GREEN SYNTHESIS OF GOLD NANOPARTICLES AS PHOTOSENSITIZER FOR PHOTOTHERAPY BREAST CANCER TREATMENT

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by

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LIST OF SYMBOLS

µg	microgram
ml	Milliliter
mW	milliwatt
nm	nanometer
°C	degree Celsius
g	gram
cm	centemeter
m	meter
s	second
eV	electron volt
K	Kelvin
J	joule
V	volt
ms	millisecond
mJ	Millijoules
cm^2	square centimeter
W	Watt
mm	millimeter
λ	wavelength
Å	Angstrom
kV	kilovolts
mA	Milliamperes

θ	Theta
h,k,l	Miller indices
μA	Microampere
ζ	Zeta potential
m/z	mass to charge ratio
cm^2	Square centemeter
mg	milligram
P	Statistical probability of the hypothesis
mV	millivolt
μl	microliter

LIST OF ABBREVIATIONS

Ag	Silver
AgNPs	silver nanoparticles
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	One-way analysis of variance
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
ATR	attenuated total reflectance
Au	gold
$AuCl_4$	Tetrachloroaurate ion
AuNPs	gold nanoparticles
Be	Beryllium
C	Carbon
$C_6H_8O_7$	citric acid
Ca	Calcium
CMC	carboxy methyl cellulose
CO_2	Carbon dioxide
Cr^{3+}	Chromium (III)
CT	Computed tomography
CW	continuous wave
DC	Diclofenac,
DLS	Dynamic light scattering
DMEM	Dulbecco’s modified Eagle’s medium-high glucose

DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDGR	Potential of hydrogen
EPR	retention effect
ER	Oestrogen receptor
EDX	Energy dispersive X-ray
ESI	electron spectroscopy images
etc	et cetera
FBS	Fetal bovine serum
FCC	Face centered cubic
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
FWHM	Full width at half maximum
GC	gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GC-MS	Gas Chromatography-Mass Spectroscopy
$HAuCl_4$	chloroauric acid
$HAuCl_4 \cdot 4H2O$	Chloroauric acid
He	helium
He-Ne	Helium–neon
HEPA	high efficiency particulate air
HER2	Human epidermal growth factor receptor 2
IR	infrared
Hg	Mercury
IC_{50}	half maximal inhibitory concentration

K	potassium
LDA	Doppler anemometry
LILI	low-intensity laser irradiation
LLI	low-power laser
LLLT	low-level laser therapy
M/F	molecular formula
miRNAs	MicroRNA
MRI	magnetic resonance imaging
MS	mass spectrometry
MTS	5-(3carboxymethoxyphenyl)-2-(4,5-dimethylthiazoly)-3-(4-sulfophenyl) tetrazolium, inner salt
MTT	3-(4,5-dimethylthi-azol-2-yl)-2,5-diphenyltetrazolium bromide
Na(Acac)	sodium acetylacetonate
$NaBH_4$	sodium borohydride
NaOH	sodium hydroxide
Nd:YAG	neodymium-doped yttrium aluminum garnet
NIR	near infrared
NOS	nitric oxide species
O	Oxygen
OD	optical density
PBS	Phosphate-buffered saline
Pd	palladium
PDGF	Platelet-derived growth factor
PDI	polydispersity index

PEG	polyethylene glycol
PET	positron emission tomography
pH	Potential of hydrogen
PR	Progesterone receptor
PBM	photobiomodulation
Pt	platinum
PTT	photothermal therapy
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	Revolutions Per Minute
RT	Radiation therapy
RT	retention time
SD	standard deviation
SEM	scanning electron microscope
SERS	Surface-enhanced Raman scattering
siRNA	small interfering RNA
SPR	surface plasmon resonance
STM	scanning tunnelling microscope
TBI	traumatic brain injury
TEM	Transmission electron microscopy
TNF-α	Tumour necrosis factor α
TOAB	tetraoctylammonium bromide
U	Uranium
UV-Vis	Ultraviolet–visible spectroscopy
VEGF	Vascular endothelial growth factor

w/v	Weight per volume
WHO	World Health Organization
WST-1	(4-[3-4-iodophenyl]-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate
XPS	X-ray photoelectron spectroscopy
XRD	X-Ray Diffraction
XTT	2,3-bis (2-methoxy- 4-nitro-5-sulphophenyl)-5-carboxanilide-2H-tetrazolium
$(COOH)_2$	oxalic acid

SINTESIS HIJAU NANOZARAH EMAS SEBAGAI FOTOPEMEKA UNTUK RAWATAN FOTOTERAPI KANSER PAYUDARA

ABSTRAK

Kanser payudara adalah punca utama kematian di kalangan wanita, dan rawatan semasa seperti kemoterapi dan radioterapi boleh membawa kepada kesan sampingan jangka panjang yang teruk. Oleh itu, strategi alternatif adalah perlu. Kajian ini menyiasat penggunaan gabungan terapi laser peringkat rendah (LLLT) dan nanopartikel emas (AuNPs) untuk meningkatkan kesan hipertermia secara selektif dalam sel-sel kanser payudara. AuNPs telah disintesis melalui kaedah kos efektif yang mudah menggunakan ekstrak tumbuhan berair, bertujuan untuk mengurangkan isu ketoksikan dan penggumpalan. Kaedah sintesis hijau baru yang menggunakan daun *Coleus scutellarioides* (L.) Benth telah dibangunkan, dan pencirian menyeluruh telah dilakukan. Sitotoksisiti AuNPs yang disintesis telah dinilai menggunakan ujian MTT pada garisan sel kanser payudara manusia (MDA-MB-231) dan fibroblas kulup manusia (Hs-27), mendedahkan pengurangan dalam daya hidup sel kanser yang bergantung kepada dos dalam masa 24 jam. Selepas itu, kajian ini menyiasat kesan penyinaran laser inframerah berhampiran (NIR) 808 nm tahap rendah pada kedua-dua saluran sel, dengan dan tanpa AuNPs. Pelbagai parameter telah diterokai, dan kehadiran AuNP dengan ketara meningkatkan keberkesanan penyinaran laser NIR dalam menghalang pertumbuhan sel MDA-MB-231 yang berkemungkinan menjadi jalan rawatan yang berpotensi tinggi. Pendedahan sel Hs-27 kepada laser 300 mW untuk tempoh dua kali (10 dan 15 minit) membawa kepada daya hidup sel kurang daripada 50% berbanding dengan kesan kuasa dan masa laser lain yang digunakan.

