AMERICAN UNIVERSITY OF BEIRUT

BIOGENIC SYNTHESIS OF GOLD NANO-PARTICLES USING ORIGANUM SYRIACUM EXTRACT FOR CATALYTICAL, ANTIOXIDANT AND QUENCHING APPLICATIONS

by
SAEEDEH ALI MOHAMMAD MARVI

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ABSTRACT
OF THE THESIS OF

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Major: Chemistry

Title: Biogenic Synthesis of Gold Nano-Particles Using Origanum Syriacum Extract for catalytical, antioxidant and quenching applications

The interest in using nano-particles (NPs) has increased in recent years due to the wide range of applications. Gold nano-particles (AuNPs) are metallic NPs with a wide variety of applications in several scientific and technological domains that is gaining popularity in demand recently.

To fulfill this, an in-depth study of optimizing synthesis techniques for AuNPs is required. Among the various methods reported for the synthesis of AuNPs including chemical, physical, and biological (green) methods; biogenic synthesis using plant extract has been found to be the fastest, easiest, most cost-effective, and environmentally friendly method. The antibacterial, analytical, antioxidant, anticancer, and catalytic capabilities of AuNPs generated from plant extract have demonstrated remarkable results in a variety of biological applications.

In this thesis for biosynthesizing and optimizing AuNPs; Origanum syriacum plant extract is used. Formed AuNPs have been characterized using several spectroscopic and microscopic techniques such as UV-Visible, DLS, SEM, and EDX. The formed NPs then have been used as catalysts for the reduction of different organic dyes, and recycled for three times to catalyze the reduction of CR. Furthermore, the AuNPs generated exhibited notable capabilities in scavenging free radicals, demonstrated by a substantial 62.1% inhibition of DPPH only using 900 µl of AuNPs. Additionally, the impact of AuNPs on the fluorescence intensity of Rh. B was investigated. It was observed that the most significant quenching effect occurred with 200 µl of AuNPs. The quenching was not interfered by uric acid, ascorbic acid, glucose, ATP, phenol, and melamine.

Keywords: OSWE, AuNPs, reduction, catalyst, recycling, antioxidant, quenching, DPPH.

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ABBREVIATIONS

AuNPs: Gold Nano-Particles

C: Concentration

CR: Congo red

DDW: doubled distilled water

DLS: Dynamic Light Scattering

DPPH: 2,2-diphenyl-1-picrylhydrazyl

FRET: Fluorescence Resonance Energy Transfer

MO: Methyl Orange

NPs: Nano-Particles

OS: Origanum syriacum

OSWE: Origanum syriacum water extracted

ROS: Reactive oxygen species

Rh. B: Rhodamine B

Rh. 6G: Rhodamine 6G

SEM: Scanning Electron Microscopy

SET: Surface Energy Transfer

CHAPTER I

INTRODUCTION

A nano-particle is a small particle that ranges in size from 1 to 100 nanometers. NPs, which are invisible to the naked eye, can have significantly different physical and chemical properties than their larger material counterparts. The majority of NPs are composed of only a few hundred atoms.

NPs have shown great potential for a variety of applications due to their small size and exceptional surface properties. In general, NPs are the most basic component of nanomaterials, serving as a link between atomic/molecular structures and nanomaterials. The properties of the NPs are size and shape dependent and can be tailored over a wide range. Other structural analogs of NPs include dendrimers, liposomes, polymer micelles, and quantum dots, which are used in a variety of applications. NPs are used in a wide range of applications, including drug delivery, biosensors, bioimaging, molecular tagging, food technology, textile manufacturing, antimicrobial coatings, quantum computers, quantum lasers, energy, and the environment, depending on the type of material [1].

NPs are mainly classified into three categories, organic, inorganic and carbon-based NPs. There are various techniques to synthesize NPs such as chemical, physical and biological (green method) techniques. Nowadays the biological techniques have become popular to synthesize and fabricate the NPs because they offer environmentally friendly, low-toxic, cost-effective, and efficient ways. Biosynthesis of NPs using plant extracts or biomass is one of the most effective, fast, clean, none toxic, and environmentally friendly biological methods that has primarily been used to produce

NPs of noble metals, metal oxides, bimetallic alloys, and other materials such as AuNPs [2].

A. Nano-Particles

Nanoparticles (NPs) are a broad class of materials that comprise particulate compounds (particulate dispersions or solid particles) with a minimum diameter of 100 nm. These materials may be 0D, 1D, 2D, or 3D depending on their general form. The significance of these materials became clear when researchers discovered that size may impact a substance's physiochemical qualities, such as optical capabilities.

NPs show different colors and properties with variation of their size and shape. Considering NPs are not simple molecules, they consist three layers: (a) the surface layer, which may be functionalized with a range of small molecules, metal ions, surfactants, and polymers. (b) The shell layer, which is chemically distinct from the core in all respects, and (c) the core, which is the NP's center section and commonly refers to the NP itself [3].

1. Categorizations of NPs

Nanoparticles are classified into several categories depending on their size, shape, physical and chemical characteristics [3]. The three main categories of NPs are classified into organic, inorganic, and carbon-based are listed below [4].

a. Organic NPs

Organic nanoparticles or polymers include dendrimers, micelles and liposomes (See figure 1), among others. These nanoparticles are biodegradable, non-toxic, and some include a hollow core, also known as Nano capsules, which are sensitive to thermal and electromagnetic radiation such as heat and light.

Because of their distinguishing properties besides their usual properties like size, composition, surface and shape, they are an excellent candidate for drug delivery. Organic nanoparticles are often used in biomedical fields, such as medication delivery systems, since they are efficient and can be injected into particular regions of the body, a process called as targeted drug delivery [4].

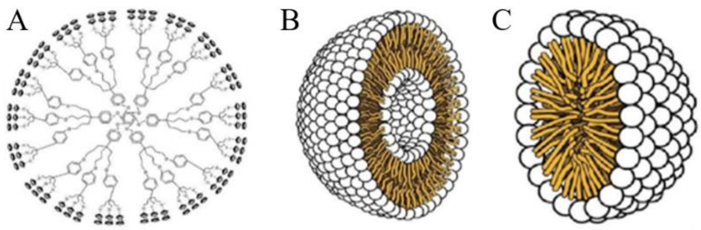


Figure 1. Type of Organic nanoparticles: (A) Dendrimer; (B) Liposomes; and (C) Micelles [4].

b. Inorganic NPs

Inorganic nanoparticles are divided into main classifications including metal and metal oxide-based nanoparticles (non-carbon components).

i. Metal-Based NPs

Metal-based nanoparticles are those that are synthesized to nanometric sizes from metals, either destructively or constructively. Almost any metal may be synthesized into nanoparticles. Metals often utilized for nanoparticle synthesis include Al, Cd, Co, Cu, Au, Fe, Pb, Ag, Zn, etc. In comparison to bulk metal crystals, metal nanoparticles have distinct electrical, optical, and chemical characteristics. Nanoparticles have distinct properties such as sizes ranging from 10 to 100 nm, surface characteristics such as high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline and amorphous structures, shapes such as spherical and cylindrical, and color, reactivity and sensitivity to environmental factors such as air, moisture, heat, and sunlight, and so on [4, 5].

ii. Metal Oxides-Based NPs

Metal oxide-based nanoparticles are created to adjust the characteristics of metal-based nanoparticles. In the presence of oxygen, for example, Fe nanoparticles rapidly oxidize to Fe_2O_3, boosting their reactivity compared to iron nanoparticles. Metal oxide nanoparticles are made largely for their increased reactivity and efficiency. The most often produced minerals are Al_2O_3, CeO_2, Fe_2O_3, Fe_3O_4, SiO_2, TiO_2 and ZnO. These nanoparticles outperform their metal counterparts in terms of properties [4].

c. Carbon Based NPs

Carbon-based nanoparticles are those that are entirely composed of carbon. They are categorized as fullerenes, graphene, carbon nanotubes, carbon nanofibers, and carbon black (See figure 2) [4].

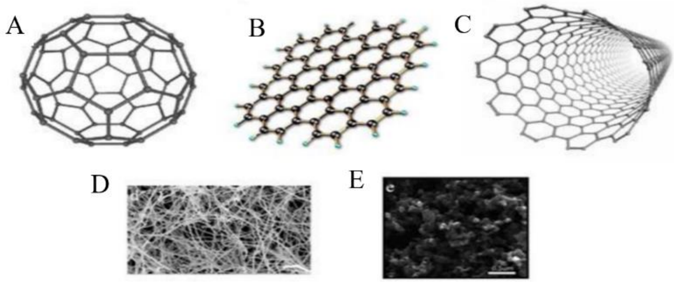


Figure 2. Different type of carbon-based nanoparticles. (A) Fullerenes; (B) Graphene; (C) Carbon nanotubes; (D) Carbon nanofibers: and (E) Carbon black [4].

2. Synthesis Methods of NPs

There are different methods to synthesis NPs which lead to various properties for NPs. These methods are classified into three main categories below.

a. <u>Physical Methods</u>

To manufacture NPs, physical techniques utilize mechanical pressure, high energy radiations, thermal energy, or electrical energy to cause material abrasion, melting, evaporation, or condensation. These approaches, which primarily use a top-down approach, are desirable because they are solvent-free and create uniform monodisperse NPs. At the same time, the considerable waste generated during synthesis makes physical methods less cost-effective. Some of the most often used physical processes for producing NPs are high energy ball milling, laser ablation, electrospraying, inert gas condensation, physical vapor deposition, laser pyrolysis, ash spray pyrolysis, and melt mixing [1].

b. Chemical Methods

There are various methods to synthesis NPs and some of the most commonly used chemical methods are mentioned below [1]:

- Sol–gel
- Microemulsion
- Hydrothermal synthesis
- Polyol synthesis
- Chemical vapor synthesis
- Plasma enhanced chemical vapour deposition (PECVD)

c. Biological Methods (Green Synthesis)

Biological approaches, also known as green synthesis, offer an environmentally friendly, low-toxic, cost-effective, and efficient way for synthesizing and fabricating NPs. For the generation of metal and metal oxide NPs, these approaches use biological systems such as bacteria, fungi, viruses, yeast, actinomycetes, plant extracts, and so on. biological approaches are broadly classified into three types [1].

i. Biogenic Synthesis Using Microorganisms

Prokaryotic bacteria, actinomycetes, fungi, algae, and yeast are widely utilized as bio-reactors for NP synthesis. Huge scientific efforts were expended in developing this approach for creating a diverse range of NPs such as Ag, Au, Pd, TiO_2, CdS and etc. Microorganisms capture target ions from their surroundings and convert the metal ions into the element metal using enzymes produced by cellular processes. Depending on the site of the NP synthesis, this synthesis might be characterized as intracellular or extracellular. In the presence of enzymes, metal ions are transported inside the microbial cell to generate NPs. The extracellular creation of NPs requires the trapping of metal ions on the cell surface and the reduction of ions in the presence of enzymes, also the potential of marine bacteria for the production of AuNPs was investigated in a report.

ii. Biogenic Synthesis Using Biomolecules as The Templates

To generate NPs, several biomolecules such as nucleic acids, membranes, viruses, and diatoms were utilized as templates. DNA is well recognized as an effective biomolecular template with a great attraction for transition metal ions. It was demonstrated that DNA hydrogels could be synthesized and crosslinked prior to the incorporation of transition metal ions (e.g., gold, Au^{3+} metal ions) to DNA macromolecules, resulting in the formation of Au NPs. The reduction of Au^{3+} results in the formation of Au atoms and metal clusters, which develop into AuNPs on the DNA chain.

iii. Biogenic Synthesis Using Plant Extracts

The use of plant extract as a reducing agent in the manufacture of metal nanoparticles is being considered as a possible alternative to traditional chemical and physical methods. Furthermore, when compared to previous methods, the technology allows for the mass production of metal nanoparticles at a faster rate and in a wider range of shapes and sizes [6]. Yun et al. have adequately delineated a variety of plant biometabolites that, due to their valuable roles as reducing and capping agents, could aid in the preparation of NPs. The mechanism of metal NP phytosynthesis is depicted in figure 3 [7]. The kinetics of phytosynthesis of NPs is much faster than that of other biosynthesis methods, and it is sometimes comparable to the rate of chemical routes. Shankar et al. reported the preparation of gold nanotriangles by treating lemongrass leaf extract with aqueous $AuCl^{-}$ ions [8]. Similarly, leaf extracts from various plants such as Tamarindus indica, Aloe vera, Emblica officinalis, and others have been reported to be useful for designing AuNPs [9, 10]. Another Efficient, rapid and environmentally safe phytosynthesis of gold nanoparticles (AuNPs) in a single step using stems of two halophytic species; Atriplex hal-imus and Chenopodium amperosidies (See figure 4). Flavonoids, glycosides, and alkaloids are among the phytoconstituents found in A. halimus and C. amperosidies extracts. These compounds existing in both extracts, is responsible for the reduction and stabilization of AuNPs [6].

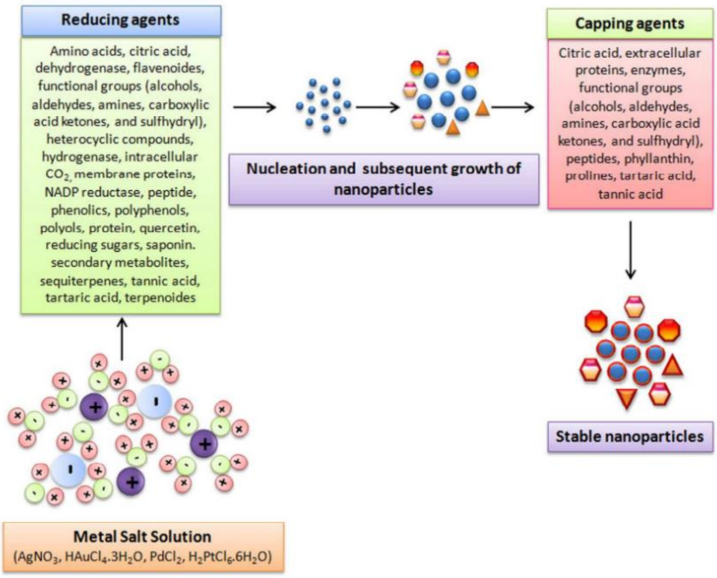


Figure 3. Mechanism for the biogenic synthesis of metal NPs using plant extract [7].

