحناء* (*Lawsonia inermis*)، *الكركديه* (*Hibiscus sabdariffa*)و*اللافندر البري* (*Lavandula stoechas*)، المعروف محليًا بالتسلغة). تم توصيف الجسيمات النانوية المُخلّقة حيويًا بدقة باستخدام تقنيات التحليل الطيفي للأشعة فوق البنفسجية-المرئية، المجهر الإلكتروني الماسح(SEM) ، وانحراف الأشعة السينية(XRD) ، وقد أظهرت النتائج أنها ذات أبعاد نانوية وطبيعة بلورية وشكل منتظم.

ثم تم دمج هذه الجسيمات النانوية في مستحضرات تجميلية، مثل كريمات العناية بالبشرة وأصباغ الشعر النباتية. تم تقييم الكريمات من حيث الخصائص الفيزيائية والكيميائية مثل الرقم الهيدروجيني، والثبات، ونوع المستحلب، بالإضافة إلى الأنشطة الدوائية مثل الفعالية المضادة للأكسدة والمضادة للبكتيريا. تم تحديد النشاط المضاد للأكسدة باستخدام اختبارDPPH ، حيث أشارت قيم IC_{50} إلى قدرة عالية على التخلص من الجذور الحرة، خاصة في الكريمات التي تحتوي على جسيمات الفضة النانوية. كما أظهرت اختبارات النشاط المضاد للبكتيريا تأثيرًا انتقائيًا ضد *Staphylococcus aureus*(بكتيريا موجبة الجرام)، دون تأثير على) *Escherichia coli*سالبة الجرام.(

كما أظهرت تجارب التلوين على الشعر الأبيض والبني التصاقًا جيدًا وثباتًا عاليًا للّون، خاصةً في التركيبات المعتمدة على الجسيمات النانوية. تؤكد هذه الدراسة على إمكانات الجسيمات النانوية المستخلصة من النباتات كمكونات متعددة الوظائف في المنتجات التجميلية الطبيعية والمستدامة، من خلال دمج الفوائد العلاجية والجمالية.

الكلمات المفتاحية : جسيمات أكسيد الزنك النانوية، جسيمات الفضة النانوية، مُخلّقة حيويًا، توصيف، مستحضرات التجميل، كريم صبغة، العناية بالبشرة

DEDICATION

To those who were the light that illuminated my path and the support that strengthened my back, I dedicate the fruit of this humble effort:

To **my mother (Kelthoum)**, the beloved of my heart and eyes, the source of tenderness and safety, whose embrace was a homeland, and whose imagination was a bomb that restored my energy whenever I faltered.

To **my father (Hossin)**, whose prayers were a guiding light accompanying me at every step, and whose determination was my role model in patience and perseverance.

To **my only brother (Khalil)**, my beloved and dear companion, the light of my path, who encouraged me silently at times, and with his words so often.

To **my late grandfather (Bouamama),** my second father, who departed in body but left a lasting imprint on my soul. I pray that this work is counted among his good deeds.

To my dear **grandmother (Yamina),** whose love and tenderness were like a second mother always present in my heart and prayers.

To my aunt and second sister **(Wahiba)** and her little one **(Aseel),** who never withheld a kind word or sincere support, always close like my own mother's heart.

To my dear friends: **Ferdous, Wafa , Bouchra** and **Sirine,** and to all the companions of the joumey – partners in effort and joy without whom this experience wouldn't have been complete, thanks to their beautiful spirits and sweet words.

To all of you.... you are the most beautiful chapter of this story.

And to everyone who supported me, even with just one kind word–thank you.

Meriem

DEDICATION

To the one whose prayers have always embraced me, To the one who stayed up through the nights to light my path, To the one who shared in my joys and sorrows, To the fountain of compassion and tenderness, To the most beautiful smile in my life,To the most wonderful woman in existence: **my beloved mother.**

To the one who taught me that life is a struggle and that knowledge is its weapon, To the one who never withheld anything from me and always strove for my comfort and success, To the greatest and dearest man in the world: **my dear father.**

To my strength, my refuge, my unwavering support, To those whom God has blessed me with — my solid bond and greatest allies in this journey: My beloved siblings: **Sara, Mati, Hammou, and Farah.**

And to the two little lights who bring joy to my days, the apples of

my sisters' eyes: **Seddik** and **Djawad.**

To the one I am proud to call my grandmother, To the one whose prayers always preceded my steps, To the one who taught me strength through patience and love through giving: **my dearest grandmother.**

To the best souls destiny has gifted me, To those who embody loyalty and sisterhood, To my companions in the journey, in learning, and in making cherished memories My dear friends: **Amal, Hadia,** and **Meriem**.

To the one who stood by me, supporting me with silent strength and kind words, To the one who walked with me in heart even before steps,

To my future life partner my dear fiancé: **Smail.**

And finally, To everyone who supported me, whether closely or from afar, even with just a prayer.

Thank you.

Wafa

Thanks

In the Name of Allah, the Most Gracious, the Most Merciful

All praise is due to Allah, who enabled us to complete this work and facilitated the paths of research and knowledge.

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Your supervision was not merely academic guidance, but rather an educational and human experience that left a lasting impact on our academic and personal development. We will never forget your continuous support and motivation, which was a powerful driver that helped us overcome challenges and elevate the quality of our work.

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May Allah reward you all on our behalf, and may this work be a blessed step on the path of knowledge and learning.

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Chapter I: Bibliographic study

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List of abbreviations

DPPH : 1,1-Diphenyl-2 picrylhydrazyl radical

UV-vis : Ultraviolet-visible spectrometer

XRD : X-ray diffraction

SEM : Scanning electron microscopy

Abs : Absorption

IC : 50% Efficiency Concentration

NPs : Nanoparticles

ZP : Zéta potential

TA : The titratable acidity of the sample

N : normality

Veq : volume at equivalence

PVP : polyvinylpyrrolidone

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Chapter II: Materials and methods

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General Introduction

General introduction

Nanotechnology has emerged as a groundbreaking tool with transformative applications across numerous scientific fields, including cosmetics and personal care. Its ability to manipulate matter at the nanoscale—typically between 1 and 100 nanometers—has opened new avenues for the development of high-performance, multifunctional cosmetic products. As one of the most innovative and rapidly evolving sectors, nanocosmetics leverage the unique physical, chemical, and biological properties of nanomaterials to achieve enhanced product performance, bioavailability, and targeted delivery.

Nanotechnology is now widely studied and implemented in the formulation of beauty products, skincare, hair lcare, and dermatological treatments [1, 2]. The incorporation of nanomaterials such as silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), gold nanoparticles, liposomes, nanoemulsions, and solid lipid nanoparticles (SLNs) has allowed manufacturers to design products with superior texture, increased stability, extended shelf life, and controlled release mechanisms.[3]

One of the key advantages of nanomaterials is their high surface-area-to-volume ratio, which enhances their interaction with biological membranes, improves skin absorption, and allows lower doses of active ingredients to be used effectively. As a result, cosmetic products can deliver prolonged and localized action, while maintaining minimal systemic exposure, reducing side effects, and maximizing therapeutic benefit [4, 5].

In addition to their aesthetic improvements—such as better skin coverage, smoother application, and long-lasting effects—nanoparticles contribute functional benefits including:

- ✓ Enhanced UV protection (via ZnO and TiO_2 NPs as physical sunscreens),
- ✓ Improved antioxidant delivery to combat aging and oxidative stress,
- ✓ Antibacterial and anti-inflammatory properties, particularly with AgNPs,
- ✓ Encapsulation of unstable or hydrophobic compounds (e.g., vitamins, plant extracts),
- ✓ Stimulation of collagen synthesis, hydration, and skin regeneration.

However, the widespread use of nanoparticles in cosmetics has also raised concerns regarding toxicity, bioaccumulation, and skin penetration, prompting regulatory bodies and researchers to conduct comprehensive safety assessments and clinical trials to ensure consumer protection.

In the context of environmentally sustainable cosmetics, there is increasing interest in the green synthesis of nanoparticles using plant-based extracts. This eco-friendly approach avoids toxic chemicals and harsh conditions traditionally used in chemical synthesis. Plants such as turmeric (Curcuma longa), henna (Lawsonia inermis), karika (Carica papaya), and oleoresins contain bioactive compounds—flavonoids, polyphenols, terpenoids, and alkaloids—that act as natural reducing and stabilizing agents for nanoparticle formation.

This study explores the synthesis and application of AgNPs and ZnO NPs prepared via green methods using medicinal plant extracts. These nanoparticles were integrated into face creams and hair dye formulations, targeting various cosmetic concerns such as skin hydration, acne, pigmentation, UV damage, and hair

thinning. Due to their antibacterial, antimicrobial, and antioxidant properties, these natural nanomaterials provide a safer and more effective alternative to synthetic ingredients in commercial products.

The global market for nanocosmetics continues to expand rapidly, driven by consumer demand for natural, high-performance, and multifunctional products. According to market research, the nanotechnology-based cosmetics industry is projected to surpass several billion dollars in value over the coming years, emphasizing the urgency for sustainable development, green innovation, and toxicological validation.

This research contributes to this growing field by presenting a holistic approach that combines green nanotechnology, medicinal plant resources, and cosmetic formulation. By using plant-derived nanoparticles in topical applications, this study aims to enhance both the therapeutic efficacy and safety profile of cosmetic products.

This chapter will be divided into three chapters :

- Chapter one provides an overview of nanoparticles and outlines the study on silver nanoparticles and zinc oxide nanoparticles, followed by skin care creams and hair dyes .
- Chapter two discusses the experimental methods for nanoparticles, creams, and various characterization techniques .
- The final chapter discusses the results of the characterization methods.

Chapter I: Bibliographic study

I.1.Introduction about nanoparticles

I.1.1. Definition of nanoparticles:

A nanoparticle is a little fragment of material having a diameter of under 100 nm. A nanoparticle operates as a singular entity regarding its properties and transport, despite its diminutive size. Due to their potential applications across various sectors, nanoparticles have become a highly researched subject.

Nanoparticles are significant as they act as a conduit linking atomic or molecular components with bulk substances. Irrespective of dimensions, bulk materials frequently preserve their characteristics. The physical and chemical properties of materials, however, fluctuate with size when reduced to the nanoscale.

The fraction of atoms on the surface area of materials over 1 μm is insignificant relative to the total number of atoms constituting the bulk of the material. The surface area of nanoparticles is significantly more critical than their mass, elucidating a substantial aspect of their unique physical properties. The rate of chemical reactions is proportional to the surface area of reactants, making a high surface area-to-volume ratio particularly significant. Consequently, nanoparticles can elicit more efficient responses.[6]

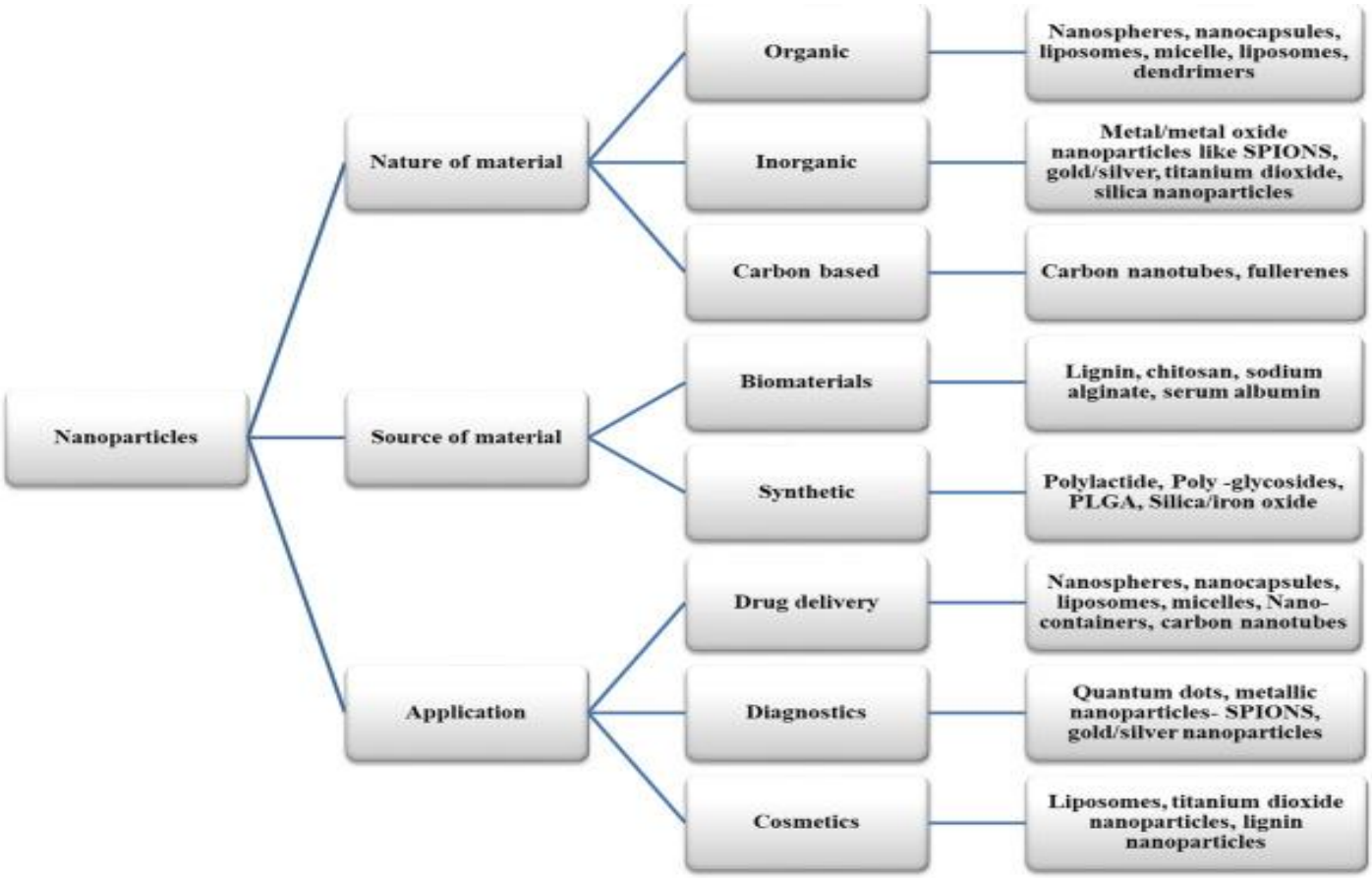


Figure I.1: general information about nanoparticle [E1]

I.1.2. Approaches for the synthesis of metal NPs

The creation of nanoparticles primarily involves three approaches: physical, chemical, and biological. The physical method is referred to as the top-down technique, whereas the chemical and biological methods are jointly termed the bottom-up approach. The biological approach is often referred to as green systems of NPs. All these approaches are further classified into numerous types based on the methods employed, Figure I.2 delineates the reported methodologies employed by each strategy for the synthesis of nanoparticles.

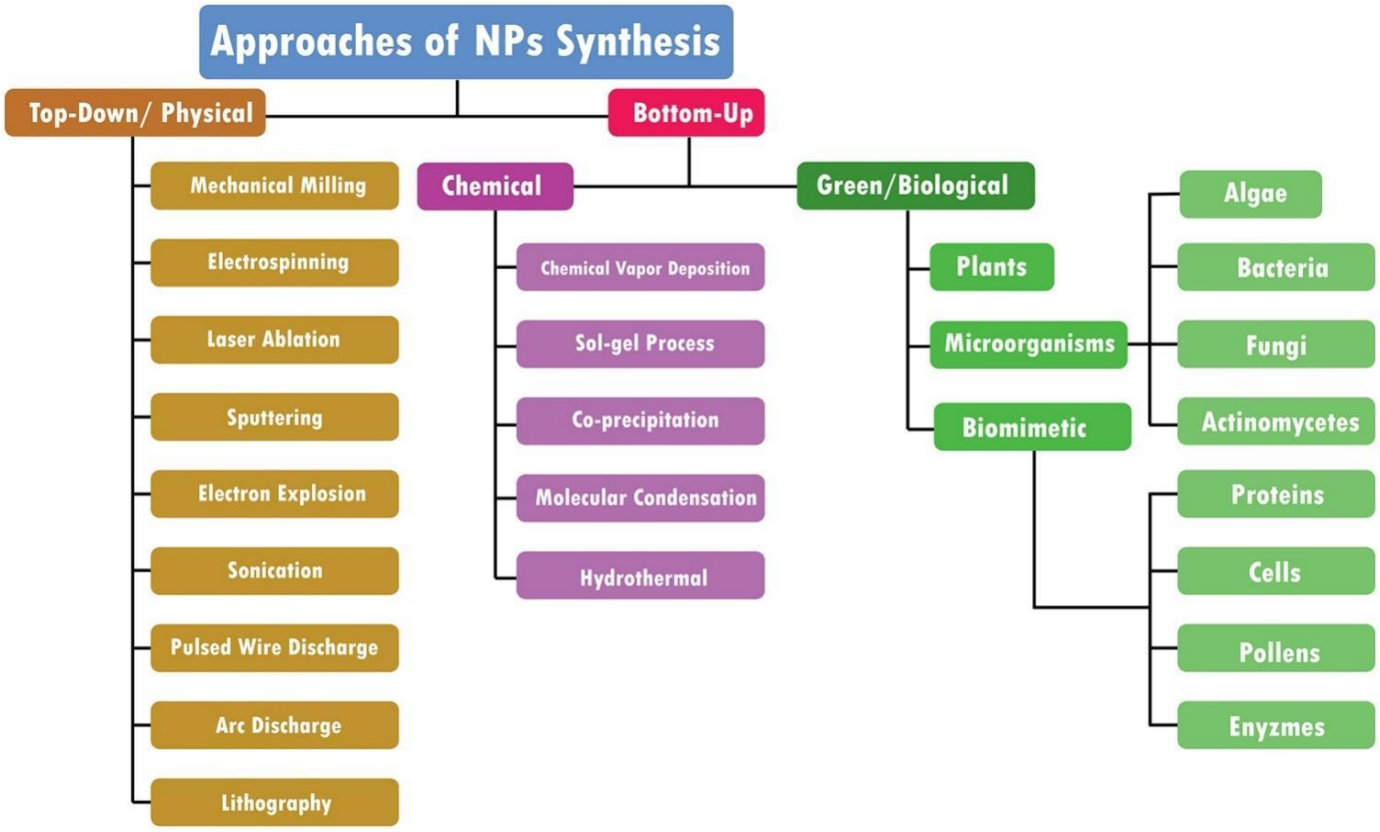


Figure I.2: Schematic diagram for different synthetic techniques of Ag NPs and ZnO NPs [E2]

- **Top down/physical approach :** Bulk materials are fragmented in top-down methods to create nano-structured materials (Figure I.3).They are additionally known as physical approaches [7]

The following techniques can achieve a top-down approach

- **Bottom-up approach**

Tiny atoms and molecules are combined in bottom-up methods to create nano-structured particles (Figure I.3) [7] These include chemical and biological approaches:

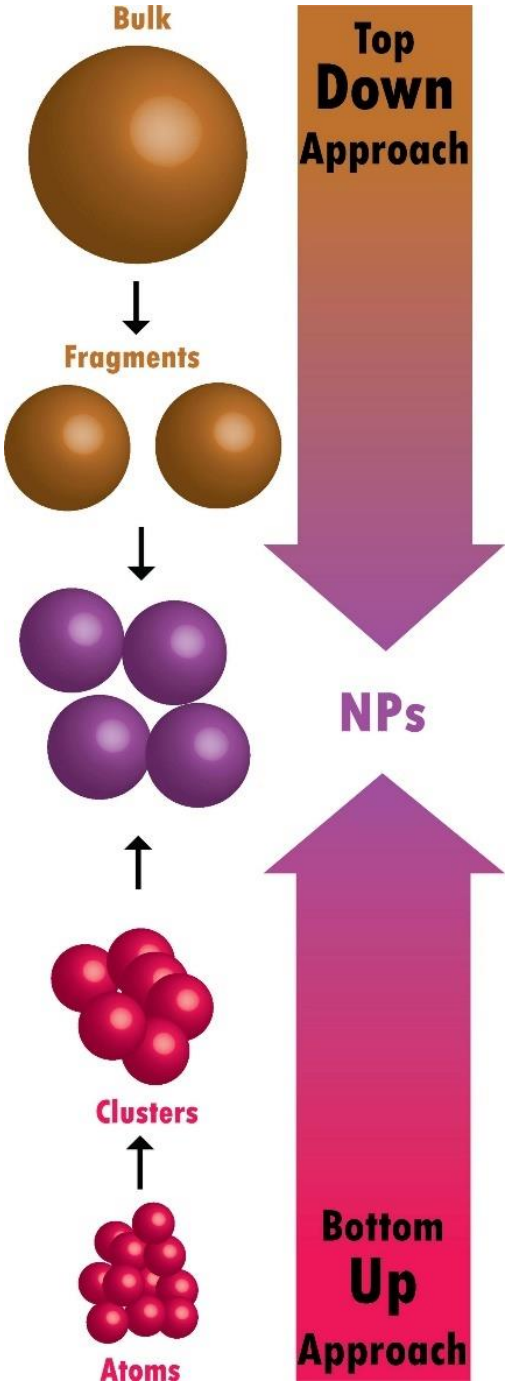


Figure I.3: Difference between top-down and bottom-up approaches [E3]

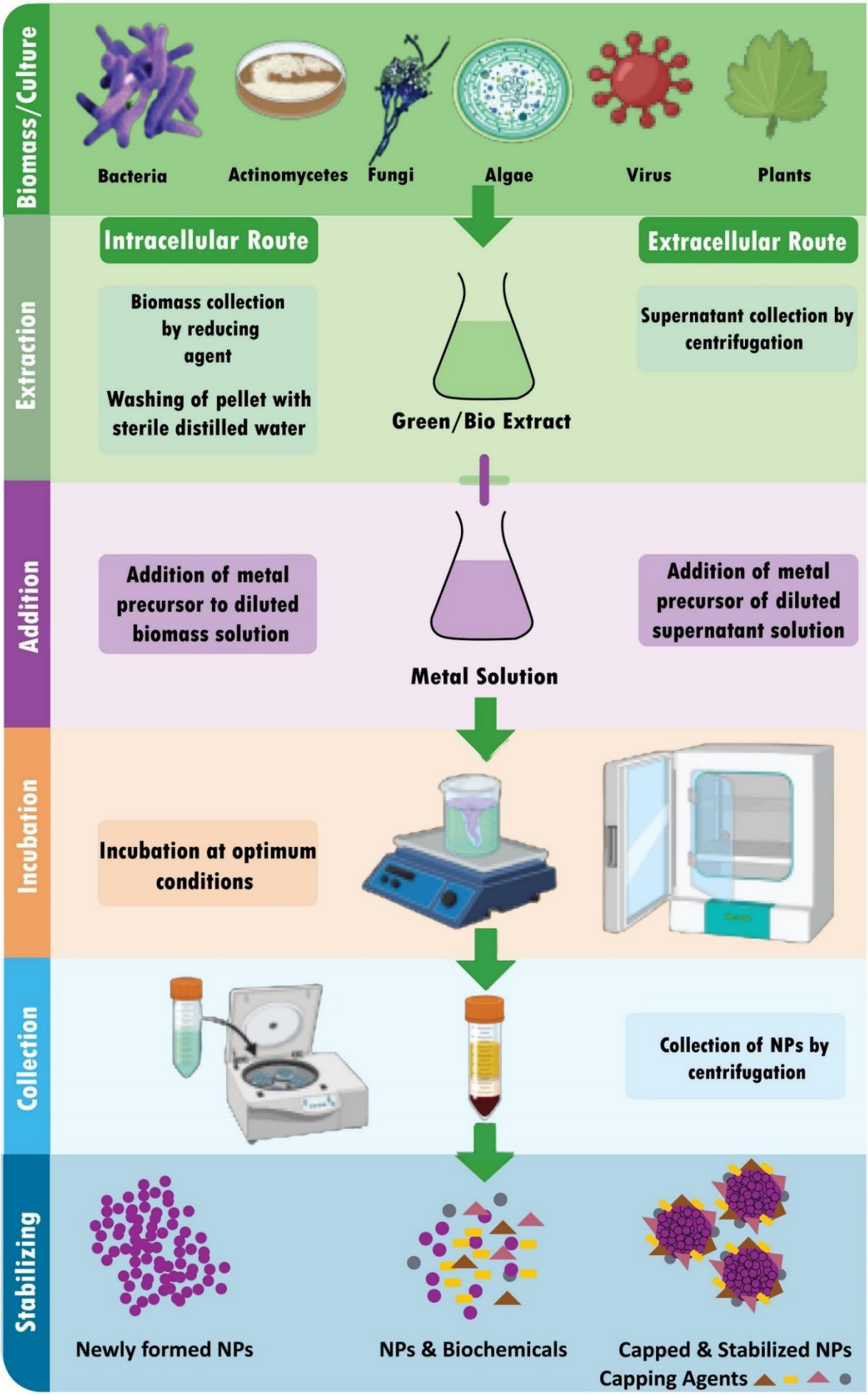


Figure I.4: Schematic diagram for biosynthesis of NPs [E4]

I.1.2.1. Chemical Methods:

Chemical synthesis or wet chemistry procedures, including the sol-gel technique, chemical vapour deposition, molecular condensation, and chemical reduction, are predominantly utilised due to the substantial yields they may achieve [8]. Nevertheless, these methods sometimes entail extended hours, the utilisation of hazardous chemicals, and limitations on the types of metal alloys permissible for BNP production [9]. Noble metal-based BNPs, including those composed of Pt, Pd, Au, Ag, and Zn, are the most chemically synthesised varieties. The majority of studies concentrate on drug transport, catalytic processes, and antibacterial applications, as these are frequently employed in biological and catalytic contexts.