GREEN SYNTHESIS OF GOLD NANOPARTICLES AS PHOTOSENSITIZER FOR PHOTOTHERAPY BREAST CANCER TREATMENT

ABSTRACT

Breast cancer is a significant cause of mortality among women, and current treatments like chemotherapy and radiotherapy can lead to severe long-term side effects. Therefore, alternative strategies are necessary. This study investigated the combined use of low-level laser therapy (LLLT) and gold nanoparticles (AuNPs) to enhance hyperthermia effects selectively in breast cancer cells. AuNPs were synthesized through a simple cost-effective method using aqueous plant extract, aiming to reduce toxicity and agglomeration issues. A novel green synthesis method utilizing *Coleus scutellarioides* (L.) Benth leaves was developed, and comprehensive characterization was performed. The cytotoxicity of synthesized AuNPs was evaluated using the MTT assay on human breast cancer (MDA-MB-231) and human foreskin fibroblast (Hs-27) cell lines, revealing a dose-dependent reduction in cancer cell viability within 24 hours. Subsequently, this study investigated the impact of low-level near infrared (NIR) 808 nm laser irradiation on both cell lines, with and without AuNPs. Various parameters were explored, and the presence of AuNPs significantly enhanced the effectiveness of NIR laser irradiation in inhibiting MDA-MB-231 cell growth, suggesting a promising treatment avenue. Notably, exposure of Hs-27 cells to a 300 mW laser for the double duration (10 and 15 minutes) led to less than 50% cell viability compared to the effects of other output laser powers and times used.

CHAPTER 1

INTRODUCTION

1.1 Overview

Cancer is a large group of diseases characterized by the uncontrolled growth of abnormal cells, which can originate in almost any organ or tissue within the body. Cancer is one of the top causes of mortality, accounting for around 10 million deaths in 2020 (Sung et al., 2021). Furthermore, according to the World Health Organization (WHO), it is expected to be more than three folds by the end of 2040; it is expected to increase by 27.5 million new cases and 16.3 million deaths due to population growth and aging (Ferlay et al., 2019). Cancer is responsible for the loss of life in one out of every six individuals globally, surpassing the combined fatalities caused by malaria, tuberculosis, and acquired immunodeficiency syndrome (AIDS) (Jemal et al., 2011). The work in the technical advancement of cancer treatment is focused on eliminating the side effects, enhancing the efficacy of the treatment and survival. However, applications commonly used in physics, such as lasers, can help develop an effective strategy for a cure. Laser device application within oncology presents promise for non-invasive therapeutic option for addressing breast cancer.

For more than 50 years, laser therapy or also known as LLLT, has been widely used (Ginani et al., 2015). Emerging in its modern form post the invention of the laser in 1960, laser therapy has evolved as a widely utilized treatment method across diverse clinical applications (Karu, 1989; Kreisler et al., 2003; Posten et al., 2005). Biostimulation and photobioactivation were frequently employed for the stimulation effect of low-level lasers (King, 1989; Wu et al., 2012). Subsequently, an inhibitory impact of this radiation was discovered, leading to the development of the concept of biomodulation (Schindl et al., 2000). However, photobiomodulation

(PBM) is also suggested as a more appropriate term, offering a particular definition for this light application and can confirm its scientific principle (Anders et al., 2015). The interaction of laser irradiation with biological tissues has attracted the interest of researchers (Jawad et al., 2011). The PBM effect of low-intensity laser irradiation (LILI) is based on the sensitivity of specific cellular components (chromophores) to the applied light photons. According to studies, after photon absorption by photoreceptors in the mitochondrial respiratory chain, cellular activity can be affected in one of the following ways: To mention a few, cell growth stimulation, anti-inflammatory response production, enhanced cell regeneration, and stimulation of long-term production of intracellular or extracellular reactive oxygen species (ROS) overload (Hamblin & Demidova, 2006; Kiro et al., 2017). These photobiological responses to LILI do not cause a significant increase in temperature. They depend on different factors, such as the intensity and wavelength of light used and the cell type. As a result, it can be defined as a non-thermal PBM technique that employs optical waves that usually correspond to visible red or NIR light and low fluencies to induce photobiological processes at the cellular level (Abrahamse, 2011; Crous & Abrahamse, 2016).

In the context of cancer therapy, a specific application of laser therapy revolves around exploiting the photothermal effect to precisely target and eliminate cancer cells. A prominent method involves the utilization of NIR lasers. In photothermal therapy (PTT), optical properties such as scattering, and absorption are crucial. Noble nanoparticles' conduction electrons exhibit strong resonant oscillations within the visible light frequency; thus, AuNPs absorb visible light strongly (Zeng et al., 2013). To increase penetration, the maximal absorption wavelength of nanoparticles must be in the NIR light range, which is between 600 to 1400 nm. The

absorption range might be altered because the surface plasmon resonance (SPR) band of noble nanoparticles is tunable. The unique property SPR of noble nanoparticles enhances optical absorption and scattering properties. The photothermal properties of AuNPs indicate that if AuNPs enter cancerous cells and are irradiated with a light source, they will cause photothermal effects. This AuNPs property has been studied to enhance PTT cancer treatment (Huang et al., 2007).

