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**DEPARTMENT OF NANOSCIENCE AND NANOTECHNOLOGY**



**ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC  
ACTIVITIES OF CHRYSIN-CONJUGATED GOLD  
NANOPARTICLES AS DRUG DELIVERY SYSTEM**

Master's Thesis

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## ACCEPTANCE AND APPROVAL OF THE THESIS

This study entitled “ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF CHRYSIN-CONJUGATED GOLD NANOPARTICLES AS DRUG DELIVERY SYSTEM” prepared by **Ahmed JAMAL** and supervised by **Assoc. Prof. Dr. Hilal AY** was found successful and unanimously accepted by committee members as Master thesis of the Department of Nanoscience and Nanotechnology, following the examination on the date 24.06.2022.

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## ÖZET

### İLAÇ İLETİM SİSTEMİ OLARAK KRİSİN-YÜKLÜ ALTIN NANOPARTİKÜLLERİNİN ANTIMİKROBİYAL, ANTİOKSİDAN VE SİTOTOKSİK AKTİVİTESİ

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Krisin (5,7-dihidroksiflavon), birçok bitki özütünde, bal ve propoliste yüksek konsantrasyonlarda bulunan doğal bir flavonoid bileşiktir. Krisin bileşiğinin antimikrobiyal, antioksidan ve antikanser gibi birçok biyolojik ve farmakolojik aktivitesinin bulunduğu bilinmektedir. Ancak, biyolojik olarak erişilebilirliği ve düşük çözünürlüğü nedeniyle, bu bileşiğin terapötik potansiyelinden tam olarak yararlanabilmek için bir ilaç iletim sistemi gerekmektedir. Bu çalışmada chrysin, kimyasal sentez yoluyla sodyum borohidritin ( $\text{NaBH}_4$ ) indirgeyici ajan olarak kullanılmasıyla sentezlenen altın nanopartiküllerine yüklenmiştir. Krisin-yüklü altın nanopartiküllerinin fizikokimyasal karakterizasyonu UV-Vis spektrometri, alan salım taramalı elektron mikroskopisi (FESEM), Fourier dönüşümlü kızılötesi spektroskopisi (FTIR), X-ışını difraksiyonu (XRD), enerji-dağılımlı X-ışını spektrumu (EDX) ve zeta potansiyeli analizleri ile gerçekleştirilmiştir. Krisin-yüklü altın nanopartiküllerinin absorpsiyon spektrumu 550 nm'de ölçülmüştür. FTIR analizi, krisin-yüklü altın nanopartikülleri üzerindeki fonksiyonel ve moleküler grupları belirlemek için yapılmıştır. XRD ve FESEM analizleri ile krisin-yüklü altın nanopartiküllerinin düzenli küresel biçimde ve ortalama 30-50 nm boyutunda olduğu doğrulanmıştır. Krisin-yüklü altın nanopartiküllerinin antioksidan aktivitesi DPPH süpürme testi ile analiz edilmiş olup krisin-yüklü altın nanopartiküllerinin krisin veya altın nanopartiküllerine kıyasla daha güçlü antioksidan aktiviteye sahip olduğu gösterilmiştir. Altın nanopartiküllerinin, krisin ve krisin-yüklü altın nanopartiküllerinin antimikrobiyal aktivitesi de *Escherichia coli* ve *Staphylococcus aureus*'a karşı test edilmiştir. Krisin-yüklü altın nanopartiküllerinin antimikrobiyal aktivitesinin krisin ve altın nanopartiküllerinin antimikrobiyal aktivitesinden daha yüksek olduğu görülmüştür. Ayrıca, insan meme kanseri hücre hattına (AMJ13) karşı altın nanopartiküllerinin, krisinin ve krisin-yüklü altın nanopartiküllerinin sitotoksik aktivitesi değerlendirilmiştir. Krisinin tek başına AMJ13 hücrelerine karşı daha az toksik olduğu ve altın nanopartiküllerinden daha az etkili olduğu tespit edilmiştir. Diğer taraftan krisin-yüklü altın nanopartiküllerinin AMJ13 hücrelerine karşı daha toksik olduğu görülmüştür. Sonuç olarak, krisin-yüklü altın nanopartiküllerinin ilaç iletim uygulamaları için nano-formulasyon olarak kullanılma potansiyeli oldukça yüksektir.