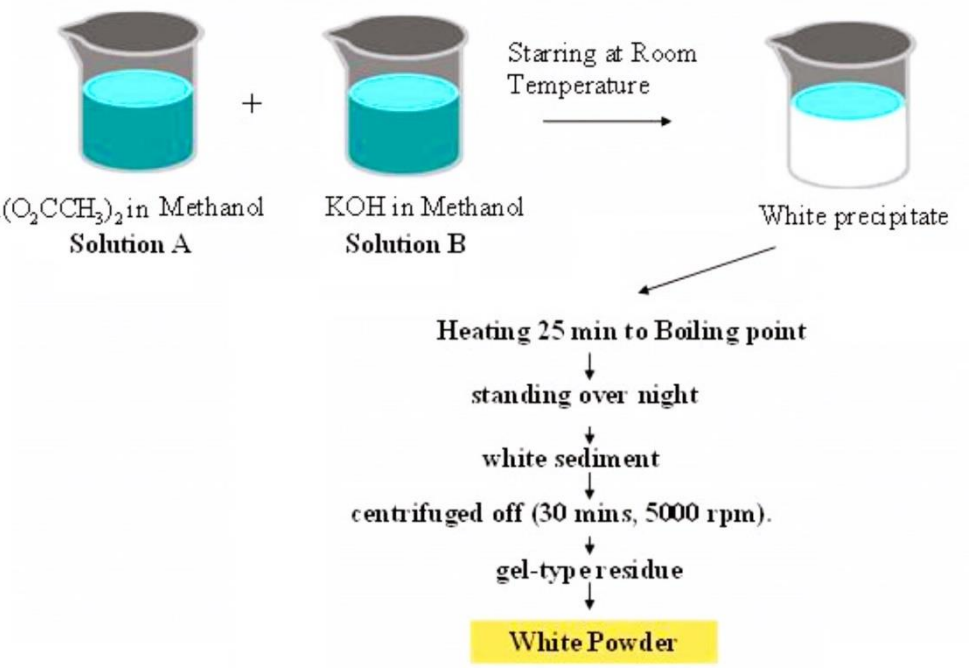


Figure I.5: Flow chart diagram showing chemical synthesis procedure [E5]

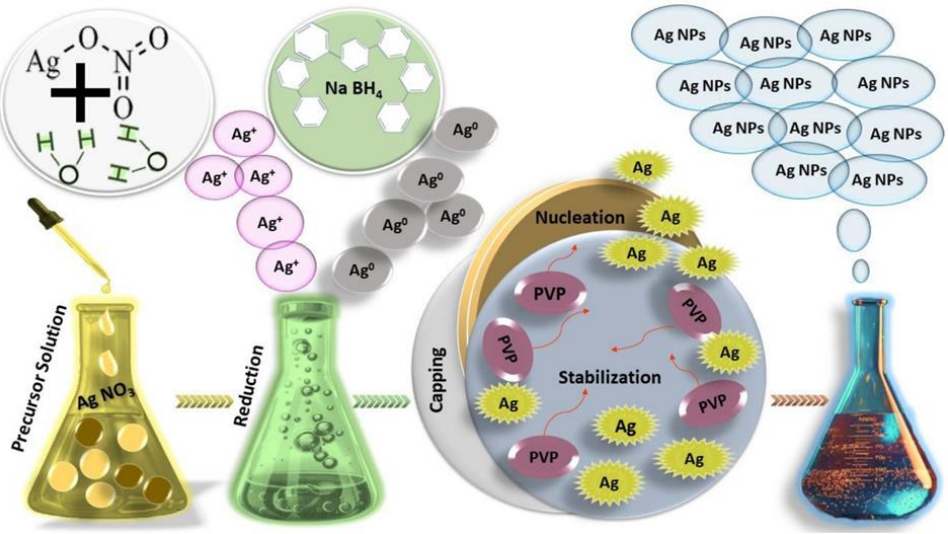


Figure I.6: Schematic diagram for chemical synthesis of Ag NPs [E6]

I.1.2.2 Physical Methods:

Physical synthesis refers to the generation of nanoparticles by procedures such as evaporation–condensation, laser ablation, and sputtering. These procedures typically include the physical transformation of bulk materials into nanoparticles through nucleation, condensation, and vaporisation [10]. The subsequent are few conventional physical synthesis methods for nanoparticles:

1. Evaporation–condensation: This technique involves heating or laser-treating a substance to induce evaporation, followed by the condensation of the resultant vapour to form nanoparticles.
2. Laser Ablation This technique generates a plasma that condenses to form nanoparticles through the ablation of a target material using a high-energy laser.
3. Sputtering: In sputtering, high-energy ions or atoms are directed towards a target material, causing the ejection of atoms that subsequently deposit as nanoparticles on a substrate [11].

Physical synthesis techniques provide benefits including elevated purity, precise control over particle dimensions and morphology, and scalability. Nonetheless, they may necessitate specialised apparatus and can be more energy-consuming than chemical synthesis techniques.

I.1.2.3. Biosynthesis of nanoparticles using natural materials

- Synthesis of green and biological

"Green" or "biological" nanoparticle synthesis refers to the fabrication of diverse metal nanoparticles utilising bioactive agents, including plant materials, microorganisms, and various biowastes such as vegetable refuse, fruit peel, eggshells, agricultural byproducts, and algae [12]. To prevent the generation of undesirable or dangerous byproducts, it is essential to establish dependable, sustainable green synthesis procedures ,The green synthesis of nanoparticles has numerous advantages, such as simplicity, cost-effectiveness, the generation of very stable nanoparticles, minimal time requirements, the production of non-toxic byproducts, and the feasibility of large-scale synthesis [13].

- Biological synthesis using microorganisms

Microorganisms synthesise nanoparticles by capping, enzymatic reduction, and metal sequestration. Metal ions are initially sequestered on the surface or within microbial cells prior to their conversion into nanoparticles by enzymes [14]. The production of metallic nanoparticles by microorganisms, especially marine bacteria, is rapid, cost-effective, and environmentally friendly [15]. Metal nanoparticles are synthesised utilising several microorganisms, including:

- Biosynthesis of NPs by bacteria
- Extracellular synthesis of NPs by bacteria
- Intracellular synthesis of NPs by bacteria
- Biosynthesis of NPs by fungi
- Biosynthesis of NPs using actinomycetes
- Biosynthesis of NPs using algae

- Intracellular synthesis of NPs using algae
- Extracellular synthesis of NPs using algae

➢ Biological synthesis using plant extracts:

Plant extracts are chemicals or active components of suitable quality derived from plant tissue through particular treatment for designated purposes [16]. Nanoparticles are synthesised by combining plant extracts with a metal salt solution at ambient temperature. The response is finalised within minutes. This technique has been employed to synthesise nanoparticles of silver, gold, and various other metals [17]. Biosynthesis of nanoparticles is accomplished through many plant species. The type of plant extract, its content, the concentration of the metal salt, pH, temperature, and duration of contact all influence the rate of nanoparticle synthesis, their quantity, and other characteristics [18]. A leaf extract from Polyalthia longifolia was utilised to synthesise silver nanoparticles, with an average particle size of approximately 58 nm [19; 20].

➢ Biological synthesis using biomimetic

"Biomimetic synthesis" typically refers to chemical reactions that replicate the biological synthesis occurring in living organisms [21]. The biomimetic approach utilises waste biomass, viruses, cells, proteins, enzymes, and pollen to synthesise nanoparticles. Biomimetic synthesis is categorised into two groups:

Functional biomimetic synthesis emulates specific characteristics of natural materials, structures, and systems through diverse materials and methodologies [22].

The objective of process biomimetic synthesis is to replicate the synthesis pathways, techniques, or protocols of natural chemicals and materials/structures to generate various desired nanomaterials/structures. Numerous distinctive nano-superstructures have been synthesised in vitro by emulating protein production, including satellite structures, dendrimer-like formations, pyramids, cubes, 2D nanoparticle arrays, and 3D AuNP tubes [22].

I.1.3. Structure and Properties of metal Ag NPs and metal oxide ZnO NPs:

Nanoparticles display distinct physical and chemical characteristics relative to their bulk equivalents, attributable to their diminutive size (1–100 nm) and elevated surface area-to-volume ratio. These characteristics encompass heightened reactivity, augmented strength, and modified optical, electrical, and magnetic properties. For instance, gold nanoparticles exhibit red or purple hues based on their dimensions, whereas zinc oxide has enhanced efficacy as antibacterial agents at the nanoscale. The unique properties of nanoparticles render them exceptionally important in disciplines such as medicine, energy, catalysis, and environmental science.[23]

I.1.3.1. ZnO NPs:

Because of its distinct physical and chemical properties, zinc oxide nanoparticles (ZnO-NPs) are a desirable and adaptable inorganic compound and one of the metal oxide nanomaterials. With the molecular formula

ZnO, they have a wide radiation absorption spectrum, a high electrochemical coupling coefficient, good photostability, and high chemical stability [24]. Zinc oxide nanoparticles (ZnO-NPs) are widely produced and used in a wide range of commercial and additive products, such as food as a zinc nutrient and in ceramics, cement, plastics, glass, ointments, lubricants, adhesives, sealants, pigments, batteries, ferrites, fire retardants, cosmetics, and sunscreens [25, 26]. Because of their small size, nanosized ZnO particles exhibit strong antibacterial properties. Once inside the bacterial cell, they can stimulate various bactericidal mechanisms, such as the bacterial core or surface, produce ROS (reactive oxygen species), release Zn2+, and even be endocytosed by cells, (figure I.7) [25,27,28].

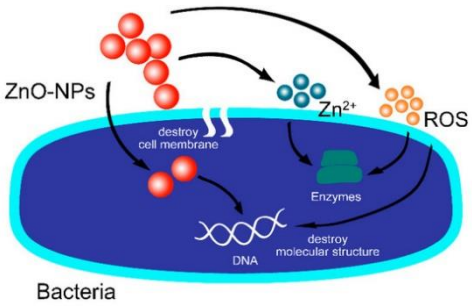


Figure I.7 :Antibacterial mechanism of ZnO-NPs. (ROS formation, Zn2+ release, internalized ZnO-NPs, and electrostatic interactions [E7]

- **Structure:**

In materials science, zinc oxide (ZnO) is a known II-VI semiconductor since it belongs to the second and sixth groups of the periodic table, respectively. Good transparency, antibacterial agents, high electron mobility, a broad bandgap, great mechanical and thermal stability at room temperature, and strong room-temperature luminescence are just a few of the remarkable and advantageous qualities of the ZnO semiconductor. It lies on the edge of the ionic and covalent semiconductor spectrum due to its wide bandgap of 3.37 eV [24,29].

With two lattice parameters (a = 0.325 nm and c = 0.521 nm), the hexagonal unit cell of ZnO crystals has a wurtzite (B4) crystal structure. Each anion in the hexagonal wurtzite structure is encircled by four cations at the tetrahedron's corners, exhibiting tetrahedral coordination and, consequently, sp3 covalent bonding. A non-centrosymmetric structure is produced by ZnO's tetrahedral form (Figure I.8) [30,31].

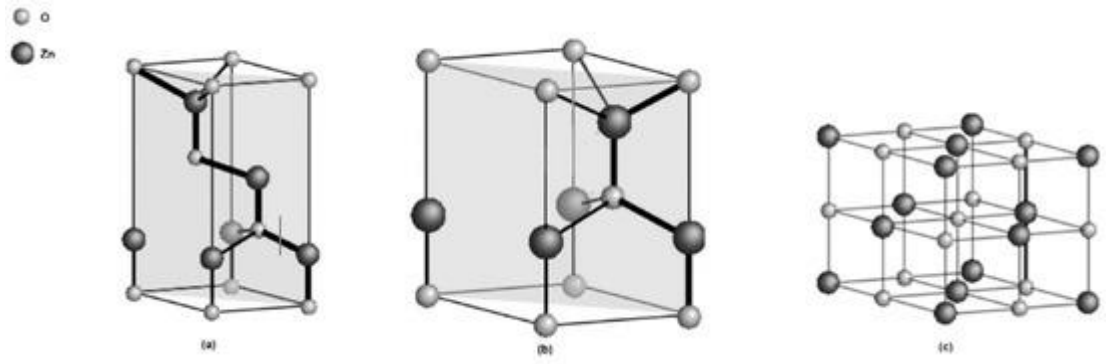


Figure I.8: Crystal structure models of ZnO (a) zinc blende (b) wurtzite and (c) rock salt [32].

I.1.3.2. Ag NPs:

A type of metallic colloidal nanoparticle, silver nanoparticles have sizes between 1 and 100 nm. Particular attention was paid to the biomedicine-related characterisation of silver nanoparticles (AgNPs), which initially gained extensive attention as unusual antibacterial agents , AgNPs are the most important type of nanoparticle for a number of reasons, including their capacity to be made in a variety of geometries, their affordable production costs, and their antibacterial efficacy against a wide range of taxa, AgNPs have demonstrated efficacy against a variety of cancers in both in vitro and in vivo studies, including breast cancer, cervical cancer, lung cancer, nasopharyngeal carcinoma, hepatocellular carcinoma, glioblastoma, prostate carcinoma, and colorectal adenocarcinoma.

This nanomaterial is gaining scientific attention because of its versatility in chemistry, engineering, medicine, and physics , It has a balanced physicochemical performance, works well in optics, electronics, and catalysis, and has strong antibacterial activity , AgNPs are increasingly being used in biomedicine for molecular imaging, medication delivery, wound healing, diagnostics, and the creation of antimicrobial materials and medical devices. Additional therapeutic uses of AgNPs have been studied, such as their use as active molecules or carriers for cancer treatment and diagnosis, as well as antiviral, antifungal, and antibacterial medications [33].

- **Structure:**

Silver nanoparticles (Ag NPs) are particles of silver measuring between 1 and 100 nanometres in size. Their diminutive size, elevated surface area-to-volume ratio, and ability to absorb and scatter visible and near-infrared light confer unique physical and chemical properties. Silver nanoparticles may possess supplementary antibacterial capabilities absent in ionic silver, attributable to their elevated surface-to-volume ratios and diminutive size, leading to distinct chemical and physical characteristics in comparison to their bulk equivalents [34].

Furthermore, the production process—chemical reduction being the most popular, can produce AgNPs in a variety of sizes and shapes. A 12 mM AgNO3 aqueous solution was chemically reduced to produce the AgNPs. Using 70 mL of this solution containing PVP (maintaining the molar ratio of the repeating unit of PVP and Ag equal to 34) and 21 mL of Aloe Vera, the reaction was conducted in an argon atmosphere. After 45 minutes of ultrasonic agitation at room temperature, the mixture was heated at a rate of 2°C per minute to 80°C and allowed to sit for two hours. This produced a clear solution with small suspended particles that needed to be eliminated by straightforward filtration [34,35].

I.1.4.Application of NPs in cosmetics:

The cosmetics industry has shown a tremendous deal of interest in nanotechnology. Nanotechnology is used in the production, packaging, and formulation of cosmetics. Cosmetic formulations have included a variety of nanoparticles. They are transparent or translucent, have a wide surface area, a high number of particles per unit weight, are well soluble, and have good skin-penetration qualities. Additionally, several of them have outstanding antibacterial, antioxidant, and UV protecting qualities. In a variety of cosmetic compositions, they serve as transporters, active agents, skin moisturizers, UV filters, and free radical scavengers. They can greatly enhance skin penetration, stability, controlled release of active ingredients, and the solubility of poorly soluble substances. The various kinds of nanoparticles and their uses in cosmetics are covered in this chapter [36].

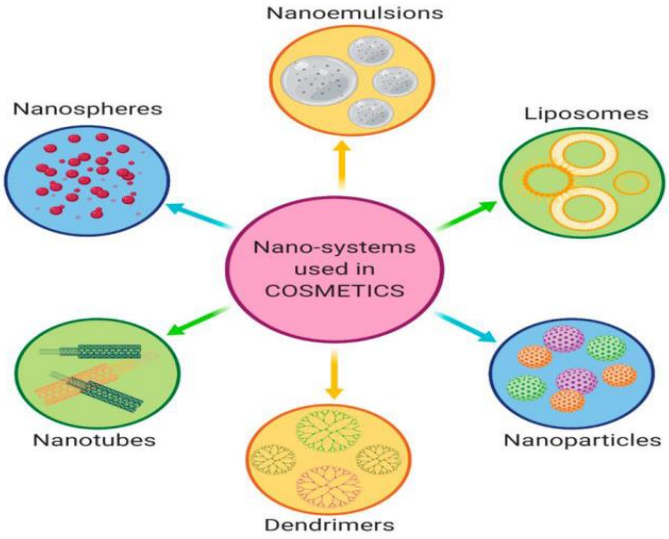


Figure I.9: Diverse range of nanomaterials in cosmetics. [37]

-The dimensions of nanoparticles must be taken into account when utilising nanomaterials. Particle size, colour, structural integrity, transparency, optical activity, solubility, and chemical composition can all influence a material's properties. The reactivity of nanoscale particles differs from that of their larger counterparts. Nanoscale materials has unique dimensions that enhance solubility by aligning the size of cosmetic compounds with the biological structure of skin cells. This results in instances of contact and selectively affects cellular activities at naturally occurring dimensions [38]. Cosmetics utilising nanoparticles

can enhance skin penetration, provide prolonged effects, provide improved finish quality, and deliver UV protection [39,40]

-Extended exposure to ultraviolet radiation from the sun induces oxidative stress and alters the structure and composition of the skin. Acute changes such as erythema, pigmentation, and photosensitivity may occur, along with long-term effects including skin cancer and premature ageing. Sunlight, characterised by a restricted radiation range of 200–400 nm and a moderate component of the electromagnetic spectrum, is referred to as UV light. The ultraviolet spectrum comprises three wavelengths: UV F (290–320 nm), UV G (320–400 nm), and UV A (320–400 nm). Melanin synthesis, keratinocyte proliferation, and perspiration can all mitigate the detrimental effects of solar exposure.

[41-42] Nonetheless, due to concerns regarding the potential adverse effects of synthetic sunscreens on human skin, some researchers have asserted in recent years that cosmetics using herbal compounds are safer for hypersensitive skin. This is due to the reduced likelihood of skin irritation from natural substances and their superior compatibility with the skin. Moreover, sunscreens with natural components are more benign for the skin. [40,43-44].