Nanotechnology-based therapeutic and diagnostic techniques have shown significant potential to enhance cancer therapy (Vickers, 2004). Cancer nanotechnology developed a new area of integrated study in chemistry, biology, engineering, and medicine concerned with significant advancements in cancer diagnosis, prevention, and treatment (Wang & Thanou, 2010).

1.2 Problem Statement

There were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. As of the end of 2020, 7.8 million women have been diagnosed with breast cancer in the previous five years, making it the most prevalent cancer globally (World Health Organization (WHO), 2020). Therefore, breast cancer has become a significant health concern, and appropriate breast cancer treatment is necessary.

Despite advancements in medical technology and cancer research, the burden of cancer persists, prompting the need for enhanced diagnostic and treatment methods. Although surgery, chemotherapy, and radiation therapy constitute the main therapeutic approaches, the concurrent use of radiation therapy and chemotherapy raises concerns about normal tissue toxicity, given the potential harm to healthy tissues (Herscher et al., 1999; Ma et al., 2003). Moreover, this not only complicates therapeutic outcomes but also adversely impacts patients' quality of life.

The challenges of conventional cancer therapies underscore the urgent need for more selective and less harmful treatment strategies. In this context, the field of nanomedicine has emerged as a highly promising area, offering innovative approaches to cancer treatment that could potentially minimize side effects while enhancing efficacy (Brannon-Peppas & Blanchette, 2004; Iancu et al., 2011; Jain, 2005; Jain et al., 2012; Llevot & Astruc, 2012; Thakor & Gambhir, 2013). Among the various strategies explored, the use of AuNPs combined with laser therapy has garnered significant attention. This combination leverages the unique properties of AuNPs such as their facile conjugation with antibodies, photostability, non-toxicity, and ease of fabrication (Zhao & Wu, 2010). In addition to the tunable optical resonance wavelength especially within the NIR range (Chen et al., 2005; Chou et al., 2005; Oldenburg et al., 1999), make them particularly suited for targeted PTT in breast cancer treatment.

Despite the potential of AuNPs in enhancing cancer treatment, the synthesis methods typically employed pose environmental and health risks, involving toxic chemicals and high energy processes (Sharma et al., 2015). This brings to light the critical need for developing safer, more sustainable methods for nanoparticle production. The biosynthesis of AuNPs using plant derived materials presents a promising solution, utilizing the natural reducing and stabilizing capabilities of phytoconstituents found in various plant parts such as stems, roots, flowers, and leaves. This green synthesis approach not only reduces environmental impact but also aligns with the principles of simple, rapid, eco-friendly technology and cost-effectiveness (Mohanpuria et al., 2008).

Hence, this study aims to explore the potential of utilizing a combination of laser therapy and biosynthesized AuNPs, synthesized through a simple and eco-

friendly method employing *Coleus scutellarioides* (L.) Benth leaves as a reducing and stabilizing agent, to provide another alternative for targeting photothermal destruction on MDA-MB-231 cells. This study will investigate whether such a novel therapeutic approach can provide a viable alternative treatment to conventional therapy, by offering localized treatment that could potentially result in fewer side effects, overcome treatment resistance, and improve early cancer detection.

1.3 Research objectives

The main objectives of this research are summarized in the points below:

1. To synthesis AuNPs by using *Coleus scutellarioides* (L.) Benth leaves extract via green synthesis method with different analytical techniques.
2. To evaluate the *in vitro* cytotoxicity effect of the synthesized AuNPs against MDA-MB-231 and Hs-27 cell lines by MTT assay.
3. To compare the effectiveness of low-level NIR laser of 808 nm wavelength with single and double irradiation in MDA-MB-231 and Hs-27 cell lines.
4. To study the potential cytotoxicity effect of the synthesized AuNPs combined with a low-level NIR laser of 808 nm wavelength on MDA-MB-231 and Hs-27 cell lines.

1.4 Scope of the study

This research aims to conduct a green synthesis of AuNPs using *Coleus scutellarioides* (L.) Benth leaves extract, resulting in AuNPs with an average particle size of 40.10 nm. Additionally, the study focuses on investigating the potential of LLLT using an 808 nm NIR laser at four different output powers: 25, 43, 120, and

300 mW. The irradiation process involves various exposure times of 1, 5, 10, and 15 minutes. Both single and double irradiation, separated by 5-minute intervals, are employed to assess their effectiveness in destroying MDA-MB-231 cells and compare the results with Hs-27 cells. The MDA-MB-231 and Hs-27 cells are subjected to different powers of the NIR laser and exposure times, both in the presence and absence of the synthesized AuNPs.

1.5 Organization of the thesis

This thesis is divided into five chapters, each addressing specific aspects of the research. Chapter 1 serves as the introduction, presenting the study's problem statement, objectives, and aims. In Chapter 2, a comprehensive literature review is conducted, covering the properties of AuNPs, different synthesis methods, and the theoretical background of AuNPs in nanomedicine. The chapter also explores different laser applications in therapy, discussing their mechanisms and types. It also includes previous and current reviews on laser and nanoparticle treatments, with a particular focus on *in vitro* and *in vivo* studies.