**Anahtar Kelimeler:** Altın nanoparçacıklar, Krisin, Antioksidan, Antibakteriyel, Sitotoksikite, AMJ13 hücreleri

## ABSTRACT

### ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF CHRYSIN-CONJUGATED GOLD NANOPARTICLES AS DRUG DELIVERY SYSTEM

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Chrysin (5,7-dihydroxyflavone) is a naturally distributed flavonoid found in high concentrations in many plant extracts, honey, and propolis. It is known that chrysin has many biological and pharmacological activities such as antimicrobial, antioxidant and anticancer. However, due to its bioavailability and low solubility, a delivery system is necessary to achieve the full therapeutic potential of chrysin. In this study, chrysin was loaded on gold nano-particles (CHR-AuNPs) prepared via chemical syntheses using sodium borohydride ( $\text{NaBH}_4$ ) as reducing agent. Physicochemical characterization of chrysin-loaded Au NPs (CHR-AuNPs) has been achieved with the use of the UV-Vis spectroscopy, field emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy dispersive X-ray (EDX) spectrum, and zeta potential analyses. The absorption spectra regarding chrysin on AuNPs showed a curved absorption at 550 nm. FTIR was utilized for evaluating the functional and molecular groups of chrysin, which were loaded onto gold nanoparticles. XRD and FESEM studies confirmed the presence of chrysin loaded on gold nanoparticles with a regular spherical shape and 30-50 nm average size. The anti-oxidant activity of CHR-AuNPS has been tested with the use of the DPPH scavenging assay, and the results revealed that CHR-AuNPs showed a stronger antioxidant effect compared to chrysin or AuNPs. The antimicrobial activity of AuNPs, chrysin and CHR-AuNPs against *Escherichia coli* and *Staphylococcus aureus* was also examined. The anti-microbial activity of CHR-AuNPs has been found to be higher than that of chrysin and AuNPs. In addition to that, cytotoxic activity of chrysin, AuNPs and CHR-AuNPs against the human breast cancer cell line (AMJ13) has been assessed by utilizing MTT assay. The results showed that chrysin alone was less cytotoxic to AMJ13 cells and was less effective than AuNPs. CHR-AuNPs, on the other hand, were found to be significantly more toxic to AMJ13 cells. In conclusion, chrysin-loaded gold nanoparticles have a high potential to be utilized as a nano-formulation for drug delivery applications.

**Keywords:** Gold nanoparticles, Chrysin, Antioxidant, Antibacterial, Cytotoxicity, AMJ13cells

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## ABBREVIATIONS OF TERMS

AuNPs	: Gold nanoparticles
CHR	: Chrysin
NP	: Nanoparticles
CHR-AuNPs	: Gold nanoparticles conjugated with chrysin
AMJ13cells	: Cancer cell line
FESEM	: Field Emission Scanning Electron Microscopy
XRD	: X-Ray Diffraction
ZP	: Zeta Potential Analysis
EDX	: X-ray Spectroscopy With Energy Dispersive
FTIR	: Fourier Transform Infrared
UVS	: UV-Vis Spectrometry

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## 1. INTRODUCTION

Nanotechnology is defined as an evolving and new field of applied science that combines chemistry and physics for various applications like electronics, food processing, and wastewater treatment packaging (Krishnan *et al.*, 2018). The incorporation of nanotechnology into pharmaceuticals and medicine, known as nanomedicine, has raised expectations about its potential as new diagnostic tools and therapies (Aw-Yong *et al.*, 2018). Nanomedicine is defined as the technology and science of disease treatment and prevention, pain relief and management, improvement and preservation of human health, traumatic injuries, all employing molecular knowledge and tools of the human body. The capacity to modify nanoparticle qualities like chemical, physical, and biological properties opens up a world of possibilities for drug delivery, imaging, diagnostic contrast and clarifying agents (Chithrakumar *et al.*, 2021).

Because of their multi-surface functions and distinctive properties, gold nanoparticles (AuNPs) were widely used in biotechnology. It is widely utilized in drug delivery systems as a result of its biocompatibility properties and high surface area ratio, the surface area greatly increases drug density on the surface, allowing to increase drug concentration or load multiple drugs onto single molecules (Lee *et al.*, 2020). Some studies currently suggest that using AuNPs as drug carriers for cancer treatment yields significantly better results than anticancer drug therapy (Kong *et al.*, 2017 & Jain *et al.*, 2012). Therefore, extensive research has been conducted on development of gold nano-particles and their utilization as a nanocarrier in nanomedicine applications.