Table I.1: Type nanomaterial in cosmetic

Type materials		**Properties**		**Reference**
Inorganic Nanomaterilas		The characteristics of inorganic nanoparticles include being non-toxic, hydrophilic, biocompatible, and highly stable	Ti, Ag, Au. TiO2, For Suncreen as UV Filter	[45]
Carbon Black Carbon	**(Nano)**	cytotoxicity, inflammation.	Eye cosmetics, skin care products, and mascaras often use this cosmetic ingredient as a colorant.	[46]
Silica. SiO2		hydrophilic surface favors prolonged circulation and low production cost	products for hair, skin, lips, face, and nails contain SiO2 for Silica nanoparticles.	[47]
Nano-Organic Tris-Biphenyl	**Materials**	Tris-Biphenyl Triazine is	Ingredient for the formulation of sunscreens	[48]
Silver and Nanoparticles	**Gold**	Used in cosmetics that include deodorants and antiaging creams. In	Antibacterial and antifungal	[49]

Inorganic nanoparticles are widely used in cosmetic products due to their UV protection, antimicrobial properties, and optical effects. The most commonly used inorganic nanoparticles and their approximate usage proportions in cosmetic formulations are:

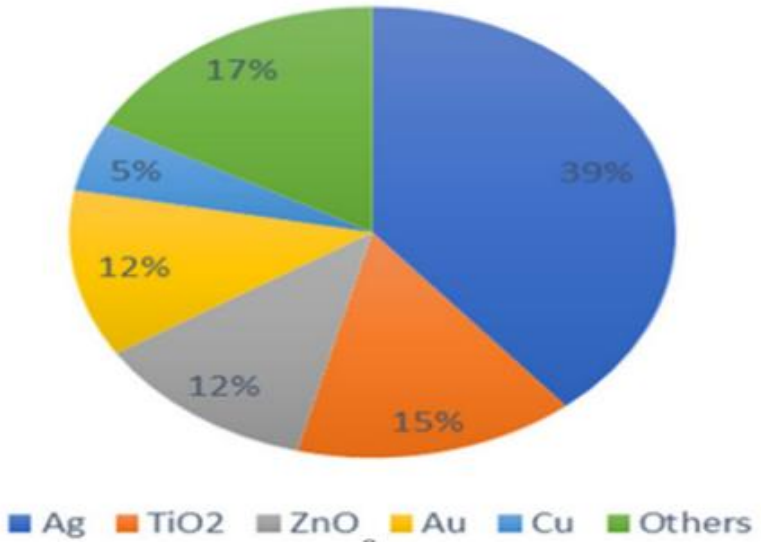


Figure I.10: Proportions of different inorganic nanoparticles in cosmetics [E8]

Melanin is thus a unique substance in products intended for direct application to the human body. It fulfils essential roles in nature that cosmetics want to replicate, such as skin and hair pigmentation and ultraviolet protection. Moreover, melanin predominantly exists in nature as nanoparticles.

In recent decades, nanotechnology has been employed to develop advanced hair and scalp care products. Approximately 19% of all nanocosmetics catalogued in the StatNano database are formulated for hair and scalp care, corroborating this trend.

I.1.4.1 Silver nanoparticles and Zinc oxide as cosmetic ingredients :

Nanomaterials serve as active carriers and formulation supports in cosmetic products, enhancing the efficacy, stability, and functional performance of active ingredients. Among these, silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnO-NPs) are widely used due to their antimicrobial, UV-protective, and anti-inflammatory properties.

The use of silver nanoparticles in cosmetics is gaining increasing popularity. According to N. Manosalva et al., biogenic silver nanoparticles have been effectively assessed as preservatives and in sunscreen formulations, offering natural alternatives to synthetic chemicals [50]. Colloidal silver (AgNPs) is now among the most frequently used nanomaterials in cosmetics, acting both as a chemopreventive agent in sunscreens and as a preservative in various personal care products.

The Scientific Committee on Consumer Safety (SCCS) considers silver nanoparticles safe as a preservative, provided they are used within specific concentration limits and do not penetrate beyond the viable layers of the skin [51]. The primary appeal of silver nanoparticles in nanocosmeceutical formulations lies in their broad-spectrum antibacterial, antifungal, and anti-inflammatory properties. These features make them ideal for use in acne treatments, deodorants, moisturizers, and products targeting sensitive or inflamed skin.

Moreover, silver nanoparticles are used as:

- Pigments in colored cosmetics such as lipsticks and eyeshadows,
- Antifungal agents in nail polish,
- Preservatives that reduce microbial contamination and prolong shelf life.

Despite their wide application, the mechanisms of action of AgNPs on human skin and biological systems are not yet fully understood. Their toxicity, biological interaction, and penetration potential depend heavily on particle size, shape, surface coating, and method of application. Recent studies highlight that smaller particles (<20 nm) may penetrate deeper into the skin, especially if the skin barrier is compromised, potentially leading to cytotoxic and genotoxic effects [52- 53].

On the other hand, Zinc Oxide Nanoparticles (ZnO-NPs) are primarily used in sunscreens due to their excellent UV-blocking ability, covering both UVA and UVB spectra. Unlike chemical UV filters, ZnO acts as a physical blocker, reflecting and scattering harmful radiation. At the nanoscale, ZnO particles form transparent films, improving aesthetic appeal without leaving a white residue on the skin.

ZnO-NPs also exhibit:

- Mild antimicrobial activity,
- Sebum-regulating properties, making them suitable for oily and acne-prone skin,
- Anti-irritant effects, particularly when combined with soothing agents.

Importantly, several studies have shown that ZnO nanoparticles remain on the surface or in the stratum corneum and do not penetrate into deeper viable layers of skin under normal usage conditions, making them generally safe for topical application [54].

Silver and zinc oxide nanoparticles play key roles in the next generation of cosmeceutical formulations by enhancing product safety, stability, and performance. However, their long-term effects, penetration behavior, and ecotoxicity remain areas of active investigation. Regulatory bodies such as the SCCS continue to update safety assessments to ensure consumer protection [55-56-57].

I.1.4.2. For Sunscreen:

In personal care products like sunscreens, nanoparticles (NPs) that are particularly made from leaf extracts that have a high concentration of phytonutrients have become more important. Based on their stability and capping agents, the aim of this work was to determine the in vitro sunscreen activity of an emulsion made of ZnO and Ag NPs generated with the extract of Laurus nobilis and Citrus limon leaves. The Mansur equation and an ultraviolet spectrophotometric method were used to determine the sun protection factor (SPF) of a cream emulsion that included ZnO and Ag NPs. The synthesized ZnO-NPs and Ag-NPs were finely characterized at specific and controlled parameters using scanning electron microscopy, Fourier transform infrared spectroscopy, ultraviolet–visible spectroscopy, and an energy-dispersive x-ray spectroscopy technique. Some physicochemical parameters were then analyzed, including the hydrogen ion concentration (pH), stability test, viscosity analysis, irritancy patch test, and physical texture verification. The SPF values of the cream emulsion containing ZnO and Ag NPs were recorded as 0.573 and 0.601, respectively

I.1.4.3. Anti aging:

Aging is defined as a continuous process by a consistent functional decline in physiological markers. Early aging symptoms are mostly caused by internal variables including hormones, gender, mood, enzymes, and hereditary vulnerability, as well as exterior elements like heat, air pollution, and radiation exposure. The skin is one of the most susceptible organs. Nanoparticles are regarded as a powerful tool in the cosmetics industry for improving the efficacy of medications that are currently successful as well as creating novel stand-alone therapies. The activity of anti-aging drugs is enhanced by nanoparticles, which focus on specific cellular and chemical processes. On the other hand, nanoparticle platforms have also gained popularity as a skin protection against environmental factors including pollution and UV radiation [58]. Table I.2 explains the different NPs from various sources and their anti-aging properties with the suitable outcome.

Table I.2 : Effect of organic and inorganic nanoparticles on anti-aging

Nanoparticles	Resources	Antiaging property	Outcome	References
Liposomes	Phosphatidylcholine	Vitamin D3	Anti-wrinkle	[58]
Solid-lipid NPs	Lecithin and glycerol	Peptide	UV protection	[59]
Nanostructured lipid carriers	Compritol 888	Idebenone	Anti-oxidant	[60]
Silver NPs	Rutin	–	Anti-wrinkle	[61]
Platinum and palladium NPs	–	–	Protect skin from thinning	[61]
Gold NPs	Ginseng	–	Anti-oxidant and whitening	[62]
Zinc oxide NPs	Oxalic acid	–	Skin whitening	[62]

I.1.4.4. ZnO NPs as Physical UV Filters

UV radiation, which is classified as UVA (λ = 320–400 nm), UVB (λ = 280–320 nm), and UVC (λ = 100–280 nm), accounts for approximately 10% of all light output [63]. The only radiation that may really penetrate the atmosphere and endanger human health are those with the longest wavelengths, such as UVA and UVB. UV filters, which are typically categorized as either inorganic or organic, can be used to achieve photoprotection [64]. In cosmetics, minimizing UV-induced skin damage is crucial for avoiding sunburn and long-term impacts (such skin aging and skin cancer), and the use of filters has now been shown in everyday products as well as sunscreens [65]. These days, formulations with filters are made to provide protection from UVA and UVB rays. UVA penetrates deeper into the layers of the skin, causing immune system suppression and precancerous alterations, even though the latter has more energy and is significantly involved in carcinogenesis [66]. Furthermore, by increasing the synthesis of matrix metalloproteinases that break down collagen and elastin, UVA is the main cause of the loss of skin elasticity [67].

Insoluble titanium oxide and zinc oxide particles are widely used as inorganic filters, particularly when they are in their nanometric form (30–150 nm) [68].

Despite having a wide absorption spectrum spanning both UVA and UVB, ZnO NPs are more effective as UVA filters [69]. When applied, NPs smaller than 200 nm are visible as translucent [70]. Because these NPs might produce reactive oxygen species (ROS) when exposed to UV light, there is discussion over their possible long-term toxicity (see section 5 below) [71]. Coatings like silica, polymethylacrylic acid, aluminum hydroxide, aluminum oxide, and methicone are frequently used to reduce their photocatalytic activity without compromising UV attenuation [72]. These coatings can work by capturing ROS or by reducing interactions with the surrounding media.

On the other hand, Zaccariello et al. grew bismuth titanate in mesoporous silica NPs to create a unique inorganic filter that both increases the absorption window and inhibits photocatalytic activity [73].

I.2. Skin care cream: Formulation and mechanism

I.2.1. overview of skin care formulations:

The epidermis, dermis, hypodermis, and its appendages (sweat glands, sebaceous glands, and hair follicles) are the several diverse layers that make up the skin. Keratinocytes that are stratified from a base layer of viable cells to an outermost layer of terminally developed keratinocytes make up the epidermis. Natural-moisturizing-factor (NMF)-laden and lipid-bound corneocytes (differentiated keratinocytes), corneodesmosomes (proteinaceous rivets binding corneocytes together), and lipids make up the stratum corneum (SC), a very thin layer of around 10 μm. Lipid bilayers in the SC are well-structured and have a lamellar structure. The SC performs a barrier function thanks to the high content of proteins and lipids, i.e., ceramides (50%) containing phytosphingosine, fatty acids (10–20%, highly enriched in linoleic acid), and cholesterol (25%) [74].

- Types of Skin Creams:

They are separated into two categories: water-in-oil (W/O) creams, which are made up of tiny water droplets scattered throughout an oily phase, and oil-in-water (O/W) creams, which are made up of tiny oil droplets scattered throughout a continuous phase. The emulsifying agent employed and the proportions of the two liquid phases determine whether the oil or aqueous phase becomes the dispersed phase. Therefore, an oil-in-water (O/W) emulsion is one in which the oil is distributed as droplets throughout the aqueous phase. The emulsion is of the water-in-oil (W/O) type when the dispersed phase is water and the dispersion medium is oil. Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. Water-in-oil creams are also more moisturizing as they provide an oily barrier which reduces water loss from the stratum corneum, the outermost layer of the skin

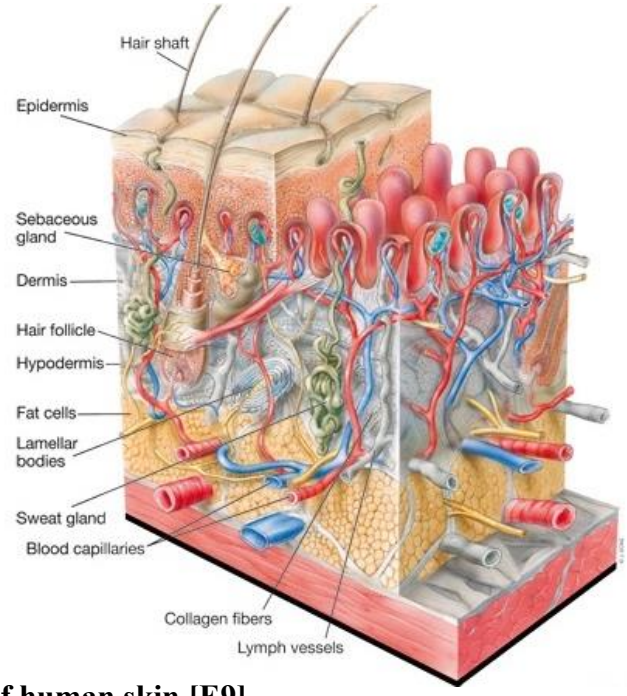


Figure I.11: Structure of human skin [E9]

The outer layer of the epidermis, the external layer of human skin, is made up primarily of corneocytes which provide a barrier function. Underlying the corneocytes are viable keratinocytes, which migrate outward and terminally differentiate to become corneocytes. The epidermis is organized into extensions called rete ridges that project between dermal papille (pink) into the underlying connective tissue. Underlying the epidermis is the dermis, which is primarily made up of collagen, elastin fibers, and other extracellular matrix components. Collagen and elastin fibers are synthesized by fibroblasts to provide tensile strength, firmness, and elasticity to the skin. The innermost layer of the skin, the hypodermis, is composed largely of fat cells, which helps provide structure to the skin. Blood capillaries, lymph vessels, sweat glands, sebaceous glands, hair follicles, and lamellar bodies lie within the dermis and hypodermis

I.2.2. The Penetration of NPs through the Skin

I.2.2.1. The Routes of Penetration:

NPs with insufficient penetration capacity can be applied to the skin's surface as antibacterial and photoprotective agents (Ag-NPs, ZnO-NPs, etc.).

Regarding the penetration process, the skin is a porous barrier with many semi-circular channels that range in diameter from 0.4 to 36.0 nm. The SC is the main obstacle that NPs have to go beyond. NPs can enter the skin in three different ways: through the lipid matrix of the SC, sweat gland pores (60–80 μm in diameter), hair follicles, pilosebaceous pores (10–70 μm in diameter), and sebaceous glands.

Hair follicles, a key region for NP translocation and storage, offer an intriguing method of delivering the medication through the skin and transdermal route utilizing NPs. [75-79].

Topical medication distribution occurs along the skin appendages via the transfollicular pathway or across the SC via the intracellular and intercellular channels (Figure I. 12). The deeper layers provide a more watery

environment, whereas the SC is a lipidic acidic compartment. Therefore, polar molecules diffuse into the deeper tissues, while tiny (<10 nm) lipophilic particles with a positive charge can passively pass through the epidermis into the deeper layers of the skin [80]. Effective skin absorption is considered to be provided by partition coefficients (log P) between 1 and 3. [81].

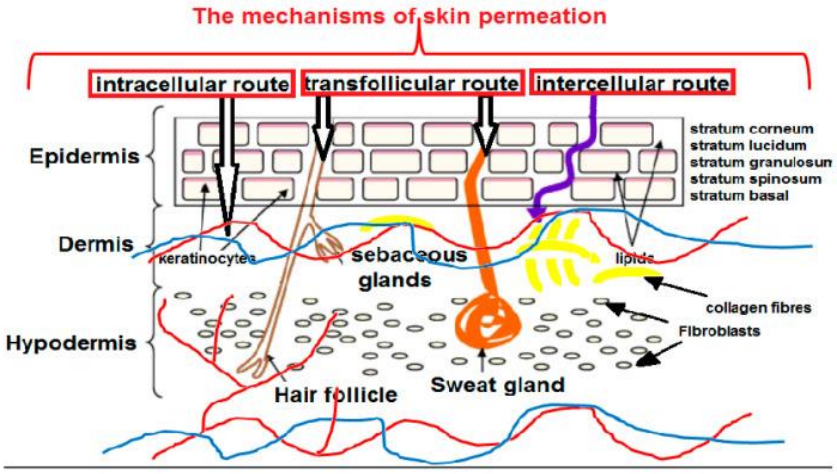


Figure I.12: the mechanisms of skin permeation [E10]

I.3. Hair dye:

-A widespread technique for both men and women is to use chemical dye to modify the original color of their hair. Based on its ability to retain color, hair dyes are divided into four categories: temporary, semi-permanent, demi-permanent, and permanent. Temporary dyes, also known as non-oxidative dyes, had a shorter stay period on the fiber and removed the hair during the first shampoo wash since the coloring procedure was done without the use of an oxidizing agent. Nitro aromatic amines and aromatic amino nitroanthroquinone dyes are examples of semi-permanent chemicals; they diffuse into the hair and bond to it, but they do not adhere permanently. Because of the oxidizing ingredient used to generate the color, permanent hair dyes are known as oxidation hair dyes.

In general, oxidation hair dyes produce shampoo-resistant hair colors, and an active intermediate further interacts with the coupler to give the hair its color. Skin irritation and hair loss may arise from allergic reactions or sensitization caused by some hair colors. Repeated exposure can cause sensitivity in people. Formulations can also evolve over time. The primary toxicological concerns of hair dyes, primarily oxidation hair dyes, are with contact dermatitis and long-term "potential" systemic effects. The para diamine oxidation derivative dyes reported to having more sensitizing potential when compared to other amine derivatives.

I.3.1. Human hair structure:

-Human hair fibers are made up of four components: the cuticle, cortex, medulla, and cell membrane complex. They are around 30 to 120 µm broad. The protective layer known as the outer cuticle is made up of the low cysteine endocuticle below, the cysteine-rich, cross-linked exocuticle, and the outer membrane epicuticle.

The majority of the fiber mass, which is made up of matrix, macrofibrils, and microfibrils, is concentrated in the cortex and contributes to the swelling behavior of hair. The medulla, the center portion of the hair fiber, is made up of keratin fibers and gives hair its reflective quality. The cell membrane complex is made up of cell membranes and binding materials, and it opens pathways for diffusion of molecules through the hair fiber fiber. [82,83].

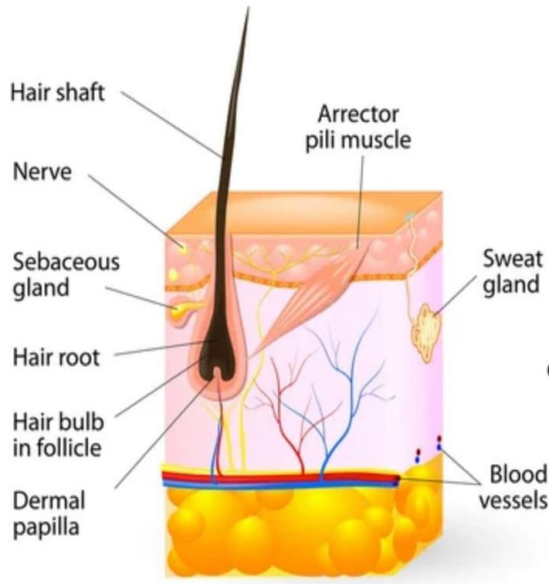


Figure I.13: Human hair structure [E11]

I.3.2. Mechanisms of Nanoparticle-Based Hair Coloring:

Direct dyeing and mordant dyeing are the two methods used to color hair with the majority of natural plant-based hair colors. In a nutshell, there are two processes in the hair coloring procedure.

i. Dye molecules diffuse into the keratinous hair fiber from the dye bath.

ii. With or without the help of auxiliary mordanting chemicals, chemical bonds (hydrogen, ionic, and covalent) are formed between the carboxyl or hydroxyl groups found in the dye molecules and the amino/sulfhydryl groups in hair keratin. [84]

• Diffusion is a three stage process [85]:

1. The first stage is the transport of dye molecules to the fiber/water interface by a combination of aqueous diffusion and agitation
2. In the second stage, dyestuffs are adsorbed onto the outer layer of the hair cuticle
3. The final stage is the diffusion of dye molecules of low molecular weight into inner hair structures (cuticle and cortex) and can be characterized by the change to the cell membrane complex (CMC) present in the hair cuticle. The CMC is a continuous phase of intercellular matters that binds the

cuticle and cortical cells together [86]. Studies have shown that penetration though CMC is the main transport pa thway for dye substances to reach the hair cortex [87,88]. Less ionized small molecules are more likely to penetrate through and spread over the lipid bilayer of CMC [89]. Besides, the condition of hair fibers also affects the absorption and diffusion of external dye s substances

I. 3. 3. Extraction methods for natural dyes:

Following the drying and grinding of plant material, a suitable solvent is selected based on the desired dye and extracted using both conventional and new procedures to extract natural colorants from natural sources. Chromatographic or other separation techniques are used to separate the target isolated compounds utilized for dyeing, and spectrum techniques are employed to describe the separated compounds. Figure I.14 [90], [91]

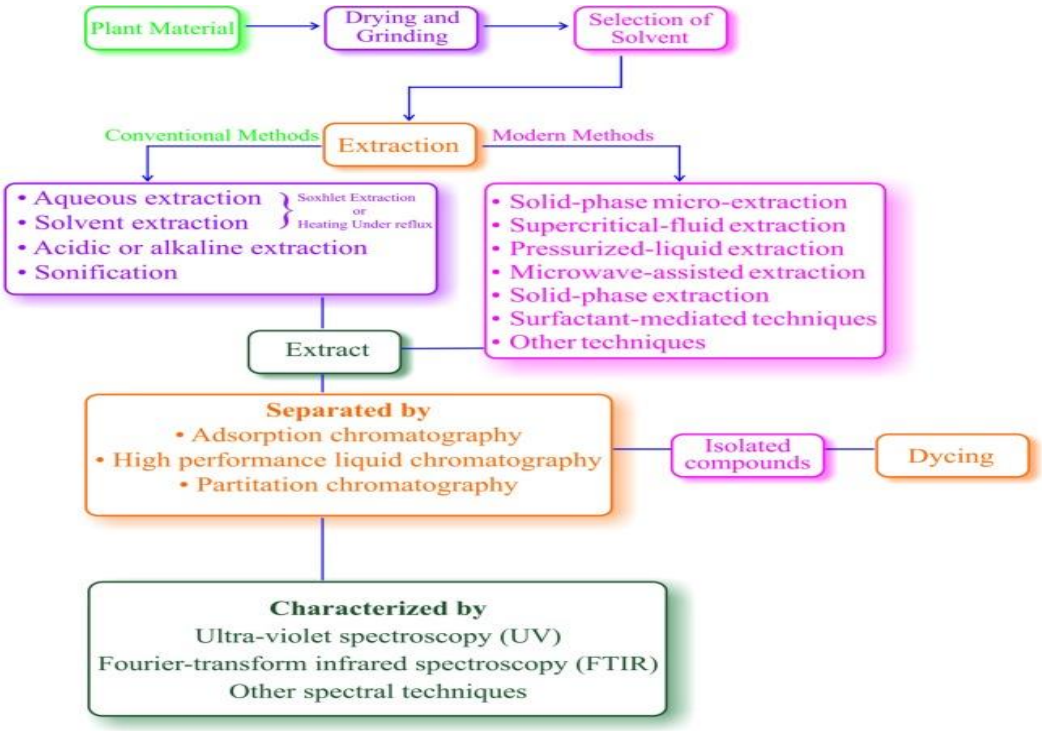


Figure I.14: Extraction methods of natural dye [E12]

I. 3.4. formulation strategies:

I. 3.4.1 Natural Pigments vs. Synthetic Dyes :

Environmentally friendly synthetic dyes versus natural dyes

Promoting environmentally friendly synthetic dyes in favor of dangerous ones is another method of being sustainable in the dying industry. These environmentally acceptable synthetic dyes are less common because of a number of problems, including their high cost and non-green synthesis pathways and application processes. With improvements in extraction and application techniques, natural dyes can be promoted with sustainability comparable to that of environmentally friendly synthetic dyes [92- 97].