Chapter 3 details the experimental procedures and characterization techniques employed for AuNP synthesis using *Coleus scutellarioides* (L.) Benth leaves extract as a reducing agent. The step-by-step synthesis process and characterization methods to confirm the formation and properties of the AuNPs are thoroughly described. The chapter also outlines the protocols for conducting biological studies using cell culture to evaluate the cytotoxicity of the synthesized AuNPs. It explains the methods for culturing different cell lines and assessing the effects of AuNPs on cell viability. Moreover, it compares the effects of 808 nm NIR laser with different powers and exposure times.

Moving on to Chapter 4, the specific results obtained throughout the study are highlighted and discussed. This includes the characterization of the synthesized AuNPs using *Coleus scutellarioides* (L.) Benth leaves extract, encompassing their physical and chemical properties. The chapter also examines the cytotoxicity of AuNPs on different cell lines and their potential for phototherapy using laser irradiation. Finally, Chapter 5 concludes the thesis by summarizing the key findings and their implications. It suggests future research directions and areas that could benefit from further investigation based on the outcomes of this study.

CHAPTER 2

LITERATURE REVIEW

2.1 A brief overview of nanoparticles

The concept of "smallness" had already developed since its inception on December 29, 1959, when Nobel Prize winner in Physics Richard Feynman presented the speech titled "There's Plenty of Room at the Bottom" at an American Physical Society conference at Caltech (Feynman, 2018). Dr. Feynman described a process by which individual atoms and molecules could be manipulated, serving as potential candidates for future innovation and development. Nanotechnology refers to the study of matter on the atomic, molecular, and supramolecular levels (size of 1-100 nm) with the aim of understanding, creating, and using materials, structures, devices, and systems with fundamentally new properties and functions due to their small structure (Roco, 2003).

Oxford English Dictionary defines nanotechnology as a technology field concentrating on precision and measurements smaller than 100 nanometers. This field specifically involves the manipulation of individual atoms and molecules. The inception of modern nanotechnology went back to the creation of the scanning tunnelling microscope (STM), which has the ability to observe individual atoms. This breakthrough invention paved the way for the development of nanotechnology.

The unique characteristic of nanotechnology lies in the considerable variation of physicochemical properties exhibited by nanoscaled materials compared to bulk materials of similar composition. Bulk gold, for example, is considered to be inert and unreactive as a catalyst. However, AuNPs show exceptionally high catalytic reactivity in a wide range of procedures, including carbon monoxide and alcohol oxidation in the gas phase (Haruta et al., 1987). Another example of nanomaterials

with different properties than their bulk counterparts is non-magnetic bulk materials such as platinum (Pt), gold (Au), and palladium (Pd) nanomaterials embedded in polymer, which exhibit magnetic moments at the nanometric size of nanomaterials but are non-magnetic in bulk counterparts (Nakae et al., 2000; Yamamoto et al., 2003). This variation in physicochemical properties can be attributed to two factors: (1) the surface effect of nanomaterials, where the fraction of atoms at the surface has fewer adjacent atoms than their bulk counterpart, and (2) quantum effects that show discontinuous behaviour, which is attributable to the completion of shells in systems by delocalized electron (Roduner, 2006).

2.2 The role of nanotechnology in medicine

Nanoparticles, ranging in size from 1 to 100 nm, possess distinct properties that make them highly suitable for investigating and controlling a wide range of biological processes. These minuscule materials are essentially commensurate with biomacromolecules such as proteins and deoxyribonucleic acid (DNA), making them promising candidates for mimicking the structure and function of individual proteins, an endeavor that is challenging to achieve using traditional "small molecule" systems (Saha et al., 2011; You et al., 2007). Furthermore, the nanoparticles' high surface-to-volume ratio, along with the facile tuning of surface properties, offers effective surface area and creates receptors in a highly divergent fashion. Given this foundation, several nanomaterials have been employed in a wide range of highly innovative applications, including chemical/biological sensing (Saha et al., 2012; So et al., 2005), imaging (Agasti et al., 2010; Hahn et al., 2011), cancer treatment (Kennedy et al., 2011; Lal et al., 2008), and delivery of drugs (Mura et al., 2013; Yezhelyev et al., 2008). In recent times, the use of engineered nanoparticles for

delivering antibiotics has become an emerging and realistic area of research (Fang et al., 2014; Zhao et al., 2010). Nanoparticle-based antibiotic-carriers have been engineered to overcome antibiotic resistance that was developed with the use of traditional antibiotics (Duncan et al., 2015). With the advancement of these new technologies, the interface between nanomaterials and biological systems has merged into a new science concerned with the safe use of nanotechnology and nanomaterial design for biological purposes. The study of nano-bio interactions enables the establishment of predicted relationships between structure and activity that are affected by the physicochemical characteristics of nanomaterials. Furthermore, nanoparticles show particular promise in the targeted delivery of antineoplastic drugs/agents to cancerous tissues. Nanoparticles can penetrate deep into the tissues and deliver drugs to specific targeted sites, making them an attractive option for treating cancer (Telrandhe, 2019). Nanoparticles have been shown to increase the intracellular concentration of drugs in cancerous cells through active or passive targeting while minimizing toxicity to normal cells (Nguyen, 2011).

Moreover, as a targeted drug delivery system, nanoparticles have also been engineered to respond to changes in temperature or pH, allowing for more precise and controlled drug release. As a temperature-sensitive drug delivery system, these nanoparticles may deliver and release drugs in the tumour region by undergoing local temperature changes through ultrasound waves or magnetic fields. Nanoparticles can also be designed to respond to changes in pH, making them efficient carriers for delivering drugs to the acidic environment of cancerous cells (Saad et al., 2008). Furthermore, these nanoparticles can be modified with specific targeting moieties, such as antibody fragments, antibodies, specific molecules, ribonucleic acid (RNA)

aptamers, and small peptides, to enhance their ability to selectively bind to cancerous cells and tissues (Subbiah et al., 2010).