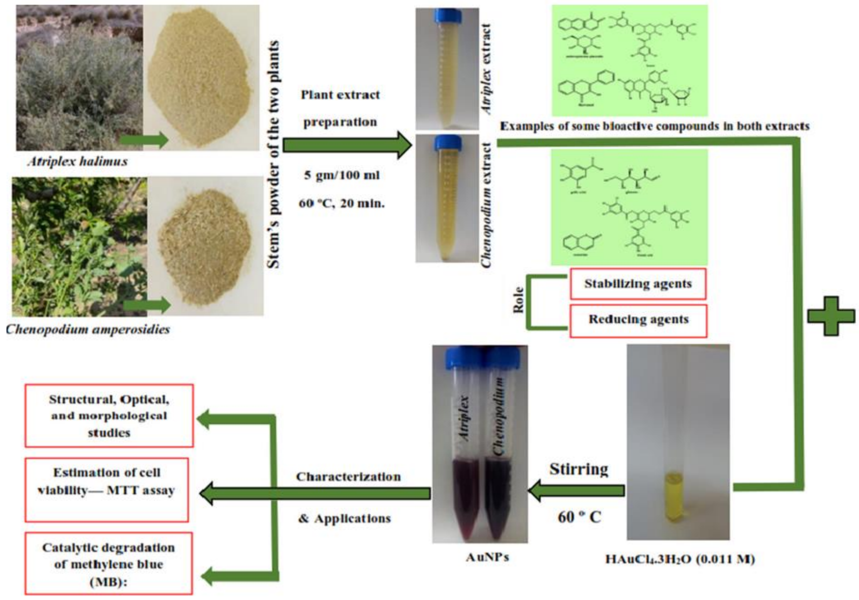


Figure 4. Displays a schematic design for the green production of AuNPs utilizing extracts of A. halimus and C. amperosidies [6].

3. Gold Nano-Particles

Gold nanoparticles (AuNPs) are small gold particles with diameters ranging from 1 nm to 100 nm that, when dispersed in water, are also referred to as colloidal gold. Spherical AuNPs have advantageous properties such as size- and shape-related optoelectronic properties, a high surface-to-volume ratio, excellent biocompatibility, and low toxicity. AuNPs are an important tool in bio-nanotechnology because of these properties (See table 1). Surface plasmon resonance (SPR) and the ability to quench fluorescence are two important physical properties of AuNPs. Spherical AuNPs exhibit a variety of colors in aqueous solution, including brown, orange, red, and purple as the core size increases from 1 to 100 nm, and generally exhibit a size-relative absorption peak from 500 to 550 nm. This absorption band is created by the collective oscillation of conduction electrons caused by resonant excitation by incident photons, and it is referred to as a "surface plasmon band S figure 5). This band, however, is missing in both small nanoparticles (d 2 nm) and the bulk material. This phenomenon is influenced not only by size, but also by shape, solvent, surface ligand, core charge, temperature, and proximity to other nanoparticles. Because of interparticle plasmon coupling, nanoparticle aggregation causes a significant red-shifting of the SPR frequency, a broadening of the surface plasmon band, and a change in the color of the solution from red to blue. The excellent quenching ability of AuNPs for proximal fluorophores is due to the deactivation pathway, which is based on the high overlap between the emission spectrum of excited fluorophores and the surface plasmon band of the AuNPs. This FRET phenomenon can be observed even in the presence of 1 nm AuNPs because the nanoparticles affect both the radiative and nonradiative decay rates of fluorescent molecules. In the photoinduced electron transfer (PET) process, AuNPs can also act as electron acceptors, quenching fluorophores. This PET process is controlled by charging and discharging the gold core, which can be used to make sensors [11].

Properties	**Application area**
Redox activity	Electronic devices, electrochemical sensing
Surface-enhanced Raman scattering (SERS)	Imaging, sensing
Surface plasmon resonance (SPR)	Colorimetric sensing, Photothermal therapy
Fluorescence quenching	Sensor fabrication, materials science

Table 1. AuNPs properties and application areas

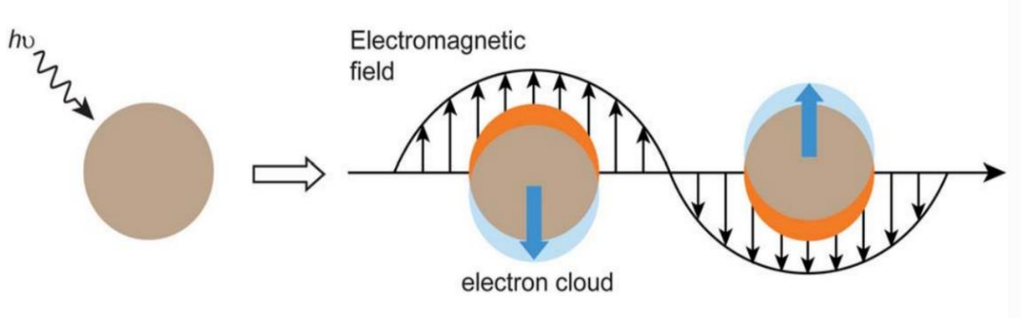


Figure 5. In the electromagnetic field of incident light, conduction electrons oscillate across the nanoparticle in a schematic representation [11].

4. Applications of AuNPs

Gold nanoparticles have been extensively used in sensing analysis, catalysis, environmental monitoring, and disease therapy because of their distinct optical properties, narrow size distributions, and high biological affinity [12].

a. <u>Sensing</u>

Utilizing gold nanoparticles (AuNPs) in colorimetric sensing represents a highly promising analytical method for the identification of analytes and the detection of biomolecules, including amino acids, peptides, proteins, nucleic acids, inorganic ions, and enzymes. The fundamental principle relies on the alteration of color from red to blue when the distances between nanoparticles are shorter than the average diameter of AuNPs. This transformation is easily perceptible to the naked eye. The strategies employed in these sensors can be categorized into two approaches: inducing a change towards red or a shift towards blue in absorption, leading to either AuNP aggregation or disaggregation. The outcomes can be quantified using a UV–visible spectrophotometer [13].

b. Therapeutics

The delivery of therapeutic agents to cells by AuNPs is an important step in biomedical treatment. So many research teams have investigated the interactions of functionalized AuNPs with cell membranes in order to improve delivery efficiency [11]. As an example, Stellacci et al. showcased that the organization of surface ligands on AuNPs can govern cell membranes penetration [14]. In their study, they found that AuNPs with an organized arrangement of amphiphilic molecules were capable of entering cell membranes. In contrast, AuNPs coated with the same molecules arranged in a random fashion ended up being confined within vesicular structures.

c. Imaging

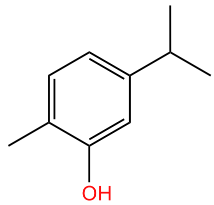
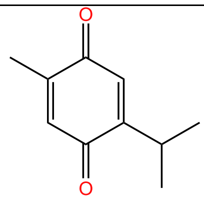
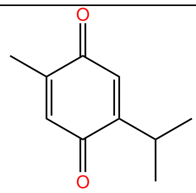
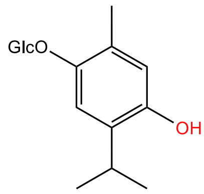
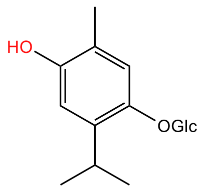
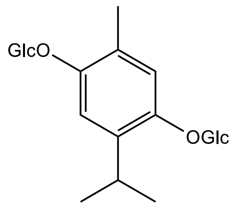
Optical imaging is one of the most important tools in biological research. Despite significant advancements, bio-optical imaging still has issues with resolution, sensitivity, speed, and penetration depth. Because of their distinct optical properties, such as surface plasmon resonance, gold nanoparticles (AuNPs) can be easily used to boost optical imaging through absorption, scattering, fluorescence, Raman scattering, and other means. The following are some of the most recent achievements and challenges related to using AuNPs to improve resolution and sensitivity in biological imaging in vitro and in vivo: 1) Direct visualization of AuNPs within biosystems using i) dark field (DF) microscopy, ii) differential interference contrast (DIC) microscopy, and iii) other techniques like interferometric scattering (iSCAT) microscopy and photothermal imaging. 2) Biomolecular event and physiological process monitoring using i) surface enhanced Raman spectroscopy (SERS) and ii) plasmon enhanced fluorescence (PEF) for ultra-sensitive detection of biomolecules such as proteins, metabolites, DNA, RNA, and so on. 3) In vivo deep tissue imaging for disease diagnosis, such as detecting tumors and other diseases in the eye, brain, and bone, using i) two-photon and/or multiphoton imaging, ii) optical coherence tomography (OCT), and iii) photoacoustic (PA) imaging. Finally, AuNPs-assisted bioimaging is a promising tool for investigating fundamental biological questions and early disease detection [15].

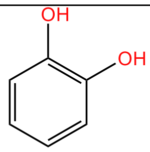
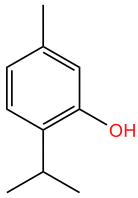
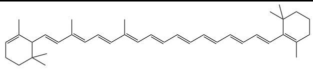
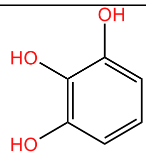
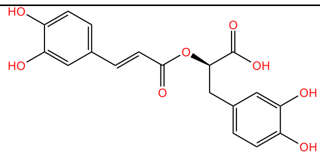
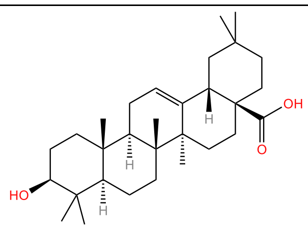
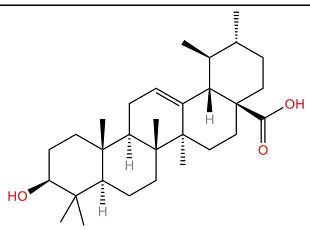
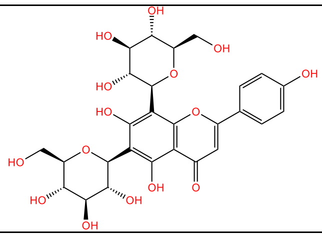
B. Origanum Syriacum

OS is a perennial plant coming from lamiaceae family, it can be found in the eastern Mediterranean region such as Lebanon, Jordan, and Syria. OS is small and bushy plant and has grayish-green leaves covered by fine hairs [16]. OS is famous because of its unique aroma and flavor, due to the presence of carvacrol and thymol in this plant, OS leaves have warm, spicy, and a bit bitter flavor, which makes it very popular as a culinary herb. basically, the dried and crushed leaves of OS are used as a spice in salads, sauces, and different dishes. Additionally, it has been used as a traditional medicine to relieve pain and treatment of colds and coughs [17]. Due to the presence of bioactive compounds such as phenols, glycosides, terpenes and flavonoids [18], it possesses antioxidant, antimicrobial, anticancer and anti-inflammatory properties, making it a traditional herbal medicine [19]. Moreover, Research on toxicity has demonstrated that the consumption of this plant does not produce negative effects on animals. This quality makes it an appealing natural candidate for use in the development of new drugs [20]. However, further researches are needed to confirm and understand its potential medical properties.

1. *Phytochemical Constituents of OS Extracts*

Despite the presence of various phytochemical compounds in OS extracts, limited research has been conducted to analyze their composition thoroughly. Depending on the specific extract, distinct constituents have been identified. For instance, an ethanolic extract of this plant has yielded several terpenoids or monoterpene glucosides through isolation [21]. Furthermore, this extract is abundant in volatile oils and phenolic compounds, with flavonoids being the primary components. Carotenoids, primarily β-carotene, are also present in significant quantities [22]. Additional investigations have demonstrated that the ethanolic extract of the plant contains other phenolic compounds, specifically thymol and carvacrol, as its main constituents [23]. Conversely, the aqueous extract of the plant is found to be rich in only three compounds: carvacrol, carveol, and thymoquinone [23]. Interestingly, the methanolic extract of the plant stands out due to its distinctive flavonoids. Notably, a previously unknown prenylated biflavone was successfully isolated from the extract [24], alongside the discovery of a novel flavone glycoside [25]. Additionally, the methanolic extract of this plant is notable for its high content of rosmarinic acid, oleanolic acid, and ursolic acid, which serve as major constituents [26]. Table 2 provide a comprehensive overview of the primary phytochemical constituents found in various extract types of OS.

Name	Chemical formula	Chemical structure	Type of extract
Carvacrol	$C_{10}H_{14}O$		Ethanolic and aqueous
Carveol	$C_{10}H_{16}O$		Aqueous
Thymoquinone	$C_{10}H_{12}O_2$		Aqueous
Thymoquinol 2-O-beta-glucopyranoside	$C_{16}H_{24}O_7$		Ethanolic
Thymoquinol 5-O-beta-glucopyranoside	$C_{16}H_{24}O_7$		Ethanolic
Thymoquinol 2,5-O-beta-diglucopyranoside	$C_{22}H_{34}O_{12}$		Ethanolic

Catechol	$C_6H_6O_2$		Ethanolic
Thymol	$C_{10}H_{14}O$		Ethanolic
Beta-carotene	$C_{40}H_{56}$		Ethanolic
Pyrogallol	$C_6H_6O_3$		Ethanolic
Rosmarinic acid	$C_{18}H_{16}O_8$		Methanolic and ethanolic
Oleanolic acid	$C_{30}H_{48}O_3$		Methanolic
Ursolic acid	$C_{30}H_{48}O_3$		Methanolic
Vicenin	$C_{21}H_{20}O_{10}$		Methanolic

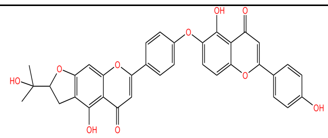
5-hydroxy-6,7-(2"-isopropanol-dihydrofuran)-flavonyl-(4'-7''') 5''',4''', dihydroxy flavone	$C_{35}H_{26}O_{10}$		Methanolic

Table 2. Major phytochemical components of OS aqueous, ethanolic and methanolic extracts

2. *Extraction Methods for OS*

The extraction methods for essential oils from OS significantly influence their composition. Several studies have employed steam distillation to extract the oils, as it has shown higher yields compared to other methods [17, 22, 27-34]. Steam distillation involves three physiochemical processes: hydro-diffusion, hydrolysis, and decomposition by heat. In this method, the whole plant, dried and shredded, is immersed in water in a steam distillation clevenger apparatus. The mixture is then boiled for 3 hours, and the essential oil is collected with water vapor and purified with anhydrous sodium bicarbonate before being stored in a sealed glass vessel at 4 °C [34]. Research comparing conventional steam distillation to microwave-assisted steam distillation found that the latter was three times faster, required less time to reach the desired temperature, and yielded more essential oil. In the conventional method, the plant was heated at 100 °C for 3 hours using a heat mantle, while in the microwave-assisted method, a focused microwave apparatus was utilized, and the extraction took around 10 minutes [35]. In the conventional steam distillation method, the plant material was placed in a rounded flask connected to a Clevenger-type apparatus and heated at 100 °C for 3 hours using a heat mantle. On the other hand, in the microwave-assisted steam distillation method, a focused microwave apparatus was utilized, and the plant was extracted using a Clevenger-type apparatus connected outside of the microwave reactor. The temperature and power of the microwave were adjusted to 100 °C and 800 W, respectively [36]. Another study using ultrasound microwave-assisted steam distillation revealed different yields based on the plant sample's location. Comparisons were also made using solid-phase micro-extraction (SPME) and supercritical fluid extraction (SFE) with steam distillation [37]. While steam distillation predominantly yielded thymol and carvacrol as the main components, SPME identified only low concentrations of these compounds. Instead, monoterpene hydrocarbons were identified as the major components in the emitted aroma [37]. Additionally, carvacrol was found as the major oxygenated terpene in SFE, while SPME indicated very low concentrations of oxygenated monoterpenes [37]. Moreover, volatile oils were better preserved using the static headspace (HS) method compared to steam distillation (SD), confirming that extraction methods do affect the constituents of the essential oils [38]. These findings emphasize the importance of selecting appropriate extraction methods to obtain desired compositions of essential oils from OS.