I.3.4.2 Herbal-NP Hybrid Dyes (e.g., Henna + ZnO NPs):

Natural pigments and dyes: Present situation

Humans are already using a greater quantity of synthetic colorants for a variety of beneficial purposes in modern civilization [98]. However, the environment and human health are at risk due to the overuse of synthetic dyes that are azo and benzidine-like, non-biodegradable, and carcinogenic [99]. Even if natural dyes don't have these negative impacts, they nevertheless need to be addressed because of their weak binding ability and low yield. Therefore, it is imperative that more study be done and that natural resource output be optimized. From an Indian point of view, there are enough plants, minerals, and animals that produce dyes , and they may be cultivated or processed to produce huge quantities [100]. It is possible to investigate using insects and bacteria to produce colorants.

The performance of colorants can also be enhanced by investigating the usage of natural mordants. Nowadays, synthetic dyes play a prominent role in all industries (Figure I.15), whereas natural dyes are utilized in certain of them to replace synthetic dyes with the required sustainability [101], [102].

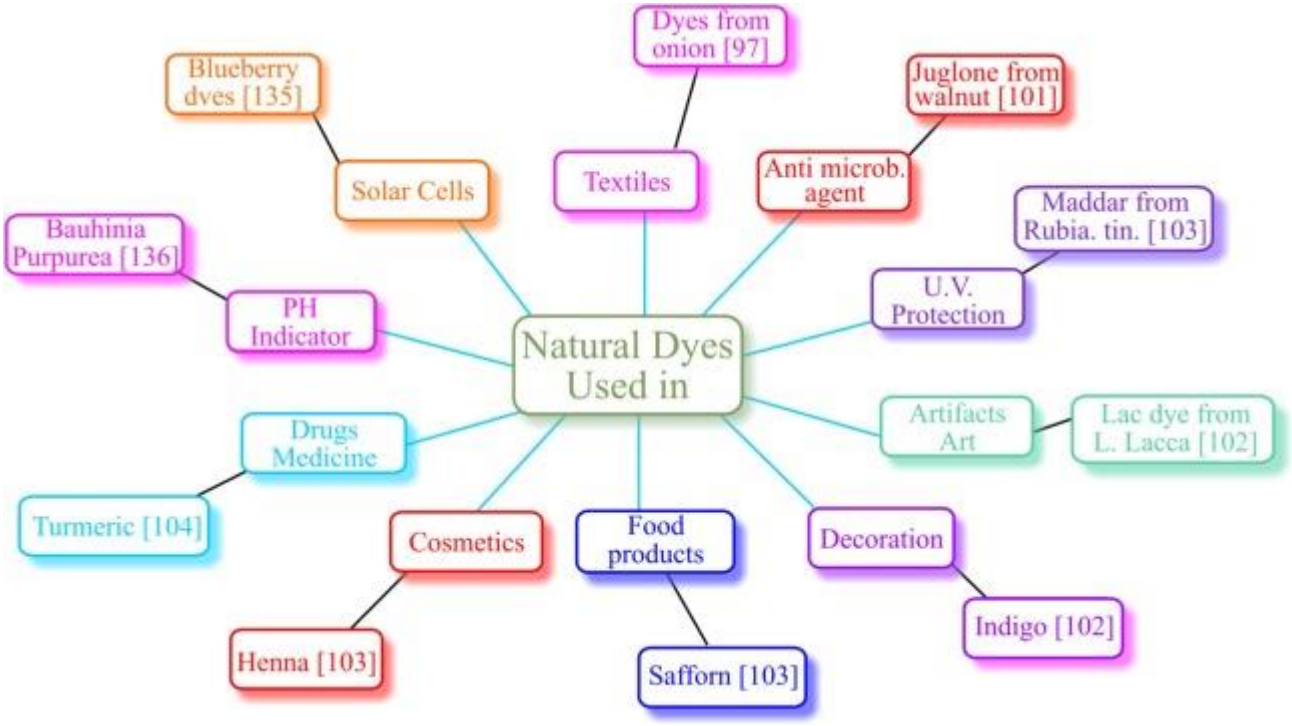


Figure I.15: Use of natural dyes in various fields [102 - 111].

I.4. Advantage of Nanoparticles in hair dye and skin care formulation:

Hair Dye and Skincare Nanoparticles

• Nanoparticles improve hair dye's penetration and absorption, guaranteeing greater color coverage and more durable effects.

• They keep dye molecules stable throughout time, avoiding oxidation or degradation.

• Active compounds in skincare products are encapsulated by nanoparticles, which shield them from environmental deterioration.

• By offering more equal and comprehensive coverage, they improve the effectiveness of hair dyes.

• By encasing color or chemicals in nanoparticles, they lessen sensitivity and discomfort.

• Following hair coloring, they enhance the hair's appearance and texture.

• For focused therapies, nanoparticles can be employed to target certain regions of the skin or hair layers.

• They are transparent on the skin and provide excellent protection from UV rays**.**

I. 4.1.Antioxidant and anti inflammatory activities of Ag NPs and ZnO NPs:

It has been demonstrated that bioactive ingredients in plant root extract and/or synthetic NPs increase their anti-inflammatory and antioxidant properties. Plant root extracts are antioxidants because they include compounds including phenols, flavonoids, vitamins, and terpenoids that scavenge free radicals (Singh and Dhaliwal, 2019). Ag-NPs were produced from N. leucophylla root extract by Singh and Dhaliwal (2019), and their antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique. The produced Ag-NPs shown more antioxidant activity (79.41± 0.004 in 250 µg mL) than the root extract of N. leucophylla (68.29 ± 0.004 in 250 µg mL), according to the DPPH test. Ponvel et al. (2015) used the DPPH technique to examine the antioxidant activity of Ag-NPs made from the root extract of the medicinal plant Justicia adhatoda, and the findings validated the synthetic Ag-NPs' antioxidant properties, Using root extract from Helicteres isora, Bhakya et al. (2016) investigated the antioxidant efficacy of biogenically produced Ag-NPs. Three distinct techniques—DPPH, hydrogen peroxide, and nitric oxide assay—were used to conduct the test. Every result from the radical scavenging experiment showed that the phytoconstituents in the H. isora root extract significantly contribute to the reduction of Ag metal to Ag-NPs, which have greater antioxidant activity. evaluated the anti-inflammatory and antioxidant properties of green generated zinc oxide nanoparticles (NPs) made from P. tenuifolia root extract using the DPPH technique and lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. While significant anti-inflammatory action was seen in a dose-dependent manner, the results demonstrated that zinc oxide nanoparticles had modest antioxidant activity. The green synthesis of Ag-NPs also made use of the root of Lovoa trichilioides (Sidjui et al., 2016). The DPPH technique was used to examine the produced Ag-NPs for antioxidant activity. In this work, the inhibitory action was detected between 100 and 25 µg mL, and the antioxidant activity (in vitro) was shown to be biphasic.

I.4.2. Acne Treatment & Antimicrobial Effects:

I. 4. 2.1 Ag NPs in Anti-Acne Formulations:

Endogenous (such as breakdown products and cellular debris) and exogenous (such as pollutants and pathogens) impurities build up in the hydrolipidic layer that covers the skin and is made up of secretions from the sweat and sebaceous glands. Therefore, washing is essential to maintaining skin health in order to eliminate pollutants and manage smells and skin bacteria.

The removal of dirt on the skin is mainly accomplished by using surfactants that are able to bind lipophilic substances not eliminated by simple washing with water. However, surfactants may also impair the skin barrier function by solubilizing a number of skin components and/or accumulating in the SC matrix [112]. Thus, novel formulations have been developed as alternatives to conventional soap bars and detergents, especially for facial cleaning. Micellar systems and nanoemulsions have especially found application in this area [113,114]. A widely used product that exploits the first is so-called micellar water—it contains water and small amounts of mild surfactant and is able to efficiently remove dirt from skin without foaming and/or disrupting the skin barrier [115]. The removal of harmful microorganisms is another benefit of skin washing, and as was already noted, certain metal-based NPs are employed as decontaminants and disinfectants. Ag NPs are therefore included into goods like fluid detergents and soap bars. These solutions also promise to effectively cure acne and sun-damaged skin by utilizing silver's anti-inflammatory qualities [116].

I.4.2.2 Antibacterial and antifungal agents:

Commonly used antimicrobial medicines have a number of drawbacks, such as toxicity and limited potency and efficiency brought on by the emergence of resistant strains [117]. In order to examine their antibacterial and antifungal activities for a variety of applications (such as the textile, food, and cosmetics sectors), several metal-based NPs, including Ag NPs, Au NPs, ZnO NPs, TiO2 NPs, and copper-based nanomaterials, have been developed [118].

Since bacterial and fungal environments have been found to be a common cause of several cutaneous manifestations and an imbalance in the skin microbiota has been linked to a number of disorders, including acne and atopic dermatitis, antimicrobial agents may be included in the final formulation of cosmetics as an active ingredient to prevent and treat skin infections [119,120]. As an alternative, they can be employed as preservatives to stop microbiological contamination of the product while it is being manufactured and utilized by consumers [121].

Ag NPs are arguably the most widely employed nanomaterials for antibacterial applications among all of them. The usage of silver compounds has attracted a lot of attention lately since it has broad-spectrum activity and inhibits the formation of resistant strains through a variety of modes of action, including DNA damage, cell membrane leakage, and protein denaturation [122]. Ag NPs often exhibit more antibacterial activity than silver ions, as our group previously showed. This result is probably caused by special microbial-nanomaterial interactions associated with the prolonged release of silver [123-125]. Ag NPs have also been shown to disrupt the production of biofilms, which is frequently the cause of infection recurrence [126,127]. Both conventional

and "green" (i.e., silver reduction carried out by microorganisms or using nontoxic reducing agents) chemistry can be used to create Ag NPs; the former is inexpensive and highly effective, but a number of examples of the latter have been published recently, suggesting a growing interest in environmentally friendly procedures and bio-manufactured goods [128]. Semisolid formulations like gels and creams can contain silver nanoparticles (Ag NPs) [121,129]. Because of their ability to combat bacterial and fungal infections, Ag NPs are used in a wide range of cosmetic and personal care products, such as face cleanser, creams, masks, foot balms, deodorants, shower gels, and shampoos. These products work just as well as or even better than traditional antimicrobials. A recent example is a randomized study that compared the efficacy of Ag NPs and clindamycin in treating acne vulgaris. The results showed that an Ag NP-based gel was just as effective as one that contained an antibiotic, with good tolerability and a higher satisfaction rating [130].

I.5.Challenges & Future Perspectives:

I.5.1 Regulatory and Safety Concerns:

Nanocosmeceuticals' Regulatory Characteristics. The quick development of nanotechnology in the cosmetics sector has raised concerns regarding safety concerns and potential negative impacts, especially on human health, as there are currently few toxicity studies. The following are the dangerous consequences [131,132].
1. Because of their small size and structure, nanoparticles may easily pass through membranes and move through the human body. They can then infiltrate blood, tissues, organs, and cells, where they can damage or destroy cells.

2. The altered physicochemical properties of nanoparticles are associated with a greater surface-to-volume ratio. In comparison to bigger particles, high reactivity and biological activity may be seen, which raises free radicals, leads to oxidative stress and skin irritation, both results in toxicity to the human system.

3. In order to assure their stability, nanocosmetics employ high surfactant concentrations, however surface active chemical exposure may cause serious skin irritation by impacting cells in the deep skin layer;

4. One common way to administer nanoparticles is via inhalation, which may cause the particles to enter the lungs, travel to the brain, and then enter the blood, organs, and nervous system, leading to a number of detrimental effects.

Other exposures include ingestion and skin contact. A variety of regulatory authorities have created suggestions to address the safety risk of nanocosmetics and help academics, researchers, the cosmetics industry, etc. Table 1 compiles important suggestions from important bodies. These guidelines must be adhered to in order to prevent any adverse side effects while applying nanocosmeceutical products [133,132].

I.5.2. Scalability of Green Synthesis:

Although green synthesis of ZnO and Ag nanoparticles offers a sustainable option for the environment, scaling up this process is still quite difficult. The composition of biological systems, including plant extracts, might differ based on species, cultivation circumstances, and extraction techniques. This can impact the consistency and repeatability of nanoparticle properties. There are also financial and technological challenges in maximizing reaction parameters on an industrial scale without sacrificing nanoparticle quality. Standardizing biosynthetic procedures, enhancing process control, and combining green synthesis with scalable technologies like flow-based systems and microreactors are some future directions. These developments are essential to bridge the gap between laboratory research and commercial production.

I.5.3. Next-Gen Trends: AI-Designed Nanoparticles & Personalized Cosmetics:

Artificial intelligence (AI) and customized product creation are propelling the area of nanotechnology into a new age. Artificial intelligence (AI) algorithms are being used to more accurately and efficiently design and forecast the best synthesis pathways, forms, and functions of nanoparticles. This data-driven method makes it possible to customize nanoparticles to meet certain biological or aesthetic requirements. Simultaneously, new opportunities for nanoparticle integration are created by the emergence of customized cosmetics, in which formulas are tailored to specific skin types, genetic profiles, or microbiomes. In this regard, environmentally friendly ZnO and Ag nanoparticles can be used as safe, adaptable building blocks for user-specific, next-generation cosmetic and medicinal solutions.

I.6. Conclusion:

The green synthesis of metallic nanoparticles, particularly zinc oxide (ZnO) and silver (Ag) nanoparticles, serves as an environmentally benign and sustainable alternative to traditional physical and chemical synthesis methods. Green synthesis minimises the utilisation of hazardous chemicals while generating biocompatible nanoparticles with optimal size, shape, and functional attributes through the use of plant extracts and other biological resources as reducing and capping agents. The robust antibacterial, antioxidant, and catalytic characteristics of ZnO and Ag nanoparticles render them particularly attractive for diverse applications.

Green synthesis methods are optimal for large-scale production owing to their simplicity, cost-effectiveness, and ecological sustainability. Industries are currently exploring the incorporation of biosynthesised ZnO and Ag nanoparticles into commercial products to meet the increasing demand for safer and more eco-friendly nanomaterials. Their versatility and pragmatic advantages offer substantial commercial prospects in sectors such as environmental cleanup, healthcare, packaging, and textiles.

To standardise synthesis protocols, ensure reproducibility, and get a comprehensive grasp of the mechanisms involved in nanoparticle creation using biological agents, additional theoretical and experimental investigations are recommended. Furthermore, attention must be directed towards evaluating long-term

stability and safety while optimising production scalability. Collaboration between academic research and industry is essential for the advancement of green nanotechnology and its practical application across various technical fields.

Chapter II :
Materials and methods

II.1 Introduction:

The several experimental tools and chemical and plant products used in this investigation are described in depth in this chapter, along with the experimental procedures and methodologies used.

II.2 Objective of the work:

Our work was conducted in the Chemistry Laboratory of the Faculty of Science and Technology at the University of Ghardaïa. This research focuses on the green synthesis and characterization of silver (Ag) and zinc oxide (ZnO) nanoparticles using plant extracts from curcuma (turmeric), hibiscus (carcadia), tasalgha, and henna. The use of these natural, biologically active resources enables the production of low-cost, non-toxic, well-crystallized nanoparticles with high specific surface areas.

The primary objective of this study is to develop a skin care cream and a hair dye formulation incorporating Ag and ZnO nanoparticles. These products aim to color hair, treat gray hair, and enhance skin health, offering a natural and eco-friendly alternative in cosmetic applications.

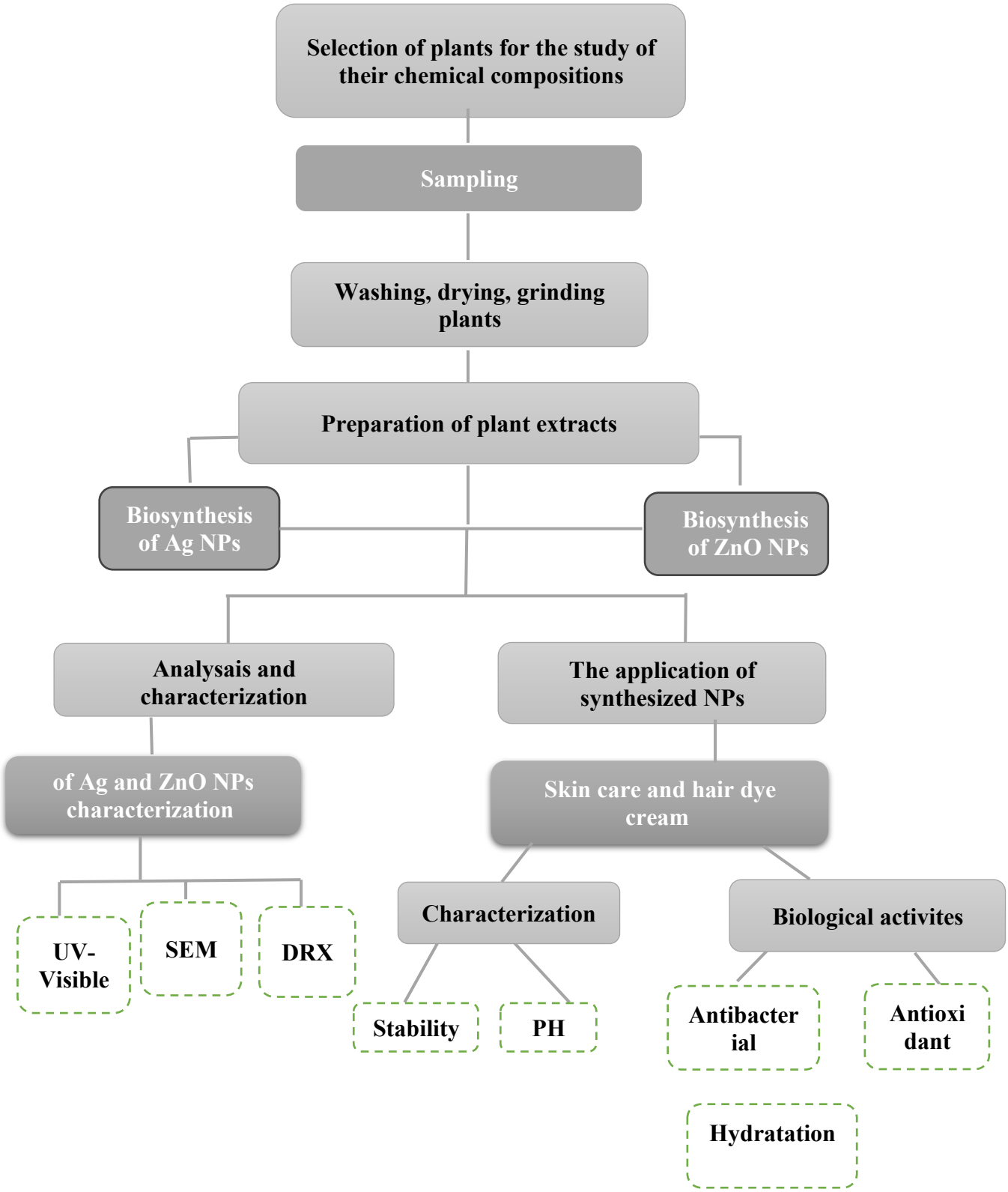


Figure II.1: Descriptive flowchart of the work methodology

II.3. Materials and Methods:

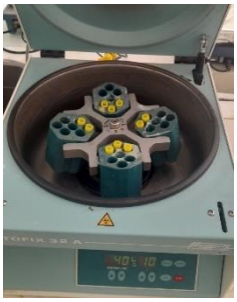
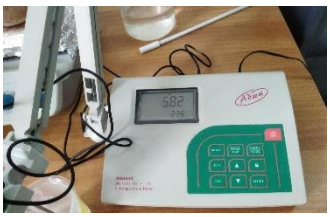
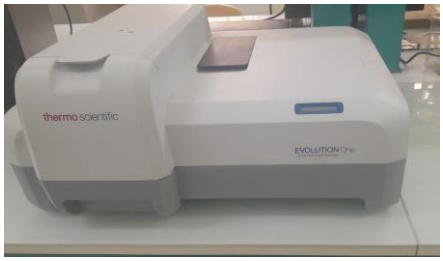
II.3.1. Materials and Chemicals products:

The following table lists all the main chemicals and materials used in our study.

Table II.1 : table of products

Produits	
○ **silver nitrate ($AgNO_3$) :** • Molar mass : 169.87 g/mol • Melting point: 212°C • Boiling point : 440°C ○ **Sodium hydroxide (NaOH) :** • Molar mass : 39.997g/mol, • Melting point : 318°C • Boiling point 1388°C ○ **Acetone ($(CH_3)2CO$) :** • Molar mass: 58.07 g/mol • Melting point: −94.9 °C • Boiling point : 56.08°C ○ **Ethanol (CH_3CH_2OH) :** • Molar mass: 46.07 g/mol • Melting point: 78.73 °C • Boiling point: 114.1 °C ○ **Distilled water** ○ **Zinc acetate dihydrate($C_4H_6O_4Zn_2H_2O$)** • Molar mass : 219.50 g/mol • Melting point : 100 °C • Boiling point : 242-4°C	○ **Sodium Alginate ($C_6H_7NaO_6$) :** • Molar mass: 288.38 g/mol • Melting point: 119°C ○ **Calcium chloride dihydrate ($CaCl_2$ $2H_2O$):** • Molar mass : 147.01g/mol • Melting point : 176 °C • Boiling point : 1670 °C ○ **Ascorbic acid ($C_6H_8O_6$) :** • Molar mass: 176.12 g/mol • Melting point : 190 °C • Boiling point : 553 °C ○ **DPPH (C18H12N5O) :** • Molar mass : 394.32 g/mol • Melting point : 135°C

Table II.2: table of materials

<table>
<tr><th colspan="3">Matériels et verreries</th></tr>
<tr><td>Oven
  </td><td>Balance analytique
  </td><td>Centrifuge
  </td></tr>
<tr><td>pH meter
 
Turbine agitator
  </td><td>Agitateur
 
Thermometer
 
Microscope
  </td><td>Furnace
 
UV- visible (UVILIINE 9400C),
 
 
Thermo Scientific™ Evolution One UV-Visible Spectrophotometer</td></tr>
</table>

II.3.2. Presentation of Plant Material

The plant material used in this study is the plants : curcuma , tasalgha, carcadia, hanna

- The leaves of the *Taslgha* plant come from the state of Sidi Belabbas. The leaves were collected in March 2025.