Cancer has been considered as one of the most serious issues related to public health worldwide. Lung cancer (12.90% of all cancers), breast cancer (11.90% of all cancers), and colorectal cancer (10% of all cancers) are the most frequently identified cancers in the world, while lung cancer (9.40% of all cancers), liver cancer (9.10% of all cancers), and stomach cancer (9.1 % of all cancers) are the most common cancers related to death (8.8 % over-all) (Siegel *et al.*, 2019).

Surgery, chemotherapy, and radiation are all common cancer treatments. Those treatments are frequently ineffectual in the treatment of cancer and come with some side effects. Because of inability to target, poor solubility, and rapid elimination

of chemotherapy, its therapeutic effects are limited and have extreme side effects, which requires creating new cancer treatment methods (Rosière *et al.*, 2019).

Recently, a lot of research was performed with the aim of identifying safer and more effective synthetic and natural compounds which could be utilized for preventing and/or treating cancer. Many animal and plant extracts showed antitumor and immunomodulatory activity, among other biological activities. Flavonoids, which are pigments present in fruits and vegetables that are secondary plant metabolites, could assist to lessen the risk of cancer. Generally, they are considered harmless and have a low toxicity level (Abbas *et al.*, 2017). Chrysin (5,7-di-hydroxyflavone) can be defined as a biologically active flavonoid that was proven to have anti-inflammatory, anti-oxidant, and anti-allergic activities in honey, plants, and propolis (Watanabe *et al.*, 2011). Various recent research showed that chrysin, by decreasing cell proliferation and inducing apoptosis, could be used for treating cancer in a wide range of the cancer cell lines. Despite its anti-cancer characteristics, chrysin's low water solubility limits its bioavailability, biocompatibility, and bio-medical uses, providing a major difficulty for the treatment of cancer due to the low absorption, quick metabolism, and rapid systemic elimination (Sulaiman *et al.*, 2018).

### **1.1. The Aims of the Study**

Chrysin is one of the flavonoid compounds found in many plants. Although this compound has significant bioactivity features, its efficacy is limited due to its pharmacokinetic characteristics such as low solubility. This study is aimed to improve medicinal efficiency of chrysin in biomedical applications by loading it on gold nanoparticles. To accomplish the objective of this study, the following analyses were employed:

- Manufacturing and preparing gold nanoparticles (AuNPs) by chemical synthesis
- Loading of chrysin onto gold nanoparticles
- Physicochemical characterization of chrysin, gold nano-particles and chrysin-loaded gold nanoparticles by UV-Vis spectroscopy, FTIR, field emission scanning electron microscopy (FESEM), XRD, EDX and zeta potential analyses

- Cytotoxic activity analysis of chrysin, AuNPs and chrysin-loaded AuNPs on AMJ13 cell line
- Antioxidant activity analysis of chrysin, AuNPs and chrysin-loaded gold nanoparticles by DPPH scavenging assay
- Anti-microbial activity of chrysin, AuNPs and chrysin-loaded gold nanoparticles against *S. aureus* and *E. coli*

## 2. NANOTECHNOLOGY

Nanotechnology emerged in the 1990s as a new method for monitoring and manipulating atoms and molecules with dimensions in range of 1 to 100 nanometers. Nanotechnology has opened new horizons for biomedical research. The overlap between nanotechnology and medicine has also led to an evolution in the medical system known as nanomedicine, in which life information is obtained at a single molecule level through the use of nanotechnology (Matteucci *et al.*, 2018). Then, in the field of nanomedicine, diseases are diagnosed, treated, and prevented. After coupling the nanopattern to the ligand, homeostasis, signaling, regulation, movement of intracellular or intracellular structures, true behavior, and pathogenesis of cells are discussed in depth to provide powerful support for early treatment and diagnosis of various tumors and diseases (Nanda, 2016).

The word "nano" comes from Greek word "nanos," meaning "small," and is utilized to denote one billion parts per billion ( $10^{-9}$ ). Nanoparticles are particles of one or more dimensions and sizes ranging from 1 to 100 nanometers. The exceptional properties of NPs are majorly determined through their size. Various stabilizers, solvents, reducing agents, and other agents were used in preparing the NPs to control their size and shape (Loeve, 2010).

According to some studies, particles with the same chemical composition yet various sizes provide many risks; smaller particles are more harmful. Certain nanotoxicity research has focused on the cytotoxicity of NPs at very high dosage concentrations, which can have long-lasting and deadly effects on cells at low dose concentrations. The presence of positive and negative charges on nanoparticle surfaces may effect the electrostatic structure of biomolecules such as DNA, proteins and lipids (Tang & Zheng, 2018). The binding of biomolecules on the surface of nanoparticles, such as proteins, peptides, and other target bonds containing specific molecules that recognize receptors on different cell lines, is important not only in their interactions with biological tissues, but also in their biological functions and cellular absorption, which can facilitate their use in drug delivery, treatments, and diagnosis (Rosen *et al.*, 2012).