Moreover, there exists an alternative method for extracting the bioactive components from OS, specifically flavonoids and phenolic acids. This extraction process involves several key steps to ensure the efficient extraction of these valuable compounds. Firstly, the aerial parts of the OS plant are meticulously prepared by carefully drying and grinding them. This preparatory step is crucial as it enhances the surface area for extraction, thereby increasing the overall efficiency of the process. Next, the selection of an appropriate solvent is of paramount importance. Solvents such as ethanol, methanol, hexane, or ethyl acetate are considered based on their polarity and ability to effectively extract the desired bioactive compounds from the plant material.

The maceration method is then employed, where the finely ground plant material is combined with the chosen solvent in glass containers. The mixture is left to stand at room temperature for a specific duration, allowing sufficient time for the bioactive compounds to dissolve into the solvent efficiently. Once the designated maceration period elapses, the liquid extract is carefully separated from any solid residues through a meticulous filtration process. This crucial step guarantees that the resulting extract is refined, devoid of impurities, and of high quality. To further concentrate the extract and eliminate any remaining solvent, precise concentration techniques such as gentle heating or vacuum evaporation are diligently employed. Finally, the concentrated extract is stored in opaque, air tight containers under controlled conditions at approximately 4 °C. This stringent storage procedure ensures the long-term preservation of the extract's bioactivity and overall quality. The solvent extraction process ultimately yields an extract enriched with a diverse array of bioactive compounds. The choice of solvent significantly impacts the composition and quality of the final extract, making it a vital consideration in the extraction process. This extraction method presents promising potential for diverse applications, depending on the specific objectives of the study or research at hand. Its ability to efficiently extract flavonoids and phenolic acids from OS makes it a valuable technique in various fields of study and research. [39-41].

3. Pharmacological Activities of OS

OS has been widely examined for its pharmacological effects, and multiple research investigations have found diverse bioactive qualities linked with this plant. Some of the prominent pharmacological actions of OS include:

a. <u>Antioxidant Activities</u>

The disproportion between the formation and aggregation of ROS and the body's ability to neutralize them through antioxidant defenses cause oxidative stress. This leads to oxidative damage, premature aging, and increased susceptibility to various diseases like cancer, inflammatory disorders, diabetes, and neurodegenerative conditions. Furthermore, oxidative reactions also impact food deterioration, affecting the storage and quality of food products, and their shelf life [42]. Recently, there has been a growing interest in the role of antioxidants in protection against oxidative damage. Plants offer a natural source of antioxidant compounds, serving as safer alternatives to synthetic preservatives such as butyl hydroxy anisole (BHA) and butylated hydroxytoluene (BHT), which are commonly used in food and consumer products but have raised concerns about potential toxic and carcinogenic effects [42-44].

Its strong antioxidant effect is attributed to the high content of phenolic components, mainly carvacrol and thymol, present in its extracts. Additionally, the specific chemical composition of the plant extracts and the relative proportions and synergy among their constituents contribute to the diverse antioxidant and biological activities observed [30, 45-47]. Notably, a recent study comparing the antioxidant capacity of OS essential oils from different regions revealed that higher antioxidant capacity, measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition, was associated with high lipophilic content, including unsaturated terpenes like γ-terpinene and α-terpinene. Conversely, a higher phenolic composition in the essential oil resulted in lower antioxidant capacity, indicating the role of unsaturated hydrocarbons in OS's scavenging ability [36].

In conclusion, OS serves as a valuable source of antioxidants, offering potential applications as natural food preservatives with strong antimicrobial properties or for managing and treating chronic and oxidative stress-related disorders. Its abundant phenolic components, particularly carvacrol, and thymol, contribute to its remarkable antioxidant potential, making it a promising natural alternative in various contexts.

b. Antimicrobial Activities

Today, combating antimicrobial resistance has emerged as a significant challenge in the treatment of microbial infections and diseases, as well as in crop protection and food preservation, posing a threat to public health and society at large. This resistance is a consequence of natural selection and is impairing the effectiveness of various antimicrobial drugs [48]. In response to this issue, researchers are actively exploring novel agents, with an increasing focus on medicinal plants due to their potent phytochemical constituents and ethnomedicinal properties [49]. Extensive research has been conducted to evaluate the efficacy of various extracts from OS against a wide range of pathogenic microorganisms, including Gram-negative and Gram-positive bacteria and fungi. Notably, extracts rich in carvacrol have demonstrated enhanced effectiveness against both bacteria and fungi. Specific investigations using the essential oil of OS have shown that increased lipophilic content contributes to heightened antibacterial activity against Gram-negative strains, whereas a thymol-rich content exhibits greater effectiveness against Gram-positive strains [36]. The antibacterial properties of thymol and carvacrol are attributed to their ability to disrupt cell membranes and inhibit ATPase activity, leading to the release of cellular components and depletion of ATP [32]. In oral hygiene and the treatment of denture stomatitis, chemical-based disinfectants, and cleansers have been commonly recommended; however, these can have adverse effects and alter the physical properties of denture bases. As a safe and efficient alternative, extracts from OS have been explored. Studies have shown that the essential oil of OS strongly inhibits the growth of bacterial strains like Staphylococcus aureus and Streptococcus pneumoniae, as well as the fungus Candida albicans commonly found in the oral cavity [20]. Furthermore, in the treatment of denture stomatitis, a prevalent mucosal disorder among denture wearers, the antimicrobial activity of OS essential oil has been confirmed, associated with the growth of oral bacterial and fungal strains such as Staphylococcus aureus, Streptococcus mutans, and Candida albicans [50]. The effectiveness of different ecotypes of OS against these microorganisms was attributed to the varying proportions of carvacrol and thymoquinone in their essential oils [50]. In another study examining the antimicrobial activity of several medicinal plant extracts against opportunistic oral infections, the methanolic extract of OS leaves exhibited the highest antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans [51]. This effect was associated with the substantial concentrations of carvacrol, thymol, and thymoquinone in the different OS extracts, which work by disrupting bacterial membranes [52]. It is noteworthy that the oral administration of OS essential oil in rats showed no adverse effects, as observed through routine hematological and biochemical assays [20]. Collectively, these studies provide further evidence supporting the incorporation of OS in antiseptic preparations for general oral hygiene and in the prevention of oral cavity infections, tooth decay, and denture stomatitis, as well as its potential use as a natural antimicrobial agent in disinfectants and food preservatives.

c. Anti-Inflammatory Activities

Inflammation is a natural process where the immune system activates to protect the body against harmful stimuli like injury, infection, and oxidative stress. However, when inflammation becomes chronic, it can lead to various diseases, including cancer, cardiovascular issues, atherosclerosis, diabetes, and obesity, as well as autoimmune and neurodegenerative disorders. Chronic inflammation is now recognized as the primary cause of global deaths, accounting for more than 50% [53]. Although anti-inflammatory drugs are widely prescribed [54], concerns over their safety, especially for chronic conditions, have prompted a focus on plants with anti-inflammatory properties to discover new therapeutic compounds [55]. In this regard, Shen et al. conducted a study quantifying the anti-inflammatory constituents in Origanum species and found that a methanolic extract of OS aerial parts contains significant levels of triterpenoid acids like oleanolic acid and ursolic acid, known for their potent anti-inflammatory activities [26, 56]. Monitoring the inflammatory response involves assessing various biomarkers, including pro-inflammatory cytokines like IL-6, IL-8, TNF-α, and the anti-inflammatory immune suppression cytokine IL-10. In a study using a methanolic extract of OS, the release of IL-6 was significantly inhibited by more than 80%, while the release of IL-10 was attenuated in human peripheral blood mononuclear cells treated with concanavalin A, a plant lectin that activates the inflammatory response through the release of pro-inflammatory cytokines by activating T cells [51]. These findings align with previous studies associating both IL-6 and IL-10 with pathogenesis and inflammation related to microbial infections, as their levels are usually elevated during infections [57, 58].

d. Anticancer Activities

Despite significant progress in cancer treatment, cancer remains a formidable global challenge and the second leading cause of death worldwide. Conventional therapies often come with undesirable side effects and are hindered by multidrug resistance, prompting an increasing interest in exploring bioactive compounds from plant sources for modern anticancer therapy [59]. In vitro studies screening different culinary herbs for cytotoxicity against human breast adenocarcinoma cells revealed that an ethanolic extract from the aerial parts of OS demonstrated antiproliferative activity against the MCF-7 breast cancer cell line, with an IC50 value of 6.40 µg/mL, while the aqueous extract and essential oil showed no cytotoxic activity [31]. Another study using the ethanolic extract confirmed moderate cytotoxicity against MCF-7 cells, causing around 40% of cell death at 500 µg/mL and an IC50 value between 500 and 600 µg/mL [60]. While antimicrobial and antioxidant activities have been extensively studied, the anticancer efficacy of OS requires further investigation.

e. Antimelanogenic Activity

Changes in skin pigmentation are caused by irregular melanin production and accumulation in the epidermis due to disturbances in pro-melanogenic factors [61]. Hyperpigmentation, resulting from various factors like hormonal changes, inflammation, age, or sun exposure, can lead to skin disorders such as melasma, freckles (lentigo simplex), and age spots (solar lentigo). In search of depigmenting and skin-lightening agents, the cosmetic industry has focused on targeting the melanogenic pathway [62]. Commonly used active ingredients in topical applications include hydroquinone, salicylic acid, kojic acid, and niacinamide. However, concerns over side effects with long-term use have prompted research into plant-based alternatives in cosmetics [63, 64].

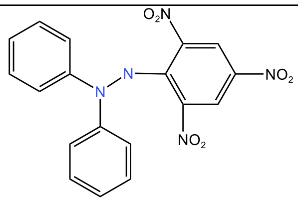
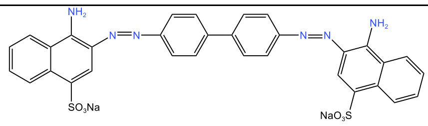
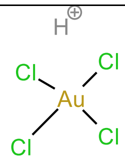
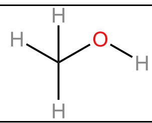
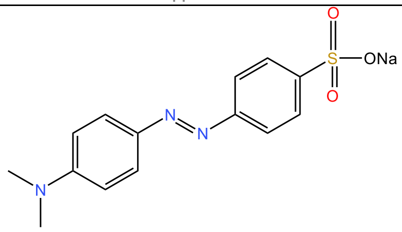
In this context, El-Koury and colleagues investigated the in vitro effects of essential oil from OS on melanin production in B16-F1 melanocytes. The essential oil reduced melanin production by 15% at 40 μg/mL in these cells. Additionally, the main component of the essential oil, carvacrol, was found to significantly decrease melanin levels, implicating carvacrol in melanogenesis [65]. Further investigations on the mechanism of action revealed that both OS oil and carvacrol inhibited the activity of tyrosinase, the key enzyme involved in melanin synthesis, without affecting its expression in B16-F1 melanocytes. This suggests that the extract reduces melanin levels by blocking melanogenesis. Interestingly, OS essential oil and carvacrol displayed a remarkable 80% and 56% inhibition of tyrosinase activity, respectively, further supporting their potential as skin-lightening agents [34]. The proposed mechanism of action involves a competitive inhibition model, where carvacrol binds to tyrosinase, preventing its usual substrate, tyrosine, from binding – similar to the mechanism seen in hydroquinone, a common skin-lightening agent used to treat hyperpigmentation [62, 65].

CHAPTER II

MATERIALS AND METHODS

A. Chemicals

Table 3 includes an extensive compilation of the chemicals employed in the research. The table provides essential information such as chemical formulas, structures, purity, and the sources from which these chemicals were obtained.

Nomenclature	**Chemical formula**	**Chemical structure**	**Purity (%)**	**Source**
2,2-diphenyl-1-picrylhydrazyl (DPPH)	$C_{18}H_{12}N_5O_6$			Aldrich
Congo red	$C_{32}H_{22}N_6Na_2O_6S_2$		97	Acros Organic
Gold (III) chloride trihydrate	$HAuCl_4.3H2O$			Acros Organic
Methanol	CH_3OH			Sigma
Methyl orange	$C_{14}H_{14}N_3NaO_3S$		98	Acros Organic

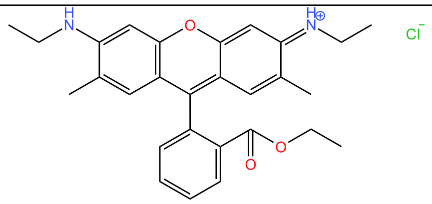
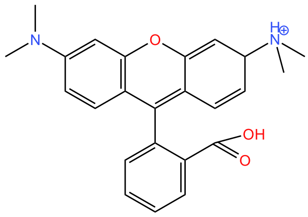
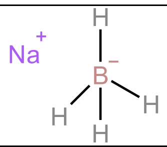
Rhodamine 6G	$C_{28}H_{31}N_2O_3Cl$		97	Sigma Aldrich
Rhodamine B	$C_{28}H_{31}ClN_2O_3$		98	Sigma Aldrich
Sodium borohydride	$NaBH_4$			Fisher Chemical

Table 3. List of chemicals used

B. Characterizations

The optical characteristics of the synthesized AuNPs with a 2:1 ratio of OSWE to $HAuCl_4$ were investigated using ultraviolet-visible (UV-VIS) spectroscopy. The absorption spectra were recorded at room temperature in the wavelength range of 500-650 nm, utilizing a JASCO V-570 UV-VIS-NIR spectrophotometer and a 3 mL cuvette. To analyze the size distribution and zeta potential of the AuNPs, dynamic light scattering (DLS) was employed. The DLS measurements were conducted using a Brookhaven Instruments Corps instrument, with a 658 nm laser source and a PMT detector from HAMAMATSU (HC120-30). The data analysis was performed with 90Plus Particle Sizing Software Ver. 5.23, and the dust setting was adjusted to 40.

