Democratic and Popular Republic of Algeria
Ministry of Higher Education and Scientific Research
University of AKLI MOHAND OULHADJ-BOUIRA

جامعة البويرة

Faculty of natural and life sciences and earth sciences
Department of Biology

THESIS

SUBMITTED FOR OBTAINING A MASTER DEGREE

Domain: Science of Nature and Life **Field:** Biological Sciences
Option: Microbial Biotechnology

Presented by

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Thesis Title

Exploring the effects of synthesized molecules in the water treatment process

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Academic Year: 2023/2024

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جامعة البويرة

FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE ET DES SCIENCES DE LA TERRE
DEPARTEMENT DE BIOLOGIE

Réf : ……./UAMOB/F.SNV.ST/DEP.BIO/22

MEMOIRE DE FIN D’ETUDES

EN VUE DE L’OBTENTION DU DIPLOME MASTER

Domaine : SNV **Filière** : Sciences Biologiques
Spécialité : Biotechnologie Microbienne

Présenté par :

SAIB Nada Yasmine & MENAOUI Houda

Thème

Exploration de l’effet des molécules synthétisées dans le processus de traitement de l’eau

Soutenu le 26/06/2024

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Année Universitaire: 2023/2024

Acknowledgments

In the name of God, the Most Gracious, the Most Merciful,
First and foremost, we express our deepest gratitude to Almighty God for granting us the strength and perseverance to complete this work.
Alhamdulillah
We would like to extend our sincere appreciation to our supervisor, Mrs. Amal MAMERI, and co-supervisor, Mr. Hamza MOUSSA, for their unwavering support and encouragement throughout the preparation of our thesis. Their expertise, valuable advice, and availability have been indispensable in the completion of this project.
Their engagement and dedication have been of immeasurable value. Thanks to their insightful feedback and constructive suggestions, we were able to deepen our research and refine our analyses.
We would like to extend our deepest gratitude to the thesis committee members , Pr Farid DAHMOUNE and Souhila BENSMAIL , Your expertise and dedication are essential to enrich this work, and we are profoundly grateful for the countless hours you have invested to examine this work.

We would also like to express our gratitude to the entire academic team for their continuous support and guidance throughout our university journey. Their dedication and expertise have served as a constant source of inspiration and motivation.
It is with great pride and a profound sense of accomplishment that we present to you this thesis, which is the culmination of several months of diligent effort and passion for our field of study.

Dedication

What could be more fulfilling than cherishing life's joyful moments with our loved ones? As I near the completion of my studies, I am deeply honored to dedicate this humble endeavor:

To my parents,

Mom and Dad, your endless love, unwavering support, and boundless encouragement have been the cornerstone of my journey to this moment. From the earliest days of my education to the culmination of this thesis, you have been my steadfast champions, guiding me through challenges and celebrating every milestone.

***Mom**, your nurturing spirit and selflessness have been a constant source of inspiration. Your sacrifices and tireless dedication to my well-being have shaped me into the person I am today. **Dad**, your wisdom, resilience, and unwavering belief in my abilities have instilled in me the courage to pursue my dreams fearlessly.*

*To my beloved siblings, **Sara**, **Besma** and **Ali,***

Your love, companionship, and unwavering support have been my greatest blessings

*To my dear friends, **Ouiam** and **Amira,***

Your unwavering support, encouragement, and belief in me have been a constant source of strength and inspiration throughout this journey

*To my dedicated study partner, **Houda,***

Your unwavering support has been invaluable, profoundly impacting my progress and success throughout this academic journey.

*And to **myself**,*

I acknowledge the challenges I have faced, the obstacles I have overcome, and the growth I have experienced throughout this journey. This thesis is a testament to my perseverance, resilience, and dedication to achieving my goals.

This dedication is to all of you, as a token of my deepest gratitude and appreciation for your unwavering support. Your presence in my life has made all the difference, and I am forever grateful for each and every one of you.

With heartfelt thanks and immense gratitude,

Nada

Dedication

Praise be to Almighty God for allowing me to see this long-awaited day.

To my dearest ***father****, you have always been an example to me of a respectful, honest and hardworking father, and I want to honor the man you are. Thanks to you, Dad, I learned the meaning of work and responsibility. I'd like to thank you for your love, your generosity, your understanding... Your support has been a beacon throughout my life. No dedication can express the love, esteem and respect I've always had for you. This modest work is the fruit of all the sacrifices you made for my education and training. I love you, Dad, and I pray to the Almighty to grant you good health and a long and happy life.*

To the immaculate soul of ***my mother****, I wish you were with me.*

To my dear ***second mother,*** *no dedication can express the depth of my feelings for you, your countless sacrifices and devotion have been and continue to be my greatest support. You have watched my every step and nurtured me with your tenderness, and your prayers and blessings have been a great help to me in my studies. You have helped and supported me for many years, each time with renewed interest. I ask Almighty God to grant your health, happiness and long life.*

To my dear sister ***Sanaa****, my dear brothers* ***Mahdi*** *and* ***Tarek****, I love you very much.*

To my friends, ***Amira*** *and* ***Ouiam*** *no dedication could express my deep affection and immense gratitude for all. the encouragement and support they have given me,*

to my beloved partner ***Nada*** *and her family.*

To all my colleagues and those who know

Houda

Acronyms

Ag-NPs: Silver nanoparticles

Au: Aurum, the Latin name for gold.

Cu: Cuprum, the Latin name for copper.

Al: Aluminium

ZnS: Zinc sulfide

ZnO: Zinc oxide

CdS: Cadmium sulfide

Fe: Ferrum, the Latin name for iron

Co: Cobalt

Ni: Nickel

AChE: Acetylcholinesterase

ATP-aze: ATP hydrolyzing enzyme

DMF: N,N-dimethylformamide

NaBH4: Sodium borohydride

$\mathrm{DPPH}^{\bullet}$: 2,2-diphenyl-1-picrylhydrazyl

$\mathrm{ABTS}^{+\bullet}$: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

MIC: Minimum inhibitory concentration

IC_{50}: Concentration inhibiting 50% of activity

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INTRODUCTION

INTRODUCTION

Wastewater includes water contaminated by various sources such as organic pollutants, bacteria, industrial effluents, and other compounds, thus diminishing its original quality. They are classified into two main categories: municipal wastewater, which comes from residential and commercial sources, and industrial wastewater, which is discharged by industrial and agricultural operations. While municipal wastewater tends to have a relatively homogeneous composition, industrial wastewater varies considerably depending on the specific industrial activities that generate it (El Saliby, et al., 2008). The dairy industry faces significant challenges in wastewater management. Effectively treating dairy effluents is crucial not only for environmental conservation but also for water reuse within industrial operations (Oueslati et al., 2020; Sajjad et al., 2020).

The composition of industrial wastewater varies significantly based on the specific industrial activity, necessitating selective treatment methods to ensure optimal water quality. In this regard, treatment processes are tailored to target the contaminants present, balancing effectiveness with the cost of the treatment process (El Saliby, et al., 2008). Industrial wastewater treatment uses methods such as coagulation, activated carbon adsorption, and membrane processes to ensure water quality meets standards at a reasonable cost (Tahraoui et al., 2024). These processes effectively treat wastewater. Moreover, a collaborative effort is essential to develop environmentally friendly methods to prevent further discharge of pollutants into water, addressing the limitations of current wastewater management systems (C. Singh et al., 2019).

It is also important to consider the economic and social impacts of these new methods, ensuring they are accessible and beneficial to a wide range of communities. The success of nanotechnologies can be attributed to numerous factors, and ongoing research aims to further improve their applications. As a result, nanotechnologies are poised to play a crucial role in addressing critical issues such as healthcare, energy, and water management (Qu et al., 2013). Metallic silver nanoparticles (Ag-NPs), in particular, exhibit exceptional properties applicable across numerous fields beyond wastewater treatment, including biology, coating, food products, drug therapy, cosmetics, and biomedicine. Moreover, a considerable portion of Ag-NP research centers on their antimicrobial activity against various microorganisms, particularly in the context of water purification, dye removal, and enhancing the efficiency and cost-effectiveness of water recycling processes (Palani et al., 2023). Numerous research studies have explored the synthesis of Ag-NPs from various natural or biological sources and their applications in effluent and wastewater treatment (Cavassin et al., 2015; Chaloupka et al., 2010; Valkovszki et al., 2023). On the other hand, silver nanoparticles (Ag-NPs) are particularly notable for their interactions with three essential cell components: the peptidoglycan cell wall, cytoplasmic membrane, and ribosomal DNA. These interactions induce modifications in chemical and physical properties, disrupting osmolality, permeability, electron transport, cellular respiration, and molecular sites involved in protein synthesis, particularly in enzymes related to the electron transport chain. These advantages are due to the silver nanoparticle's (Ag-NP) properties, including high electrical and thermal conductivity, a large surface area, catalytic activity, and significant antibacterial properties.

Ag-NPs can be synthesized through various methods, yielding distinct morphologies and unique characteristics. Traditional methods for synthesizing metallic nanoparticles often require sophisticated and expensive equipment or costly chemicals, with potential environmental safety concerns. Hence, there is a preference for "green" technologies in nanoparticle synthesis. These methods are simple, convenient, non-toxic, environmentally friendly, cost-effective, scalable, and offer greater stability compared to other biological, physical, and chemical approaches (Vijayaram et al., 2024). Green synthesis of nanoparticles represents an innovative approach utilizing biological sources. Different plant parts, including leaves, flowers, fruits, and seeds, have been effectively utilized for the biosynthesis of silver nanoparticles (Ag-NPs). This method offers a low-cost process with higher yields, attributed to the abundant phytochemical components in plant extracts. Plants rich in polyphenols are particularly valuable sources because these compounds are excellent reducing and stabilizing agents, facilitating the conversion of metal ions into metallic nanoparticles (Vijayaram et al., 2024).

The green synthesis of silver nanoparticles (Ag-NPs) using plant extracts has garnered significant attention due to its eco-friendly and sustainable approach. Among various plants, *Salvia officinalis* L., commonly known as sage, is recognized for its rich phytochemical composition and potential biomedical applications. However, there is a paucity of studies focusing on the optimal extract of *Salvia officinalis* L. for the green synthesis of Ag-NPs and evaluating their effect on $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ free radicals. Additionally, the antimicrobial activity of these nanoparticles, particularly against bacterial strains isolated from industrial effluent, remains underexplored.

This research addresses these gaps by focusing on the green synthesis of silver nanoparticles using *Salvia officinalis* L. extract. The study aims to evaluate the synthesized nanoparticles for their antioxidant activity using $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ assays. Furthermore, it assesses the antimicrobial efficacy of these Ag-NPs against bacteria isolated from industrial wastewater. By exploring these aspects, this work contributes to the understanding of the potential applications of Salvia officinalis L. in the green synthesis of silver nanoparticles and their subsequent biomedical and environmental benefits.

The master thesis is divided into two main parts, each addressing critical aspects of the study. The first part, the literature review, is structured into three chapters. The first chapter provides comprehensive general information on *S. officinalis* L., highlighting its phytochemical properties and potential applications. The second chapter reviews the biosynthesis of silver nanoparticles, emphasizing green synthesis methods and the role of plant extracts. The third chapter discusses the applications of these nanoparticles in wastewater treatment, outlining their efficacy and advantages over traditional methods.

The second part of the thesis encompasses the experimental work and is divided into two chapters. The first chapter details the materials and methods used in the study. It includes the extraction of phenolic compounds from *Salvia officinalis* L, the green synthesis of silver nanoparticles, and the evaluation of their antioxidant and antimicrobial activities. The second chapter presents the results obtained from the experimental work and discusses their implications. It explores the health benefits of the synthesized nanoparticles, particularly their antioxidant properties, and assesses their potential in wastewater treatment by evaluating their antimicrobial activity against bacterial strains isolated from industrial effluent.