- The *hibiscus* plant was purchased from the local market in Ghardaïa in February 2023.
- The *henna* plant leaves come from the state of Adrar and were collected during the month of March.
- The *turmeric* plant is from India.

Figure II.2: The geographical location of plants

II.3.2.2.1. The plant of *Lawsonia inermis (hanna):*

Henna (Lawsonia inermis) is a perennial shrub belonging to the Lythraceae family. Henna is a considerable part of different industries for many clinical, cosmetic, and pharmaceutical applications. However, more applications and uses of the henna by-products are added constantly. Henna plant shows a variety of antimicrobial activities like strong antioxidant and antiinflammatory activities. Henna grows to 2.4-5 m and is a heavily branched deciduous, glabrous, occasionally spinescent shrub. It is grown for its dye and as a commercial crop in various Indian states being a hedge plant. Since the henna plant needs little irrigation, it may thrive well in semiarid and arid environments. In areas with limited water resources, it offers a special potential for agriculture. During the months of March and April, its seeds are sowed [134]. Three to five times a year, henna is collected, with the best products coming in early July and the worst in February, and multiple harvests of the crop are possible each year [135]. Every part of the plant has been found to be important, and every of its parts have been reported to be used in the treatment of diseases just like every other [136-137] Use of the leaves was advised by the Indian Ayurvedic Pharmacopoeia for pruritus, bleeding issues, dysuria, and other difficult skin diseases [138]. The leaf has a bitter taste and is used to treat scabies, boils, ophthalmia, syphilitic sores, amenorrhea, lumbago, headache, and hemicrania. Additionally, it favors hair growth. The leaves are traditionally applied to wounds and ulcers as well as to prevent skin inflammation, and they are used to treat febrile disorders, hemicranias, cephalalgia, leukoderma, and ophthalmia [139].

Figure II.3: *Lawsonia inermis* pant

Table II.3: properties of *Lawsonia inermis* plant

Information Common Name	**Henna**
Scientific Name	*Lawsonia inermis*
Family	Lythraceae (Loosestrife family)
Origin	North Africa, the Middle East, South Asia
Habitat	Dry, hot climates; cultivated in semi-arid and tropical regions
Appearance	Small shrub or tree with green leaves and small fragrant white or pinkish flowers
Flowering Season	Late spring to early summer
Traditional Uses	Dye for skin, hair, nails, leather, and fabrics

Medicinal Benefits	Antimicrobial, antifungal, used to treat skin conditions and wounds
Cultural Significance	Important in weddings, religious festivals, and body art traditions
Main active ingredient	lawsone, also known as 2-hydroxy-1,4-naphthoquinone

II.3.2.2.2. The plant *of Lavandula stoechas (Tasalgha):*

Globularia alypum L. (G. A.) is a wild perennial shrub belonging to the Plantaginaceae family, which is found throughout the Mediterranean region. The plant, locally named "Ain Larnab" or "Tasselgha," is known for a variety of purposes in Moroccan traditional medicine [140]. Leaves of G. A. are traditionally used as an antidiabetic, laxative, stomachic, and purgative agent [141]. In Algeria, this plant has also been used in the treatment of urinary incontinence and skin problems, such as eczemas, according to an ethnobotanical survey, which showed that G. a. is one of the most important medicinal plants used in traditional remedies by Algerian people [142-143].

Figure II.4 : *Lavandula stoechas* plant

Table II.4: properties of *Lavandula stoechas* plant

Information Common Name	Taselgha (Tasselgha, Taselgha)
Scientific Name	*Lavandula stoechas* (commonly identified)
Family	Lamiaceae (Mint family)
Origin	Mediterranean region (North Africa, Southern Europe)
Habitat	Rocky hillsides, dry grasslands, mountainous regions
Appearance	Small shrub with woody stems, narrow leaves, and purple-violet flowers
Flowering Season	Spring to early summer
Traditional Uses	Herbal medicine (anti-inflammatory, soothing), perfumes, essential oils
Medicinal Benefits	Treats respiratory issues, digestive problems, and used for wound healing
Cultural Significance	Used in traditional rituals and local folk medicine
Main active ingredients	fenchone (31.81 %) and camphor (29.60 %), followed by terpineol (13.14 %) and menthone (8.96 %)

II.3.2.2.3. The plant of *Curcuma longa (curcuma):*

Curcuma longa, a plant in the ginger family, is the source of the herb turmeric ,It is taken out of rhizomes, or underground stems, by boiling and purifying them with alkaline water ,Curcuma longa is indigenous to regions with high temperatures (20–30 degrees Celsius) and heavy rains , Turmeric is supposed to taste bitter once it is prepared. Although it is primarily grown in India, turmeric has been used for 4,000 years as a dietary supplement, medicinal, and culinary spice worldwide. In Ayurvedic medicine, it was first used to reduce gas, enhance digestion, dissolve gallstones, get rid of worms, and ease arthritis. [144]

Figure II.5 : *Curcuma longa* plant

Table II.5: properties of *Curcuma longa* plant

Common name	Turmeric, Indian saffron
Other name(s)	Turmeric (English), jianghuang
Scientific name(s)	*Curcuma longa*
Family	Zingiberaceae
Origin	India and Southeast Asia

Part(s) used	Rhizomes
Main active ingredients	Curcumin
Associated property(ies)	Anti-inflammatory | Antioxidant | Anti-ulcer | Hepatoprotective

II.3.2.2.4. The plant of *Hibiscus sabdariffa (carcadia):*

Known locally as "karkade," roselle *(Hibiscus sabdariffa L.),* a member of the Malvaceae family, is a significant annual crop that thrives in tropical and sub-tropical regions [145]. The fleshy calyx (sepals) that envelops the fruit (capsules) is the plant's most valuable economic component. The entire plant can be used as a beverage, or the dried calyces can be boiled in water and consumed hot or soaked in water to make a vibrant cold beverage. It has certain therapeutic qualities as well [146]. The seeds are occasionally used as animal feed [147] and contain 17.8–21% non-edible oil [148] and 20% protein. Roselle is a flexible plant with a number of uses. It is intercropped with crop staples such as sorghum and sesame, or planted along field margins. It requires little care. Its leaves, seeds, capsules and stems are used in traditional medicines.

Figure II.6: *Hibiscus sabdariffa* plant

Table II.6: properties of *Hibiscus sadbariffa* plant

Common name	**Hibiscus**
Other name(s)	Roselle, Red sorrel, Jamaican sorrel, Karkadeh (كركديه), Sour tea
Scientific name(s)	*Hibiscus sabdariffa*
Family	Malvaceae
Origin	Likely native to Africa (especially West Africa), but now widely cultivated in tropical and subtropical regions (e.g., Sudan, Egypt, Thailand, Mexico)

Part(s) used	Mainly the calyces (the outer floral parts), sometimes leaves and seeds
Main active ingredients	Anthocyanins (e.g., delphinidin and cyanidin derivatives), flavonoids, organic acids (e.g., citric acid, malic acid), vitamin C (ascorbic acid), polysaccharides
Associated property(ies)	Antioxidant, antihypertensive (lowers blood pressure), diuretic, antimicrobial, hepatoprotective (liver protection), mild laxative, cholesterol-lowering

II.4.preparation of plants extracts:

The plant materials are thoroughly washed several times with distilled water to eliminate dust and other impurities.

Following the washing step, the samples are dried in an oven for 24 hours to significantly reduce their moisture content. This step is essential to facilitate the subsequent grinding process.

Once dried, the plant material is ground using a mortar and pestle to obtain a uniform, fine powder. This increases the surface area, enhancing its interaction and reactivity with the extraction solvent.

Grinding

Drying

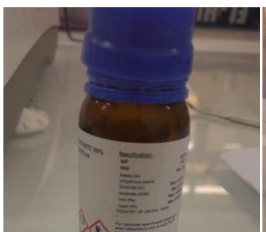
Washing

Figure II.7 : preparation of plant extracts

II.5.Biosynthesis of de nanoparticles :

II.5.1 Ag NPs :

The AgNO3 solution: We prepared 100 ml of a Sodium Nitrate (AgNO3) solution with a concentration of (10^{-3} M).



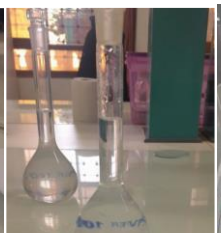


Figure.II.8 : Synthesis of silver nanoparticles (AgNPS).

In a typical reaction to prepare secondary silver particles, 10 ml of the plant extract is gradually added (dropwise) to 90 ml of a previously prepared 1 mM silver nitrate solution. The reaction mixture is kept at 60°C for 15 minutes under continuous mechanical stirring stirring.

-The formation of secondary silver particles is visually observed by the gradual color change of the mixture, which turns from light yellow to brown, indicating the formation of AgNPs. After several hours, a significant color change in the solution is observed, further confirming particle formationformation.

-The formation of AgNPs is subsequently confirmed using ultraviolet visible (UV Vis) spectroscopy. The resulting secondary particles are then precipitated by centrifugation at 15,000 rpm for 20 minutes. The resulting pellets are washed with a high ethanolic solution (10% ethanol) to obtain a fine powder of secondary silver particles particles.

- The AgNPS synthesis process involves three main stages:

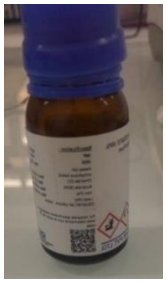
1) Dissolving the mineral salts in the plant extract in the presence of a specific amount of surfactant surfactant.

2)Heating the mixture with continuous mechanical stirring until the optimum temperature is reached reached.

3) Gradually cooling the mixture to room temperature, resulting in the formation of a colloidal solution containing silver nanoparticles (AgNPs).

Preparation of an AgNO3 solution

Preparation of plants extracts



Heating at 60°C for 1 hour

Figure II.9: preparation of Ag NPs

II.5.2.ZnO NPs :

Zinc oxide nanoparticles were synthesized by reducing zinc ions with the phytochemical compounds from the 4 plants extract and sing:

- Zinc acetate solution: 2.195 g of zinc acetate dihydrate stirred with 100 ml of distilled water in a heating system with agitation until 36 °C.

- NaOH solution: prepared with 8g of NaOH in 100 ml of distilled water.This preparation was placed under agitation and heating until the temperature reached 36°C.

The reaction was carried out by stirring 80 ml of zinc solution with 20 ml of the extract and titrating it with NaOH solution until the pH reached 10.Upon completion of the titration, the mixture was stirred for one hour, after which a precipitate formed and was separated using a centrifuge, Pure ZnO nanoparticles were obtained after calcination at 650 degrees Celsius for two hours to enhance the crystallinity of the nanoparticles.The protocol for the biosynthesis of ZnO nanoparticles can be summarized in this diagram:

Preparation of zinc acetate dihydrate solution

Preparation of plants extracts

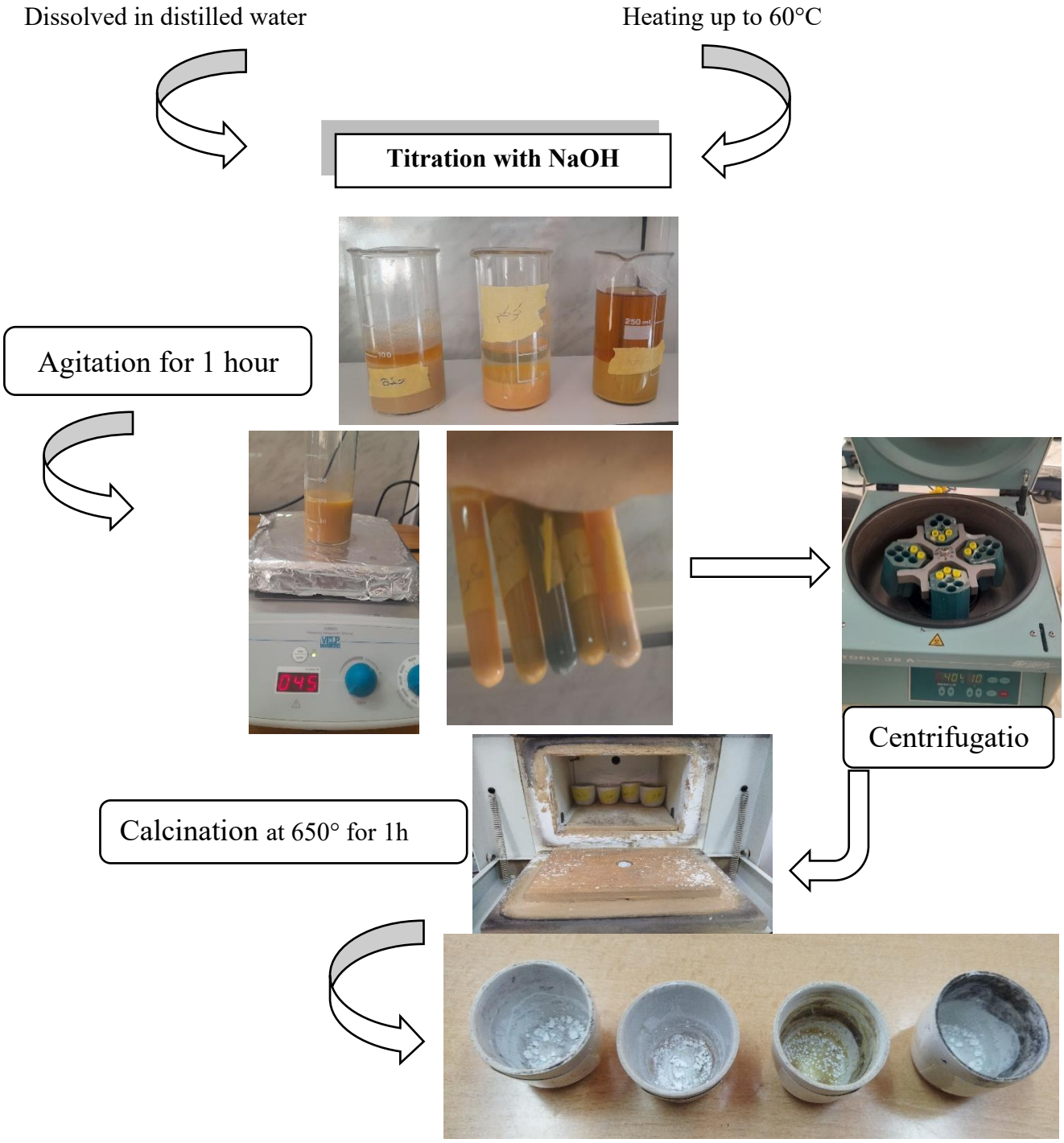


Figure II.10: preparation of ZnO NPs

II.6. The application of synthesized ZnO and Ag NPs:

II.6. 1.Preparation of creams:

The oily phase: This is the oil we prepared. In our work, we chose corn oil as the base for the cream.

The aqueous phase: This consists of distilled water and plant extracts.

Surface-active agents (SA): In our preparation, we used a hydrophilic surface-active agent, namely glycerin.

Anti-inflammatory and antibacterial agents

Binding and stabilizing agents: We used shea butter as a stabilizer and beeswax as an emulsifier.

Fragrance: Lavender essential oil was used to add fragrance

- operating procedure:
  - The different ingredients using a scale;
  - Heating the 2 phases separately until reaching 70°C;
  - Add the surfactants to the different phases;
  - Slowly pour the two phases into a beaker under agitation;
  - Once all the phases are added, leave it open to the air with a turbine stirrer until completely cooled.The resulting mixture is homogeneous and has a creamy texture.
  - Add additives to correct the viscosity and odor.
  - We divided the cream into three creams, considering the first one as a skin care cream and the remaining two as hair dye creams.
  - We divided the cream into three creams, considering the first one as a skin care cream and the remaining two as hair dye creams. Then we added plant extracts, ZnO NPs , and Ag NPs.

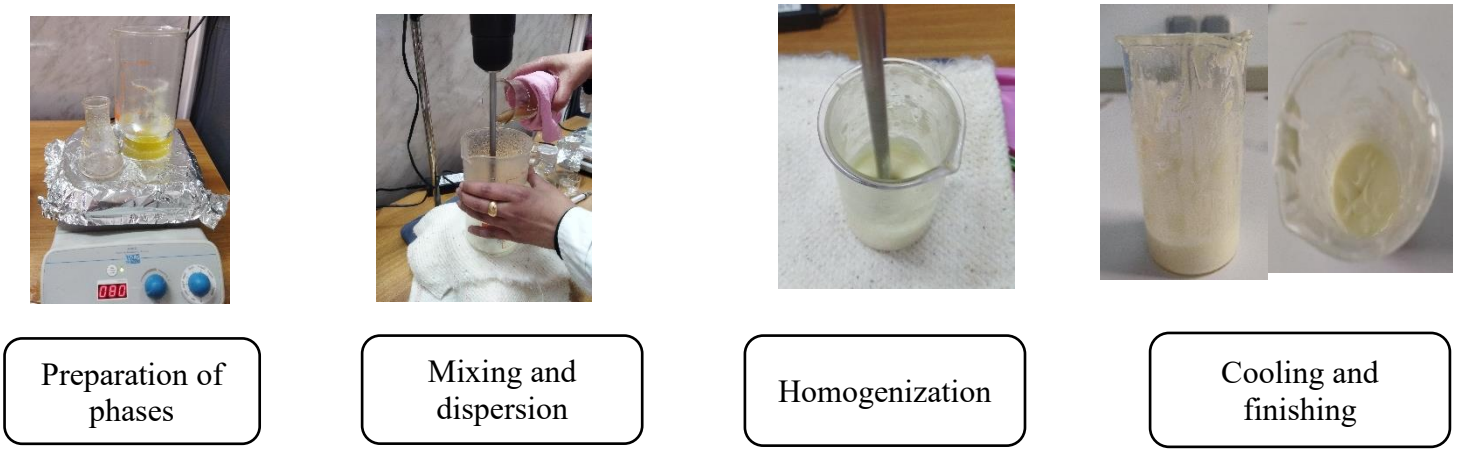


Figure II.11: Emulsion Preparation Process

II.6.1.1. Preparation of skin care Creams:

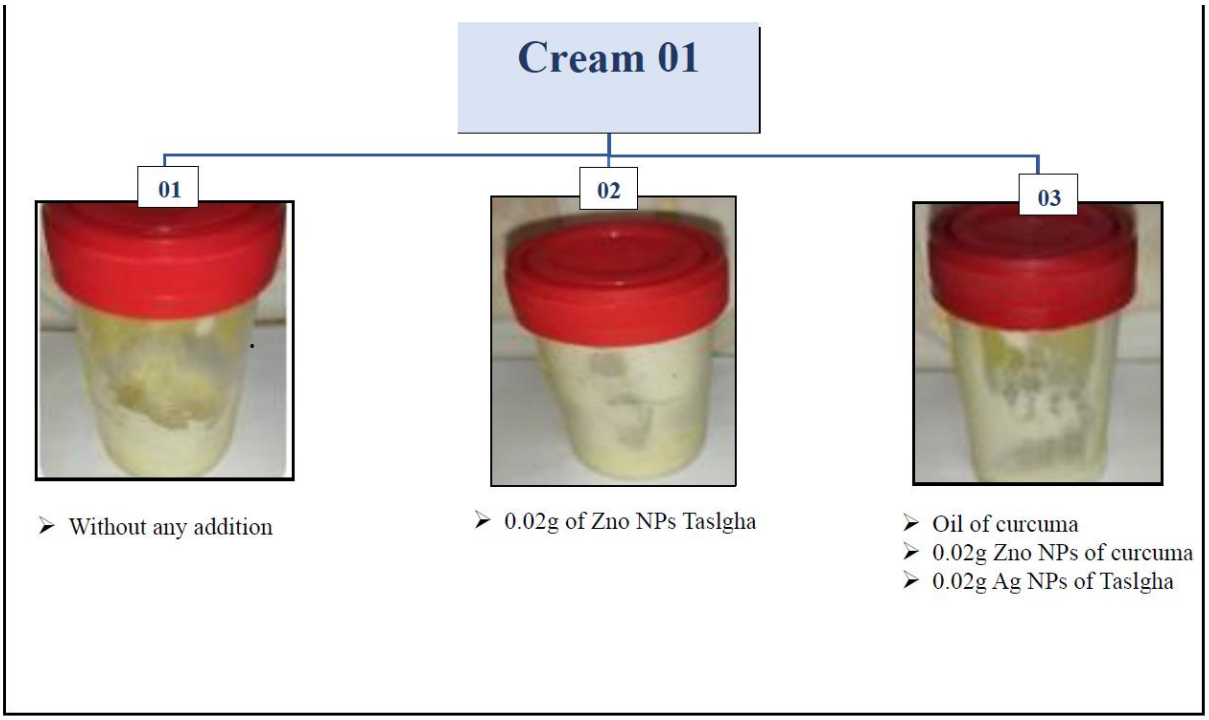


Figure II.12 : Preparing the skincare cream

II.6.1.2 Preparation of Hair Dye Creams:

In this step, a portion of the base cream was taken and mixed with a small amount of henna powder, which serves as the primary natural coloring agent for hair. Subsequently, plant extracts, zinc oxide nanoparticles (ZnO NPs), and silver nanoparticles (Ag NPs) were added to enhance the formulation.

To achieve a range of hair colors, different mixtures were prepared using:

- *Curcuma (turmeric)* for a light brown shade
- *Carcadia (hibiscus)* for a reddish-brown tone
- *Henna(Lawsonia)* for a classic brown color

Additionally, *Tasalgha* and *Moringa* plant extracts were incorporated to boost the protective and nourishing effects of the cream, enhancing hair strength and scalp health.