Physical, chemical, optical, and electrical properties separate inorganic NPs and hybrids created by merging inorganic NPs with organic materials, making them more

suitable compared to large-scale materials (Xia *et al.*, 2011). Controlling the shape and the size of metal nanostructures has attracted great interest since shape and size have an impact on all of metal nanostructures' magnetic, catalytic, electrical, and optical capabilities (Singh and Bhateria, 2019). Nanoparticles have a broad variety of the applications in bio-medicine, which include the delivery of pharmaceuticals and diagnostic techniques, along with therapeutic purposes. They are extremely small particles and could be utilized for delivering targeted drugs. Moreover, metallic NPs respond resonantly to a magnetic field that changes over time, transferring enough toxic thermal energy to the tumor cells as the hyperthermic agents (Khan *et al.*, 2014).

## **2.1. Nanoparticles**

Nanoscale materials offer a wide range of uses due to the production of stronger materials, advanced medical treatments, more memory capacity, smaller electronics, and better sensors, according to various researchers. To be considered "nano," the side of a device or material should be at a nanometer scale of 100 nm or less.

Nanoparticles are fascinating because they have properties that connect atomic structures to bulk materials. The characteristics of the nanoscale materials differ from their bulk counterparts (Heiligtag & Niederberger, 2013). Nanoparticles differ from bulk materials in terms of the size, shape and other properties (Abo El-Nour *et al.*, 2010).

The surface area to volume ratio of NPs is very high. Furthermore, the number of atoms inside a bulk material is comparable to the number of atoms on its surface, whereas the number of atoms inside nano-particles is negligible in comparison to the number of nanoparticles' surface atoms. Surface plasmon resonance and optical properties are examples of such properties (Moliner *et al.*, 2016). A solution of gold nanoparticles at 20 nm, for example, appears ruby red, while nanoparticles at 200 nm appear bluish, but a solution of gold at a larger scale appears golden yellow. Chemical and physical interactions of the particles with the surrounding environment, which can be a liquid, solid, or even a polymeric matrix, are improved by the ratio of surface area to volume. Because of a higher surface area to volume ratio at lower temperatures, diffusion becomes more practicable and faster (Singh and Bhateria, 2019).

Particles can be formed at the nanoscale in a variety of ways, which could be divided into two categories: the first method involves obtaining nanoparticles from

building molecules or atoms interacting with each other to form NPs of the required size and shape. The second method involves obtaining nanoparticles from bulk materials using various methods such as laser ablation. This necessitates the formation of NPs of different shapes and sizes, such as nanoshells, nanodisks, nanowires, nanocomposites, and hybrid nanostructures, among others, due to the need for multiple applications in various fields (Enza Fazio *et al.*, 2020)

Since nanoparticles serve as drug-release mediators, they were utilized for improving the efficiency and selectivity of drug delivery systems (Liu *et al.*, 2016). Because nanoparticles have large surface area and they are small in size, their surfaces can now be further modified with electrical, hydrophobic, anionic, cationic or other environmentally neutral materials that have many applications in the biological sciences (Juárez-Maldonado *et al.*, 2019). Different physical and chemical approaches were deployed for creating NPs which are expensive and contain chemicals harmful to the environment. Metallic nanoparticles, especially gold nanoparticles, are widely used in biomedicine and biotechnology since they have high surface bio-pairing with molecular probes and a variety of optical properties centered on local plasmon resonance (PR) (Panahi *et al.*, 2017).

### **2.1.1. Gold Nanoparticles (AuNPs)**

Due to the latest advancements in medical research and nanotechnology, various nanomaterials and NPs have evolved from numerous bulk elements, like silver, gold, platinum, copper, iron, cobalt, etc. (Dreaden *et al.*, 2012). The capacity to control NP qualities, like their chemical, physical, and biological capabilities, brings up some new options for drug delivery, diagnostic applications, and image contrast agents. Gold, a multifunctional inorganic and organic NP with anti-corrosive, antibacterial, and anti-oxidant characteristics, was employed in medical applications for centuries. AuNPs have been shown to be the least harmful and safest drug delivery and high-temperature cancer treatment agents. Furthermore, as a result of their high surface area ratio and biocompatibility, AuNPs are commonly deployed in the drug delivery systems (Biffi *et al.*, 2015).