For the scanning electron microscopy (SEM) analysis, a Tescan Vega 3 LMU instrument equipped with an Oxford EDX detector (Inca XmaW20) was used. A few drops of the diluted AuNPs solution were carefully deposited onto an aluminum stub and coated with carbon conductive adhesive tape before SEM examination. The emission spectral measurements were recorded using a Jobin-Yvon-Horiba Fluorolog III fluorometer and the FluorEssence program. The measurements were taken with a resolution increment of 1 nm and a slit width of 5 nm. For excitation, a 100 W Xenon lamp was employed as the light source. The detection was carried out using an R-928 detector operating at a voltage of 950 V. To capture the resonance Rayleigh scattering spectrum, a synchronous fluorescence scan was conducted on the same instrument. This was achieved by maintaining a zero nm interval between the excitation and emission wavelengths.

This characterization process provides valuable insights into the optical properties, size distribution, and morphology of the produced AuNPs, contributing to a comprehensive understanding of their potential applications in various fields.

C. Applications of Green Synthesized AuNPs

Gold nanoparticles have gained significant importance in various fields due to their versatile properties. These nanoparticles have found applications as catalysts, antioxidants, anti-cancer agents, quenching agents, and in numerous sensing applications, among others. In our research, we conducted two distinct investigations to assess the effectiveness and suitability of these nanoparticles. The main areas of focus were:

a) Exploring the catalytic reactivity of AuNPs and studying their recycling effect.

b) Analyzing the antioxidant reactivity of the synthesized AuNPs.

c) Determining the fluorescence quenching impact of AuNPs on Rh. B.

It is worth noting that each application's sample preparation has been elaborated in dedicated chapters of this thesis.

CHAPTER III

CATALYSIS REACTIVITY OF PREPARED GOLD NANOPARTICLES USING OSWE

A. Introduction

Due to the prevalence of chemical contaminants, clean air, sufficient electricity, and safe drinking water have all become serious issues [66]. CR [67], as well as other organic dyes such as Rh. 6G, Rh. B, and MO, contribute significantly to environmental contamination [68-70]. Synthetic organic pollutants emitted by numerous industries such as paper, plastic, pharmaceutical, food, textiles, and cosmetics can directly result in ground or surface water pollution, affecting humans and other living species [71, 72]. These organic pollutants may cause skin irritation, liver, and kidney, damage as well as the poisoning of the central nervous system in animals and humans [73]. For that reason, a lot of efforts have been made to develop newer technologies for catalytic reduction/degradation of organic pollutants from water/wastewater to reduce ill effects on the environment and humans. Although treating organic pollutants involves biological, physical, and chemical methods, bio-treatment of organic pollutants is less adapted due to their resistance to aerobic degradation while anaerobic degradation may lead to the development of carcinogenic aromatic amines [74, 75]. Conversely, physical and chemical adsorption only relocate pollutants from one phase to another, possibly exacerbating secondary pollution. Moreover, the subsequent post-treatment and recovery of adsorbent materials are financially demanding, hazardous, and intricate [76-78]. Au nano catalysts for the reduction of organic dyes were developed and used [79]. Garg et al. prepared L-asparagine functionalized gold nanoparticles (Asp-AuNPs) which is not an environmentally-friendly method due to the usage of NaOH and also it is time-consuming due to the long preparation time for AuNPs. So, it is a challenge to build up a simple, non-toxic, environmentally friendly, and fast method to prepare metal NPs to overcome the above-mentioned difficulties. The present scientific climate places significant emphasis on green technologies for producing a range of metal nanoparticles. Many studies focused on plant extract as a reducing and capping agent to synthesize metal nanoparticle. Among these nanoparticles, AuNPs hold a unique position due to their distinct properties and pronounced affinity for biological macromolecules, rendering them versatile for applications such as photo-thermal therapy, tissue imaging [80], immunoassays [81], biosensors [82], cancer treatment [83], and drug delivery systems [80].

The use of non-toxic solvents, and reactions carried out in aqueous solutions under favorable conditions remains a priority to address both environmental and industrial imperatives. This study employs a strategy to engineer AuNPs by OS plant extract. The study introduces a straightforward and effective methodology for synthesizing AuNPs, involving the direct reduction of $HAuCl_4$ in the presence of OS plant extract, followed by their catalytic reduction of CO and degradation of Rh. 6G, Rh. B, and MO using $NaBH_4$. Although the reaction is thermodynamically favorable in the absence of a catalyst, it presents kinetic complexities. The synthesized AuNPs by lowering the activation energy, make the reaction thermodynamically and kinetically favorable.

B. Methods and Preparation

1. Extract of OS in Water

The preparation process for OSWE was conducted within the Biology Department of the American University of Beirut. Initially, OS leaves were brought from south of Lebanon, subsequently washed and dried in a shaded area, and then ground. Next, 30 grams of the powdered OS leaves were suspended in 900 mL of distilled water and allowed to stay in darkness on a shaker for three days. Following this, the mixture underwent filtration using layers of cheesecloth. The suspension and filtration procedure were repeated twice, and the resultant filtrate was stored at 4 ^{o}C. The second mixture of the filtrate was added to the first filtrate, and the combined 2700 mL filtrate was subjected to evaporation using a rotary evaporator. The concentrated mixture was then freeze-dried at -20 ^{o}C and around 1.8 g plant extract were collected. Upon completing these steps, the OSWE was prepared and is now ready for subsequent applications.

2. Synthesis of AuNPs

The synthesis of AuNPs was performed in a single step under neutral conditions (See figure 6). Initially, 5 mg of $HAuCl_4$ was dissolved in 1 mL of heated double-distilled water and then added to 10 mg of OSWE (Origanum syriacum water extract), dissolved in 5 mL of heated double-distilled water at 70 ^{o}C to have 1:2 ratio of $HAuCl_4$: OSWE. The resulting solution was further diluted to a final volume of 50 mL to ensure complete dissolution of the reactants. Next, the solution was subjected to a bath-type ultrasonic sonicator for 30 minutes with 30 kHz frequency and RPM of 300 min^{-1}. until the color changed from yellowish to dark purple, indicating the successful formation of AuNPs. Afterward, the solution was centrifuged at 15000 rpm for 15 minutes to collect the AuNPs. The collected nanoparticles were then sonicated and re-dissolved in 15 mL of double-distilled water and stored at room temperature and for further studies it was shaken for 1 to 2 minutes then used. This efficient synthesis process offers a promising approach for producing well-defined gold nanoparticles with potential applications in various fields.

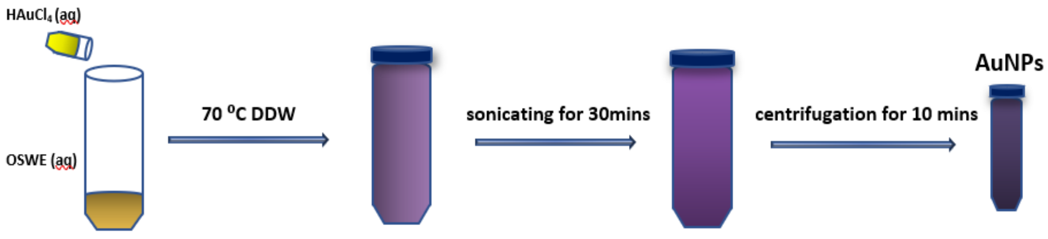


Figure 6. Schematic representation of the AuNPs synthesis.

3. Characterizations

A fast and simple green method was used to synthesize AuNPs using OS plant extract. The bioactive elements found in OSWE, such as flavonoids, tannins, terpenoids, and others, contain functional groups like carboxylic acid, hydroxyl, and phenolic groups. These functional groups play a role in reducing Au^{3+} to Au^0 and can also act as capping agents, stabilizing the formed AuNPs. In more detail, our hypothesis suggests that when bioactive compounds in OSWE are dissolved in water, their functional groups can be dissociated into carboxylate and phenolate groups, carrying a negative charge. This negative charge then facilitates the reduction of Au^{3+} to Au0. Subsequently, these negatively charged components remain on the surface of AuNPs, effectively capping them, essentially acting as reducing agents (See figure 7). The successful reduction is confirmed visually when the color turned from yellowish to dark purple (See Figure 8). Furthermore, some characteristics of the produced AuNPs with ratio 1:2 of $HAuCl_4$:OSWE were studied.

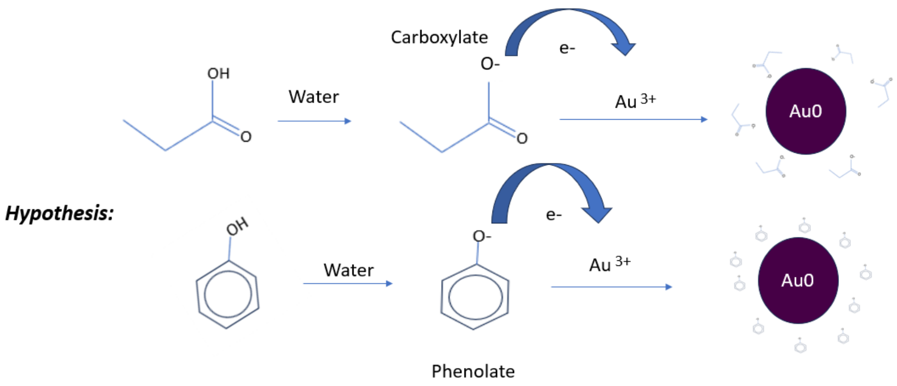


Figure 7. Bioactive components in OSWE reducing Au^{3+} to Au^0, then stabilizing formed AuNPs.

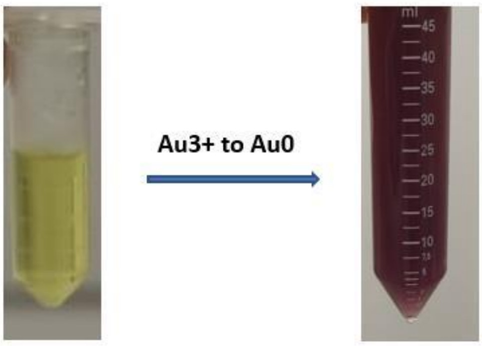


Figure 8. Reduction of Au^{3+} to Au^0.

a. <u>Optical Properties Analysis</u>

The optical characteristics of the produced gold nanoparticles (AuNPs) were examined using UV-VIS spectroscopy and Synchronous fluorescence spectroscopy methods. To do this, a volume of 500 µ of the prepared AuNPs was combined with 2.5

mL of DDW in a 3 mL cuvette for characterization. Resonance Rayleigh Scattering of prepared AuNPs using OSWE were measured by applying synchronous fluorescence scan (SFS), more in fluorometer during such measurements the wavelength interval between excitation and emission monochromator was kept zero ($\Delta\lambda = 0$), then the Resonance Rayleigh Scattering spectrum of prepared AuNPs resembled similar to often Resonance Rayleigh Scattering in a prior study that was done by El Kurdi et al. [84] and the Resonance Rayleigh Scattering peak of prepared AuNPs was between 350 nm to 450 nm (See figure 9), similar peaks were observed in earlier study [84], confirming the formation of AuNPs using OSWE.

The synthesized AuNPs were assessed using the UV-VIS technique, revealing a distinct peak at approximately 530 nm, which affirms the presence of AuNPs (Refer to figure 10).

To study the effect of OSWE acting as a reducing agent, three different ratios of $HAuCl_4$ to OSWE were tested. The 3 samples were kept under sonication and the absorbance of the different solutions was measured at different time before centrifugation. Subsequently, the absorbance of AuNPs in the three reaction mixtures with ratios of 1:1, 1:2 and 2:1 of $HAuCl_4$ and OSWE, before centrifugation, was monitored for 100 minutes at 530 nm. As expected, the absorbance of AuNPs increased as the time increased at λ_{abs}=530 nm and afterwards it becomes saturated. The continuous enhancement in the absorbance is related to the formation of more AuNPs, and therefore Au^{3+} ions present in the solution are being highly reduced. El-Kurdi et al. when using curcumin as reducing agent obtained same results; where on a first hand, the absorbance of curcumin decreases and on the other hand, the absorbance of the AuNPs increases simultaneously [85]. Interestingly, the reaction time decreases when the concentration of OSWE increases. This is because the excess of the reducing agent encourages the reduction of Au^{3+} to Au^{0} and consequently accelerating the rate of the reaction (Figure 11).

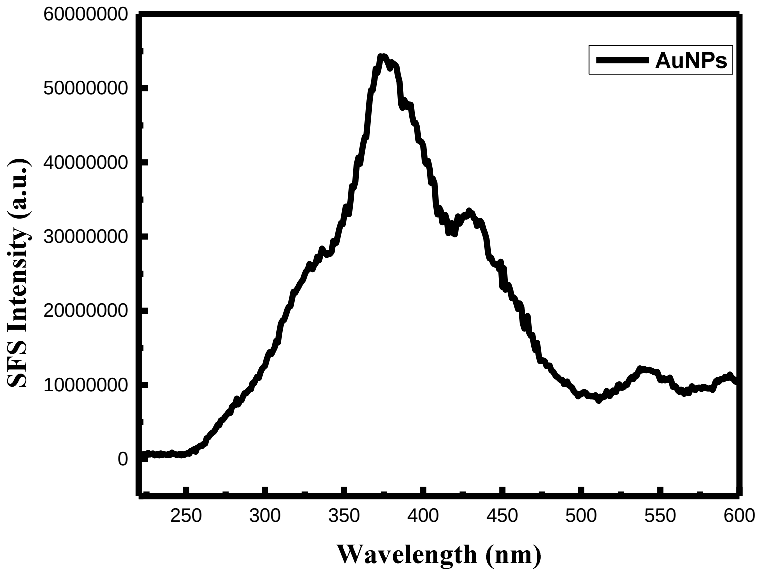


Figure 9. Synchronous fluorescence spectra at $\Delta\lambda$= 0 nm.