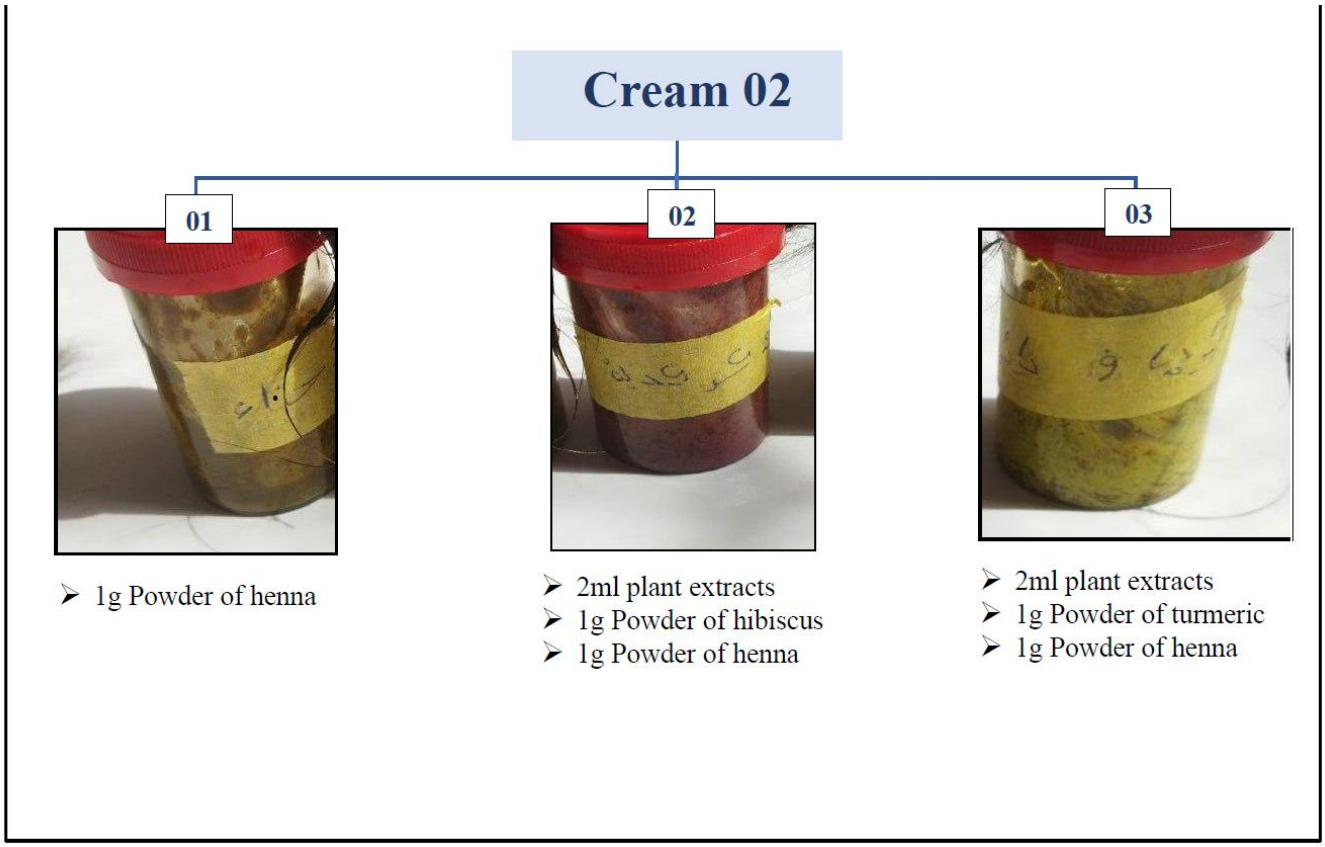


Figure II.13: Preparing hair dye cream 2.

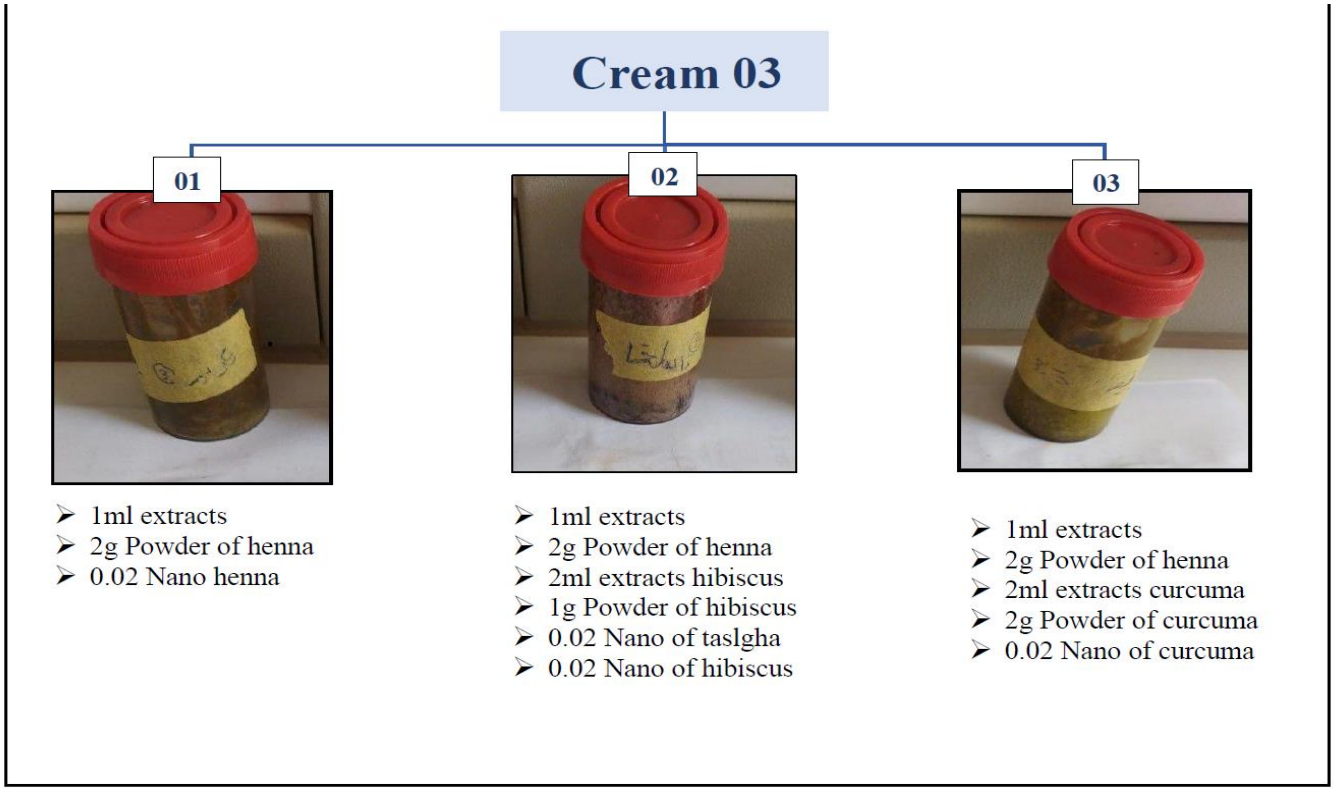


Figure II.14: Preparing hair dye cream 3.

II.6. 2.The Mask:

II.6.2.1. Preparation of solutions:

Sodium alginate solution:

A quantity of sodium alginate powder was dissolved in 100 ml of distilled water. The mixture is stirred for 24 hours until the alginate is completely dissolved and a homogeneous solution is obtained.

The mixture is stirred for 24 hours until the alginate is completely dissolved and a homogeneous solution is obtained.

Calcium chloride ($CaCl_2$) solution:

A quantity of calcium chloride (13.78g) was dissolved in 250 ml of distilled water, and the mixture was stirred until the calcium chloride was completely dissolved.

II.6.2.2. Preparation of mask:

In this practice, we designed a face mask.

A sodium alginate solution is mixed with a small amount of skin care cream (cream 1) under magnetic stirring until homogeneous. The solution is then poured into Petri dishes. The dishes are frozen for 24 hours, then thawed at room temperature, and finally placed in a CaCl2 solution to obtain a homogeneous and good mask.

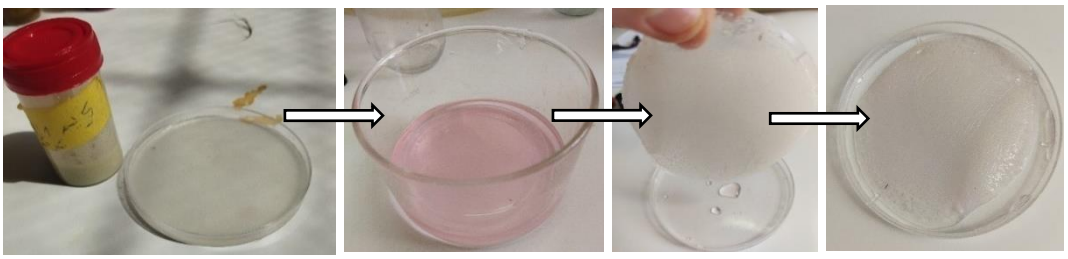


Figure II.15: process of preparation of mask

II.7. Characterization of ZnO and Ag NPs:

II.7. 1. UV-visible Spectroscopy:

UV-Visible spectrophotometry analysis was carried out at the University of Ghardaia, to evaluate the optical properties and absorbance behavior of the synthesized samples.

This technique is employed to assess transparency and examine the energy band gap of materials, which helps in understanding their electronic structure and optical behavior

- In this study, a UV-Vis spectrometer was used. This device is capable of measuring absorbance across a wide wavelength range. This range allows materials to be analyzed in both the UV and Vis regions of the spectrum, providing accurate and comprehensive information about the optical properties of the sample under study.

Figure II.16: UV-Vis spectrometer

II.7. 2 X ray Diffraction (XRD) :

XRD measurements were performed at the PTAPC research laboratory affiliated with CRAPC in Laghouat, Algeria, to identify the crystalline structure of the synthesized nanoparticles.

It is an effective tool for understanding the atomic arrangement within secondary particles

-In this research, a Malvem Panalytical XRD instrument was used to study the crystalline structure of secondary silver (Ag) and zinc oxide (ZnO) particles.

The instrument is equipped with a LYNXEYE scintillation detector and uses an X ray tube containing Cu/Ka rays with a wavelength of 1.54184 A , operating at a voltage of 30 kV and a current of 10 mA.

-The secondary samples were placed in a special powder holder and then the secondary samples were placed in a special powder holder and then scanned using Xscanned using X--rays over an angular range of 25 to 40 degrees. The scanning rays over an angular range of 25 to 40 degrees. The scanning was carried out with a precise step of 0.0202 was carried out with a precise step of 0.0202 degrees per second at each stage, degrees per second at each stage, for a total of 1,733 stages. In addition, the sample rotated at 15 rpm during the for a total of 1,733 stages. In addition, the sample rotated at 15 rpm during the scan to obtain a more homogeneous and accurate diffraction pattern, which aids scan to obtain a more homogeneous and accurate diffraction pattern, which aids in a comprehensive analysis of the grain structure.in a comprehensive analysis of the grain structure.

Figure II.17: Malvern Panalytical model diffractometer

II.7. 3 SEM (electron microscopy with bleaching)

Scanning Electron Microscopy (SEM) analysis was performed at the PTAPC research laboratory affiliated with CRAPC in Laghouat, Algeria, to examine the surface morphology and particle size of the synthesized nanoparticles. a SEM equipped with an accelerating voltage of 30 kV was used to provide high resolution imaging For analytical purposes, the sample was prepared by placing a small amount of biosynthesized secondary particles on a conductive coated grid to ensure the particles were fixed to the sample surface and improve image quality, The sample was then left to dry naturally at room temperature before being inserted into a scanning electron microscope to obtain detailed images showing the morphological characteristics of the particles and the extent to which they were clustered or dispersed.

Figure.II.18: The SEM device model Thermo Scientific

II.8. Characterization of cream:

II.8.1. Determining the type of emulsion:

We use two methods to determine the type of emulsion:

Dilution method: an L/W emulsion easily dilutes with water but not with oil. It's the opposite for an H/L emulsion.

In a test tube, we pour 1ml of the emulsion and add 9ml of distilled water, then we note the presence or absence of the dilution.

II.8.2. pH Measurement:

To determine the pH of the mixture, it was done twice by immersing the pH meter electrode into the preparations to be examined and taking the reading.

II.8.3. Centrifugation stability:

subject the emulsions to relatively high accelerations (around 3000 rpm for 6 minutes) using a centrifuge. Then observe the appearance of the emulsions, noting any potential instabilities. [124]

II.8.4.Acidity activity:

A- Totale acidity:

Total amount of organic acids in a solution (which can include lactic, acetic, tartaric, phosphoric, succinic, citric, etc.) , Typically each acid is reported as a concentration in grams per liter (g/L)[6]

B- Titratable acidity:

An approximation of total acidity, measures both associated and dissociated hydrogen ions , Measures how much a strong base (ex. sodium hydroxide (NaOH)), it takes to reach a basic pH (typically pH 8.2) , This details the total available hydrogen ions and is a more accurate to measure of perceived sourness , Is typically reported in either g/L or a percent TA, g/100ml.[7]

C- Procedure:

Titration was used in our investigation to ascertain the titrable acidity of the skin care and hair dye creams that were nano formulated, We reported the acidity in terms of NaOH consumption because the formulations lacked a single specified acid and the sample mass was not accurately assessed because we distributed a random fraction in distilled water.

We titrated each sample with 0.1N NaOH until the equivalence point (Veq), which we determined using a pH meter. We also measured the initial pH of each cream before titration in order to assess both the free acidity (pH) and the total acidity (titrable).

This approach allowed us to compare the acidity levels between different cream formulations. For example, a higher NaOH volume at the equivalence point indicates a higher total acid content in the formulation.[8]

D- Expression :

The titrable acidity was calculated as:

$$\textbf{TA (relative) = Veq × NNaOH}$$

- **TA** = The titratable acidity of the sample
- **Veq** = volume of NaOH at equivalence point (in liters)
- **NNaOH** = normality (concentration) of NaOH (mol/L)

II.8.5. Washing activity:

Washing and Coloring Activity of Hair Dye Cream Based on Nanoparticles

We evaluated the coloring and washing stability of the nanoparticle-based hair dye and skin care creams on two types of hair: white and brown.

We applied the dye cream and left it for a specific contact time before rinsing. Afterward, we washed the dyed hair four consecutive times to assess the color stability.

II.9. Pharmacological Activities:

II.9.1.Activity antioxidant:

There are various analytical techniques available for determining the antioxidant activity of biological samples, including food and plant extracts. The different methods are categorized into three main groups, such as spectrometry, chromatography, and electrochemistry techniques**.**

II.9.1.1.Test DPPH:

The DPPH (diphenylpicrylhydrazyl) test is a widely used method in the analysis of antioxidant activity.

A. Principe:

The DPPH° test is used to measure the antiradical power of pure molecules or plant extracts in a model system (organic solvent, room temperature). It measures the capacity of an antioxidant (AH, generally phenolic compounds) to reduce the chemical radical DPPH° (2,2-diphenyl-1-picrylhydrazyl) by hydrogen transfer.

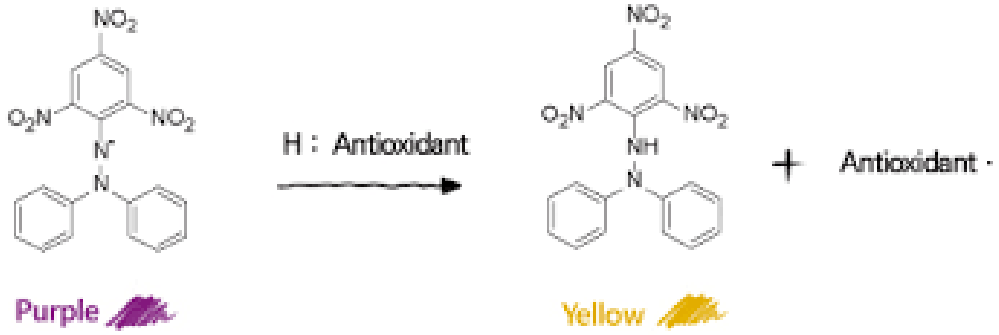


Figure .II.19: Chemical structure of the DPPH˙ radical and its reduced form

B. Procedure

In a volume of 1 ml, different concentrations of the extract to be tested in methanol are prepared, then 2 ml of the 0.1 mM DPPH· solution is added.After vigorous shaking, the mixture is incubated for 1 hour in the dark at room temperature, and then the absorbance is measured at 517 nm using a UV-visible spectrophotometer.A solution containing 1 ml of methanol and 1 ml of DPPH·, considered as an analytical blank, is prepared in parallel.

this DPPH radical scavenging activity was determined based on the assays described by Brand-Williams et al with some modifications.[149]

C. Expression:

The following formula was used to calculate the percentage of antioxidants:

% of antioxidant activity= [(Ac−As) ÷Ac] × 100

- ✓ Ac—Control reaction absorbance;
- ✓ As—Testing specimen absorbance.

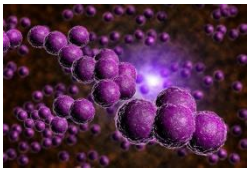
D. determination of IC50:

Inversely correlated with antioxidant capacity, this metric is defined as the concentration of antioxidant needed to reduce the initial concentration by 50%. [150].

II.9.2. Antibacterial Activity:

Antibacterial tests are conducted to evaluate the bactericidal effect of each prepared product. Antibacterial activity is evaluated using the method of diffusion of the antimicrobial compound in solid medium in a Petri dish, thereby creating a concentration gradient after a certain contact time between the product and the target microorganism. The effect of the antimicrobial product on the target is evaluated by measuring the zone of inhibition and taking its diameter into account. The strain is classified as sensitive, very sensitive, extremely sensitive, or resistant based on these measurements.

Table II.7: General Information on the Bacterial Strains Used

The Strains tested	**Characteristics bacteriological**	**Habitats**	**Power pathogen**
Escherichia coli* ATCC *25922.	Gram – 	-The digestive tract	Escherichia coli ATCC 25922 is a Gram-negative bacterium that is the most common aerobic host of the colon. Some strains cause diarrhea, and all strains are infectious when they invade sterile sites, causing active infection. Diagnosis is based on standard culture techniques or molecular testing. Toxin testing helps determine the cause of diarrhea. Treatment is with antibiotics, guided by the antibiotic regimen.
Staphylococcus aureus* ATCC *25923.	Gram + 	-The nasal cavities -The throat -The digestive tract	Staphylococcus aureus is an opportunistic pathogen that can cause food poisoning, as well as a variety of infections (1). Staphylococcus aureus subsp. aureus ATCC is a clinical isolate bearing the designation Seattle 1945, and 25923 is used as a standard control strain for laboratory testing. It is susceptible to a variety of antibiotics, including methicillin. The isolate contains a cassette chromosome-like element that lacks both the SCC and meca recombination (2, 3). We present the complete genome of the isolate, which consists of a 2,778,854 base pair chromosome and a 27,491 base pair plasmid, pS1945

The solid culture medium for the bacteria was Mueller Hinton agar (MHA) (Sigma-Aldrich). It has the following characteristics:

- Beef extract: 300.0 ml

- Casein peptone: 17.5 g
- Cornstarch: 1.5 g
- Agar: 17.0 g
- pH = 7.4.

So: 38g of GMH powder in 1L of distilled water are sterilized in an autoclave for 90 minutes at 120°C, then stored at room temperature.

II.9.2.1. Antibacterial activity test by diffusion in solid medium:

A culture of the test microorganism is prepared by incubating it for 18 to 20 hours to reach a concentration of approximately 10^5–10^6 CFU/mL. Using a sterile cotton swab, the bacterial suspension is evenly spread across the surface of Mueller-Hinton agar that has been previously poured into Petri dishes, forming a uniform lawn. The plates are then left at room temperature for 15 minutes to allow the bacteria to adhere to the agar surface. Wells of 6 mm diameter are created in the agar using the thick end of a sterile Pasteur pipette, and the bottom of each well is sealed with a drop of molten agar to prevent the diffusion of the test sample beneath the agar layer.

Once the disks are prepared, the test substance is carefully added to the respective disks. Alternatively, instead of using wells, the agar spot assay offers the option to place filter paper disks (disk diffusion assay; also known as Kirby-Bauer method) or agar plugs (agar plug assay) impregnated with the test compound onto the agar surface. Another approach involves directly spotting a small volume of the test compound onto the agar surface using a sterile inoculating loop (agar spot assay). Subsequently, the plate is incubated at the appropriate temperature for a defined period to allow the compound to diffuse into the surrounding medium. Following incubation, the plates are carefully examined for the presence of inhibition zones. The area or diameter of the inhibition zones is measured to assess the antimicrobial activity of the tested substances , Control plates containing only the solvent, culture filtrate or buffer are included to account for any potential effects of the vehicle on microbial growth.

Chapter III:
Results and Discussion

III.1.Introduction:

This chapter is entirely dedicated to presenting the results of an environmentally friendly biosynthesis of silver and zinc oxide nanoparticles using extracts from different plants for use in the preparation of skin care creams and hair dye creams.

III.2. Characterization of Ag and ZnO Nanoparticles:

III.2.1.UV Spectroscopy-Vis:

As part of a study on the ability of certain aqueous plant extracts to reduce silver ions (Ag+) to metallic silver (Ag^o), aqueous extracts were prepared from four plants*: Henna (Lawsonia inermis), Hibiscus (Hibiscus sabdariffa), Turmeric (Curcuma longa), and Taselgha (Lavandula stoechas)* , Each extract was individually mixed with a silver nitrate ($AgNO_3$) solution, and the following observations were made:

- Henna, Hibiscus, and Turmeric: No noticeable color change was observed upon addition of the extracts, indicating no significant reduction of silver ions occurred in these cases.
- Lavandula stoechas : When the aqueous extract of Taselgha was added to the silver nitrate solution, a distinct color change from yellow to dark brown was observed. This indicates an effective reduction of Ag+ ions to metallic silver (Ag^o), likely due to the presence of reducing compounds in the extract.

Using a UV-visible spectrophotometer, a spectrum was obtained in the visible range of 380 to 420 nm to confirm the presence of nanoparticles. The UV-Vis absorption spectrum of the synthesized Ag-NPs is presented in figure III.2, where a well-defined surface plasmon band is observed, centered around different time intervals showing a peak at 418.16 nm, characteristic of Ag-NPs.

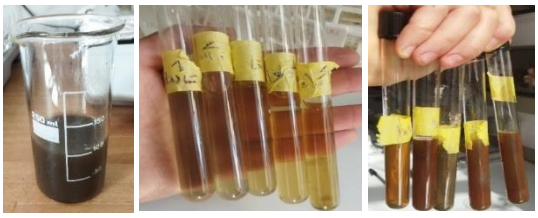
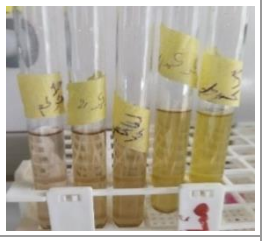
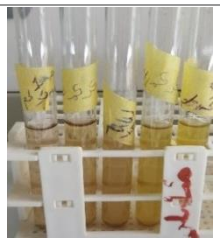
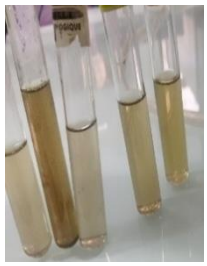


Figure III.1: Mechanism of Ag NPs synthesis.