The field of cancer nanotechnology has opened up a new area of integrative research in spans biology, chemistry, engineering, and medicine, with a focus on achieving significant advancements in cancer detection, diagnosis, prevention, and treatment (Huang et al., 2007; Wang & Thanou, 2010).

On the other hand, the development of new blood vessels, or angiogenesis, is crucial to the progression of a tumour toward metastasis. The abnormal membrane structure of cancer cells is a result of the upregulated expression of angiogenic factors, which leads to enhanced blood vasculature (Day et al., 2009; Trédan et al., 2007). This dysregulated membrane architecture can be of great interest to delivering anti-angiogenic nano-based targets into the tumour microenvironment to inhibit the excess production of angiogenic stimulators (Abdalla et al., 2018). Due to this therapy's effectiveness has been reported in several studies to block the signaling of VEGF, PDGF, EDGR, and angiopoietin key contributors of neovascularization (Jain, 2005). Nano anti-angiogenic therapy could represent an effective delivery approach for drugs with poor oral availability, short half-life, as well as distribution throughout the tumour area (Day et al., 2009). At the same time, the nanoparticles can penetrate the tumour microenvironment and deliver antiangiogenic drugs effectively, depending on their size. The enhanced permeability and retention effect (EPR) allows nanoparticles of the appropriate size to approach metastasized tumours and release the loaded drugs efficiently (Abdalla et al., 2018).

In human clinical trials aimed at treating various types of cancer, colloidal AuNPs containing tumour necrosis factor-α (TNF-α) were used (Visaria et al., 2006). The use of nanoparticles as a delivery system has resulted in improved delivery of

TNF-α to cancerous tumours, as demonstrated by clinical studies. Furthermore, this approach has managed to avoid the systemic toxicities that typically limit the clinical usefulness of this biologic agent (Farma et al., 2007). Many studies have been conducted to explore the use of targeting agents conjugated with nanoparticles to deliver therapeutic consignment to cancerous cells (Opanasopit et al., 2008; Richard et al., 2008). Additionally, carbon nanotubes have been utilized to deliver genes or proteins to cancer cells through non-specific endocytosis (Kam et al., 2006).

Accumulating to the therapeutic practices, recent research has revealed that magnetic iron nanoparticles can significantly improve the diagnostic capabilities of magnetic resonance imaging (MRI) compared to the existing contrast agents currently used for imaging cancer patients (Bárcena et al., 2008; Kou et al., 2008). Furthermore, by conjugating iron magnetic nanoparticles with antibodies that specifically target proteins articulated on the surface of human cancer cells, the accuracy of MRI in diagnosing early-stage cancer could be further enhanced (Neumaier et al., 2008). In preclinical models, a study was conducted to investigate the potential of carbon or polymeric nanoparticles labelled with fluorine-18 deoxyglucose for enhancing tumour diagnosis and improving detection rates using positron emission tomography (PET) (Liu et al., 2007; Matson & Grubbs, 2009). The researchers are currently studying the emission of fluorescence on excitation with the proper light wavelength from the semiconductor nanocrystals of the surface modification of quantum dots. This investigation aims to improve the detection of lymph nodes and other regions of metastases during surgical procedures (Misra, 2008; Zhang et al., 2008).

2.3 AuNPs and properties

Gold has unique properties that have fascinated humans since its discovery. Its unreactive nature means it does not tarnish in the atmosphere, allowing it to maintain its attractive colour indefinitely (Hutchings et al., 2008). This characteristic has made gold a popular material for crafting jewellery, other decorative, ceremonial, and religious artifacts and metal of high monetary value. The use of gold colloids to create colourful aqueous solutions dates back to Roman times, and these solutions were known to medieval alchemists as "aurum potabile" (Mellor, 1923). The Lycurgus cup, a Roman cup, utilized nanosized (ca 50 nm) gold and silver alloys, along with some Cu clusters, to create different colours depending on the illumination angle.

The origin of a certain effect was not understood by its users until Michael Faraday discovered that it was caused by the incredibly small size of gold particles (Faraday, 1857). In 1857, during the Bakerian Lecture of the Royal Society in London on February 5th of that year, Faraday discussed the "Experimental Relations of Gold (and other metals) to Light" and noted that alterations in particle size could result in a range of colours, as seen in the ruby glass. Decades later, examination with electron microscopes revealed that Faraday's fluid preparations had particles with an average diameter of 6 ± 2 nm (Turkevich et al., 1951). While some consider this experiment a breakthrough in the history of nanoscience and nanotechnology (Edwards & Thomas, 2007), gold's chemical inertness as a bulk metal appeared to provide possibilities for new and exciting chemistries (Hutchings et al., 2008).