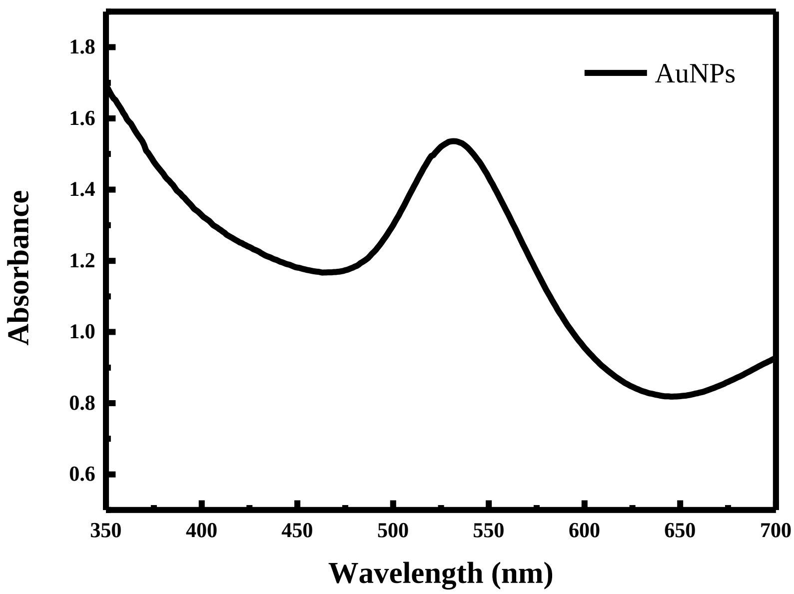


Figure 10. UV-VIS spectra of produced AuNPs using OSWE.

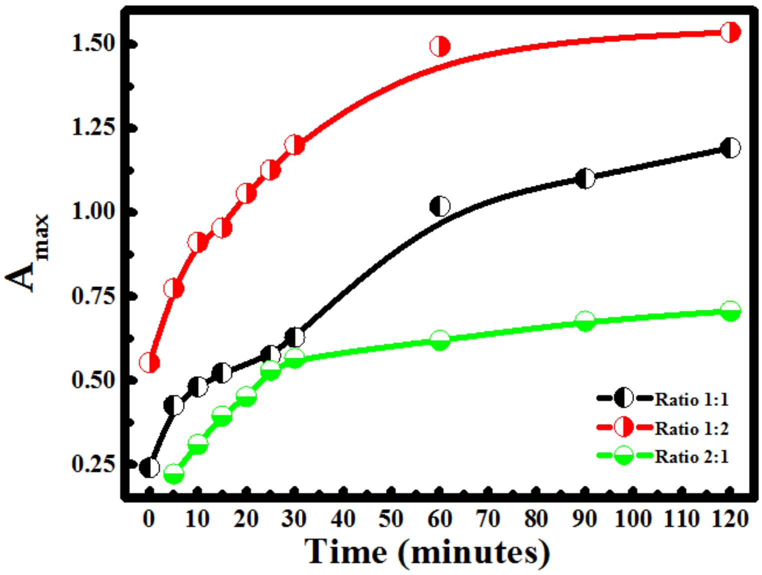


Figure 11. Maximum absorbance of three ratios of $HAuCl_4$ and OSWE versus time during 100 minutes at λ= 530 nm.

b. Size Analysis

The size distribution of the produced AuNPs was analyzed using DLS technique. To obtain the hydrodynamic size distribution of AuNPs and to determine their aggregation state, dynamic light scattering (DLS) is an appropriate and non-destructive technique to use [86]. According to Kwon et al. AuNPs were synthesized in range of 10 to 90 nm through seed growth method, in which sodium borohydride, was used as reducing agent. Hence, it is well known that $NaBH_4$ is toxic reducing agent; therefore, it can be easily substituted with OS inhibiting thereby the use of non-eco-friendly reagent [87]. However, the produced AuNPs using OSWE were in the range of 10-70 and 100-180 nm with a polydispersity index equal to 0.102 as shown in Figure 12. In fact, the produced NPs were small between 10 nm and 70 nm. The presence of bigger nanoparticles in the size range 100-180 nm can be due to the formation of aggregations. Hence, utilizing green synthesis method to produce AuNPs by OSWE as a reducing and capping agent offers various advantages including non-toxicity, cost-effectiveness, environmental friendliness, simplicity, stability over other synthesis methods such as chemicals methods using $NaBH_4$ as a reducing agent, was resulted in the same size distribution for AuNPs [87, 88].

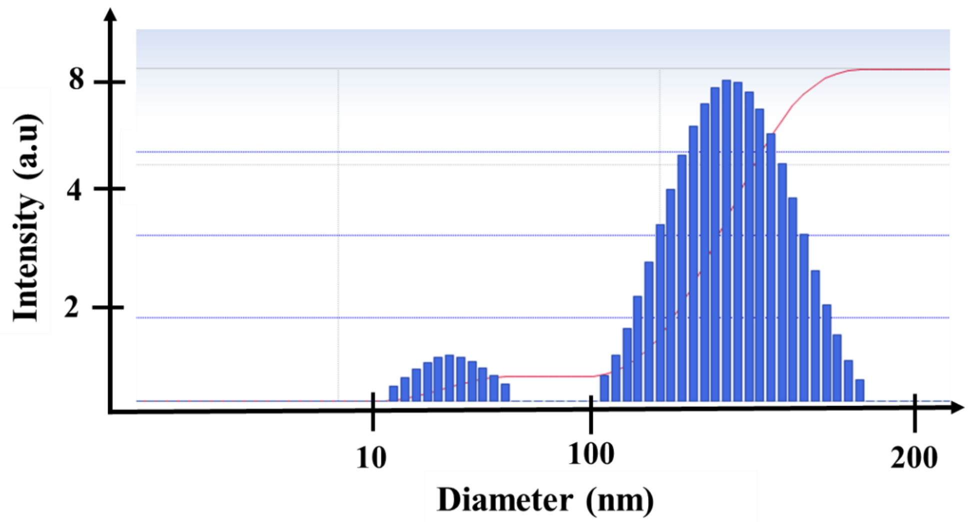


Figure 12. Size distribution of the prepared AuNPs using OSWE extract in the ratio of 2:1 (OSWE:$HAuCl_4$).

c. <u>Morphology and Elemental Mapping Analysis</u>

The morphology and the elemental composition of the obtained AuNPs were investigated using SEM and EDX analysis. The shape and the morphological characteristics of the prepared AuNPs were illustrated. Remarkably, small nanoparticles in a spherical shape were obtained (See Figure 13A&B). In fact, as shown in Figure 13C the nanoparticles were found in the size range 20-30 nm. Thus, according to Figure 13D; when the nanoparticles aggregate, their size increased consequently to the range of 90- 110 nm. These results are in accordance with the DLS measurements.

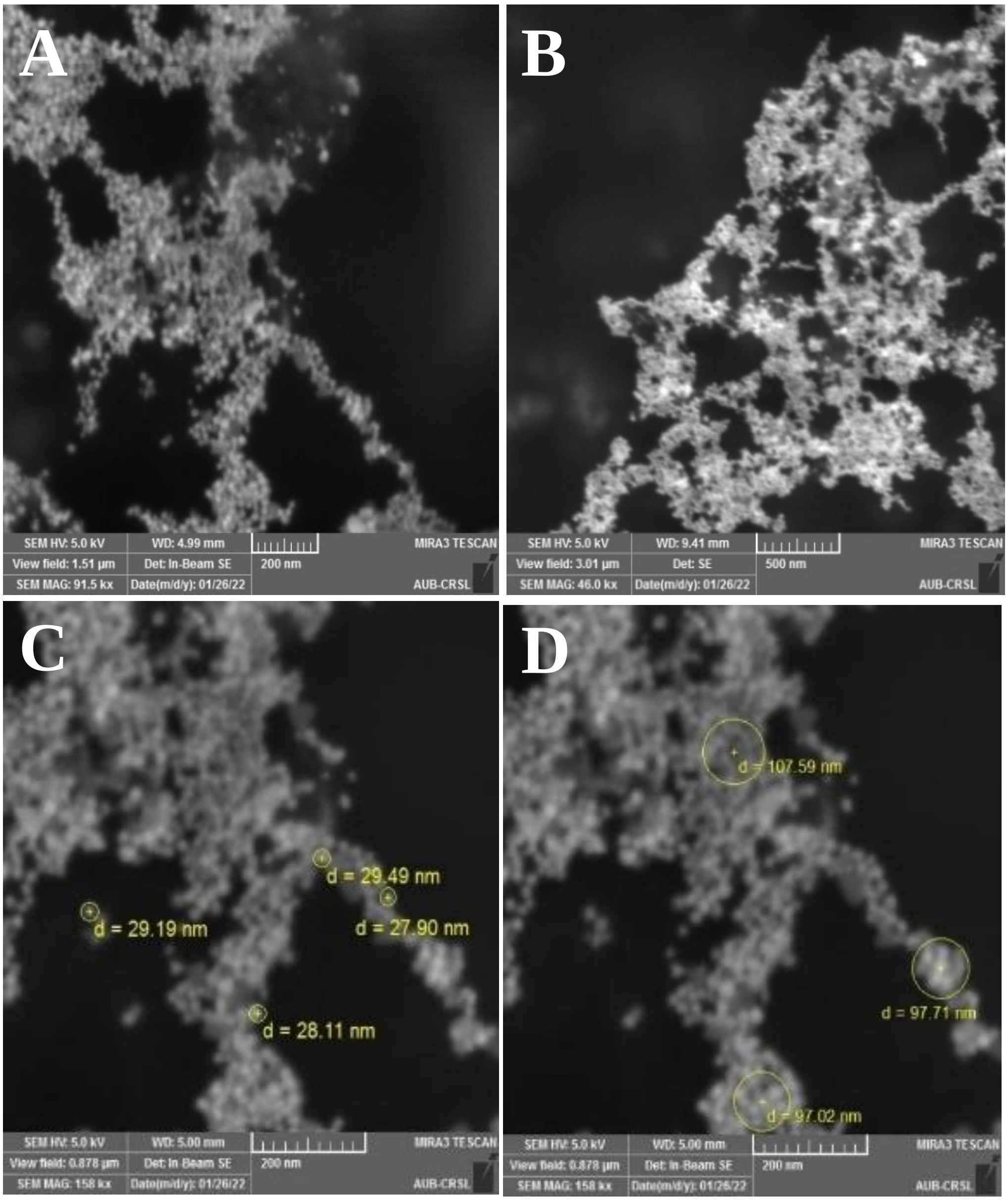


Figure 13. SEM images of the prepared AuNPs using OSWE extract in the ratio of 2:1 (OSWE:$HAuCl_4$).

Moreover, the EDX analysis of the produced AuNPs was investigated (See Figure 14).The results confirm the presence of AuNPs stabilized through green synthesis method using OSWE.

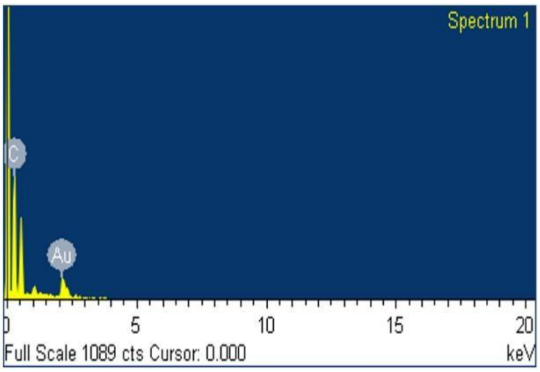


Figure 14. Elemental analysis for AuNPs using EDX.

d. <u>Zeta Potential</u>

The zeta potential was used to measure the surface charge of the prepared AuNPs in order to maintain their stability. According to Bac et el. the greater the zeta-potential value is, the more stable the suspension will be. In fact, the high surface charge of the AuNPs will create a repulsive force, and keep the gold nanoparticles apart, resulting in high stability of the suspension. Actually, nanoparticles with zeta potentials of greater than 20 mV or below -20 mV exhibit enough electrostatic repulsion to maintain their stability in solutions [89]. The measured zeta potential of the generated AuNPs is shown in figure 15. Remarkably, the surface charge of the prepared NPs was equal to -24.6 mV, supporting the earlier hypothesis, that components is OSWE act as reducing agent producing gold nanoparticles moreover, the negative charge on formed AuNPs was attributed to the attachment of phenolate and carboxylate ions from dissociation of bioactive components in OSWE to the surface of AuNPs. These components not only acted as stabilizers but also maintained the negative charge of the nanoparticles [90].

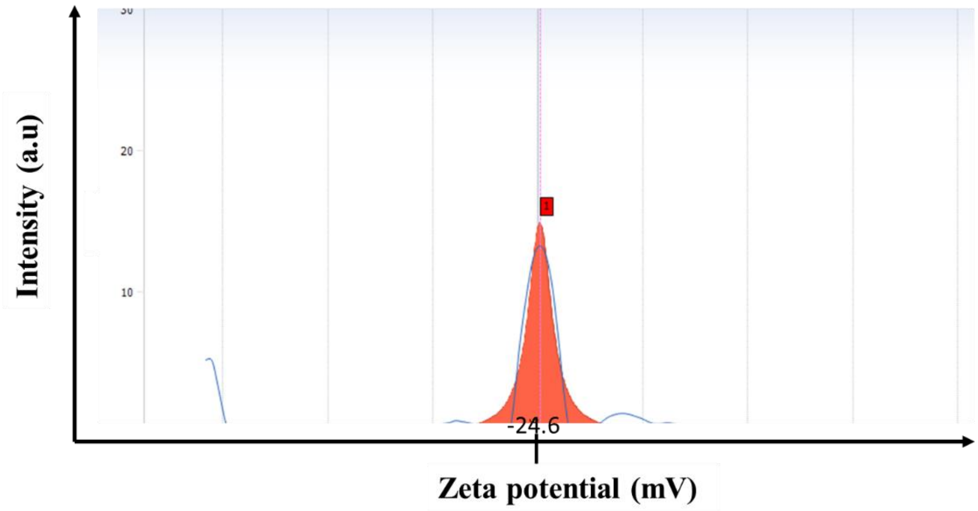


Figure 15. Zeta potential of the prepared AuNPs using OSWE in the ratio of 2:1 ($OSWE:HAuCl_4$).

C. Catalysis Reactivity Sample Preparation

1. Catalysis Reactivity of AuNPs Analysis

The study examines the catalytic activity of the produced AuNPs in the presence and absence of AuNPs. The experiment involved mixing 500 μM of different organic dyes with 15 mM of $NaBH_4$ (fresh solution). The AuNPs were used as a catalyst, with a quantity of 0.5 mL unless stated otherwise. The reaction kinetics were analyzed by observing the optical absorption peak of each dye used in the study. The results could have implications for potential applications of AuNPs as catalysts in various chemical reactions.

2. Multistage Recycling of AuNPs as a Catalyst in Reduction of CR Analysis

In this thesis, the reusability of AuNPs as a catalyst in the reduction reaction of Congo-red (CR) is investigated through multiple cycles. The experimental procedure involved preparing a 3 mL cuvette with a mixture consisting of 1440 μL of DDW, 500 μM of Congo-red, and 15 mM of freshly prepared $NaBH_4$ (designated as mixture A).