Part I: Literature

CHAPTER I: *SALVIA OFFICINALIS* L.

I. Salvia officinalis L.

I.1 Overview of *Salvia officinalis* L.

Salvia officinalis L., widely known as sage, is a prominent member of the *Lamiaceae* family, specifically within the subfamily *Nepetoideae*, tribe *Mentheae*, and genus *Salvia*. With around 1000 species, *Salvia* represents the largest genus in the *Lamiaceae* family, and it is native to diverse regions including Southeast Asia, Europe around the Mediterranean, and Central and South America (Jakovljević et al., 2019). This versatile herb is recognized by numerous common names such as common sage, golden sage, garden sage, kitchen sage, culinary sage, true sage. There are also various cultivated varieties, including red sage and purple sage, which expand its range of uses. In Turkey, sage is referred to as adaçayı, meaning 'island tea'. In the Levant, it is referred to as maramia (المريمية) (Hamidpour et al., 2014).

Figure 01: Salvia officinalis L. (sage)

(Ghorbani & Esmaeilizadeh, 2017).

I.1.1 Traditional uses

S. officinalis L. has garnered centuries of recognition in traditional medicine for its remarkable medicinal properties, as evidenced by the Latin derivation of its genus name from "salvere," meaning "to save." Sage tea, renowned since ancient times for its therapeutic benefits, holds a prominent place in the annals of natural remedies. Particularly esteemed for its efficacy in treating gingivitis, mouth cavities, and sore throats, it was revered by the Romans as "the holy plant" (GRDIŠA et al., 2015).

In traditional medicine in Europe, *Salvia officinalis* L. has been employed to treat a wide range of ulcers, rheumatism, gout, dizziness, inflammation, hyperglycemia, and tremors. Its applications extend to alleviating paralysis, diarrhea, age-related cognitive disorders, excessive sweating, and skin inflammation. The German Committee E has recommended the therapeutic use of *S. officinalis* for multiple medical conditions, dyspepsia, and particularly inflammation, encompassing symptoms like heartburn and bloating (Ghorbani & Esmaeilizadeh, 2017).

I.1.2 Botanical description

Salvia officinalis L. is a robust perennial subshrub that can grow up to 60 cm tall. Its stems are either erect or tapering, featuring numerous fines, deep green, and hairy branches. The leaves are oppositely arranged along the stem, petiolate, and elongated. While typically simple, the leaves can sometimes have basal lobes during the juvenile stages. They have a serrate margin and a rugose surface, with varying degrees of base contraction. The bottom surface of the leaf is adorned with white bristles, and the upper surface has greenish or greenish-grey hairs, giving the plant its distinctive appearance (GRDIŠA et al., 2015) as illustrated in Figure 02a.

The flowers of *S. officinalis* L. are arranged in pseudo-verticillasters, presenting clusters of 5-10 flowers on 2-4 mm long pedicels, collectively forming a spurious composed spike. The calyx, covered in fine hairs, measures 10-14 mm in length and is characterized by five teeth. The corolla typically displays hues of rosy or violet-blue, although occasional occurrences of white blooms are noted. With an approximate length of 35 mm, the corolla adds a striking visual element to the plant's aesthetic (GRDIŠA et al., 2015), as depicted in Figure 02b.

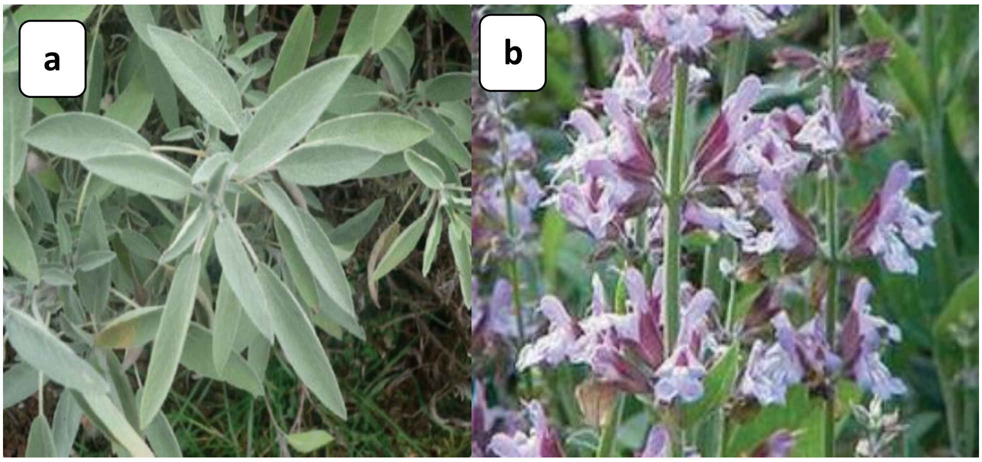

Figure 02: Pictures showing sage leaves (a) and flowers (b) (Hamidpour et al., 2014).

I.1.3 Chemical composition

Approximately 160 polyphenolic compounds have been identified in sage (*Salvia officinalis* L.) leaves, showcasing a rich diversity of phenolic acids and flavonoids. Key phenolic compounds include rosmarinic acid, caffeic acid and its derivatives, salvianolic acids, sagernic acid, lithospermic acids, sage coumarin, and yunnaneic acids. Among the most significant flavonoids present in sage leaves are hispidulin, luteolin 7-O-glucoside, apigenin, cirsimaritin, kaempferol, and quercetin. This comprehensive polyphenol profile underscores the potent bioactive potential of sage leaf extracts (Sharma et al., 2019) diterpenes (tanshinones, carnosic acid, and carnosol), triterpenes (ursolic acid and oleanolic acid) (Moussa et al., 2022).

I.1.4 Pharmacological properties and their application

The species enjoys widespread utilization across various industries including food, pharmaceuticals, and cosmetics, owing to its dual functionality in both medicinal and culinary domains. The aerial parts, referred to as *Salvia folium*, hold significance in European Pharmacopeias and are recognized by the Council of Europe as a natural source of food flavoring (GRDIŠA et al., 2015).

Antioxidants serve as crucial defenders against oxidative stress and the detrimental effects of free radicals, which are implicated in various health conditions such as diabetes, heart disorders, cancer, cognitive decline, and compromised immune function (GRDIŠA et al., 2015). In an extensive study investigating the antioxidant characteristics of diverse extracts from plants including *S. officinalis* L., it was revealed that their potent. The effects of antioxidants and free radical scavenging are primarily attributed to phenolic and flavonoid compounds (Hamidpour et al., 2014). Phenolic compounds, notably carnosic acid, carnosol, rosmarinic acid, rosmadial, epirosmanol, rosmanol luteolin-7-O-beta-glucopyranoside, and methyl carnosate, are key contributors to the high antioxidative activity observed in sage. These compounds, typically extracted using ethanol, demonstrate the ability to support endogenous antioxidant defense mechanisms and neutralize reactive species (GRDIŠA et al., 2015).

The leaves of *Salvia officinalis* L. have a longstanding traditional use as a remedy for diabetes (GRDIŠA et al., 2015). Clinical studies involving patients who received *S. officinalis* extract showed a significant reduction in blood sugar and levels of cholesterol compared to control groups. While no significant changes were observed in glycosylated hemoglobin levels, there was a notable increase in the pancreatic activities’ catalase, glutathione-S-transferase, superoxide, and glutathione peroxidase, dismutase. These changes resulted in reduced insulin resistance in diabetic rats induced by streptozotocin (Sharma et al., 2019). Moreover, research indicates that the methanolic extract of sage exhibits promising effects in improving sensitivity to insulin, inhibiting lipogenesis in adipocytes, and reducing inflammation as evidenced by plasma cytokine levels. These findings suggest its potential as an alternative treatment for diabetes and associated inflammation in pharmaceutical applications (Sharma et al., 2019).

Phenolics and flavonoids are renowned for their properties that counter inflammation and fight microbes, rendering *Salvia officinalis* L. a promising substitute for conventional antibiotics and food preservatives. Studies have demonstrated the efficacy of the hydroalcoholic extract of *S. officinalis* effectively inhibits the development of bacteria such as *Streptococcus mutans*, *Actinomyces viscosus* and *Lactobacillus rhamnosus*, known culprits in tooth caries. These findings suggest the potential of *S. officinalis* as a natural treatment for oral, and dental ailments, offering a viable alternative to chemical solutions (Sharma et al., 2019). Furthermore, research has highlighted the efficacy of herbal toothpaste formulations containing *S. officinalis* L. extract in gingivitis and controlling plaque. Both aqueous and ethanolic extracts from *S. officinalis* have demonstrated positive outcomes in this regard. Additionally, Cinalis, a component of *S. officinalis* L. has exhibited effectiveness against the herpes simplex virus types 1, 2 (GRDIŠA et al., 2015).

Sage has emerged as a subject of extensive study for its potential as an anti-cancer agent, investigated across different cells of cancer lines using animal models and in vitro. Notably, the main component of the sesquiterpene part in *Salvia officinalis*, trans-caryophyllene, has exhibited robust cytotoxic activity against human renal adenocarcinoma, and melanotic melanoma cells. Additionally, sage extract containing α-humulene has a strong cytotoxic effect. on human prostate cancer LNCaP cells, as well as on renal adenocarcinoma human, and melanotic melanoma cells (Sharma et al., 2019).

Alternatively, *Salvia officinalis* L. is rich in active ingredients renowned for their ability to enhance cognitive function and offer protection against neurodegenerative diseases. Traditionally, sage has been utilized for memory restoration and cognitive enhancement, particularly in cases of Alzheimer's disease-associated impairment. In Alzheimer's disease, the body's enzyme of Acetylcholinesterase (AChE) plays a crucial role in degrading acetylcholine, a key neurotransmitter implicated in transmitting neural signals. Sage essential oil has demonstrated remarkable potential in inhibiting AChE activity, with a notable 46% reduction observed at a concentration of 0.5 mg/ml (Sharma et al., 2019).

Furthermore, the gastro-protective properties of hydroalcoholic extracts derived from *Salvia officinalis* L. were thoroughly examined. These extracts were found to effectively inhibit gastric mucosal lesions, decrease gastric secretion, and inhibit the activity of H^+, K^--ATP-aze. Additionally, the medicinal application of *S. officinalis* L. has been explored for its efficacy in treating diarrhea and abdominal spasms, employing both in vivo and in vitro methodologies. The study unveiled that crude *S. officinalis* L. extracts possess the ability to prevent diarrhea by exerting a relaxing effect on the gastrointestinal tract. These findings offer a solid pharmacological foundation for the traditional medical use of *S. officinalis* L. in managing gastrointestinal ailments like diarrhea and abdominal colic (GRDIŠA et al., 2015).

In addition, *Salvia officinalis* L. has garnered attention both in folk medicine and clinical trials for its efficacy in alleviating symptoms of menopause, including insomnia, headaches, hot flashes, dizziness, night sweats, and palpitations. It is recommended to apply a fresh extract of *S. officinalis* once daily for optimal results. Beyond addressing menopausal discomfort, *S. officinalis* has showcased promising clinical value in treating depression, memory disorders and cerebral ischemia (GRDIŠA et al., 2015).

I.1.5 Cytotoxicity

Salvia officinalis L. is considered safe, with no reported negative side effects. However, it's crucial to adhere strictly to recommended doses and avoid prolonged use of *S. officinalis* L. preparations. This caution is particularly warranted due to the high content of thujones in sage, which can lead to adverse effects if exceeded. Thujones, in excessive amounts, may lead to lasting harm to the nervous system, seizures, and dementia. The allowable proportion of thujones is 0.0005 g/kg. It's imperative to emphasize that ingesting pure essential oil is not recommended, and it's advised to refrain from using sage preparations during pregnancy and lactation (GRDIŠA et al., 2015).