In the 21st century, gold chemistry has progressed significantly, with a solid foundation in preparing and characterizing various fundamental compounds featuring gold atoms and clusters as core units (Murray, 2000). Extensive study of AuNPs

across numerous scientific domains has yielded a profound understanding of the physio-chemical characteristics that govern their behaviour and has resulted in the development and testing of reliable new methods for preparing, synthesizing, and characterizing AuNPs of nearly any desired size and shape. One characteristic shared by many nanostructures is a lower melting point compared to their bulk counterparts. This is due to the fact that the transition from solid to liquid initiates at the interfaces (Amendola, 2008). For instance, the melting point of gold decreases by roughly 20°C as it transitions from bulk to 20 nm and by around 400 °C as it shifts from 20 nm to 5 nm (Burda et al., 2005). Among the most prominent changes in the properties of gold, as summarized in (Table 2.1) is its shift in colour from yellow at the bulk scale to purple-red at the nanoscale. This occurs as a result of the red shift of the plasmon band to visible frequencies; the “Surface Plasmonic” theory explains that. According to the theory, when an electromagnetic field hits the surface of AuNPs, the free electrons on the surface oscillate back and forth, resulting in a plasmon band with an absorption peak ranging from 530-540 nm, as shown in Figure 2.1. This phenomenon is due to the presence of six free electrons in the conduction band on the surface of the AuNPs. As the size of spherical AuNPs increases, the plasmon absorption red shift also increases, leading to a change in colour from red to purple of the AuNPs.

Table 2.1: Bulk properties of gold (Louis & Pluchery, 2017)

No.	Bulk Properties of Gold	Magnitude
1	Group of chemical elements	11
2	Electronic configaration	[Xe] $4f^{14}5d^{10}6s^{1}$
2	Atomic number	79
3	Atomic mass	196.966 a.u
4	Structure	fcc
5	Metallic radius	0.14420 nm
6	Density	19.32 g cm^{-3}
7	Melting temperature	1337K
8	Sublimation enthalpy	343 ± 11 kJ mol^{-1}
9	1st ionisation energy	890 kJ mol^{-1}
10	Electrical resistivity at 293 K	2.35 μΩ cm
11	Interband transition	5d → 6s
12	Threshold energy	1.84 eV
13	Interband Wavelength	674 nm

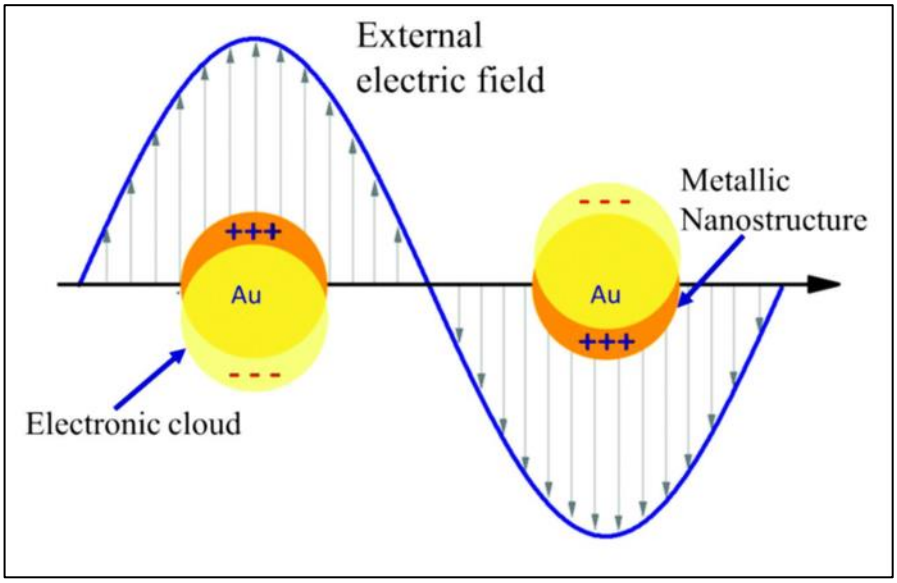

Figure 2.1: Oscillation of electron clouds caused by the interaction with electromagnetic waves on AuNPs (Choi et al., 2011)

2.4 Synthesis of AuNPs

In the field of metal nanoparticle generation, particularly AuNPs, many techniques have been developed and utilized. These techniques have rapidly advanced in recent decades and are continuously evolving, resulting in better control over the size and shape of the particles produced. As a consequence of these advancements, nanoparticle preparation methods have significantly improved. Regardless of the field or discipline, two distinct approaches have emerged for the controlled generation of nanostructures (Shenhar & Rotello, 2003). The first method is the bottom-up approach, which involves assembling atoms produced from ion reduction to generate nanostructures through chemical means. The second approach is the top-down method, also known as the physical method, which involves removing material from the bulk substance through processes such as grinding, milling, chemical techniques, or volatilization of solid material. This is followed by the condensation of the vaporized components, resulting in the desired nanostructures.

The bottom-up and top-down approaches can be employed in various states such as gas, liquid, supercritical fluids, solid, or vacuum. Manufacturers are typically interested in controlling one or more of the following aspects of nanoparticles: particle size, particle shape, size distribution, particle composition, and degree of particle agglomeration. Preventing aggregation and coalescence is crucial in both the bottom-up and top-down approaches to nanoparticle generation (Ju-Nam & Lead, 2008). Stabilization can be achieved through various means, but primarily by electrostatic repulsion or steric hindrance.

The control of nanocrystal size and the production of narrow size distributions are two critical challenges in nanocrystal synthesis (Shields et al., 2010). The production of small, monodisperse nanoparticles is a significant challenge in nanotechnology research because they tend to aggregate due to increased driving forces that reduce surface energy. To prevent this, it is essential to use a protective coating or capping during synthesis, which keeps the particles in a finely dispersed state (Sardar et al., 2009).

The bottom-up process is the prevalent and efficient approach in nano-science and nanoengineering (Sardar et al., 2009). This method has gained popularity due to its several potentially appealing advantages, including experimental simplicity extending down to the atomic size scale, the ability for three-dimensional assembly, and the potential for cost-effective mass production (Brust & Kiely, 2002). The most commonly used and straightforward bottom-up approach for producing AuNPs with varying sizes involves the reduction of Au (III) salt, typically chloroauric acid ($HAuCl_4$), by sodium citrate in water. This technique was first introduced by Turkevich and colleagues in 1951 (Turkevich et al., 1951), and later improved upon

by Frens in the 1970s (Frens, 1973). Kumar and co-workers have advanced this method more recently (Kumar et al., 2007).