Several researchers have noted various applications for gold nanoparticles (AuNPs) in drug delivery, environments, electronics, photonics, medical diagnostics, biosensors, and catalyst (Szunerits & Boukherroub, 2016). The shape and size of nano-

particles are affected by a variety of factors, one of which is temperature and the electric field of light, and gold nanoparticles interact strongly with these factors. The characteristics and applications of AuNPs are strongly impacted by their size and shape, and many approaches were created for producing AuNPs of various sizes and shapes. Various physical and chemical deposition techniques, such as simple chemical reduction in solution, synthesis under hydrothermal conditions, two-stage synthesis, evaporation, plasma synthesis and laser ablation, can be used to make AuNPs (Li *et al.*, 2014).

Gold nanoparticles in particular have biological properties such as anti-oxidant, anti-inflammatory, anti-angiogenic and anti-carcinogenic (Heiligtag and Niederberger, 2013). Gold nanoparticles have long been thought to be one of potential tools for drug delivery and cancer diagnosis due to their remarkable properties (Fig. 2.1)(Singh *et al.*, 2018) Among the most important of these are biocompatibility, high surface area-volume ratio, surface chemistry, ease of installation, and natural stability (De Matteis *et al.*, 2021).

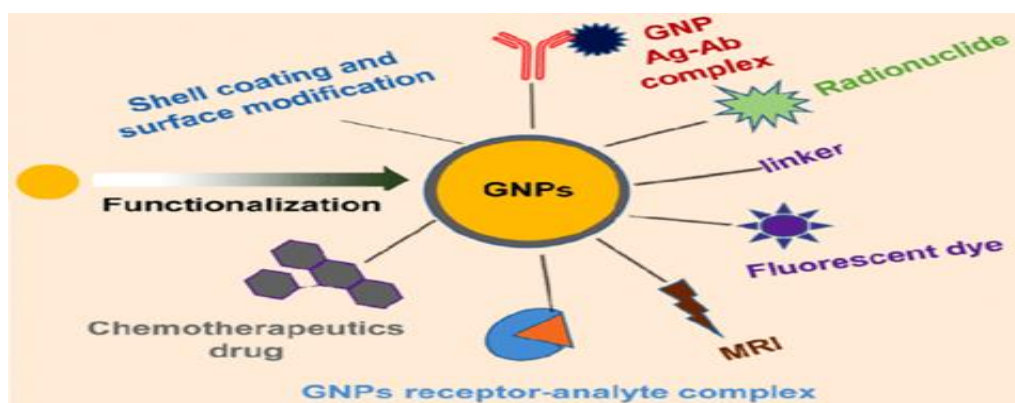


Figure 2.1. The properties of gold nanoparticles (Singh *et al.*, 2018).

### 2.1.2. Nano-Medicine

Nanomedicine can be defined as application of nano-technology for diagnosing, preventing, and treating diseases. Nanomedicine is a sort of micrometer-sized carrier material used to optimize the bio-distribution of encapsulated compounds through delivering those compounds in a more selective and effective way to the pathological site which is referred to as the site-specific drug delivery. This approach tries to increase the therapeutic compound's efficacy while lowering its toxicity. Since developing an effective drug delivery system is a priority, it was

proposed that the development of efficient nanomedicine needs a disease-driven approach, instead of the conventional setting-by-setting-driven method. This includes a thorough understanding regarding the interaction between technology and biology, including effect of disease physiology on nano-medicine distribution and retention, efficacy, and accumulation, along with the relation between the conduction system characteristics and *in vivo* behavior in the animals vs. humans (Athar & Das, 2014).

Nanoparticle-based drug delivery systems were shown to be an efficient way to target malignant brain tumors where conventional treatment is ineffective. Nanoparticles with enhanced permeability and retention (EPR) accumulate and interact with cancer cells (Feng *et al.*, 2011). Nanotechnology has heralded advanced approaches in the field of biomedical sciences (Figure 2.2), particularly in cancer treatment and diagnostic applications that include drug delivery with efficiency and fewer side effects than conventional cancer treatments. The major properties of NPs are considered as effective drug delivery agent (Biswas *et al.*, 2014).