CHAPTER II: SILVER NANOPARTICLES

II. Silver nanoparticles

II.1 Silver nanoparticles (Ag-Nps) overview

Nanotechnology encompasses the fabrication, characterization, manipulation, and application of structures at the nanoscale, making it one of the most dynamic fields in material sciences. Nanoparticles typically range in size from 1 to 100 nanometers and exhibit unique or enhanced properties based on specific characteristics like size, shape, and structure. They can be broadly classified as inorganic or organic, with examples including semiconductor nanoparticles (e.g., ZnS, ZnO, CdS), metal nanoparticles (e.g., Au, Cu, Ag, Al), magnetic nanoparticles (e.g., Fe, Co, Ni) among inorganics, and carbon nanoparticles (e.g., fullerenes, quantum dots, carbon nanotubes) among organics (Rafique et al., 2016).

One particular significance among metallic nanoparticles is silver nanoparticles (Ag-NPs), renowned for their chemical stability, conductivity, and versatile properties such as antiviral, antibacterial, cytotoxic, and antifungal effects (Özyürek et al., 2012).

While nanoparticles can be synthesized through physical or chemical methods, each approach has its drawbacks. Chemically synthesized nanoparticles may contain hazardous chemicals on their surface and produce harmful by-products, making them unsuitable for medical use and detrimental to the environment. Physical synthesis methods, though avoiding chemical hazards, can be energy-intensive, space-consuming, and costly. Recognizing these limitations, experts advocate for the utilization of biological systems (green synthesis), including microorganisms, plants, and animal cell cultures, as a promising alternative for nanoparticle production (Pirtarighat et al., 2019).

II.1.1 Silver nanoparticle principles

In the synthesis of silver nanoparticles (Ag-NPs), key concepts align with these principles. Preferably, water serves as the solvent medium, though alcohol may also suffice. Moreover, environmentally friendly reducing agents and nontoxic stabilizers are imperative for producing safe and sustainable nanoparticles. The synthesis process ideally occurs at ambient temperature and pressure, maintaining a neutral pH to mitigate environmental impact. By adhering to these principles, researchers strive to develop Ag-NPs synthesis methods that balance effectiveness with environmental responsibility (Rafique et al., 2016).

II.1.2 Methods for synthesizing Ag-NPs

Ag-NPs can be produced through unconventional or conventional methods, employing two distinct approaches: 'bottom-up' and 'top-down' as shown in Table 1. Conventional methods encompass a variety of techniques such as solution-based, chemical/photochemical reactions within thermal decomposition or via the reverse micelles of silver compounds, and sonochemical, electrochemical, microwave-assisted, radiation routes.

However, such methods often require dangerous chemicals, low compound conversions, wasteful purifications, and high energy requirements. In the last few years, alternative methods have emerged, including green chemistry and biosynthetic approaches. These methods harness biological microorganisms like fungi, bacteria, yeasts, marine algae, or various aqueous or alcoholic plant extracts to synthesize Ag-NPs (Vijayaram et al., 2024).

Tableau 01: Different techniques for synthesis of Ag-NPs

(Rafique et al., 2016).

Synthesis of nanoparticles		
Bottom-up approach		Top-down approach
Green methods	Chemical methods	Physical methods
• Using bacteria	• Chemical reduction	• Pulsed laser ablation
• Using fungi	• Sonochemical	• Evaporation-condensation
• Using plant and their extracts	• Microemulsion	• Arc discharge
• Using yeast	• Photochemical	• Spray pyrolysis
• Using enzymes and biomolecules	• Electrochemical	• Ball milling
• Using microorganism	• Pyrolysis	• Vapour and gas phase
	• Microwave	• Pulse wire discharge
	• Solvothermal	• Lithography
	• coprecipition	
Non-toxic		Toxic

The 'top-down' method entails breaking down bulk materials into smaller particles using techniques like evaporation-condensation, pulse laser ablation, pulse wire discharge, and ball milling. Conversely, the 'bottom-up' method entails synthesizing nanoparticles through biological and chemical methods, where atoms spontaneously into new nuclei that eventually grow into nanoscale particles, as illustrated in Table 1.

As depicted in Table 1, the synthesis of Ag-NPs predominantly employs the bottom-up method, with chemical reductions emerging as the most prevalent method. This entails reducing Ag ions using a variety of inorganic and organic reducing agents, either in non-aqueous or aqueous solutions. Examples of such agents encompass polyethylene glycol block copolymers, ascorbate, Tollen's reagent, N, N-dimethylformamide (DMF), sodium citrate, essential hydrogen, and sodium borohydride (NaBH4). Additionally, coverage agents are utilized to stabilize nanoparticle size. One advantage of this method lies in its capacity to swiftly synthesize a significant quantity of nanoparticles. However, it's essential to recognize that the chemicals employed in this synthesis process have been associated with toxicity and the generation of non-eco-friendly byproducts (Rafique et al., 2016).

On the other hand, green synthesis can be achieved through various approaches, including the use of microorganisms like yeasts, fungi, actinomycetes, and bacteria, as well as plant extracts, plants, and templates such as viruses DNA, membranes, and diatoms (Rafique et al., 2016). Green synthesis provides an eco-friendly and cost-efficient option. Moreover, it is easily scalable and doesn't require high temperatures, energy, pressure, or harmful chemicals (Nikam & Chaudhari, 2022). Metal oxide nanoparticle synthesis using green methodologies, which rely on natural reducing agents, is influenced by different reaction parameters such as temperature, solvent, pH, and pressure conditions (neutral, acidic, or basic). Plant biodiversity has attracted considerable interest in this realm because of the presence of potent phytochemicals in different plant parts, particularly leaves. These phytochemicals, including aldehydes, ketones, flavones, terpenoids, amides, carboxylic acids, ascorbic acids, and phenols, have demonstrated the ability to reduce metal salts into NPs. In the green synthesis of Ag-NPs, essential components typically involve bio-reducing agents and silver salts. Bio-reducing agents, which encompass different cellular constituents, serve as capping agents or stabilizers, thereby minimizing the necessity for external additives (Rathi & Jeice, 2024). The common sage is one of the most widely consumed plants globally due to its rich polyphenol content. The metabolites derived from sage exhibit dual functionality as reducing agents, facilitating the conversion of silver ions into Ag-NPs, and as covering agents, ensuring the morphology and stability of resulting nanoparticles. While various categories of plant metabolites, including proteins, carbohydrates, terpenoids, and alkaloids, contribute to the green synthesis method of metallic nanoparticles, it's particularly noteworthy that polyphenol compounds, such as flavonoids, play a central role (Swilam & Nematallah, 2020).

II.1.3 Applications of silver nanoparticles

Ag-NPs have garnered considerable benefit owing to their distinct characteristics, rendering them applicable across a wide array of fields including biotechnology, bioengineering, pharmaceutics, agriculture, water purification, air filtration, textile manufacturing, electronics, optics, and as catalysts in oxidation reactions as depicted in Fig. 3. Notably, they demonstrate potent antibacterial activity against a diverse spectrum of bacteria (T. Galatage et al., 2021).

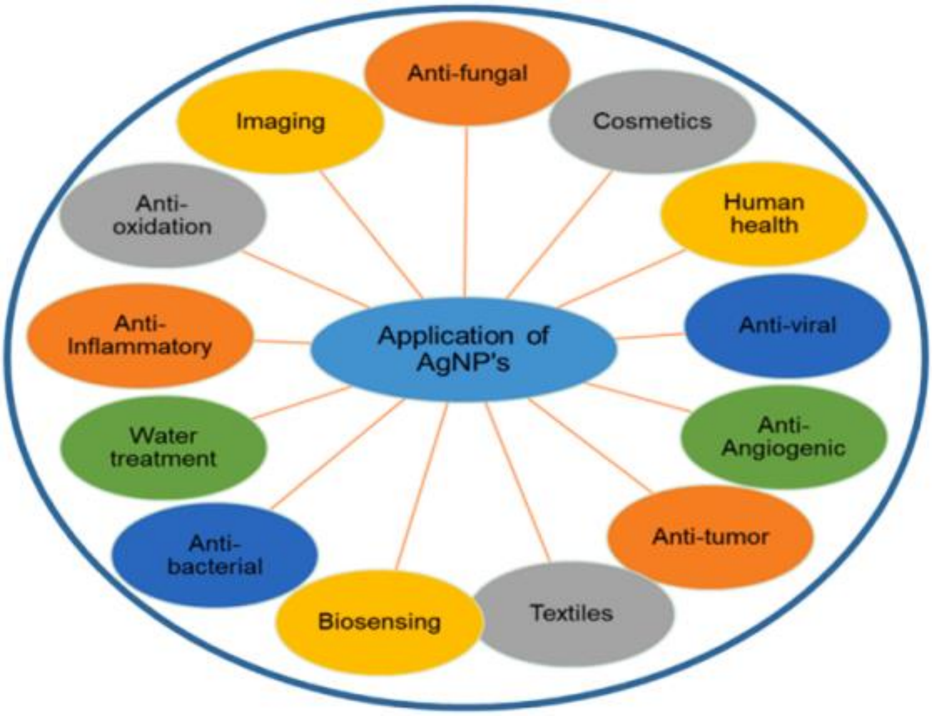

Figure 03: Application of Ag-NPs

(Abbas et al., 2024).

Lately, considerable research focus has been devoted to exploring the utilization of peptide-capped silver nanoparticles for colorimetric sensing applications. This research has primarily centered on investigating the interaction between peptides and silver, along with assessing the impact of peptides on the formation of Ag-NPs. Moreover, silver nanoparticle-based fluorescent sensors have demonstrated remarkable efficacy and have shown promise in surpassing detection limits (Sehnal et al., 2019).

Additionally, Ag-NPs are commonly employed as probes in metal-enhanced fluorescence and surface-enhanced Raman scattering applications. In comparison to other noble metal nanoparticles, silver nanoparticles present several advantages like probes, including sharper extinction bands, higher extinction coefficients, and superior field improvements (T. Galatage et al., 2021). Additionally, silver nanoparticles serve as a prevalent sterilizing agent in an array of consumer and medical items, food storage bags, spanning textiles, personal care products, and refrigerator surfaces. The antibacterial efficacy of Ag-NPs is attributed to the gradual emission of liberated silver ions from the NPs themselves (BEN ABDALLAH et al., 2015).

In recent years, photocatalytic reduction employing nanoparticles has emerged as a popular method for eliminating organic pollutants like dyes and nitroarenes from wastewater. Ag-NPs exhibit catalyzed redox properties towards both biological substances, like dyes, benzene, and chemical compounds. The catalyzed activity of these NPs is intricately influenced by their chemical environment. Notably, catalysis occurs through the adsorption of reactive species onto the catalyzed substrate. However, it's important to highlight that the catalytic efficacy might diminish when surfactants, complex ligands, or polymers are used as stabilizers or to inhibit nanoparticle fusion, leading to a decrease in adsorption. A common practice involves utilizing silver nanoparticles alongside with titanium dioxide as a catalyst in diverse chemical reactions (Vijayaram et al., 2024).

II.1.4 Mechanism of Ag-NPs antibacterial activity

The mechanism underlying the antimicrobial activity of silver nanoparticles (Ag-NPs) involves their capacity to alter bacterial membrane permeability, allowing them to enter the cell. Several studies support the idea that Ag-NPs penetrate the bacterial wall and dissolve into silver ions (Ag^+). Once inside the cell, these Ag^+ ions interact with thiol (SH) groups, which are crucial components of enzymes and cell membranes, forming stable bonds with Ag-NPs. This interaction leads to the inactivation of membrane proteins, denaturation of essential enzymes, reduction of intracellular ATP levels, and subsequent bactericidal activity. This multifaceted process effectively disrupts bacterial cellular functions, resulting in microbial death, as illustrated in Figure 04 (Nisar et al., 2019).