It is widely acknowledged that the reduction of $AuCl_4$-ions to atomic Au occurs first, causing the concentration of Au to rapidly reach the supersaturation level. The collision of these Au atoms leads to a sudden burst of nuclei formation, signifying the onset of the nucleation stage. The attachment and coalescence of these nuclei then drive the growth and formation of the desired nanoparticles (Pong et al., 2007). The reduction and nucleation phases occur rapidly, in under 200 ms. However, the growth stage is comparatively slower, making it the rate-limiting step. Controlling the nucleation and growth stages, which are intermediate steps in the particle formation process, can be challenging and may often result in a wider distribution of particle sizes (Belloni, 1996). When various reactive polymers with functional groups are present in the reaction medium, the growing metallic particles are stabilized through the adsorption of the polymer chains onto the metal surface. This reduces the surface energy and creates a barrier to further aggregation (King et al., 2003).

Both top-down and bottom-up assembly methods have their own advantages. Top-down methods are currently superior for the possibility of interconnection and integration, as in electronic circuitry. In contrast, bottom-up assembly is very powerful in creating identical structures with atomic precision, such as the supramolecular functional entities in living organisms (Puolamaa, 2006). Moreover, these two methods can be combined to achieve materials with specific physicochemical properties (Xu et al., 2006).

2.4.1 Turkevich method

The synthesis of AuNPs is typically accomplished using a method that was initially described by John Turkevich and colleagues in 1951. In this method, the formation kinetics of AuNPs are examined by reducing $HAuCl_4$ using citric acid ($C_6H_8O_7$) (Turkevich et al., 1951). Later, in the 1970s, Frens improved on Turkevich's method and developed a technique to synthesize monodisperse spherical AuNPs in water, with particle sizes ranging from 10 nm to 20 nm (Armbrüster & Cardoso-Gil, 2015; Cativo et al., 2014). Bigger particles can be synthesized by reducing the amount of sodium citrate, possibly down to 0.05%, and the reduction of gold may not be complete. This process can potentially result in the aggregation of smaller particles, leading to the formation of larger particles until the available citrate ions coat the entire surface area of all the particles (Kuo et al., 2004). Stabilizing agents are commonly used in nanoparticle synthesis to regulate particle growth and prevent aggregation. By employing a suitable stabilizing agent, the surface energy of newly formed nuclei can be stabilized, effectively preventing them from binding with other nuclei (Niu & Li, 2014). Among the various methods for synthesizing spherical AuNPs, the Turkevich method remained the simplest method (Frens, 1973; Herizchi et al., 2016; Kuo et al., 2004; Wuithschick et al., 2015).

2.4.2 Brust method

During the 1990s, Brust and Schiffrin introduced a novel technique for synthesizing AuNPs in organic liquids, which are typically immiscible with water, such as toluene via ultrasonic techniques (Kettemann et al., 2016; Shi et al., 2017). This approach involves the use of ultrasonic techniques and entails reacting $HAuCl_4$ with tetraoctylammonium bromide (TOAB) and a sodium borohydride ($NaBH_4$) solution in toluene. TOAB functions as an anti-coagulant, while $NaBH_4$ serves as the

reducing agent. The AuNPs synthesized through this method have a size range of 5 to 6 nm. However, the TOAB used in the synthesis process does not form a stable bond with the AuNPs, which causes the solution to aggregate within two weeks. A strong binding agent such as thiol, particularly alkanethiols, can be added to the solution to prevent this effect. Adding thiol yields a virtually permanent solution (Brust et al., 1994; Pol, 1989). Effectively avoiding the issue of aggregation. Preventing the aggregation of AuNPs can be achieved by precipitating and dissolving alkanethiols. When alkanethiols react with gold, they form strong gold-sulfur bonds, which is facilitated by phase transfer catalyst activities (Eckelman et al., 2008). However, this approach may have an impact on the physical properties of the particles, such as solubility. To avoid this effect, purification of the nanoparticles can be carried out using Soxhlet extraction.

2.4.3 Perrault method

In 2009, Perrault and Chan discovered a novel technique for synthesizing AuNPs within an aqueous solution. Their method involved using hydroquinone to reduce $HAuCl_4$ and utilizing 15 nm AuNPs seeds to produce nanoparticles with a size range between 30 to 300 nm (Perrault & Chan, 2009). The procedure was based on the photographic film development process, where the addition of reduced silver onto their surface led to the development of silver grains within the film. Similarly, the reduction of Au^+ within the surface of AuNPs can be stimulated by reacting with hydroquinone. The hydroquinone-based method has superseded the technique introduced by Frens (Frens, 1972; Frens, 1973) by allowing for a broader range of nanoparticle diameters to be synthesized. While Frens' method is most suitable for nanoparticles ranging from 12 to 20 nm in size, adding citrate as a capping agent

controls the removal of Au atoms from the particles and governs their growth into nanoparticles.

2.4.4 Martin method

The production of nearly monodisperse and reproducible stable AuNPs in water can be achieved through the regulation of the reduction stoichiometry of the gold precursor using sodium hydroxide (NaOH) and $NaBH_4$ ions. This process occurs at an elevated temperature range and results in particles with sizes ranging from 3 to 6 nm (Martin et al., 2010). The chemicals used in this method are similar to those used in the Brust method but without the use of TOAB (Brust et al., 1994). The reproducible colloidal nanoparticles that possess hydrophilic functionalities coated on their surface find useful applications in non-polar solvents owing to their reproducible nature. These nanoparticles can self-assemble into two-dimensional monolayer films on liquid droplets at the air-toluene interface.