Table III.1: Plant Extracts Before and After Heating for Silver Ion Reduction Assay

Plants	Before Heating	After Heating
Lavandula stoechas		
Hibiscus sabdariffa		
Curcuma longa		
Lawsonia inermis		

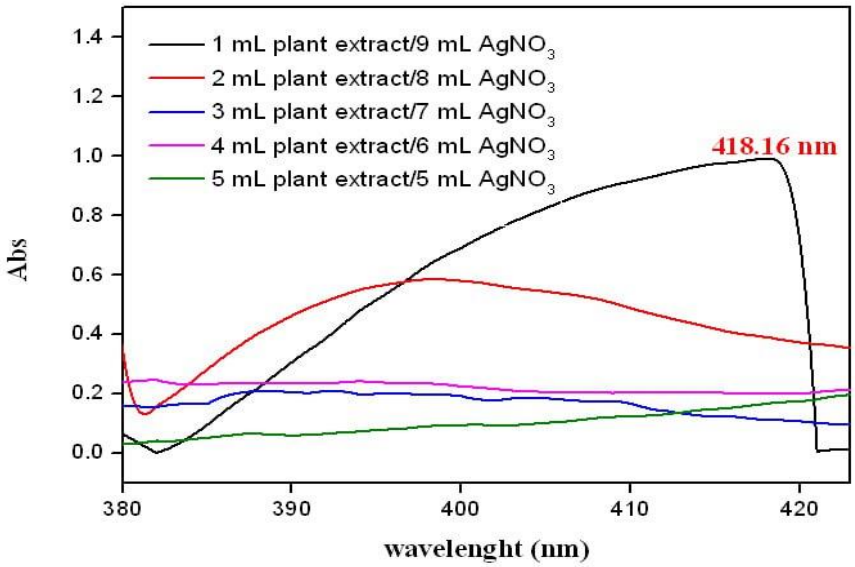


Figure.III.2 :Ultraviolet-visible (UV-Vis) spectrum of the synthesized Ag-NPs.

III.2.2. Analysis by scanning electron microscopy SEM

The morphology of all samples were analyzed using a scanning electron microscope.

III.2.2.1.Silver nanoparticle:

SEM (scanning electron microscope) was used to determine the size and morphology of the synthesized Ag NPs of Lavandula stoechas plant , SEM image and its particular magnified image along with histogram are shown in Figure.III.3 The magnified SEM image of sample S showed the agglomeration of the silver nanoparticles. Observation shown that mostly particles are of spherical in shape, the particles size distribution is in the range of 10 to 25 nm and their average size is 18 nm.

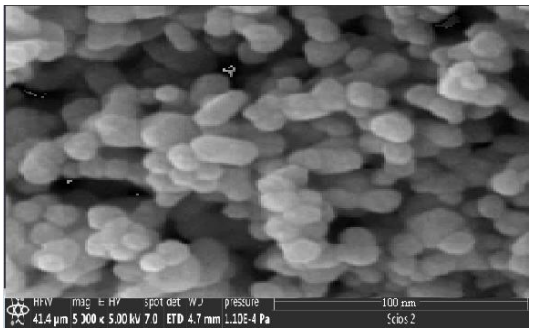
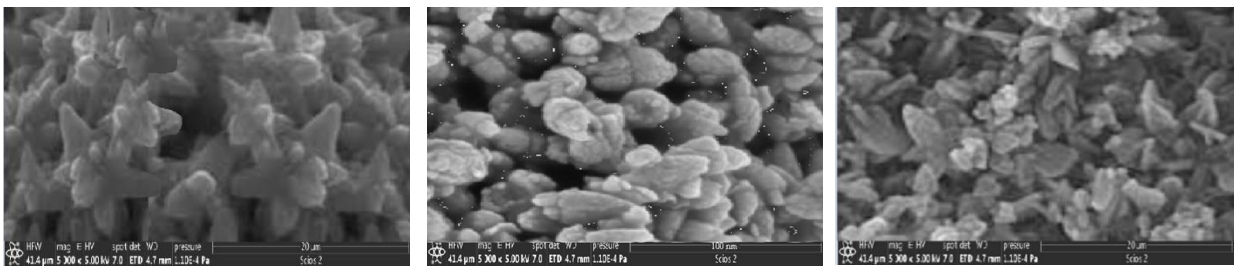


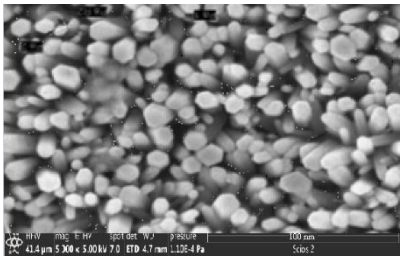
Figure.III.3: SEM images of Ag NPs of *Lavandula stoechas* plant

III.2.2.2. Zinc oxide nanoparticle:

The size and shape of the produced ZnONPs were evaluated using a scanning electron microscope, or SEM. Figure. III.7 shows an SEM image, its specified magnified image, and its graph. The agglomeration of ZnO nanoparticles was clearly visible in the magnified SEM image of sample S. The particles are mostly flower-shaped, except for *Lawsonia inermis* plants, which showed spherical particles.



a-Lavandula stoechas *b-Curcuma longa* *c- Hibiscus sabdariffa:*



d-Lawsonia inermis

Figure.III.4: SEM images of ZnO NPs of the plants

III.2.3.X-ray Diffraction (XRD)

The testing of the samples was performed in an XRD , with the model number XRD-6000

III.2.3.1.Nanoparticule de ZnO

The crystallinity, crystallization, and crystal phase of the prepared materials were determined using X-ray diffraction (XRD) analysis. The XRD patterns of ZnO NPs samples prepared with four different plant extracts showed that all of them correspond to the hexagonal wurtzite phase of zinc oxide, where the peak positions matched with JCPDS card number 36-1451, indicating successful synthesis and the absence of crystalline impurities.

Distinct diffraction peaks were observed at 2θ angles corresponding to the crystalline planes (hkl) such as: (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202), which clearly indicate the formation of a pure hexagonal structure of zinc oxide.(D) Using the Debye–Scherrer equation, And we noticed that it varies depending on each plant , the results were as follows:

Table III.2:Summary of XRD Results for ZnO Nanoparticles Synthesized Using Different Plant Extracts

Plant Extract	Number of Peaks	Main 2θ Peaks (°)	Main Miller Indices (hkl)	Average Crystallite Size (D, nm)
Lavandula stoechas	8	33.36, 35.87, 47.41, 58.53, 66.71, 73.22, 75.31	(100), (002), (101), (102), (200), (202)	18.15
Curcuma longa	8	31.89, 34.41, 39.86, 46.36, 58.53, 68.39, 72.80, 75.10	(100), (002), (102), (201), (202)	16.23
Hibiscus sabdariffa	8	31.77, 36.03, 39.68, 46.98, 58.38, 65.68, 72.38, 74.96	(100), (101), (102), (004), (202)	19.64
Lawsonia inermis	8	31.68, 34.25, 36.66, 47.08, 56.32, 66.44, 72.49, 77.02	(100), (101), (103), (200), (004), (202)	18.63

➢ *Lavandula stoechas*:

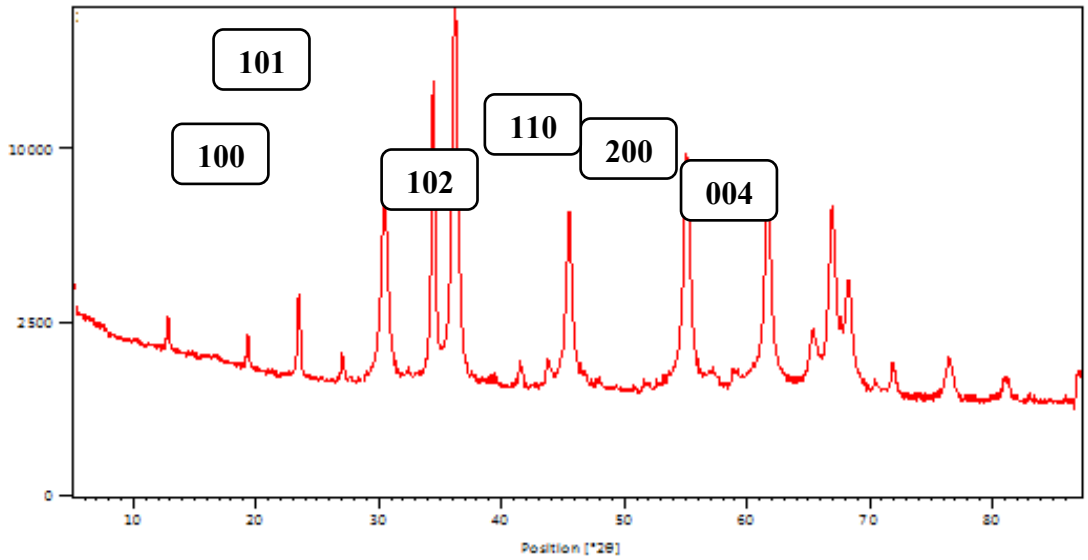


Figure.III.5: XRD patterns of ZnO NPs biosynthesized using Plant of *Lavandula stoechas* extract

Table III.3: XRD analysis data of ZnO NPs of *Lavandula stoechas* plant extract and shape description using the Scherrer equation D = Kλ/βcosθ.

Parameters		Calculations					
K	λ (Å)	Peak position 2θ (°)	FWHM β (°)		D (nm)	Average D (nm)	
0.94	1.5406	33.36	0.63	2.683727511	13.75	18.15	
		35.87	0.48	2.501478737	18.17		
		35.87	0.75	2.501478737	11.63		
		47.41	0.53	1.916037216	17.10		
		58.53	0.68	1.575740128	13.99		
		66.71	0.41	1.400991524	24.23		
		73.22	0.63	1.291658667	16.41		
		75.31	0.35	1.260915302	29.94		

➢ *Curcuma longa:*

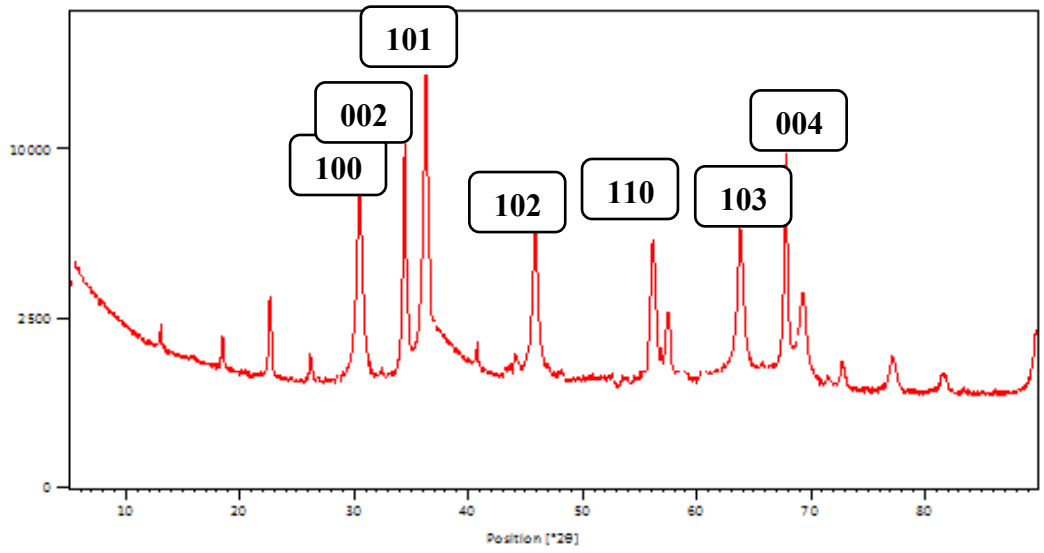


Figure.III.6: XRD patterns of ZnO NPs biosynthesized using Plant of *Curcuma longa* extract

Table III.4: XRD analysis data of ZnO NPs of *Curcuma longa* plant extract and shape description using the Scherrer equation D = Kλ/βcosθ.

Parameters		Calculations					
K	λ (Å)		Peak position 2θ (°)	FWHM β (°)		D (nm)	Average D (nm)
0.94	1.5406		31.89	0.61	2.804002137	14.15	16.23
			34.41	0.68	2.60420001	12.77	
			39.86	0.4	2.259793611	22.06	
			46.36	0.75	1.956958364	12.03	
			58.53	0.61	1.575740128	15.59	
			68.39	0.74	1.370613256	13.56	
			72.80	0.65	1.298071257	15.86	
			75.10	0.44	1.263919173	23.79	

➢ *Hibiscus sabdariffa:*

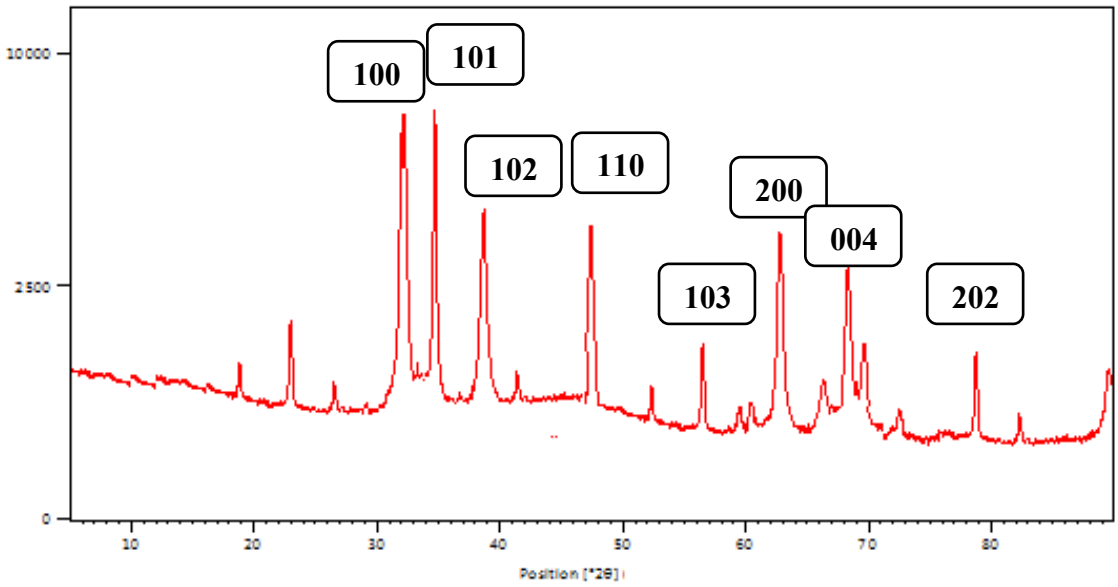


Figure.III.7: XRD patterns of ZnO NPs biosynthesized using Plant of *Hibiscus sabdariffa* extract

Table III.5: XRD analysis data of ZnO NPs of *Hibiscus sabdariffa* plant extract and shape description using the Scherrer equation D = Kλ/βcosθ.

Parameters		Calculations					
K	λ (Å)	Peak position 2θ (°)	FWHM β (°)		D (nm)	Average D (nm)	
0.94	1.5406	31.77	0.46	2.814318965	18.75	18.88	
		36.03	0.68	2.490736373	12.83		
		39.68	0.6	2.269628867	14.70		
		46.98	0.75	1.932567103	12.06		
		58.38	0.65	1.579430948	14.62		
		65.68	0.72	1.420444842	13.72		
		72.38	0.25	1.304565449	41.12		
		74.96	0.45	1.265932084	23.24		

➢ *Lawsonia inermis:*

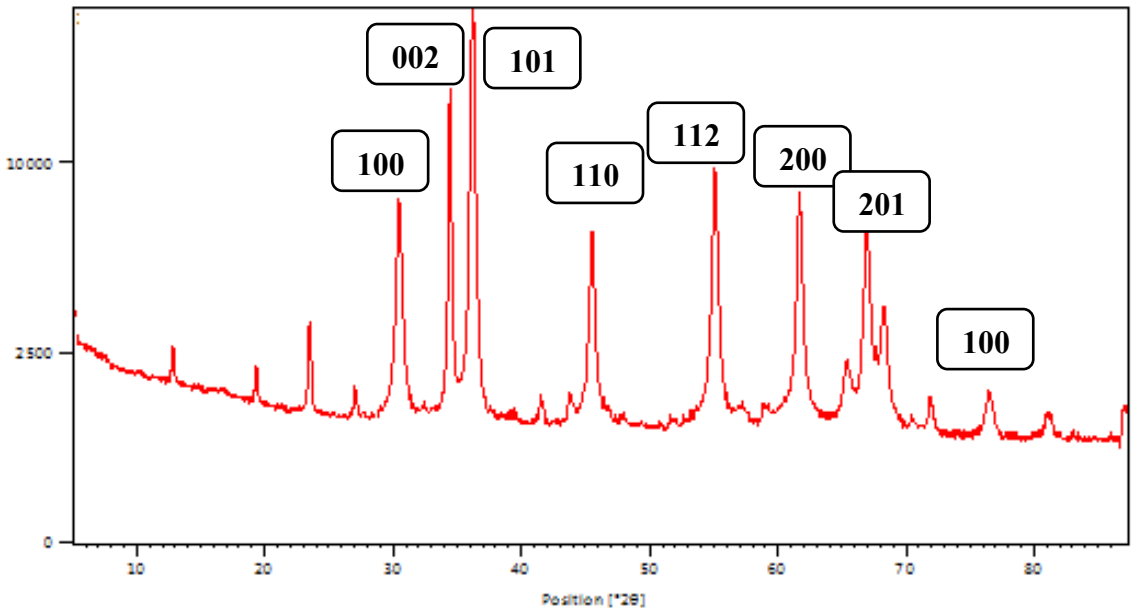


Figure.III.8: XRD patterns of ZnO NPs biosynthesized using Plant of *Lawsonia inermis* extract

Table III.6: XRD analysis data of ZnO NPs of *Lawsonia inermis* plant extract and shape description using the Scherrer equation D = Kλ/βcosθ.

Parameters		Calculations					
K	λ (Å)	Peak position 2θ (°)	FWHM β (°)		D (nm)	Average D (nm)	
0.94	1.5406	31.68	0.52	2.822108583	16.59	18.63	
		34.25	0.68	2.615998631	12.77		
		36.66	0.7	2.449366113	12.49		
		47.08	0.62	1.928695115	14.60		
		56.32	0.68	1.632214973	13.84		
		66.44	0.72	1.406028259	13.78		
		72.49	0.24	1.302856624	42.87		
		77.02	0.48	1.237129195	22.09		

III.2.3.2.Silver nanoparticle

We used X-ray diffraction (XRD) to characterize our biologically produced nanoparticles.

Fig. III.8 shows the XRD pattern of the AgNPs made from Tasalgha plant extract. The crystalline structure and average crystallite size of the silver nanoparticles were ascertained by XRD analysis.

At 2θ values of 38.32°, 44.24°, 64.84°, and 77.16°, which correlate to the crystallographic planes (111), (200), (220), and (311), respectively, distinct diffraction peaks were seen. The creation of highly crystalline AgNPs is confirmed by these peaks, which are in agreement with the typical face-centered cubic (FCC) structure of metallic silver (JCPDS No. 04-0783).

Furthermore, slight peaks were found at 2θ = 26.10° and 31.77°, which could be related to various secondary phases or organic residues or bio-components from the plant extract.

The Debye-Scherrer equation was used to determine the crystallite sizes, which ranged from 12.88 nm to 22.97 nm, with an average of roughly 17.80 nm.

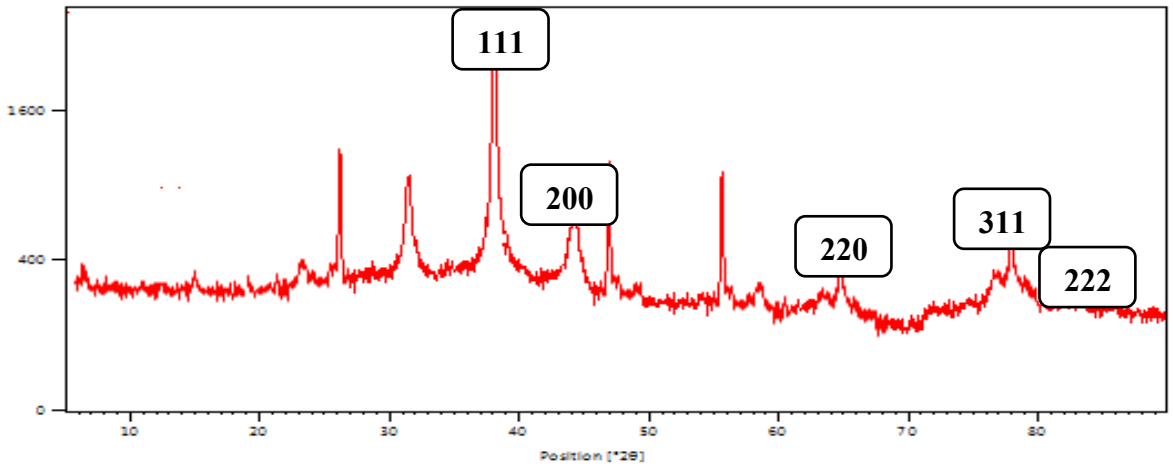


Figure.III.9: XRD patterns of Ag NPs biosynthesized using Plant of *Lavandula stoechas* extract

Table III.7: XRD analysis data of Ag NPs of *Lavandula stoechas* plant extract and shape description using the Scherrer equation D = Kλ/βcosθ

K	λ (Å)	Peak position 2θ (°)	FWHM β (°)		D (nm)	Average D (nm)
0.94	1.5406	26.1	0.45325	3.411406901	18.79	17.80
		31.77	0.57363	2.814318965	15.04	
		38.32	0.682	2.346992514	12.88	
		44.242	0.492	2.045602743	18.20	
		64.84	0.52	1.436801204	18.90	
		77.16	0.462	1.23523357	22.97	

III.3. The application of synthesized ZnO and Ag NPs

III.3.1.The skin care cream:

- Ag NPs+ ZnO NPs+ *Curcuma* Oil

Figure.III.10: skin care cream a base nanoparticles

III.3.2. the hair dye cream:

- Plant Powder + Plant Extract+ ZnO NPs + Ag NPs

Figure.III.11: hair dye cream a base nanoparticles

III.3.3. the Mask:

- sodium alginate solution + a small amount of skincare cream + $CaCl_2$ solution

Figure.III.12: face Mask a base nanoparticle

III.4. Characterization of cream:

III.4.1. The type of emulsion

- **Method by dilution**

Figure.III.13: the dilution of cream

The observation of water mixing readily with the cream suggests that the emulsion is of the oil-in-water (O/W) type.

This type of emulsion is common in cosmetic creams intended for easy skin absorption and light texture

- **Dye method:**

Figure III.14: the diffusion of methylene blue in creams

A good diffusion of the methylene blue dye in the prepared creams was observed, so these creams are of the oil-in-water type.