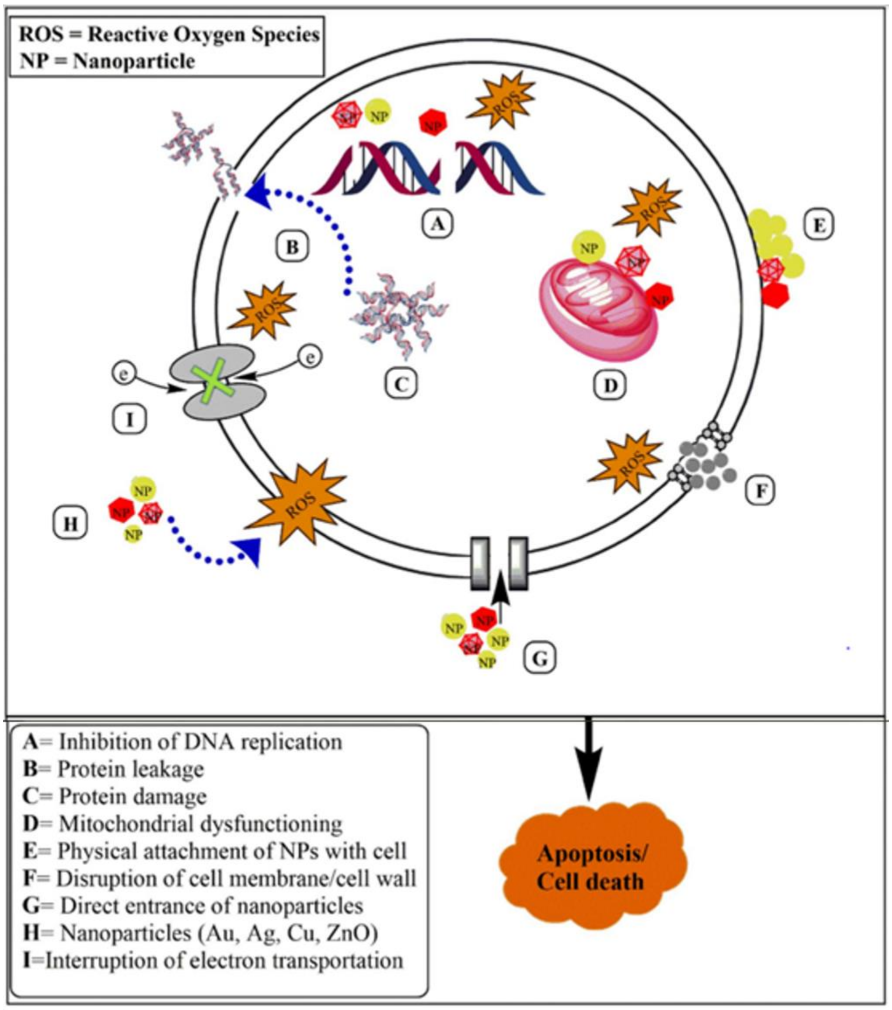

Figure 04: Illustration of possible antibacterial mechanism of action of biosynthesized metal nanoparticles

(Nisar et al., 2019).

One significant benefits of metallic nanoparticles (NPs) is their ability to act as a reservoir for Ag^+ ions, allowing for sustained antibacterial efficacy over an extended period (Prabhu & Poulose, 2012). Ag^+ ions impede cell proliferation by catalyzing DNA replication and directly targeting the DNA bases of diverse bacteria. Notably, these ions can disrupt the hydrogen bonds (H) between pyrimidine and purine base pairs, effectively inserting themselves between the parallel DNA strands. This interference can lead to various outcomes, including the induction of mutations or even the denaturation of DNA, ultimately resulting in bacterial inactivation (Li et al., 2016). This comprehensive mechanism highlights the efficacy of silver nanoparticles in combating bacterial growth and ensuring prolonged antimicrobial activity.

CHAPTER III: EFFLUENT POLLUTION

III. Effluent pollution

III.1 Effluent pollution overview

Water pollution stands as an environmental challenge, threatening ecosystems worldwide. The advent of industrialization has amplified this issue, as water resources become increasingly contaminated with toxic pollutants (Marimuthu et al., 2020). Wastewater, originating from diverse sources such as commercial, residential, agricultural, and industrial activities, represents a complex mixture whose composition varies significantly based on its origin. Industries such as battery manufacturing, and mining, etc. contribute substantially to this polluted wastewater, laden with hazardous substances. The damaging effects of these toxins react across ecosystems, posing significant risks to both aquatic life and terrestrial organisms, as well as compromising overall environmental integrity (Palani et al., 2021).

III.1.1 Industrial effluent characteristics

Industrial effluents represent a significant byproduct of human industrial activities, arising from various processes involved in processing raw materials and manufacturing. These types of wastewater encompass a wide range of activities, including washing, cooking, cooling, heating, extraction, and quality control, often leading to the rejection of products (Sathya et al., 2022).

Classifying industrial waste into hazardous and nonhazardous categories is essential for effective waste management. Hazardous waste poses risks to both environmental and human health, while nonhazardous waste includes materials like plastic, cardboard, glass, iron, organic, and stone matter, which present minimal risk. Handling hazardous materials, including flammable and biodegradable substances, requires stringent protocols to mitigate potential hazards to environment and the public health. Industrial wastes are typically categorized into three main types: solid waste, air emissions, and wastewater, each presenting distinct challenges in management and disposal (Palani et al., 2021). Wastewater from industrial processes is characterized by its diverse composition, containing a range of inorganic substances like heavy metals, solutes, gases, ammonia, and metal ions, along with sophisticated organic compounds like food, excreta, proteins, plant matter, and natural organic material. Additionally, it may also harbor pollutants commonly found in surface water, industrial water sources, or groundwater (Palani et al., 2021).

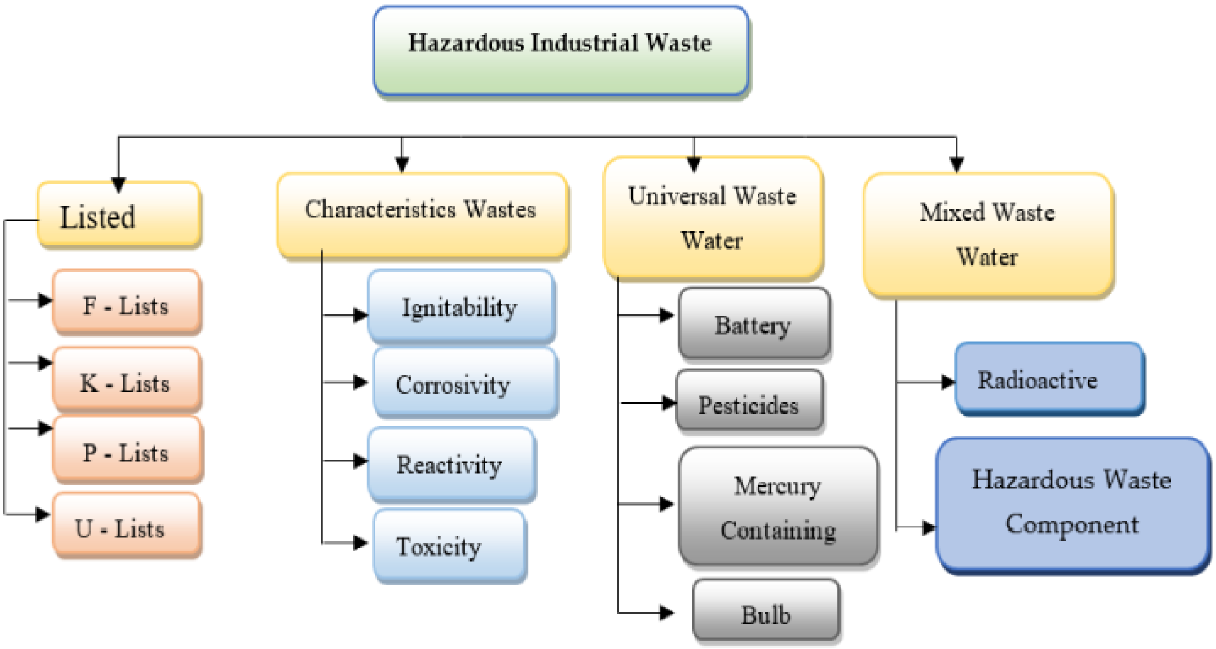

Figure 05: Industrial hazardous wastes general classification (Palani et al., 2021).

- F-list (wastes generated from typical manufacturing and industrial processes);
- K-list (wastes from particular industries);
- P-list, and U-list (wastes derived from commercial chemical products).

III.1.2 Industrial effluent composition

Industrial waste is typically characterized by its diverse composition, containing high levels of dissolved and suspended solids, organic and inorganic chemicals, oils, grease, and toxic metals, among other pollutants. These contaminants pose significant risks to freshwater ecosystems, particularly aquatic organisms like fish, when released into water bodies (Muley et al., 2007).

In recent years, there has been an increasing concern regarding the adverse effects of industrial wastewater containing pollutants containing toxic heavy metals, including nickel, cadmium, chromium, lead, mercury, and thallium. Additionally, contaminants of micro-pollutants or emerging concerns (CECs), like personal care products, pharmaceuticals, industrial substances, and pesticides, have garnered increased attention due to their potential ecological and human health impacts. Non-biogenic heavy metals, known for their persistence and toxicity, pose significant challenges in wastewater treatment systems and can have harmful effects on both aquatic ecosystems and human health (Iloms et al., 2020).

Dyes are commonly used in different industries, including textiles, pharmaceuticals, food, cosmetics, and plastics. However, their indiscriminate use can result in harmful impacts on water systems because of their chemical structure and origin. Different types of dyes, such as reactive, acidic, disperse, basic, diazo, anthraquinone-based, metal-complex dyes, and azo, are employed across industries, some of which are derived from known carcinogens like benzidine and naphthalene (Marimuthu et al., 2020). Effluents from processes like dyeing cotton with reactive dyes are heavily contaminated, contributing to color, high oxygen demand, and salt load in water bodies (Khatri et al., 2015).

III.1.3 Ag-NPs in wastewater management

Water from diverse sources often contains harmful microorganisms, heavy metals, and organic compounds, necessitating purification for safe consumption. Various methods like filtration, coagulation, and chemical treatments are employed for water purification. Silver nanoparticles (Ag-NPs) have emerged as effective agents for removing organic pollutants, heavy metals, inorganic anions, and bacteria from water. Their inherent cost-effectiveness, stability, and controllable release rate make them promising candidates for wastewater and water treatment. In the last few years, its use in water disinfection methods has surged due to its potent toxicity against microorganisms (Zahoor et al., 2021).

Silver nanoparticles offer a multitude of beneficial properties across various sectors, including biology, coatings, DNA sequencing, food, drug therapy, cosmetics, and biomedicine. While much of the research on Ag-NPs has centered on their antimicrobial activity against diverse microorganisms, their potential applications extend to water purification, dye removal, and wastewater treatment (Palani et al., 2023).

Characterization of silver nanoparticles (Ag-NPs) traditionally involves various methods, with qualitative analysis often relying on visual color changes. Ag-NPs play a crucial role in degrading toxic chemicals in aqueous solutions through two primary mechanisms. Firstly, they act as chemical reducing agents in catalytic reduction processes. Secondly, they participate in catalytic degradation processes, often in conjunction with induced light degradation methods (Sharifi et al., 2020).

Hybrid Ag-NPs materials have gained widespread use in the treatment of wastewater, often combined with materials like cellulose, chitosan, activated carbons, graphene oxides, titanium dioxides, silicon dioxide, and alginate. Water pollution stems from multiple sources, including dyes like methyl orange, Congo red, and rhodamine, so does heavy metal toxicity and bacterial contamination. Ag-NPs exhibit anti-bacterial properties and heightened adsorption capacity, making them essential for eliminating contaminants from wastewater. However, their indiscriminate use poses risks of environmental pollution. Thus, careful consideration of Ag-NP properties is essential to ensure their effective and sustainable utilization in wastewater management (Fiorati et al., 2020).