2.4.5 Navarro method

Navarro and his team utilized a modified Turkevich-Frens method to produce spherical AuNPs of varying sizes, ranging from 13 nm to 90 nm. Sodium citrate and sodium acetylacetonate (Na(Acac)) were employed as capping and reducing agents, respectively. The solution was subjected to intense stirring at high temperatures, reaching a boiling point. The formation of AuNPs was observed by a rapid colour change from yellow to red in the initial solution, and the size of the AuNPs was controlled by adjusting the amount of Na(Acac) used (Navarro et al., 2013).

2.5 Green chemistry

The development of green chemistry has gained significant attention in recent years as an alternative to traditional chemistry, primarily due to the energy crisis and its limitations, particularly in developing nations. This has led to substantial efforts in interdisciplinary scientific fields (Andraos & Matlack, 2022). Concurrently, the emergence of nanometer-sized technologies has given rise to a novel field of nanoscience comprising nanobiotechnology and nanotechnology. This field revolves around creating and utilizing materials at the nanometer scale to advance biotechnology. Integrating nanoscience in green chemistry has presented several opportunities by facilitating various chemical, physical, biochemical, and biophysical transformations in a reliable and efficient manner. The use of nanoparticles has made catalysis, synthesis, enzyme immobilization, and molecular interactions more manageable, rapid, and controlled (Dwivedi et al., 2015). The hierarchical organization of atoms or molecules is prominently observed in biological systems and agents, prompting researchers to explore the use of various biological agents as potential cell factories for producing nanomaterials (Gardea-Torresdey et al., 1999; Kasthuri et al., 2009; Singaravelu et al., 2007; Smitha et al., 2009). The use of hazardous chemicals in nanoparticle and nanomaterial synthesis has been found to pose risks of exposure to these materials through inhalation, ingestion, or dermal contact, as demonstrated by several studies (Maynard, 2000; Maynard et al., 2004; Tsai et al., 2009). Consequently, these methods are considered unsuitable for the economic and environmentally friendly synthesis of nanoparticles (Logeswari et al., 2013; Makarov et al., 2014; Verma & Mehata, 2016). To address these concerns, researchers are actively developing new strategies to generate nanoparticles without toxic chemicals. These methods prioritize green chemistry principles, including using

rapidly biodegradable reagents, minimizing waste production, synthesis at ambient temperature and pressure, and low toxicity of chemical products (Anastas & Warner, 1998). Thus, green chemistry refers to a collection of principles and practices that promote the development of products and processes which minimize or eliminate the use of harmful substances (Luque & Varma, 2012; Virkutyte & Varma, 2011). Biological methods which is also referred to as green synthesis, utilize plant extract (Akintelu & Folorunso, 2019; Akintelu et al., 2019), algae (González-Ballesteros et al., 2019), mushrooms (Owaid, 2019), bacteria (sulphate-reducing bacteria) (Assunção et al., 2016) and truffles (Owaid et al., 2018). Furthermore, the unique optical (Królikowska et al., 2003) and chemical properties (Kumar et al., 2003) exhibited by metallic nanoparticles synthesized by biological entities make their production particularly intriguing. Biologically synthesized nanomaterials have significant potential applications in various fields, such as treatment, development of surgical nanodevices, diagnosis, and manufacturing of commercial products (Bar et al., 2009). Also, Nanomedicine has made remarkable progress in treating various chronic diseases in the healthcare sector. Therefore, the eco-friendly synthesis of nanoparticles is considered a crucial element in controlling different diseases in future generations (Cruz et al., 2010). Achieving the biogenic synthesis of nanoparticles with specific shapes and sizes has been a significant challenge in biomaterial science, but it offers numerous advantages in pharmacology for the treatment of various bacterial and viral diseases (Song & Kim, 2008).

2.6 Plant extracts-based methods

In the green synthesis methods utilizing plant extracts, various plant parts such as leaves, roots, petals, fruits, and peels are used either in their fresh or dried form to prepare the extract. The plants are washed with distilled water, dried, and then cut into small pieces or ground into powder before being boiled in a universal solvent such as distilled water to extract the desired compounds. The extract is then further purified using techniques like filtration or centrifugation to obtain the reducing and capping agents required for reducing metallic ions. Dried plant material is recommended as it can be stored at room temperature for extended periods, while fresh plant material must be stored at -20 °C to prevent deterioration. Using dried samples also helps eliminate any variation in plant constituents due to seasonal effects (Huang et al., 2007; Sheny et al., 2011).

In addition, the crude extract obtained from plants contains unique secondary metabolites, such as phenolic acid, flavonoids, alkaloids, and terpenoids, which play a key role in reducing metal ions into metallic nanoparticles (Aromal & Philip, 2012). These natural compounds participate in the redox reaction and are responsible for synthesizing eco-friendly nanosized particles. External stabilizing or capping agents are not needed in this method, as the phytochemicals present in the plant extract serve as both reducing and stabilizing agents. The reaction mixture is left to incubate, causing the metal salt to be reduced, and the colour change is visually monitored. Ultimately, this leads to the formation of the nanoparticles. The biogenic reduction and stabilization of metal ions into base metal is a fast process that can be easily conducted at ambient temperatures and pressure. It is also cost-effective, environmentally friendly, and safe for clinical research, making it an ideal method for scaling up the production of metal nanoparticles (Ikram, 2015).