Subsequently, 0.5 mL of AuNPs was introduced as the catalyst. The reduction reaction was initially studied by measuring the optical absorption peak of CR. After the first reduction cycle, the solution in the cuvette was recycled by subjecting it to centrifugation for 15 minutes. The recovered AuNPs were then diluted with 0.5 mL of DDW and reintroduced as the catalyst in mixture A. The optical absorption peak of CR was monitored once again to assess the reduction process. This recycling process was repeated two more times, totaling three cycles. During each cycle, the reduction of CR was evaluated by observing changes in its optical absorption peak. The primary objective of this study is to explore the reusability and effectiveness of AuNPs as a sustainable catalyst in the reduction reaction of CR. The findings from this research could have practical implications for catalytic applications and environmental sustainability.

D. Results and Discussions

1. Catalysis Reactivity of AuNPs

In this research, the catalytic activity of the produced AuNPs is thoroughly examined concerning four different organic dyes: CR, MO, Rh. 6G, and Rh. B. The central objective is to investigate the catalytic reduction of these organic dyes using $NaBH_4$ as the reducing agent.

To conduct the experiments, two distinct solutions are prepared for each organic dye:

- Solution 1: This solution consisted of the organic dye and $NaBH_4$.
- Solution 2: This solution contains the organic dye, $NaBH_4$, and AuNPs.

Maintaining a consistent total volume of 3 mL for both solutions, double distilled water is added as needed. Throughout the experimentation, the reduction of the organic dyes is meticulously monitored by tracking the changes in their absorbance at specific wavelengths, as reported in Table 4.

Organic dye	**λ (nm)**
CR	494
MO	462
Rh. B	554
Rh. 6G	527

Table 4. Absorption wavelength maximum of tested organic dyes

To understand the role of AuNPs as catalysts, a comparison experiment was conducted without using AuNPs. In figure 17A it is shown that for all the organic dyes the reduction took 40 minutes to be done. Moreover, the results showed that only CR was completely reduced when $NaBH_4$ alone was used (See figure 16). However, when AuNPs were added as catalysts, the reduction of CR happened much faster and was completed within approximately 6 minutes, in figure 17B the comparison between two conditions (in absence and presence of AuNPs) were depicted. These findings clearly demonstrate the powerful catalytic ability of gold nanoparticles in reducing CR. Haleem et al. reported a mechanism of degradation of CR by reducing its chromophore in presence of AuNPs and $NaBH_4$ (See figure 18) [91].

These results provide strong evidence that AuNPs are effective catalysts, facilitating rapid and efficient reduction reactions. This discovery opens up exciting possibilities for using AuNPs as nano catalysts in various chemical processes, which could lead to advancements in catalysis and contribute to making chemical reactions more environmentally friendly.

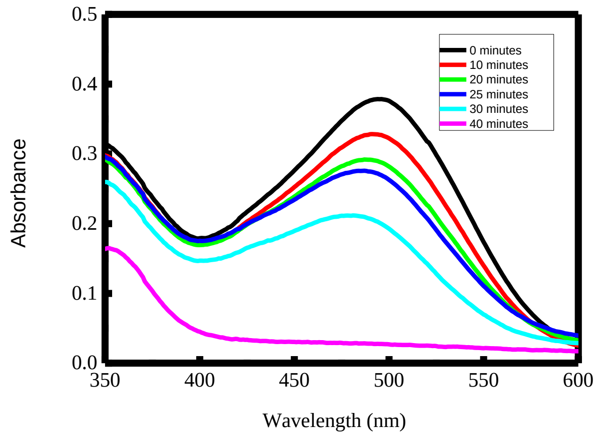


Figure 16. Absorbance of CR in presence of $NaBH_4$ only at 494 nm (complete reduction).

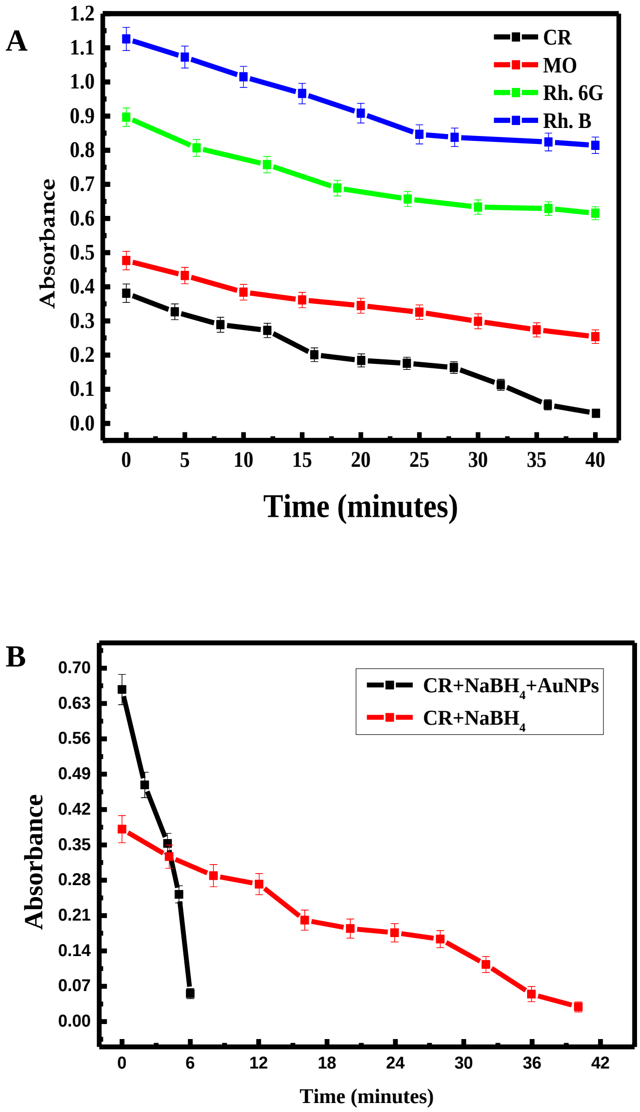


Figure 17. (A) Absorbance changes for the different organic dyes in the presence of $NaBH_4$ only; and (B) Absorbance change of CR in the presence of $NaBH_4$ and AuNPs.

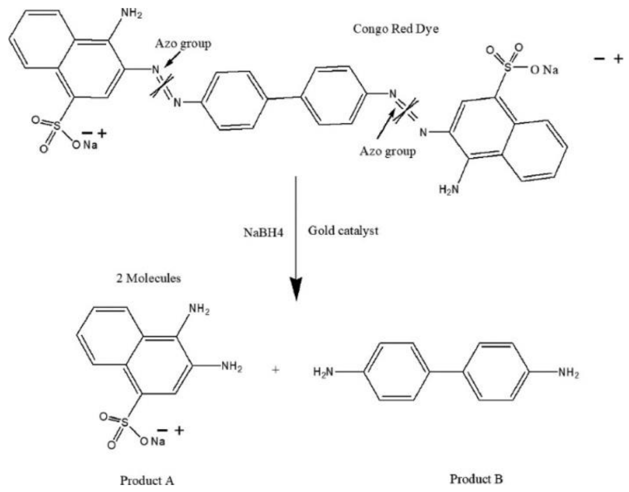


Figure 18. Degradation mechanism of CR in the presence of AuNPs and reducing agent $NaBH_4$ [91].

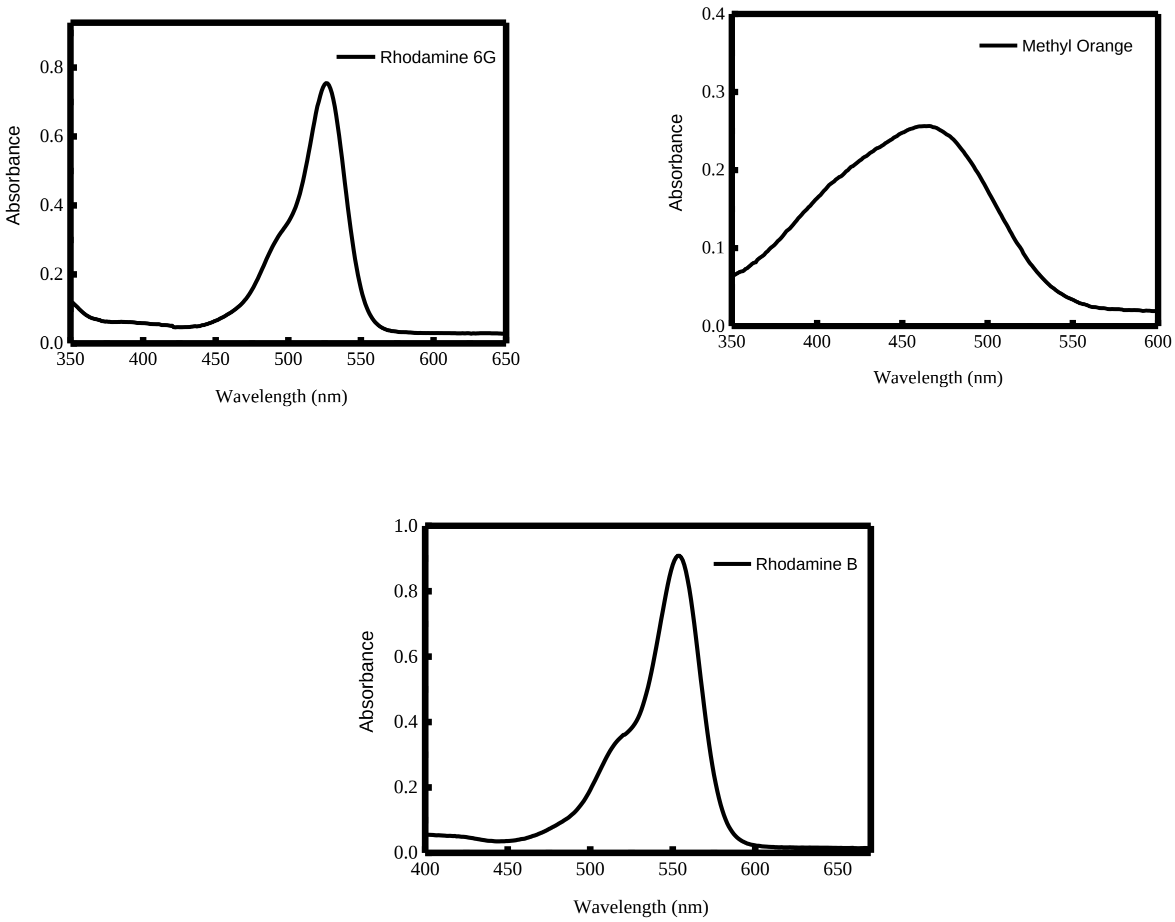


Figure 19. Absorbance of A) Rh. 6G at 527 nm, B) absorbance of MO at 462 and C) absorbance of Rh. B at 554 nm.

2. *Recycling Test of AuNPs as a Catalyst in Reduction of CR*

Among a range of organic dyes, Congo-red underwent complete reduction (chapter III, C1) in the presence of $NaBH_4$ and AuNPs. Subsequently, the AuNPs were used as catalysts in the CR reduction process through a recycling procedure. Initially, a solution containing CR, $NaBH_4$, AuNPs, and double distilled water in a 3 mL cuvette was prepared. The progress of reduction was monitored by tracking the absorbance changes of CR at 494 nm. Remarkably, a complete reduction of CR was achieved in just 6 minutes. Afterward, the solution in the cuvette was recycled for the first-time using centrifugation and reused for CR reduction, which now required 8 minutes for complete reduction. The recycling process was repeated two more times, resulting in complete reduction times of 10 and 14 minutes, respectively (Figure 20). Notably, with each recycling step, the time required for complete reduction increased, indicating the influence of AuNP concentration on the reduction process. By the third recycling, all the AuNPs were depleted and could no longer be reused. Figure 16 vividly illustrates the relationship between AuNP concentration and the reduction process. Understanding the recyclability and efficiency of AuNPs as catalysts is essential for their practical application, providing valuable insights into their optimal usage and potential lifespan in various reduction reactions.

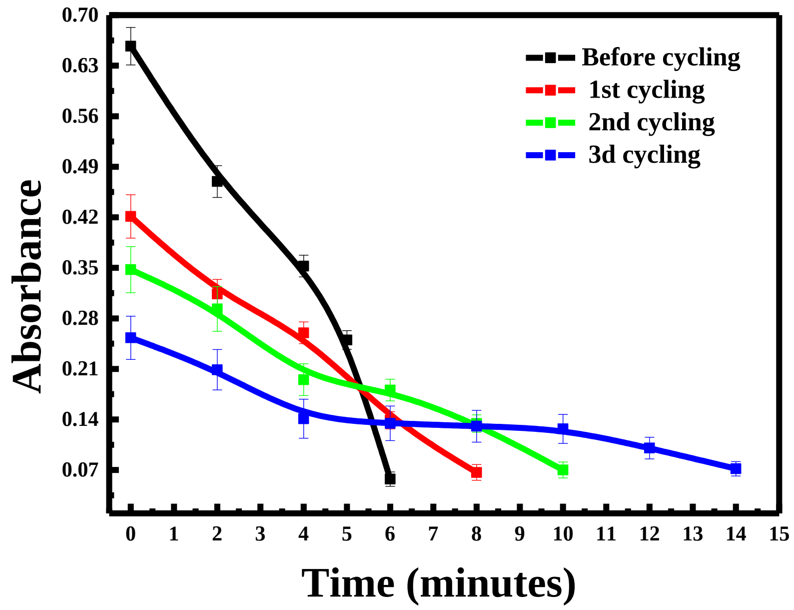


Figure 20. Absorbance changes for the CR in the presence of $NaBH_4$ and AuNPs versus time before and after 1^{st}, 2^{nd} and 3^{rd} time recycling of AuNPs.

E. Conclusion

The AuNPs produced from OSWE have been proven to possess catalytic properties in reducing organic dyes in the presence of $NaBH_4$. Notably, CR was completely reduced in the presence of AuNPs and without them, but with AuNPs, the reduction occurred nearly 7 times faster. Furthermore, AuNPs have demonstrated good recyclability as a nano catalyst for reducing CR. They can be reused three times, and even after recycling, the reduction reaction is at least 3 times faster than the normal reduction process without AuNPs. These findings highlight the potential of AuNPs as efficient and reusable catalysts for organic dye reduction reactions.