III.1.4 Silver Nanoparticles activity for wastewater treatment

The antimicrobial properties of Ag-NPs make them a promising solution for water treatment and disinfection. These properties include membrane alteration, DNA/RNA or protein damage, and Ag (I) release in the cytoplasm of the cell. Consequently, various Ag-NPs-based products are employed to disinfect water and serve as water filters to prevent the growth of pathogenic bacteria and viruses.

The use of silver has been extensively studied across various fields, particularly in medicine and water treatment. Recently, silver nanoparticles have gained prominence for their improved performance and versatility. Furthermore, the biological synthesis of Ag-NPs has emerged as a safer alternative to conventional chemical methods, which often involve harmful organic or inorganic chemicals and metal salts. It's noteworthy that silver nanoparticles can be manufactured without the use of stabilizing agents, both in chemical and physical processes.

These nanoparticles efficiently degrade organic pollutants, converting them into non-toxic substances, while also exhibiting remarkable catalytic reduction and antimicrobial activity. Fig. 5 illustrates the flowchart for the Ag-NPs synthesis for wastewater treatment across different industries. Nanocomposites based on metal nanoparticles, such as graphene oxide, hold potential applications in various material science fields. For instance, the synthesized GO-Ag nanocomposite demonstrated complete removal of MB dye after 40 minutes of sunlight irradiation. Therefore, silver-based nanocomposites could serve as viable photocatalysts for organic dyes found in wastewater and industrial effluents (S. Singh et al., 2019).

The process of dye removal using silver nanoparticles (Ag-NPs) involves several mechanisms, including adsorption onto Ag-NPs in degradation via catalytic/photocatalytic mechanism, or conjunction with loaded activated carbon, or a blend of both. To enhance catalytic efficiency and prevent poor flocculation of nanoscale materials during the catalytic degradation process employing Ag-NPs, silica spheres are often added to support the nanoparticles (Jadhav et al., 2019).

III.1.5 Potential hazardous effects of Ag-NPs

While silver nanoparticles (Ag-NPs) and their composites offer a wide array of applications across various industries, it's essential to recognize their potential adverse effects. Ag-NPs are utilized in cleaning products, digital devices, toys, the food industry, and scientific appliances, among others. However, their extensive use as antimicrobial agents and disinfectants raises concerns about potential harm to aquatic life and development of bacterial resistance (Palani et al., 2023).

Determining The toxicity threshold concentration of Ag-NPs alone may not offer a comprehensive understanding of their potential harm, as various factors come into play, including their concentration, size, shape, and surface area. Additionally, defining the toxicity range of Ag-NPs poses challenges due to the variability in their sources, methods of toxicological assessment, dose units, and how it enters the body (Zahoor et al., 2021)**.**

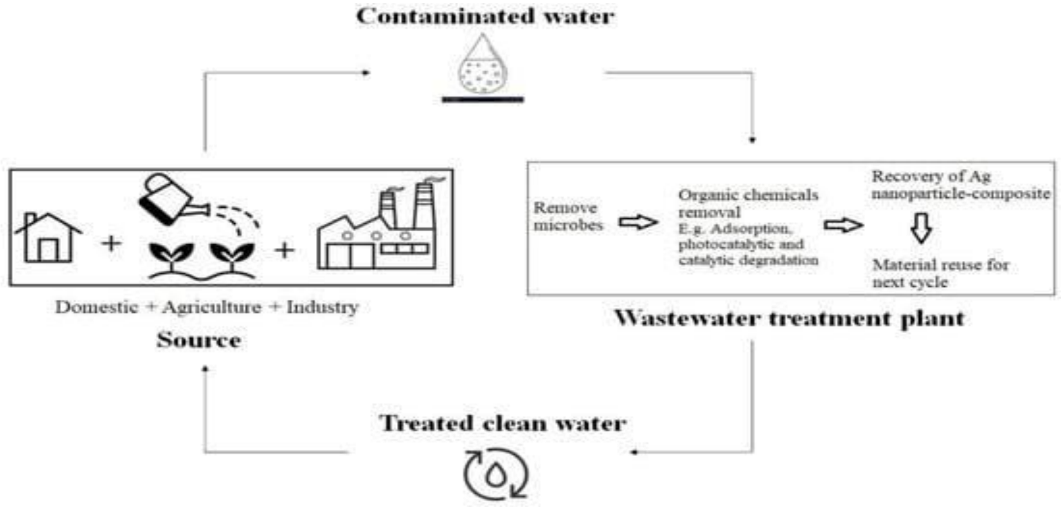

Figure 06: Flowchart for the silver nanoparticles compound for wastewater treatment (Zahoor et al., 2021b).

Part II: Experimental work

CHAPTER IV: MATERIALS AND METHODS

IV. Materials and Methods

IV.1 Study context

This study concentrates on synthesizing the nanoparticles using *Salvia officinalis* L. extract obtained via ultrasound-assisted extraction. The research aims to assess the antioxidant activity and antimicrobial properties of both the *Salvia* extract and the synthesized nanoparticles. Conducted at Bouira University in February, this work presents a novel approach to harnessing the potential of *Salvia officinalis* L. in nanotechnology and biomedical applications.

IV.2 Sample preparation

This study used a sage plant (*Salvia officinalis* L.) sourced from the SouK El-Khemiss-Bouira region, harvested on January 29th, 2024. Upon harvesting, the leaves underwent cleaning to remove any impurities. Subsequently, they were carefully collected, followed by a thorough drying process in a well-ventilated oven set at 40 °C until reaching weight stabilization as depicted in Figure 07.

Figure 07: Sample preparation procedure; a) Cleaning leaves, b) oven drying of leaves, c) Dried leaves; d) leaf powder.

This controlled drying method helps preserve the integrity of the plant material while ensuring moisture removal. Once adequately dried, the leaves of *S.officinalis* L. were finely crushed using an electric grinder to achieve a consistent particle size. To further ensure uniformity, the powdered material was sieved through a 200 µm diameter sieve, thereby enhancing homogeneity. The resulting powdered sage was then securely stored in shaded bottles at ambient room temperature, safeguarding its quality until further use.

IV.3 Ultrasound-assisted extraction of total phenolic content

Extracting phenolic compounds from sage was conducted employing an ultrasonic bath adhering to the methodology outlined by Moussa et al., (2022). In summary, 1g of sage powder was mixed with 30 mL of extraction solvent consisting of ethanol and distillated water then introduced into an ultrasonic bath. Parameters of time and temperature were carefully calibrated, with a duration of 10 minutes and a temperature of 60 °C being selected for optimal extraction efficiency.

Figure 08: UAE procedure of phenolic compounds extraction from Salvia officinalis L.: a) Extraction. b) Filtration. c) Solvent evaporation. d) Lyophilization.

Upon completion of the extraction process, the mixture underwent filtration using Watman paper to eliminate any residual plant debris. Subsequently, the resulting liquid extract was subjected to evaporation and lyophilization to remove solvent content, ensuring the preservation of the extracted phenolic compounds. Finally, the dried extract was stored in amber vials to maintain its stability until further analysis.

IV.4 Ag-NPs synthesis

Silver nanoparticle (Ag-NPs) synthesis necessitates the presence of Ag^+ ions, typically sourced from water-soluble silver salts. Among these, aqueous AgNO3 solutions are frequently utilized. Typically, within a concentration range of 0.1-10 mM, with 1 mM being the common choice. In this process, a 10% solution of the extract is combined with 1 mM silver nitrate solution (Srikar et al., 2016). The reaction mixture is then subjected to heating for 180 min (Gecer, 2021). Subsequently, the nanoparticles are washed twice with distilled water and centrifuged. This process ensures the purification of the synthesized nanoparticles, yielding SO-Ag-NPs ready for further characterization and application.

IV.5 Antioxidant activity

Several techniques are utilized to assess the antioxidant activity of pure phenolic compounds of extracts, often relying on color changes or decolorization within the reaction medium. In our investigation, we employed two distinct methods to evaluate the antioxidant capacity of sage extract and silver nanoparticles (Ag-NPs) including $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ radicals scavenging activity, as described by Singh et al., (2019). These methods offer complementary insights into the antioxidant potential of the tested samples, providing a comprehensive understanding of their radical scavenging abilities.

IV.5.1 Evaluation of antioxidant activity via $DPPH^{\bullet}$ radical scavenging activity

- **Principle**

$DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) is a stable, chromogenic free radical with a characteristic violet coloration at room temperature. The principle behind its assay lies in electron transfer; when exposed to an antioxidant molecule capable of donating a hydrogen atom (AH), $DPPH^{\bullet}$ undergoes reduction, resulting in the formation of a pale yellow-colored solution known as DPPH-H. Detection of this color change occurs spectrophotometrically at a wavelength range of 512-517 nm. Renowned for its rapidity, sensitivity, ease of implementation, and minimal reagent consumption, the $DPPH^{\bullet}$ assay holds significant appeal for the evaluation of natural antioxidants, making it a cornerstone method in antioxidant research (Habibou et al., 2019).

- **Protocol**

The assessment of free radical scavenging activity was conducted utilizing the 1-1-diphenyl-2-picryl-hydrazyl (DPPH$^{•}$) assay, with slight modifications. A 60 µM solution of DPPH$^{•}$ in ethanol was prepared. Subsequently, 1 mL of this prepared DPPH$^{•}$ solution was combined with 100 µL of extracts or Ag-NPs at varying concentrations ranging from 0.05 to 1 mg/mL. Following a 15-minute incubation period, the absorbance of the reaction mixture was recorded at 517 nm utilizing a UV/Vis spectrophotometer, following the protocol outlined by Fafal et al., (2017). The antioxidant activity was expressed as a percentage using Eq.1. In addition, the outcomes are expressed in terms of IC$_{50}$, a pivotal metric in antioxidant evaluation, where, IC$_{50}$ represents the concentration of the extract necessary to inhibit the formation of 50% of the DPPH$^{•}$ radicals within the reaction solution. Notably, a lower IC$_{50}$ value signifies a higher degree of activity and efficacy of the tested sample in scavenging DPPH$^{•}$ radicals, indicative of its potent antioxidant properties.

$$\text{Free radical inhibition (\%)} = \frac{A_{blanc} - A_{Sample}}{A_{Control}} * 100 \qquad (1)$$

IV.5.2 Evaluation of antioxidant activity via ABTS assay

- **Principle**

ABTS$^{+•}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) serves as a stable, initially colorless radical, which undergoes oxidation to ABTS$^{+•}$ in the presence of potassium persulfate ($K_2S_2O_8$). This transformation occurs through the extraction of an electron from a nitrogen atom within the ABTS$^{+•}$ molecule. The ABTS$^{+•}$ radical, characterized by its turquoise-blue coloration, is generated as a result. The ABTS assay revolves around the capacity of compounds to intercept the ABTS$^{+•}$ radical cation, resulting in the acquisition of its distinctive color. In the presence of an antioxidant acting as a hydrogen donor, ABTS$^{+•}$ is regenerated from ABTS$^{+•}$, effectively removing the coloration from the solution. The absorption of the compound is subsequently quantified at 734 nanometers, as per the methodology outlined by Shah & Modi, (2015).

- **Protocol**

The assessment of antioxidant capacity for both silver nanoparticles (Ag-NPs) and sage extract was conducted using the $ABTS^{+\bullet}$ cation radical scavenging assay as described by Fafal et al., (2017), with adaptations. Initially, a solution of 7 mM ABTS mixed with 2.45 mM potassium persulfate, prepared in ethanol, underwent a 24-hour incubation period. Subsequently, the solution was diluted until reaching an absorbance of 0.7 at 734 nm. Then 1 mL of this prepared solution was combined with 50 µL of samples and incubated at room temperature for six minutes, following which the absorbance was measured using a UV/Vis spectrophotometer, with ethanol serving as the blank. The trapping activity of the $ABTS^{+\bullet}$ radicals was quantified as a percentage of inhibition using Equation.1.