III.4.2. pH Measurement:

The pH values obtained for the emulsions range between [6.38; 7.10].

The obtained results conform to the pH standards [6.5; 7.5].

III.4.3.Centrifugation Stability:

Table.III.8: centrifugation stability test.

Tests	After centrifugation (40 rpm) for 10 min	The results
Hair dye cream		A clear and irreversible separation of the two phases.
Skin care cream		Absence of any form of instability.

III.4.4 acidity activity:

Figure III.15: acidity test of the creams

Table III.9: TA value of different creams

Sample	Cream Type	Veq (l)	TA (g acid / 100 mL)
1	Cream 1 : Skin care cream 1	$0.2 * 10^{-3}$	$2*10^{-5}$
2	Cream 1 :Skin care cream 2	$0.2* 10^{-3}$	$2*10^{-5}$
3	Cream 1 :Skin care cream 3	$0.1* 10^{-3}$	10^{-5}
4	Cream 2: Hair dye cream 1	$0.2* 10^{-3}$	$2*10^{-5}$
5	Cream 2: Hair dye cream 2	$1.7* 10^{-3}$	$1.7*10^{-4}$
6	Cream 2: Hair dye cream 3	$1.1* 10^{-3}$	$1.1*10^{-4}$
7	Cream 3:Hair dye cream 1	$1* 10^{-3}$	10^{-4}
8	Cream 3:Hair dye cream 2	$1* 10^{-3}$	10^{-4}
9	Cream 3:Hair dye cream 3	$1.2* 10^{-3}$	$1.2*10^{-4}$

The total acidity of the care creams under study varied, according to the data, which were explained by variations in the acids and active substances that made up each product. A more acidic formulation may be indicated by high TA levels, which could upset the skin's or scalp's natural equilibrium and raise the possibility of irritation.

Low acidity samples are thought to be more stable and are frequently more suited for cosmetic applications, particularly on delicate skin. These findings support the significance of TA measurement as a gauge of cosmetic stability, safety, and quality.

The results show a variation in total acidity among the studied samples. This variation is attributed to the difference in acid content (such as fatty or organic acids) present in the composition of each cream, which varies according to the active ingredients, type of oil or plant extracts used, and the degree of chemical processing.

Compared to the ideal reference values for skin and hair products (usually a pH between 4.5 and 6.5), sample number 5 shows relatively high acidity, which may indicate an imbalance in the formulation or the use of a high percentage of acidic ingredients. This could cause irritation to sensitive skin or weaken the product's stability over the long term.

As for sample number 3, which recorded the lowest TA value, it is considered more skin-friendly, due to the lower likelihood of irritation and achieving a balance close to the skin's natural pH.

III.4.5. Washing activity:

The results showed that white hair exhibited more visible and intense color uptake compared to brown hair. For the white hair, the color remained stable and did not fade significantly, indicating strong dye adherence and resistance to washing, likely due to the enhanced penetration and binding facilitated by the nanoparticles. These findings highlight the superior performance of the nano-based formulation, particularly on lighter hair types, where the color contrast is more apparent.

Figure III.16: washing test results

Microscopic Observation of the Samples:

The dyed hair samples were observed using a stereomicroscope equipped with a digital camera (Canon). This setup enabled high-resolution imaging of the hair surface and internal structure. The captured images clearly showed the penetration of the hair dye cream 3 into the hair fibers, indicating effective absorption. These observations confirm the efficiency of the nanostructured dye synthesized via green synthesis methods. The

visual evidence provided by the stereomicroscopic analysis supports the successful interaction between the dye and the hair matrix, thus validating the potential of the green-synthesized nano-dye.

Cream 3-1 **cream 3-2** **cream3-3**

Figure III.17: Microscopic image of washed hair strands under 2.5× magnification

III.5. Pharmacological Activities:

III.5.1.Antioxidant activity:

The DPPH method is used to assess the anti-radical activity of the samples (oil and cream containing nanoparticles), and the calibration range is prepared using ascorbic acid as a standard. A freshly made DPPH solution in the free radical scavenging assay shows a purple color (λmax = 517 nm), which gradually fades when a strong antioxidant, or good hydrogen donor, is present. This results in a yellow discoloration.

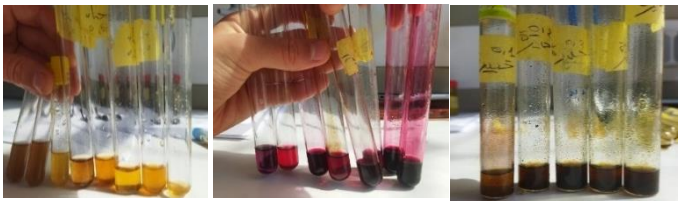


Figure. III.18: DPPH Test for the extracts

Figure. III.19: DPPH Test of ZnO NPs and Ag NPs

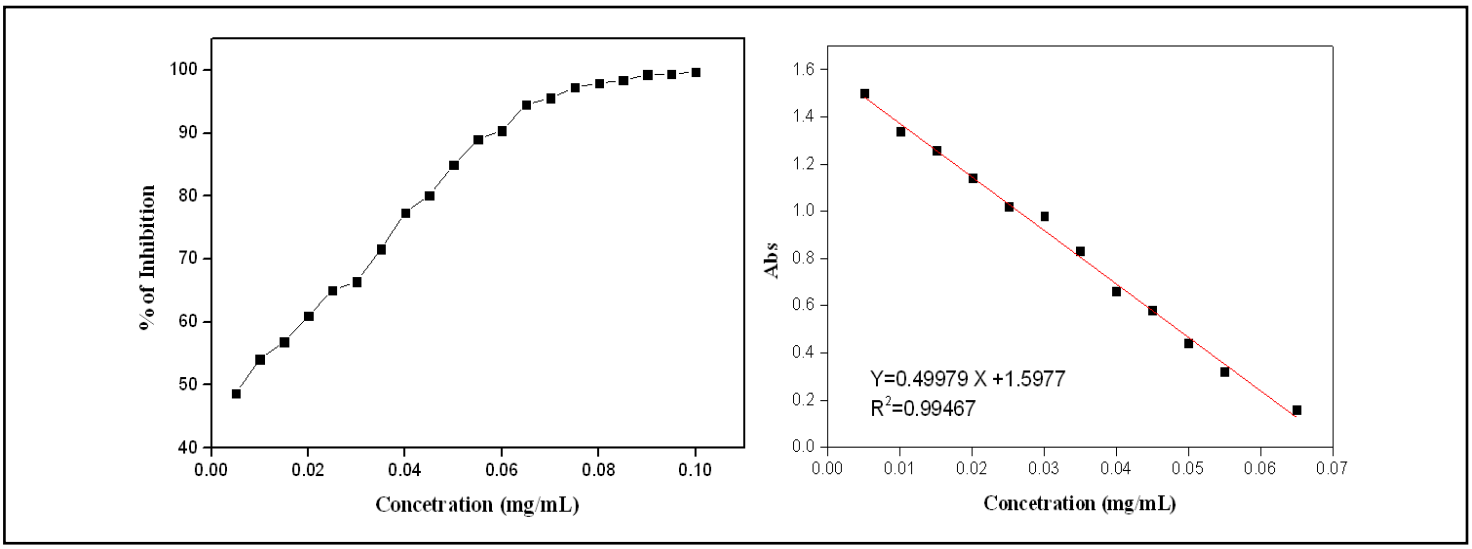


Figure III.20 :Calibration curve of ascorbic acid and Percentage of DPPH free radical inhibition with different concentrations of ascorbic acid

- **IC50 of ascorbic acid= 0.0064 mg/mL**

The results of the DPPH test showed a significant difference in antioxidant activity between plant extracts and cosmetic creams, as evidenced by the recorded IC_{50} values.

The highest antioxidant activity was attributed to both *Moringa oleifera* and *Malva parviflora,* which recorded the lowest IC_{50} value (0.02 mg/mL), followed by *Curcuma longa* and *Hibiscus sabdariffa* (0.03 mg/mL), and then Lawsonia inermis (henna) with an IC_{50} value of 0.05 mg/mL. Meanwhile, Lavandula stoechas showed relatively lower activity (0.3 mg/mL).

As for the creams, Hair dye cream 3 was the most effective (IC_{50} = 0.5 mg/mL), followed by Hair dye cream 2 (0.8 mg/mL), while Skin care cream 1 recorded the highest IC_{50} value (2.8 mg/mL), indicating lower antioxidant activiy .

Creams containing silver nanoparticles made from the extract of the Lavandula stoechas plant showed better antioxidant activity compared to the extracts due to the redox properties of important natural antioxidants, such as phenolic acids and flavonoids [151,152]. On the other hand, the antioxidant capacity of the cream containing AgNPs may be linked to the presence of phenolic compounds and flavonoids that form a layer on the silver nanoparticles, and it may also be higher due to the synergistic effect of the silver nanoparticles after the physicochemical interaction of Ag ions with the functional groups of a group of plants [153].

Given that the creams containing silver nanoparticles and the extracts prepared from a group of medicinal plants showed significantly different performances compared to ascorbic acid as a standard solution, we can say that this work highlights the therapeutic value of biosynthesized AgNPs as a source for the development of antioxidant creams for aesthetic health .

In this work, we determined the IC50 value (also known as the 50% inhibitory concentration) and grouped it in Table III.03 (Table III.9).

This parameter determines the ability to scavenge free radicals. The lower the IC50 value, the higher the antioxidant activity of the compound (reactive) [154].

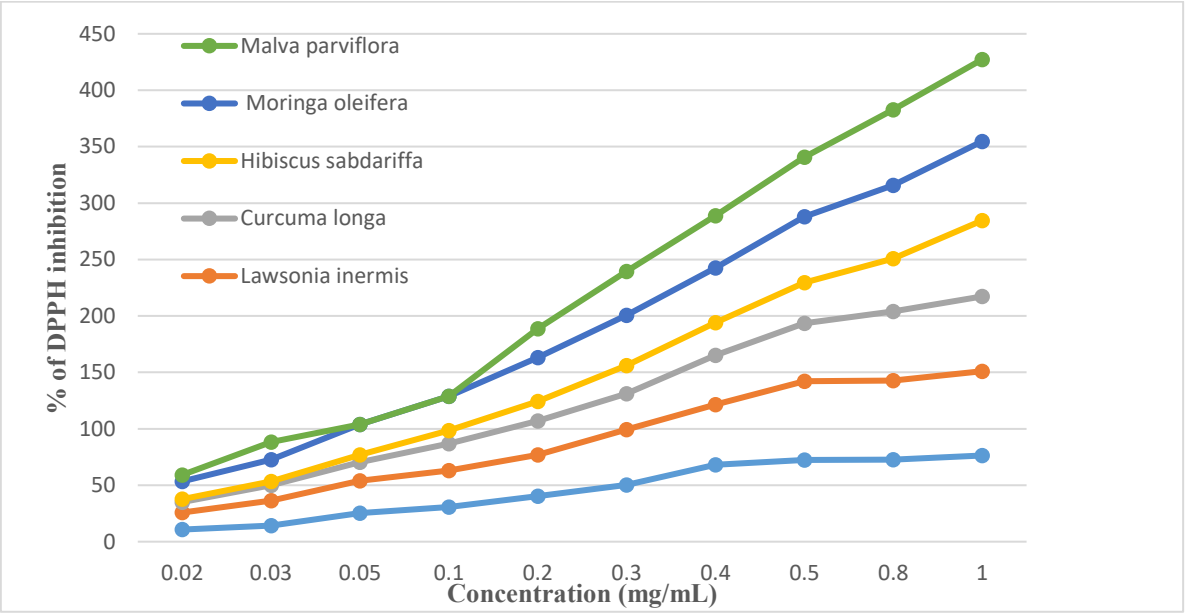


Figure III.21: Percentage of DPPH free radical inhibition with different concentrations of extracts

Table.III.10: IC50 extracts

Plant extract	*Lavandula stoechas*	*Lawsonia inermis*	*Curcuma longa*	*Hibiscus sabdariffa*	*Moringa oleifera*	*Malva parviflora*
IC50 % mg/ml	0.3	0.05	0.03	0.03	0.02	0.02

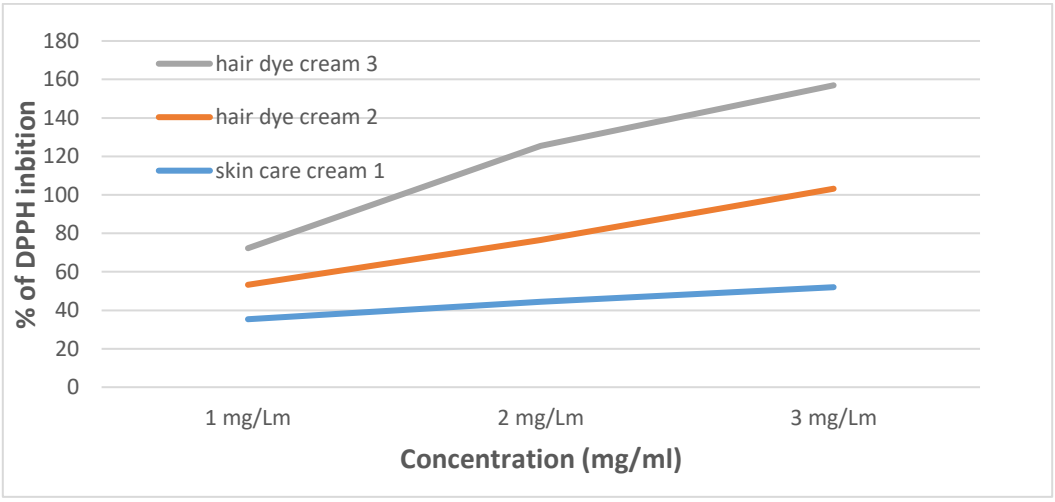


Figure III.22: Percentage of DPPH free radical inhibition with different concentrations of creams

Table.III.11: IC50 creams

Cream	Skin care cream 1	Hair dye cream 2	Hair dye cream 3
IC50% (mg/ml)	2.8	0.8	0.5

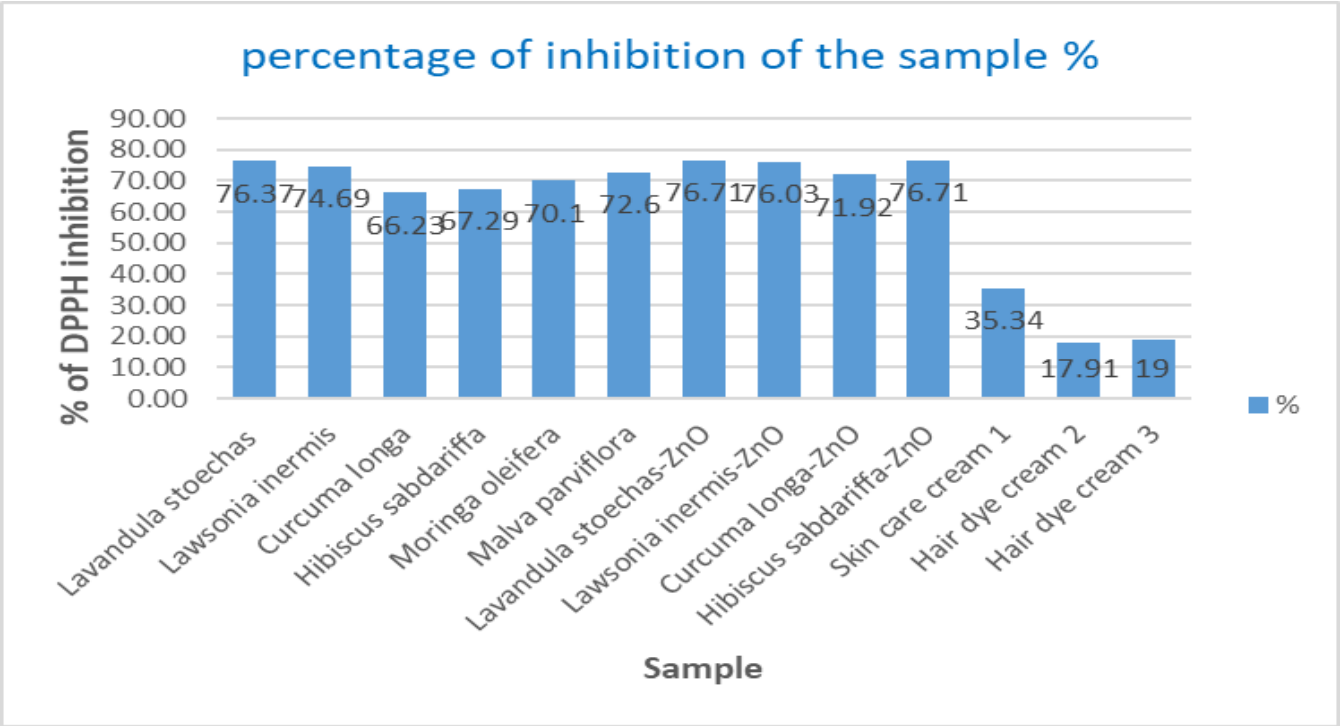


Figure: III.23: IC50 cream with NPs and extraxt (1mg/ml)

III.5.2.Antibacterial activity:

According to Ponce et al. (2003), the diameter of the inhibition zones indicates the sensitivity of a bacterium, so the results are expressed according to four levels of activity **:**

- Resistant strain (D < 8 mm)
- Sensitive strain (9mm ≤ D ≤ 14mm)
- Very sensitive strain (15mm ≤ D ≤ 19 mm)
- Extremely sensitive strain (D >20 mm) [155]**.**

Table III.12: Results of antibacterial activity.

	The bacteria used	
	Gram +	**Gram -**
	Staphylococcus aureus ATCC 25923	***E.coli ATCC 25922***
Cream 2 : Hair dye cream 1	20 mm	-
Cream 3 : Hair dye cream 3	10 mm	-
Cream 1 : Skin care cream 2	17.5 mm	-

Antibacterial Activity Against S. aureus (Gram-positive):

Cream 2 showed the highest activity, with a zone of 20 mm, bordering the “extremely sensitive” category. This suggests the presence of highly effective antimicrobial agents, possibly metal nanoparticles (ZnO, Ag, etc.), essential oils, or preservatives that are particularly active against Gram-positive bacteria.

Cream 1 (skin care cream) also demonstrated strong antibacterial activity (17.5 mm), falling into the "very sensitive" category. This suggests it contains components with notable antimicrobial potential, though perhaps at lower concentration or efficacy than Cream 2.

Cream 3 had a moderate effect (10 mm), indicating weaker antibacterial potency or possibly fewer active antimicrobial compounds compared to the others.

Lack of Activity Against E. coli (Gram-negative):

None of the creams exhibited any detectable inhibitory activity against *E. coli*. This difference between Gram-positive and Gram-negative susceptibility is commonly observed and can be explained by:

The structural differences in the bacterial cell wall: Gram-negative bacteria like *E. coli* possess an outer membrane rich in lipopolysaccharides, which acts as a barrier and reduces permeability to many antimicrobial agents.

The presence of efflux pumps and enzymes in Gram-negative bacteria that can neutralize or expel antimicrobial compounds.

It is possible that the antimicrobial agents in the creams are either not broad-spectrum or are less effective against the more resistant outer membrane of Gram-negative organisms.

Formulation Influence:

The formulation base of each cream (e.g., emollients, pH, oil content, preservatives) can greatly affect the diffusion of active agents through the agar medium and therefore impact the observed inhibition zone.

For example, Hair dye cream 1 (Cream 2) might have a more hydrophilic or nanoparticle-rich matrix that facilitates better diffusion and antimicrobial action.

Potential Role of Nanoparticles or Plant Extracts:

If the creams were formulated with zinc oxide nanoparticles, silver nanoparticles, or plant-derived bioactives, these ingredients are known to have greater efficacy against Gram-positive bacteria due to easier penetration and interaction with peptidoglycan-rich cell walls.

The better performance of Cream 2 could suggest a higher content or synergistic combination of such agents.

The antibacterial evaluation revealed that all creams exhibited selective antibacterial activity, being effective against *S. aureus* (Gram-positive) but ineffective against *E. coli* (Gram-negative). The differences in activity levels between creams are likely attributed to variations in formulation, concentration of active agents, and physicochemical properties of the creams. This highlights the importance of tailoring topical formulations to target specific bacterial profiles and selecting ingredients based on their broad-spectrum or targeted antibacterial capabilities.

General conclusion

General conclusion

This research highlights the promising potential of green synthesis as an environmentally friendly and cost-effective alternative to conventional nanoparticle fabrication methods. By employing plant extracts from turmeric, henna, hibiscus, and tasalgha, we successfully synthesized zinc oxide and silver nanoparticles with desirable physicochemical properties. These nanoparticles were characterized using advanced techniques such as SEM and XRD, revealing nanostructured particles with crystalline morphology.

The synthesized nanoparticles were then utilized in the formulation of skin care and hair dye cream**s**, which were subsequently assessed for stability, pH compatibility, and emulsion type. The biological activities of these creams were evaluated, showing:

- Strong antioxidant properties, particularly in creams with AgNPs, indicating potential in anti-aging and protective skincare.
- Selective antibacterial activity against *Staphylococcus aureus*, supporting their use in antimicrobial cosmetic formulations.
- Effective dyeing performance, especially on white hair, with color retention even after washing, thus demonstrating cosmetic applicability.

Moreover, acidity and washing tests confirmed the safety and stability of the products, with TA values and pH within acceptable ranges for dermatological use.

In conclusion, this study establishes a foundation for the integration of green-synthesized nanoparticles into natural cosmetics, combining bioactivity, environmental responsibility, and product performance. These findings open avenues for future development of nanotechnology-based skincare and hair dye products rooted in traditional botanical knowledge.

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Annexe:

University of Ghardaia
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الجمهورية الجزائرية الديمقراطية الشعبية
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قسم التعليم المشترك في العلوم والتكنولوجيا

Approval for Final Printing of a Master's Dissertation

	Name and Surname	Signature
Examiner 1	Mounir DAOUD	
Supervisor	Yasmina KHANE	
Co-Supervisor	Fares Fenniche	

I, the undersigned, Dr. Hadj Daoud BOURAS

President of the jury for the student(s): ACHOUR Wafa and HEROUINI Meriem

Field: Chemistry; **Specialization:** Analytical Chemistry

Thesis Title: Biosynthesis, Characterization, and Cosmetic Applications of Metallic Nanoparticles in Hair Dye and Skin Care.

Hereby authorize the above-mentioned student(s) to print and submit their final manuscript to the department.

Ghardaia: 26/062025

President of the jury

Head of the department

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ثنوية فيصل

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