CHAPTER IV

DETERMINATION OF THE ANTIOXIDANT IMPACT OF GREEN SYNTHESIZED GOLD NANO PARTICLES

A. Introduction

The group of compounds that neutralize the reactive oxygen species (ROS) and free radicals in a cell is called antioxidants [92]. Antioxidant activity in food and drinks has emerged as one of the most significant scientific subjects. These compounds offer crucial safeguards against the detrimental effects of free radicals, playing pivotal roles in the emergence of numerous chronic ailments like cardiovascular diseases, aging, heart issues, anemia, cancer, and inflammation [93]. DPPH is a stable compound, undergoing reduction by accepting hydrogen or electrons [94], which can be used as an assay to simply test the antioxidant property of a compound by indicating of the radical scavenging potential of that [95]. Metal nanoparticles have gained significant interest due to their considerable potential within the realm of medical nanotechnology [96]. Among various types of metal nanoparticles, gold nanoparticles have gained prominence due to their distinct attributes such as resilience against oxidation, compatibility with biological systems, adjustable surface plasmon resonance (SPR) [97], elevated surface reactivity, and promising prospects for therapeutic applications within nanomedicine [98]. Origanum Syriacum plant extract has shown promising results as an antioxidant agent due to its bioactive components such as phenolic compounds, carvacrol and, thymol [99]. This study employed an eco-friendly, rapid, and straightforward approach for generating gold nanoparticles (AuNPs) through the reduction of $HAuCl_4$ solution using OSWE. The purpose was to explore the antioxidant potential of these AuNPs by evaluating their ability to scavenge free radicals using DPPH assay.

B. Material and Preparations

1. Free Radical Scavenging Activity of AuNPs Using DPPH Assay

The antioxidant effectiveness of AuNPs synthesized through environmentally friendly method was assessed using the DPPH assay. To accomplish this, a 1 mg quantity of DPPH was dissolved in a 5 mL methanol solvent, creating a 500 µM DPPH stock solution, which was subsequently stored in a refrigerator. Control mixtures were generated by adding 600 µl of DPPH from stock solution with DDW to ascertain the optical absorbance peak of DPPH, moreover, separate cuvettes containing DDW held AuNPs in quantities of 500 µl, 700 µl, and 900 µl, were examined to measure their absorbance. Following this, 600 µl of the DPPH stock solution was combined with three different volumes of AuNPs: 500 µl, 700 µl, and 900 µl. Each sample was refrigerated for 30 minutes and stirred every 5 minutes. The ability of AuNPs to scavenge radicals was monitored by tracking alterations in the optical absorption peak of DPPH before and after adding AuNPs. The DPPH radical scavenging activity (RSA) was also represented in the percentage of inhibition using the following formula [94]:

$$\% \, RSA = [(A_D - A_{D1,2,3})/A_D]*100$$

where A_D is the absorbance of the DPPH alone and $A_{D1,2,3}$ is the absorbance of the DPPH after adding AuNPs.

C. Results and Discussion

1. *DPPH Radical Scavenging Activity of Prepared AuNPs*

The free radical scavenging property of produced AuNPs using DPPH were tested for 3 different concentrations of AuNPs (500, 700 and 900 µl). For this reason, different solutions were prepared:

- First, a control solution containing only DPPH (600 µL)
- Second, three solutions containing AuNPs only with various volumes (500, 700, 900 µL)
- Third, three solutions containing same amount of DPPH in control sample + various volumes of AuNPs (500, 700, 900 µL)

For all 7 solutions the total volume was kept equal to 3 mL within the addition of double distilled water.

In fact, the quantification of the AuNPs as a free radical scavenger was accomplished by observing alterations in DPPH absorbance. To ensure the role of the AuNPs as an antioxidant agent. a control experiment was performed. For this purpose, the maximum absorbance of DPPH was measured at 520 nm in absence of AuNPs, which was $A_D = 0.58$ (See figure 21A). Subsequently, the maximum absorbance of solutions containing only AuNPs 500 µL, 700 µL and 900 µl were calculated at 530 nm and the values are $A_1 = 0.33$, $A_2 = 0.40$ and $A_3 = 0.43$ respectively. The absorbance of the samples containing constant value of DPPH and various volumes of AuNPs was measured at 527 nm as mentioned below (See figure 21B-D):

- A_{t1} (500 µL of AuNPs+ 600 µL DPPH) = 0.74
- A_{t2} (700 µL of AuNPs+ 600 µL DPPH) = 0.67
- A_{t3} (900 µL of AuNPs+ 600 µL DPPH) = 0.65

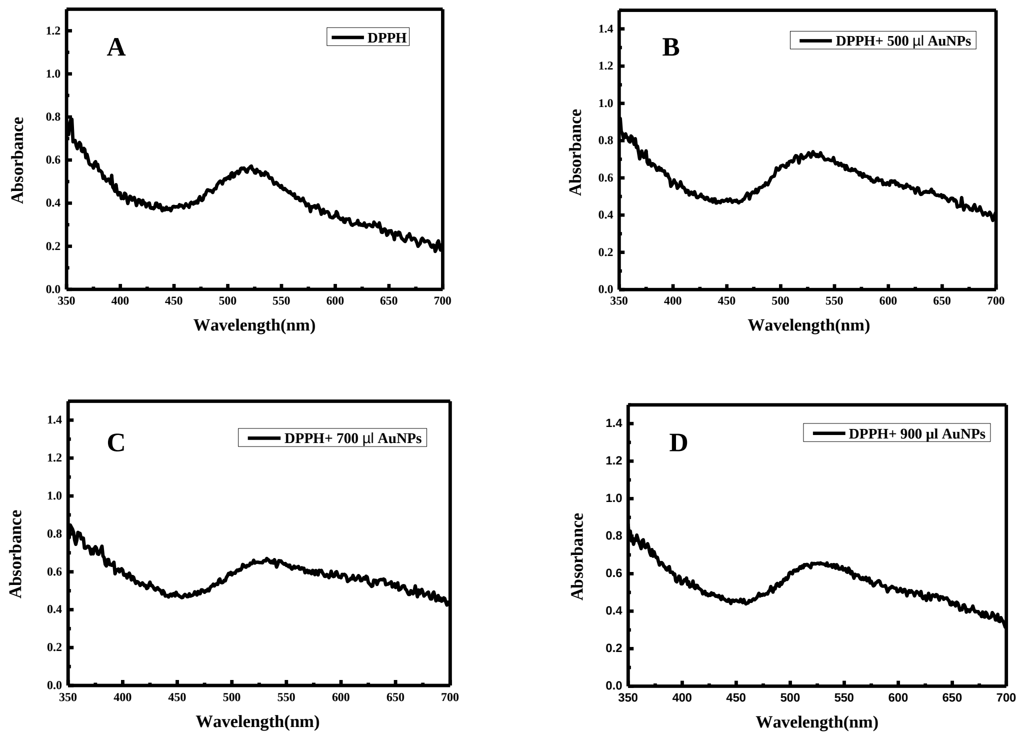


Figure 21. Absorbance of DPPH without AuNPs at 520 nm, B) Absorbance of 500 µL AuNPs + DPPH, C) Absorbance of 700 µL AuNPs + DPPH and D) Absorbance of 900 µL AuNPs + DPPH at 527 nm.

To see the changes in absorbance of DPPH before and after adding AuNPs, the subtractions of A and A_t for each volume of the AuNPs were calculated and compared to A_D:

- A_{D1} (A_{t1}- A_1) = 0.74-0.33= 0.41
- A_{D2} (A_{t2}- A_2) = 0.67-0.40 = 0.27 vs A_D = 0.58
- A_{D3} (A_{t3}- A_3) = 0.65-0.43 = 0.22

These computations indicate that as the volume of AuNPs is increased through addition, the absorbance of DPPH is diminishing. This trend underscores the free radical scavenging characteristic of the synthesized AuNPs, as depicted in Figure 22. Moreover, The, inhibition percentage of

DPPH radical scavenging activity was visually depicted in Figure 23. The DPPH radical scavenging activities were recorded as 29.31%, 53.45%, and 62.10% for AuNPs quantities of 500 µL, 700 µL, and 900 µL, respectively. The mean percentage inhibition of the synthesized AuNPs stood at 48.27%, positively correlating with increasing AuNPs concentrations. Muthuvel et al. reported a time consuming biosynthesized AuNPs using Solanum nigrum leaf extract and found similar observations with enhanced DPPH scavenging activity [94], However, in the present study, the synthesis of AuNPs using OSWE was achieved within a remarkably short span of 30 minutes, moreover, the obtained results from percentage of inhibition commend the outstanding role of these NPs as good antioxidant agent.

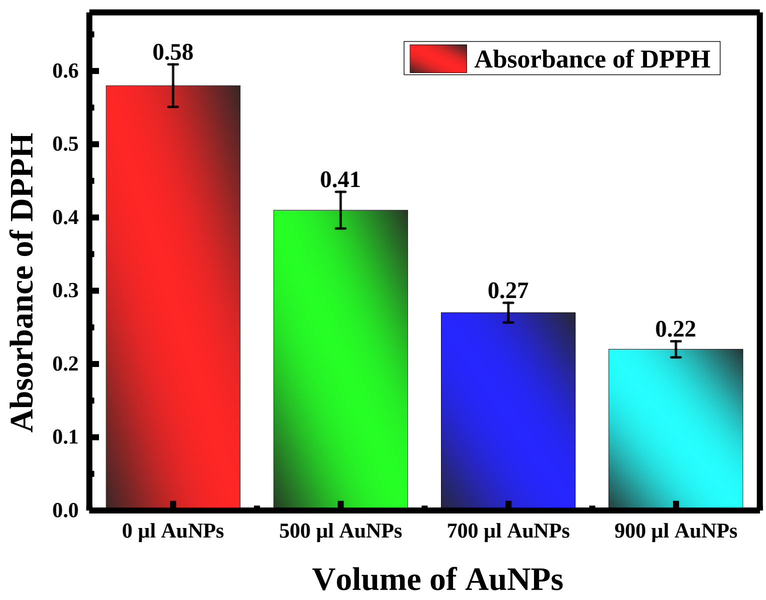


Figure 22. Absorbance of DPPH in various concentrations of the AuNPs.

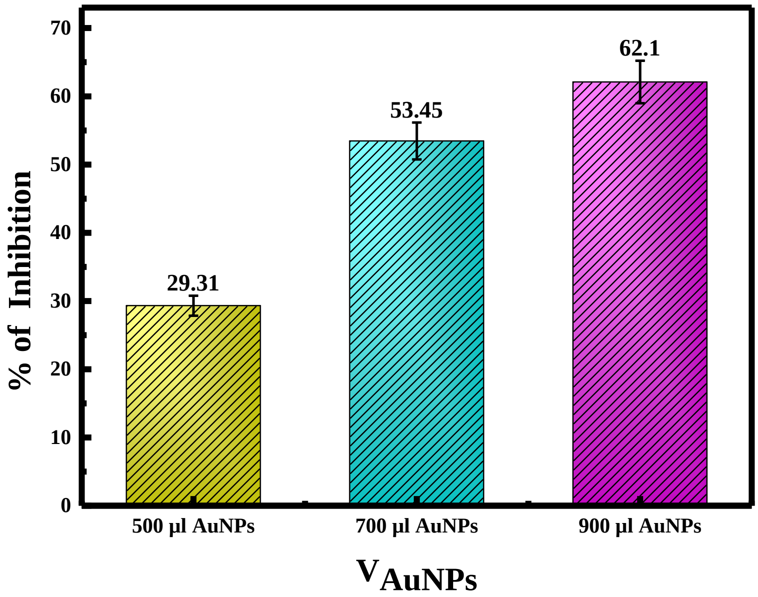


Figure 23. Percentage of inhibition vs different concentrations of AuNPs

D. Conclusion

The green synthesized AuNPs have confirmed a decreasing trend of DPPH's absorbance in relation to their concentration. The outcomes from the DPPH assay underscored the effective inhibition of free radicals by the biosynthesized AuNPs. The inhibition percentage of average and 900 µL AuNPs were reported at 48.27 % and 62.10 % respectively. This indicates a remarkable antioxidant activity of the produced AuNPs, positioning it as a potential and promising antioxidant agent in various fields including pharmaceuticals, cosmetics and skin-care, health, and nutrition and with potential applications as anti-cancer agents in the future.

CHAPTER V

INVESTIGATION OF THE FLUORESCENCE QUENCHING OF RHODAMINE B BY GREEN SYNTHESIZED GOLD NANO PARTICLES

A. Introduction

Gold nanoparticles (AuNPs) of various shapes are synthesized through chemical, physical, and biological means [100]. At specific light wavelengths, the collective electron oscillations on AuNPs' surfaces trigger surface plasmon resonance, producing robust light absorption as scattering and absorption [101]. This absorption holds significance for diverse analytical applications. Beyond their optoelectronic and biomedical uses, gold nanoparticles are renowned for their exceptional fluorescence quenching capacity across a broad wavelength range, distinguishing them from organic quenchers. This opens doors to exploring SET phenomena [102-104]. However, utilizing AuNPs as quenchers requires complex surface modifications to achieve the desired efficacy and molecule attachment [84]. This enables their absorption band to align with the fluorescence band of a chosen fluorophore, fostering efficient energy transfer that diminishes fluorescence [105]. Rhodamine, a significant dye, exemplifies this phenomenon, with successful SET established from Rh. 6G to AuNPs [104]. Extracts from plants, containing diverse components like flavonoids, tannins, phenolics, etc. These compounds can bind to metal nanoparticles through electronic interactions as stabilizing agents, resulting in inducing electrical charges on metal nanoparticles [90, 106]. this electronic charge can enable AuNPs to interact with fluorophore molecules which are electronically charged as well and causing a reduction in their fluorescence intensity without any surface modifications.

Here, a simple and environmentally friendly method for producing AuNPs using OSWE was presented to investigate their quenching effect on Rh. B without further surface modifications for AuNPs.

B. Methods of Preparation

1. Sample for Various Volumes of AuNPs

The fluorescence quenching effect of produced AuNPs was investigated by preparing 9 different samples. For this reason, various volumes of AuNPs were taken from the initial stock solution (as detailed in chapter II, C) to generate distinct samples within the 0 to 200 μL range. In parallel, a 50 μM Rh. B stock solution was created in DDW. Subsequently, a specific volume of the Rh. B solution was introduced to each individual AuNPs samples within the 0 to 200 μL range. This process aimed to achieve a final solution volume of 3 mL with a consistent Rh. B concentration of 5 μM. for calibration curve, fluorescence intensity was measured at excitation wavelengths 520 nm.

2. Sample for Various Concentrations of Rh. B

The fluorescence quenching impact of produced AuNPs was explored by preparing 10 different samples. For this reason, from the initial stock solution, 0.1 mL of AuNPs was taken for the assay. In parallel, from 50 μM Rh. B stock solution different volumes were added to AuNPs solution. This process aimed to achieve a final solution volume of 3 mL with different Rh. B concentrations ranging from 1 to 10 μM. for calibration curve, fluorescence intensity was measured at excitation wavelengths 520 nm.