The resultant outcomes are expressed in terms of IC_{50}, a pivotal metric denoting the concentration of the extract necessary to inhibit the formation of 50% of the $ABTS^{+\bullet}$ radicals within the reaction solution. A lower IC_{50} value indicates a heightened degree of activity and efficacy, underscoring the potent antioxidant capabilities of the tested sample, as established by Fafal et al., (2017).

$$\text{Free radical inhibition (\%)} = \frac{A_{blanc} - A_{Sample}}{A_{Control}} * 100 \qquad (1)$$

A_{Sample} is the absorbance of free radical solution ($ABTS^{+\bullet}$ or $DPPH^{\bullet}$) + sample extract at the required time, A_{blanc} is the absorbance of free radical solution ($ABTS^{+\bullet}$ or $DPPH^{\bullet}$) + extraction solvent, $A_{Control}$ is the absorbance of the working free radical solution.

IV.6 Antibacterial activity

IV.6.1 Water collection and storage of water samples

Effluent samples were collected from the industrial waste manufacturing facility of FAIZ LAIT, situated within the Corso Industrial Zone, Boumerdes, followed by comprehensive measurements of effluent parameters during production, revealing a temperature (T) of 18.4 °C and a pH level of 8.12.

To assess the impacts of *Salvia officinalis* L. extract, Ag-NPs, and silver nitrate on the effluent water, a thorough evaluation process was initiated. Subsequently, the collected data underwent meticulous processing and visualization to discern the efficacy and potential benefits of these substances on the effluent's composition and characteristics.

IV.6.2 Preparing the culture medium and bacterial suspension

Before initiating the experiment, “the Nutrient agar media”, “Eosin Methylene Blue agar media”, “Baird-Parker agar media”, and “Chapman Stone culture media” were prepared following the manufacturer's guidelines. Subsequently, the prepared medium underwent autoclaving at 121°C for 20 min to ensure sterilization. Post-sterilization, the culture medium was carefully dispensed into petri dishes sized at 90 mm × 45 mm, with two dishes allocated for each type of medium, as outlined by the protocol adapted from Santos et al., (2022) with necessary modifications. The culture is medias outlined above were seeded with bacteria of industrial effluent.

IV.6.3 Evaluation of antibacterial activity and treatment

The inhibitory effects of an aqueous *S.O* leaves extract, Ag-NPs, and silver were assessed against effluent-derived bacteria utilizing the well diffusion method on Mueller-Hinton agar (MHA). In this methodology, bacterial isolates were initially cultivated on nutrient agar at 37 °C for 18 to 24 hours to ensure optimal growth. Subsequently, bacterial suspensions were swabbed onto Mueller-Hinton agar (MHA) plates utilizing sterile cotton swabs under aseptic conditions. Wells with a standardized diameter of 6 mm were then filled with 50 µl of the respective test samples. Following inoculation, the plates were incubated at 37 °C for 24 h. After the incubation period, the diameter of the observed zones of growth inhibition surrounding the wells was measured to quantify the inhibitory effects of the test substances (Maqbool et al., 2020).

IV.6.4 MIC (the minimum inhibitory concentrations) activity

To determine the MIC of silver nanoparticles and sage extract, the broth dilution method was employed by Parvekar et al., (2020). A series of dilutions spanning concentrations from 1 to 0.03125 mg/mL for both Ag-NPs and silver nitrate were meticulously prepared. Additionally, dilutions of the extract ranging from 2 to 0.0625 mg/mL were also prepared. Adjusted bacterial cultures, characterized by an absorbance of 0.07 nm, were utilized to determine the minimum inhibitory concentration (MIC) in the broth Luria Bertani (LB). The control group consisted of inoculated broth incubated for 24 h at 37 °C.

CHAPTER V: RESULTS AND DISCUSSION

V. Results and discussion

V.1 Ag-NPs synthesis

Silver nanoparticles were synthesized using the extract of *Salvia officinalis* L. leaves as the primary source. The process began with heating the plant material in distilled water followed by removing solid residue. The resulting extract solution was then treated with a solution containing silver nitrate. The secondary metabolites present in the plant extract facilitated the reduction of Ag^+ ions to Ag^0. After this reduction, the Ag atoms were encapsulated and stabilized by the secondary metabolites present in the plant extract.

The synthesis process was confirmed through a distinct color change in the reaction solution, turns from light yellow to dark brown, thus affirming the successful production of silver nanoparticles derived from *Salvia officinalis* L.(Ag-NPs), as discussed by Gecer, (2021) in their research on silver nanoparticle synthesis using *Salvia aethiopis* leaves.

On the other hand, in a study conducted by Pirtarighat et al., (2019), the initial color of the fresh *Salvia spinosa* extract was yellow. However, after adding an AgNO3 solution and subsequent stirring at room temperature, the solution gradually changed color, eventually turning red. Similarly, in the research conducted by Singh et al., (2019), the leaf extract of *Premna integrifolia* L., which had a green color, was added to a colorless AgNO3 solution. After exposure o bright sunlight for 25 min, the solution color changed from dark green to dark orange. The color variation observed during the synthesis of silver nanoparticles can be attributed to the the use of different plant species used in the synthesis process.

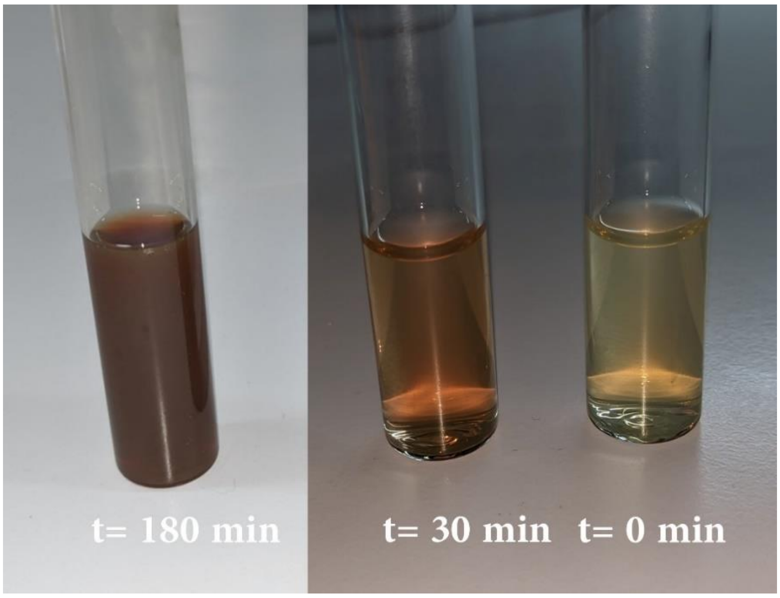

Figure 09: Ag Nanoparticles synthesis over time (t=0, 30, and 180 min), color change indicates the Ag-NPs formation.

The Silver nanoparticles (Ag-NPs) synthesis by biological entities relies on a variety of organic chemicals found within the organisms or extracts involved. These compounds, which include fats, carbohydrates, flavonoids, enzymes, proteins, coenzymes, alkaloids, phenols, gums, and terpenoids, serve crucial roles by donating electrons to reduce Ag^+ ions to Ag^0. The specific active agent responsible for this reduction varies depending on the organism or extract being utilized. Notably, for the nano-conversion of Ag-NPs, electrons are sourced from the dehydrogenation of acids such as alcohols, ascorbic acid like catechol, and undergo keto to enol conversions facilitated by compounds such as cyperaquinone, diethequinone, and remirin. Moreover, in xerophyte plants, both mechanisms may be simultaneously employed Srikar et al., (2016**)** provides further insights into these intricate processes.

V.2 Evaluation of antioxidant activity

V.2.1 DPPH• free radical activity evaluation of Sage extract and Ag-NPs

The antioxidant capacity of *Salvia officinalis* L. leaf extract and Ag-NPs was evaluated using the DPPH• free radical scavenging method. Figure 10 indicates a direct correlation between the reduction of DPPH• and the concentration of both sage extract and Ag-NPs, showing a significant enhancement in antioxidant activity with higher concentrations of both Salvia officinalis L. leaf extract and Ag-NPs. This finding emphasizes the effectiveness of both the extract and nanoparticles in neutralizing free radicals, thus highlighting their potential as potent antioxidants.

In this study, our objective was to assess the antioxidant activity of *Salvia officinalis* L. leaf extract and compare it with that of Ag-NPs, using the DPPH$^•$ assay as the main evaluation method. Our results revealed a similarity in the antioxidant efficacy between *Salvia officinalis* L. leaf extract and silver nanoparticles. The determined IC50 values further supported this similarity, with Ag-NPs exhibiting an IC50 of 185±0.01 µg/mL, while *Salvia officinalis* L. leaf extract displayed an IC50 of 173±0.002 µg/mL. Notably, at a concentration of 1 mg/mL, both *Salvia officinalis* L. leaf extract and Ag-NPs exhibited impressive percentage of antioxidant activity (95% and 90%, respectively).

Interestingly, our results suggest a a slightly higher potency antioxidant activity of *Salvia officinalis* L. leaf extract compared to Ag-NPs. The antioxidant activity of Ag-Nps can be attributed to their ability to scavenge DPPH$^•$ free radicals primarily through electron transfer and the antioxidant properties of the capping bioactive compounds. The Ag-NPs facilitate the donation of electrons or hydrogen atoms to the DPPH radicals, thus neutralizing them and reducing their reactivity. Furthermore, the bioactive compounds from the sage extract enhance this effect by directly interacting with the DPPH$^•$ radicals and stabilizing them, leading to effective antioxidant activity.

Our research findings indicate a significant enhancement in antioxidant activity compared to previous studies. For instance, Baharara et al., (2017) reported an IC50 value of 830 µg/mL for *Salvia officinalis* Ag-NPs, while Moussa et al., (2022) recorded an IC50 of 276 µg/mL for sage leaf extract. In contrast, our study demonstrated IC50 values of 185±0.01 µg/mL for Ag-NPs and 173±0.002 µg/mL for the leaf extract. Furthermore, in Singh et al., (2019) study on *Premna integrifolia* leaves, the IC50 values for *Premna integrifolia* extract (AEP) and AEP Ag-NPs were recorded as 715.23±1.26 µg/L and 524.19±2.63 µg/L, respectively.

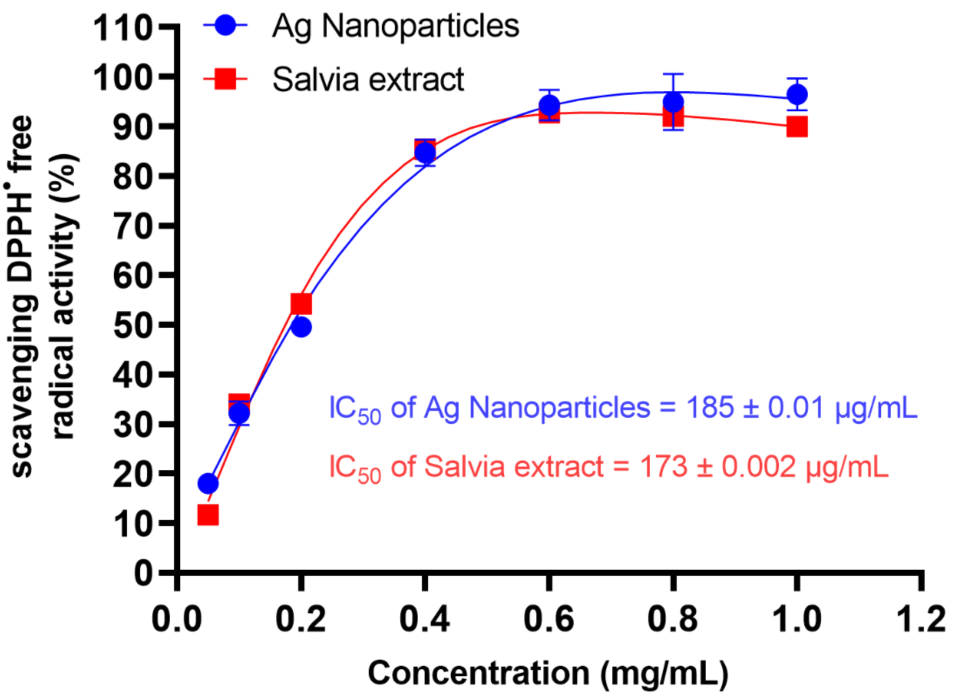

Figure 10: Scavenging DPPH• free radical activity of *Salvia officinalis* L. extract and Ag nanoparticle.

This substantial improvement underscores the superior efficacy of our method in evaluating antioxidant potential, highlighting the significant advancement in antioxidant activity assessment achieved by our approach. These varied results can be attributed to differences in DPPH concentrations, extract concentrations, silver nanoparticle concentrations, and the specific plant species used in the experiments.

V.2.2 ABTS free radical activity evaluation of *Salvia officinalis* L. extract and Ag-NPs

To further evaluate the antioxidant activity of both sage extract and Ag-Nps, the ABTS radical scavenging activity was assessed and the results were illustrated in the Figure 11. This figure illustrates the percentage of inhibition achieved by both *Salvia officinalis* L. leaf extract and Ag-NPs, along with their respective IC50 values for each were described. These metrics provide valuable insights into the antioxidant efficacy of *Salvia officinalis* L. leaf extract and Ag-NPs, offering a quantitative assessment of their potential in scavenging free radicals and mitigating oxidative stress.

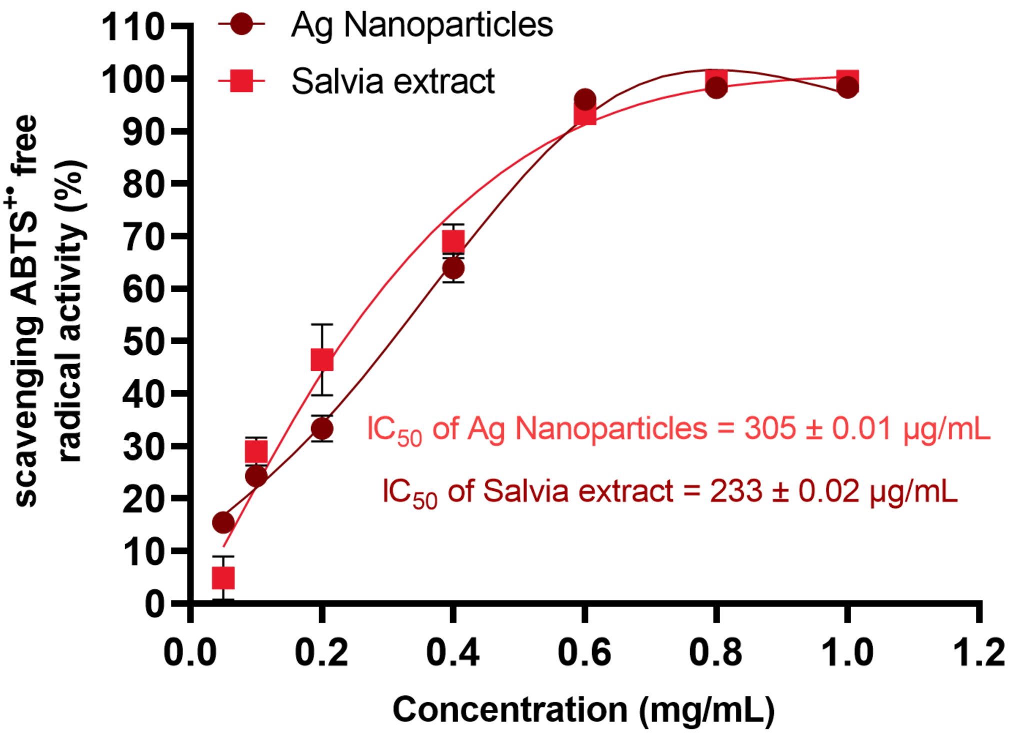

Figure 11: Scavenging ABTS+• free radical activity of *Salvia officinalis* L. extract and Ag nanoparticle.

As shown in Figure 11, there are notable differences in the IC_{50} values, with the extract having an IC50 of 233±0.02 µg/mL and the Ag-NPs having an IC50 of 305±0.01 µg/mL. At a concentration of 1 mg/mL, both *Salvia officinalis* L. leaf extract and Ag-NPs achieved 100% inhibition of antioxidant activity. These findings suggest that sage leaf extract has a slightly higher potency in terms of antioxidant activity compared to Ag-NPs.

On a molecular level, the phenolic compounds in the sage extract contain hydroxyl groups that effectively neutralize free radicals through electron transfer or hydrogen atom donation mechanisms. This high reactivity at lower concentrations accounts for the lower IC50 value observed for the extract. Conversely, Ag-NPs exhibit a different antioxidant mechanism. The silver nanoparticles act as catalysts, facilitating electron transfer to neutralize free radicals. Their surface provides a platform for redox reactions, enhancing their antioxidant capacity. However, this process may require a higher concentration of nanoparticles to achieve the same level of free radical scavenging as the sage extract, which explains the higher IC50 value for Ag-NPs.

In contrast to the findings reported by Baharara et al., (2017) on the antioxidant assay of silver nanoparticles synthesized from *Salvia officinalis* L. leaves, which an IC_{50} value of 800 µg/mL, our empirical results demonstrate a notable enhancement in antioxidant efficacy. Our approach, revealed a significant improved in free radical scavenging effect, surpassing that reported in their study. This marked advancement in antioxidant activity underscores the effectiveness of our extraction method.

On the other hand, in Srećković et al., (2023) study on the synthesis of silver nanoparticles using *Salvia pratensis* L., the results demonstrated notable differences in IC50 values between the plant extract and the synthesized nanoparticles. The IC50 value for the *Salvia pratensis* L. extract was found to be 192.44±12.55 µg/mL, whereas the silver nanoparticles exhibited an IC50 value of 74.55±9.25 µg/mL. The superior results observed in this study, compared to our findings, can be attributed to variations in the concentrations of solutions used during the synthesis of silver nanoparticles, as well as differences in the plant species utilized. The improved IC50 values in their study highlight the significant impact of these factors on the efficacy of the synthesized nanoparticles.

V.2.1 Preparing the culture medium and bacterial suspension

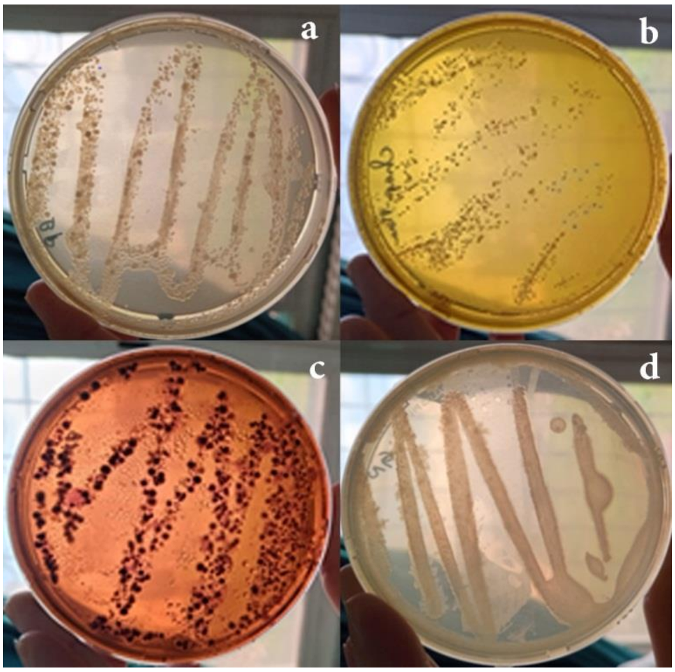

Figure 12: Obtained bacteria strains in different culture media. a) Baird-Parker agar media. b) Chapman media. c) Eosin Methylene Blue Agar media. d) Nutrient agar media.

The bacterial strains Gram negative like *Staphylococcus aureus* and *Escherichia coli* obtained from different culture media (Eosin Methylene Blue Agar, Chapman Stone media and Baird-Parker agar media) were inoculated into nutrient agar medium and subjected to incubation under suitable conditions. After this incubation period, the cultures underwent transfer into physiological water for further processing. The absorbance of the bacterial suspension was then quantified at a wavelength of 620 nm, yielding a measured value of 0.07.

V.3 Evaluation of Antibacterial Activity of Ag-NPs

Colloidal silver (Ag) has garnered historical recognition as an antimicrobial agent according to its distinct surface properties, which facilitate enhanced contact with microorganisms and promote the release of silver ions (Ag^+). These ions demonstrate a wide range of activity against various pathogens, including fungi, viruses, and a diverse array of infectious bacteria encompassing both Gram-positive and Gram-negative strains. Notable examples of susceptible bacteria include *Escherichia coli, Staphylococcus aureus,* and *Bacillus* species (Maqbool et al., 2020).

In our study, we conducted an *in vitro* assessment of the antimicrobial effect of Ag-NPs, silver nitrate, an extract derived from *Salvia officinalis* L. as a control against dairy industry effluents. As illustrated in Figure 13, our results showed distinct variations in the antibacterial effects among the tested substances. Both Sage extract and silver nitrate displayed satisfactory antibacterial activity, as indicated by the zones of bacterial inhibition observed. However, Ag-NPs demonstrated significantly stronger inhibition against all tested bacterial strains, with inhibition zones ranging from 2 mm to 9 mm (Figure 13).

Significantly, this heightened activity exceeded that of Ag-NPs biosynthesized from aqueous extracts of other plants (Vo et al., 2019). Emphasizing the profound influence of the bioactive compounds present in the extract plant on the bioactivity of biogenic Ag-NPs (Vo et al., 2019). Moreover, our results indicate consistent antibacterial activity of Ag-NPs across all four bacterial strains, even with low concentration. This consistency suggests the stability of the antibacterial properties of Ag-NPs derived from *S.O.* It has been established that the antibacterial efficacy of silver nanoparticles (Ag-NPs) is highly influenced by factors such as size, shape, synthesis method and the composition of the capping agent (Prabhu & Poulose, 2012).