3. Sample for Possible Interferes

Interference study was carried out by preparing 50 μM stock solution of uric acid, ascorbic acid, glucose, ATP, phenol and melamine. Subsequently, a defined volume of each solution was incorporated to achieve a final concentration of approximately 10 μM within the ultimate mixture. This mixture included 5 μM of Rh. B, 0.1 mL of AuNPs, and additional DDW. The fluorescence emission intensity of the AuNPs-Rh. B at excitation wavelength 520 nm was investigated in absence and presence of these analytes.

C. Results and Discussions

1. Quenching Effect of AuNPs on Rh. B's Fluorescence Intensity

The examination involved assessing the fluorescence of Rh. B molecules in the presence of varying volumes of AuNPs. The AuNPs synthesized in this study exhibited a negative charge, as confirmed by zeta potential analysis. Furthermore, the positive charge of Rh. B molecules in solution, as reported by El Kurdi et al. [84], enabled their adsorption onto the negatively charged AuNPs through electrostatic interactions. This interaction counteracted van der Waals attractions, leading to a substantial reduction in the fluorescence intensity of Rh. B. This reduction indicated an occurrence of surface energy transfer, with AuNPs acting as acceptors and Rh. B as donors [104]. This hypothesis was reinforced by the consistent decrease in Rh. B's fluorescence intensity upon AuNPs addition, as illustrated in Figure 24. Moreover, The I0/I ratio for Rh. B against different AuNPs volumes was plotted (Figure 25), revealing a sharp increase after 100 μL. This abrupt rise signified a heightened quenching effect of AuNPs on Rh. B intensity beyond 100 μL and the highest quenching effect occurred at 200 μL. While increasing Rh. B concentrations from 1 to 10 μM and keeping AuNPs concentration constant, a noticeable enhancement in fluorescence emission intensity was observed at excitation and emission wavelengths of 520 and 577 nm, respectively (See figure 26). The relationship between I_{max} (maximum emission intensity) and Rh. B concentrations was plotted (Figure 27), resulting in a linear curve. This linear relationship was fitted with the equation y = 3.84997E6 x + 1.10426E8, yielding an R^2 value of 0.95302. Furthermore, an investigation into potential interference was conducted by monitoring the fluorescence emission intensity of the AuNPs-Rh. B system in the presence of various analytes, including uric acid, ascorbic acid, glucose, ATP, phenol, and melamine. This assessment was carried out utilizing excitation and emission wavelengths of 520 nm and 577 nm, respectively. As illustrated in Figure 28, upon individually introducing each of these analytes, there is not a noticeable alteration observed in the fluorescence intensity of the Rh. B-AuNPs system. This observation reinforces the notion that the fluorescence signal remains unaffected when exposed to uric acid, ascorbic acid, glucose, ATP, phenol, and melamine.

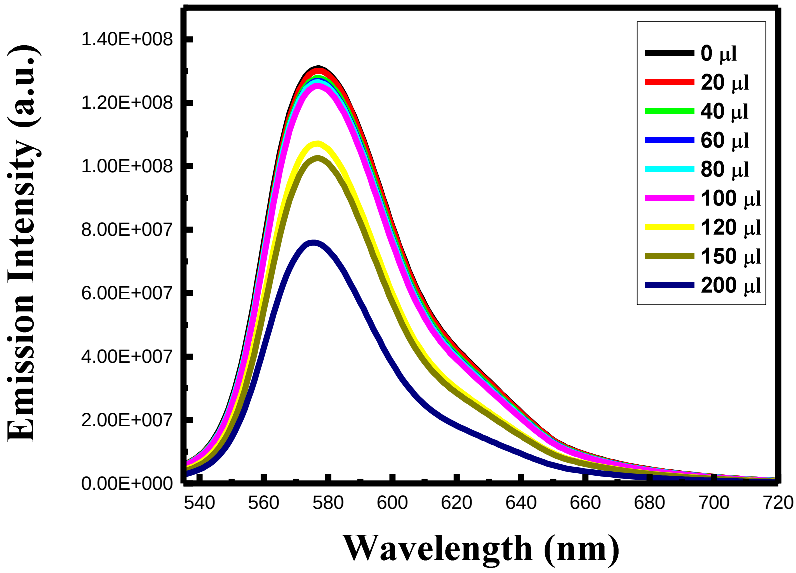


Figure 24. Emission spectrum excited at λ= 520 nm and emission λ= 577 nm of Rh. B with different volumes of AuNPs.

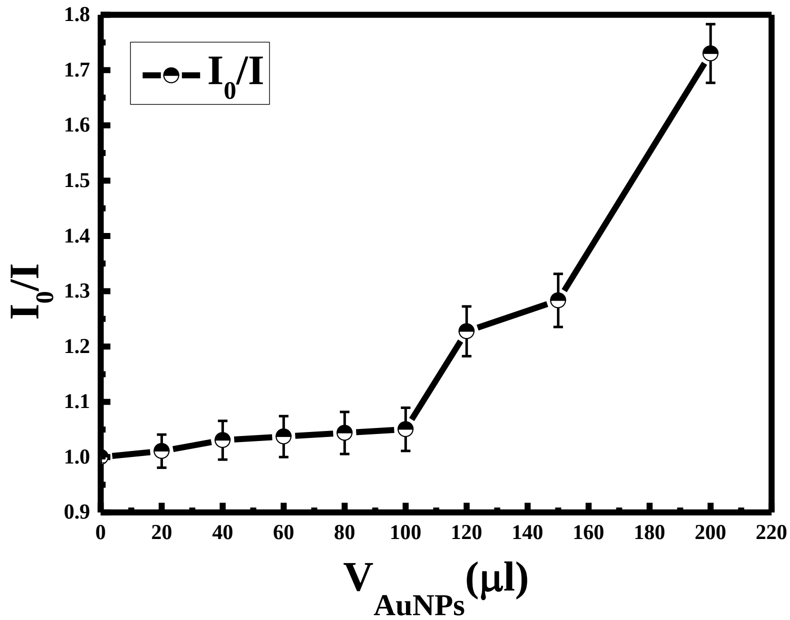


Figure 25. Linear correlation of I_0/I emission intensity of Rh. B-AuNPs (at excitation λ= 520 nm and emission at λ= 577 nm) vs. volumes AuNPs.

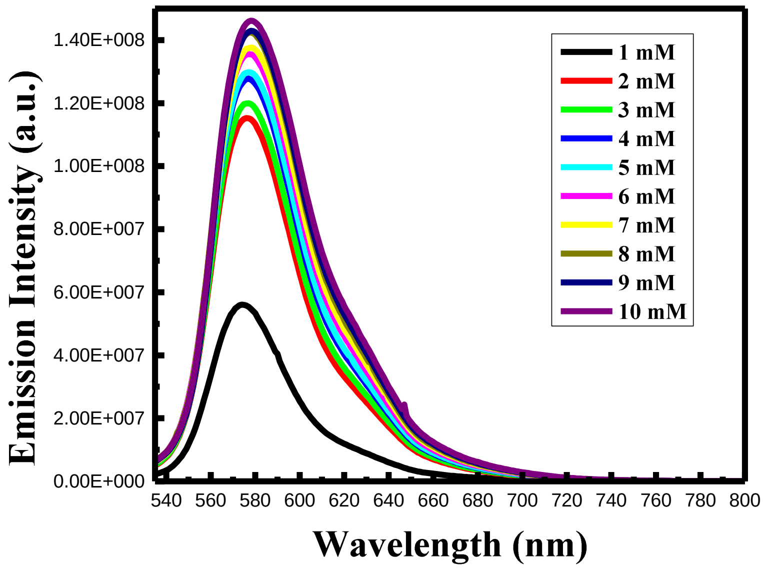


Figure 26. Emission spectrum excited at λ= 520 nm and emission at λ= 577 nm of AuNPs different concentration of Rh. B.

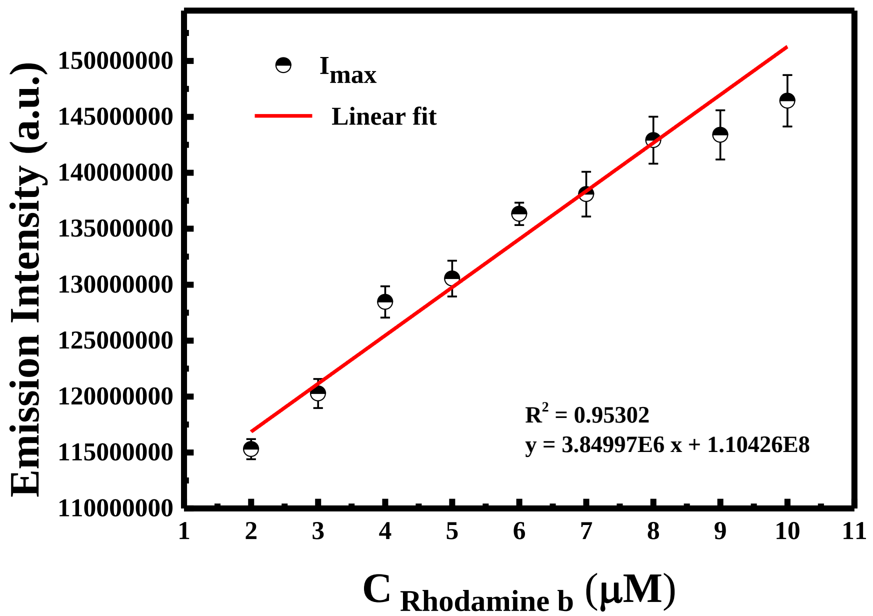


Figure 27. Linear correlation of maximum intensity of AuNPs at excitation λ= 520 nm and emission at λ= 577 nm vs. concentration of Rh. B.

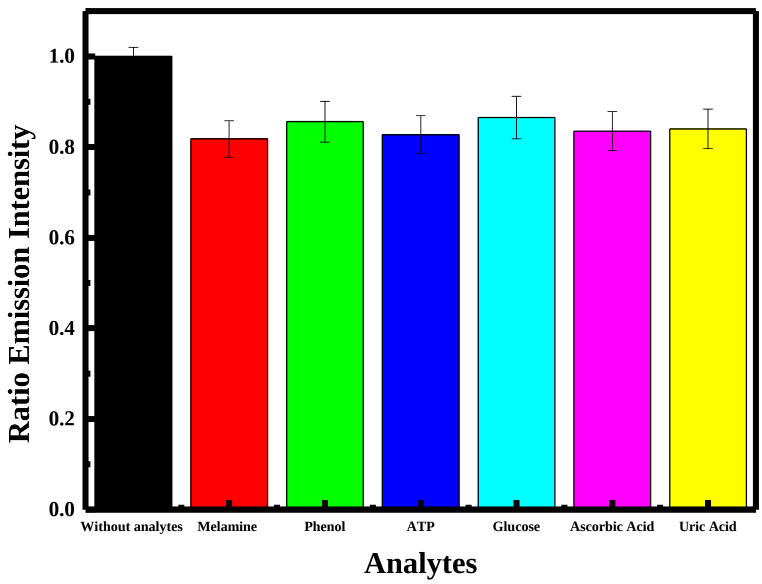


Figure 28. Ratio of emission intensity of AuNPs -Rh. B in the presence of various analytes at excitation at λ= 520 nm and emission at λ= 577 nm.

D. Conclusion

Successfully synthesized AuNPs using OSWE have yielded promising results. Notably, the negatively charged constituents such as carboxylate and hydroxyl groups from OSWE, which serve as stabilizers for AuNPs, have demonstrated their role in effectively positioning Rh. B in close proximity to the AuNPs. This proximity facilitates efficient Single Electron Transfer (SET) interactions between Rh. B and AuNPs [104]. Significantly, observations reveal a pronounced acceleration in quenching rates beyond 100 μL of AuNPs, with the optimal volume for maximal quenching of Rh. B being 200 μL. Moreover, elevating the concentration of Rh. B within a consistent volume of AuNPs has yielded a marked increase in the fluorescence intensity of Rh. B. Furthermore, the fluorescence intensity of AuNPs-Rh. B was not affected in the presence of uric acid, ascorbic acid, glucose, ATP, phenol, and melamine. These outcomes underscore the notable quenching potential of the synthesized AuNPs. This potential opens avenues for diverse applications in scientific technologies and analytical contexts. These applications include sensing and detection [107], fluorescence microscopy and imaging [108], exploration of molecular interactions [109], and analytical chemistry [110]. The implications of these findings underscore the valuable role of the produced AuNPs as a quenching tool, developed for exploitation across various scientific and technological domains.

CHAPTER VI

CONCLUSION

In summary, the synthesis of AuNPs using Origanum syriacum extracted plant (OSWE) was successfully achieved. The most stable and smallest AuNPs were formed in a 2:1 ratio (OSWE:$HAuCl_4$), displaying a negative zeta potential. This was attributed to the bioactive components from OSWE acting as stabilizing agents on the AuNPs' surface, leading to a negative charge. Furthermore, it was observed that the AuNPs exhibited catalytic properties in the complete reduction of CR, with a reduction mechanism five times faster than when $NaBH_4$ was present. The AuNPs were successfully recycled and reused as a catalyst for the reduction of CR, demonstrating their potential sustainability.

Another study focused on the free radical scavenging capacity of AuNPs using the DPPH assay. The average inhibition percentage was found to be 48.27%, with the highest inhibition at 62.1% observed for a sample containing 900 µl of AuNPs. These results indicated promising antioxidant activity of the AuNPs, suggesting potential applications in pharmaceuticals, cosmetics, skincare, health, nutrition, and even as anti-cancer agents.

Additionally, the quenching effect of the prepared AuNPs on the fluorescence intensity of Rh. B was investigated. Increasing the volume of AuNPs in a constant concentration of Rh. B resulted in a reduction of fluorescence intensity, with the most significant quenching occurring at 200 µl. Moreover, increasing the concentration of Rh. B while keeping the AuNPs concentration constant led to an enhancement in fluorescence emission intensity. This confirmed the quenching effect of AuNPs on Rh.

B intensity without any modifications to the AuNPs' surface and analytes such as uric acid, ascorbic acid, glucose, ATP, phenol, and melamine were not interfering in the fluorescence intensity. These findings highlight the multifaceted roles of the AuNPs produced by OSWE, serving as catalysts, antioxidants, and quenching agents. The potential applications span diverse scientific and technological fields, including sensing, detection, fluorescence microscopy, molecular interaction studies, and analytical chemistry. Overall, the synthesized AuNPs hold significant promise for advancements across various scientific and technological realms.

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