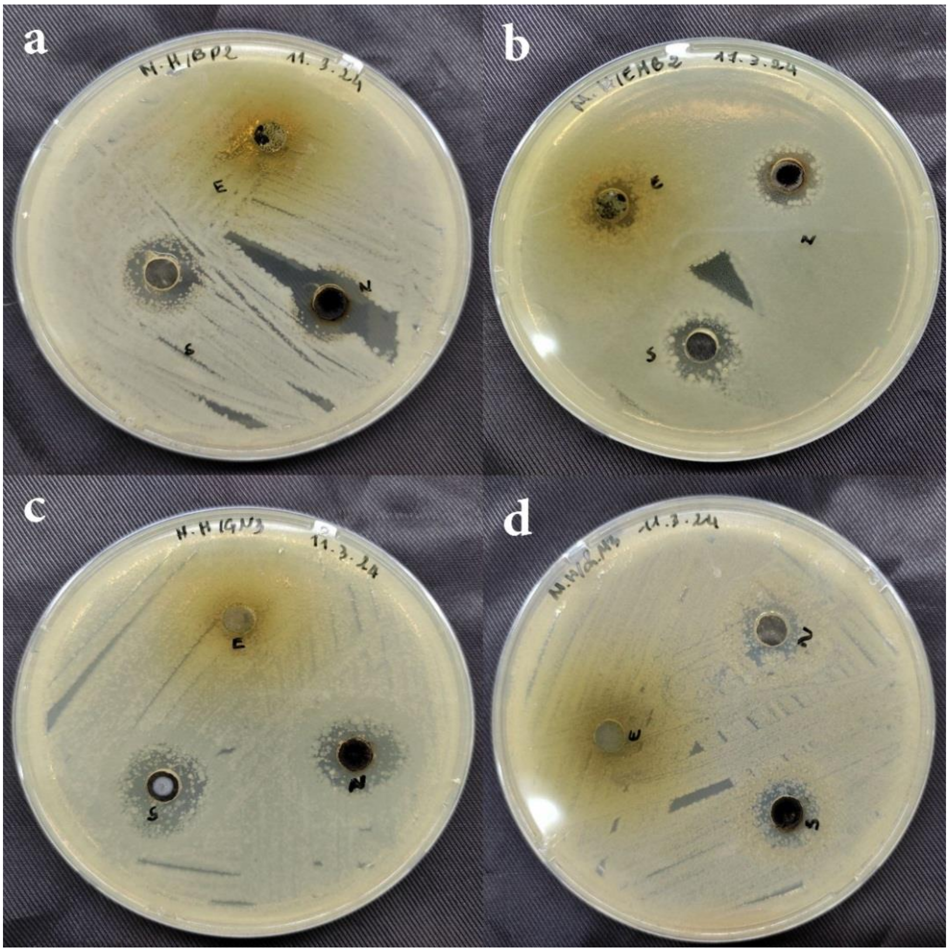

Figure 13: Antibacterial activity of sage extract (E), Ag-Nps (N) and silver nitrate (S) against bacteria strains inoculated from Baird-Parker agar media. (a). Methylene Blue Agar media. (b). nutrient agar media. (c). Chapman media (d).

V.4 Determination of minimum inhibitory concentration (MIC)

The MIC was evaluated for Ag-NPs, *Salvia officinalis* L. extract, and silver nitrate against bacteria, using Luria-Bertani (LB) medium with bacterial suspensions. Dilution plates were prepared with concentrations ranging from 1 mg/mL to 0.0156 mg/ml for Ag-NPs and silver nitrate, and from 2 mg/ml to 0.03125 mg/mL for sage extract. These plates were then incubated at 37 °C for 24 h. All samples yielded positive results, laying the foundation for subsequent analysis (Cavassin et al., 2015). The results depict bacterial growth under varied concentrations of Ag-NPs, *Salvia officinalis* L. extract, and silver nitrate.

The results illustrate bacterial growth under varying concentrations of Ag-NPs, Salvia officinalis L. extract, and silver nitrate. Each of these tests exerted different effects on growth, as evidenced by changes in color and absorbance, visually assessed for turbidity. Particularly at low concentrations of Ag-NPs, sage extract, and silver nitrate, the observed effect suggests cell death during the stationary phase. It is worth noting that the antimicrobial activity of Ag-NPs may depend on the initial bacterial concentration, as suggested by some authors.

Our study revealed MIC values for colloidal Ag-NPs synthesized, ranging from 0.062 mg/mL, with silver nitrate achieving 0.125 mg/mL, and *Salvia officinalis* L. extract at 0.5 mg/mL. These findings indicate a heightened sensitivity of bacterial suspensions across all tests. These MIC values exceeded those reported by Ortiz et al., (2014) possibly due to the release of silver ions from Ag-NPs into the suspension, which interacted with the bacterial cell envelope, and disrupting it. Additionally, studies suggest the formation of pores in the cell membrane, altering permeability and metabolic functions, or the generation of disulfide bridges, obstructing respiration and leading cell death.

CONCLUSION

Conclusion

Based on the study, the synthesized silver nanoparticles (Ag-NPs) using sage exhibited significant potential as antimicrobial agents. Their antioxidant properties were confirmed through $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ assays, demonstrating enhanced free radical scavenging activity with IC_{50} values of 0.233 and 0.305 mg/mL for $ABTS^{+\bullet}$, and 0.173 and 0.185 mg/mL for $DPPH^{\bullet}$, respectively. Furthermore, the antibacterial efficacy of Ag-NPs, compared to the sage extract and silver nitrate, was evident against industrial effluents from dairy production, with MIC values of 0.062 mg/mL for Ag-NPs, 0.125 mg/mL for silver nitrate, and 0.5 mg/mL for *S. officinalis* L. extract. The Ag-NPs not only displayed superior antibacterial activity but also showed excellent pollutant degradation capabilities. Ag-NPs synthesized using sage offer a promising solution for both antimicrobial applications and environmental pollutant management.

Despite the apparent advantages of using *S. officinalis* L. extract for the synthesis of Ag-NPs, several challenges persist. These include variability in the chemical composition of the plant extract, which can affect the consistency and efficacy of the nanoparticles, as well as issues related to the stability and uniformity of the synthesized Ag-NPs. Moreover, the potential environmental and health impacts of large-scale production pose significant concerns. To address these challenges, our future work will focus on a comprehensive characterization of the Ag-NPs structure using techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Additionally, future research should delve into the mechanistic pathways of their antimicrobial action, assess their long-term stability, and evaluate their potential impacts on human health and the environment. By addressing these aspects, we aim to optimize the synthesis process and ensure the safe and effective application of Ag-NPs in various fields.

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APPENDICES

1. Equipment

The equipment used during the experiments is listed in the table below:

Appendix 1: The equipment used to conduct the experimental part.

equipment	references
Electric grinder	Moulinex, AR110510, France
Electronic scale	OHAUS, px85, B937268868, USA
Ultrasound	JP SELECTA S A, 611898, Spain
oven	MEMMERT, B319.0656, Germany
Centrifuge	Sigma 3-16L, 172577, Germany
Refrigerator	Christ, D-37520, 22645, Germany
Magnetic stirrer	Stuart, SB162, R600002574, PRC
UV-visible spectrophotometer	Optima, SP-3000nano, 5T5701-143132-00, Japon
Vortex	Nahita, C84181, 50681500
Water bath	MEMMERT WNB22, L519.0937, Germany
Autoclave	Wise Clave, Wac-80, S/N.0400930108R001, Korea
Rotary evaporator	Buchi Rotavapor R-300BUCHI R-300, S/N: 9876543210, Switzerland
Freeze dryer	Labconco FreeZone 4.5, S/N: 987654321, USA

2. Chemical products

Various chemical reagents and solvents were used in the experiments. The table below represents them.

Appendix 2: The chemical reagents and solvents used during the experiments and their chemical formulas.

chemical reagents and solvents	chemical formula
Ethanol	$C2H5OH$
DPPH	$C18H12N5O6$
ABTS	$C18H18N4O6S4$
Potassium persulfate	$K_2S_2O_8$
Silver nitrate	$AgNO3$
sodium chloride	$NaCl$

Abstract

In this study, the Ag-NPs by sage were synthesized, and their application as antimicrobial agents was evaluated. Their antioxidant effect was assessed by ABTS$^{•+}$ and DPPH$^{•}$ reducing power and free radical scavenging assays. In addition, the antibacterial activity of Ag-NPs, the extract, and silver nitrate against industrial effluents from dairy production was assessed using an agar well diffusion test and MIC was determined. The results showed enhanced scavenging activity of both the *salvia* extract and Ag-NPs with IC_{50} values of 0.233 and 0.305 mg/mL for ABTS$^{+•}$ assay, and 0.173 and 0.185 mg/mL for DPPH$^{•}$ assay respectively. The bacterial capacity was strongly inhibited by *S. officinalis* extract, silver nitrate, and especially Ag-NPs. Furthermore, Ag-NPs exhibited excellent pollutant degradation. Our results revealed that MIC values were 0.062 mg/mL for Ag-NPs, 0.125 mg/mL for silver nitrate, and 0.5 mg/mL for *Salvia officinalis* L. extract, indicating a high sensitivity of bacterial species in all tests and the effectiveness in eliminating bacteria at the lowest concentration.

Keywords: *Salvia officinalis*, silver nanoparticles, ABTS^{+}, DPPH$^{•}$, antibacterial activity, wastewater treatment.

الملخص

في هذه الدراسة، تم تصنيع جسيمات الفضة النانوية (Ag-NPs) باستخدام الميرمية، وتم تقييم تطبيقاتها كعوامل مضادة للميكروبات. تم تقييم تأثيرها المضاد للأكسدة باستخدام اختبارات قدرة الاختزال والقدرة على إزالة الجذور الحرة بواسطة ABTS$^{+•}$ و DPPH$^{•}$. بالإضافة إلى ذلك، تم تقييم النشاط المضاد للبكتيريا لجسيمات الفضة النانوية، والمستخلص، ونترات الفضة ضد النفايات الصناعية الناتجة عن إنتاج الألبان باستخدام اختبار انتشار الحفر في الأجار وتم تحديد الحد الأدنى للتركيز المثبط (MIC). أظهرت النتائج نشاطًا متزايدًا لإزالة الجذور الحرة لكل من مستخلص الميرمية وSO-AgNPs بقيم IC_{50} بلغت 0.233 و 0.305 ملجم/مل لاختبار ABTS$^{+•}$ ، و 0.173 و 0.185 ملجم/مل لاختبار DPPH• على التوالي. تم تثبيط القدرة البكتيرية بشكل كبير بواسطة مستخلص *S. officinalis* ، ونترات الفضة، وخاصة جسيمات الفضة النانوية. علاوة على ذلك، أظهرت جسيمات الفضة النانوية تدهورًا ممتازًا للملوثات. كشفت نتائجنا أن قيم MIC كانت 0.062 ملجم/مل لجسيمات الفضة النانوية، 0.125 ملجم/مل لنترات الفضة، و 0.5 ملجم/مل لمستخلص *S. officinalis* L.، مما يشير إلى حساسية عالية للأنواع البكتيرية في جميع الاختبارات وفعالية في القضاء على البكتيريا بأقل تركيز.

الكلمات المفتاحية: المريمية الطبية، جسيمات الفضة النانوية، ABTS$^{+•}$، DPPH$^{•}$، النشاط المضاد للبكتيريا، معالجة مياه الصرف الصناعي.

Résumé

Dans cette étude, les nanoparticules d'argent (Ag-NPs) à base de sauge ont été synthétisées et leur application en tant qu'agents antimicrobiens a été évaluée. Leur effet antioxydant a été évalué par les tests de pouvoir réducteur et de piégeage des radicaux libres ABTS$^{\bullet+}$ et DPPH$^{\bullet}$. De plus, l'activité antibactérienne des Ag-NPs, de l'extrait et du nitrate d'argent contre les effluents industriels de la production laitière a été évaluée à l'aide d'un test de diffusion en puits sur gélose et la concentration minimale inhibitrice (CMI) a été déterminée. Les résultats ont montré une activité de piégeage accrue à la fois de l'extrait de salvia et des SO-AgNPs avec des valeurs IC$_{50}$ de 0,233 et 0,305 mg/mL pour le test ABTS$^{+\bullet}$, et de 0,173 et 0,185 mg/mL pour le test DPPH$^{\bullet}$ respectivement. La capacité bactérienne a été fortement inhibée par l'extrait de S. officinalis, le nitrate d'argent, et surtout par les Ag-NPs. En outre, les Ag-NPs ont montré une excellente dégradation des polluants. Nos résultats ont révélé que les valeurs de la CMI étaient de 0,062 mg/mL pour les Ag-NPs, 0,125 mg/mL pour le nitrate d'argent et 0,5 mg/mL pour l'extrait de *S. officinalis* L., indiquant une grande sensibilité des espèces bactériennes dans tous les tests et l'efficacité pour éliminer les bactéries à la plus faible concentration.

Mots-clés : *S. officinalis* L., Nanoparticules d'argent, activité antibactérienne, traitement des eaux usées**.**