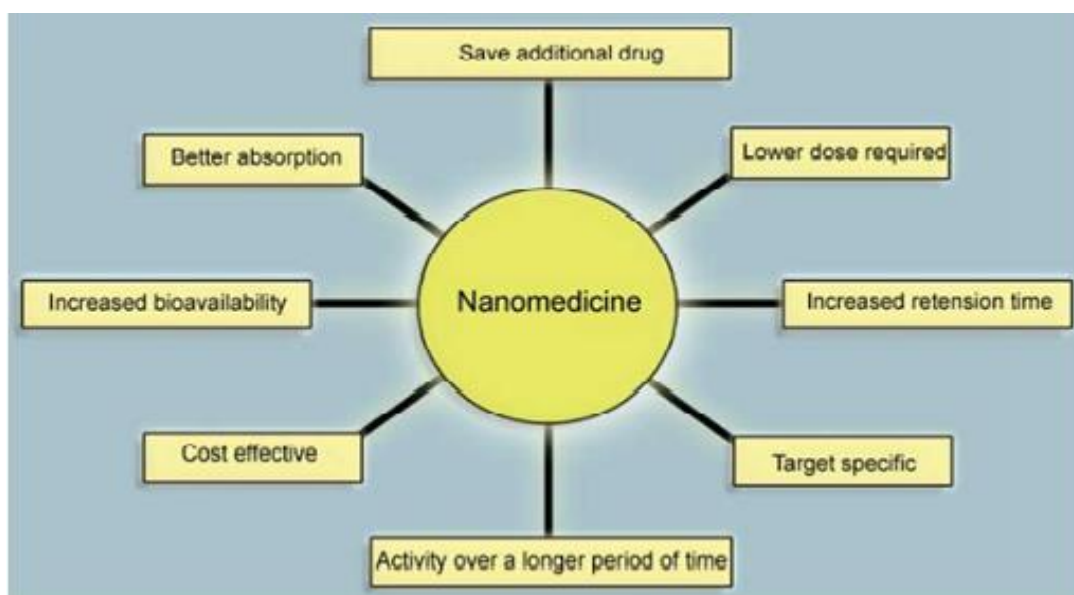


Figure 2.2. Advantages of nanomedicine (Biswas *et al.*, 2014)

## 2.2. Nano-Toxicity

Nanotoxicity, a term coined in 2004, is the study of the possible hazardous effects of NPs on environmental and biological systems. The study of nanomaterial toxicity for better understanding and assessing the health hazards related to their use is known as nanotoxicology. The chemical and physical features of NPs, like their

small size, flexible chemical composition and large surface area, have been discovered to contribute to their harmful side effects being enhanced. Particle size and surface area, in particular, are crucial aspects that influence NP toxicity significantly and directly, with smaller NPs having more hazardous effects because of their larger surface area (Arora *et al.*, 2012).

Aerosol studies that examine the effects of size-dependent particles spawned the first nanotoxicity studies; the field continues to benefit from this heritage, as well as contributions from diverse fields like material science, molecular biology, molecular toxicology, engineering, and analytical chemistry. Their anticancer, antibacterial, and antifungal properties were thought to be well-suited for this purpose, and they proved to be so. Depending on their physical and chemical characteristics, they have been classified as inorganic nanoparticles, metallic nanoparticles, or polymers (Li *et al.*, 2011). It is evident that nanoparticles increase drug solubility, stability and absorption with increasing targeting specificity. AuNPs are one of the greatest prospects for biomedical applications because of their exceptional biocompatibility and stability when put to comparison with other nanomaterials. They are simple to make and define, and they could be combined with a wide range of chemicals and biological molecules to make targeted, biocompatible, and controlled diagnostic, delivery systems, and treatment. Since their core is inert and non-toxic, NPs are believed to be relatively safe (Sztandera *et al.*, 2019).

Several gold NPs with different covering factors have been tested for cytotoxicity against a leukemia cell line in a single experiment. According to various studies, spherical gold particles enter cells and are non-toxic to cellular function (Boldeiu *et al.*, 2019). Several studies show no biologically adverse effects in both *in vivo* and *in vitro* experiments, and it has been proposed that some of the observed effects are due to conjugates or admixtures on AuNPs rather than the AuNPs themselves (Biffi *et al.*, 2015).

### **2.3. Nanoparticles and Immune System**

The immune system is tweaked to meet changing needs of the body for responding to the external and internal environment. The immune system can be harmed on multiple levels, resulting in suppression or over stimulation. As a result, before new biological and chemical entities can be used in biology, medicine, or

industry, adequate research into their interactions with the immune system is needed (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Uses, 2005). The immune system is tinkered with to meet the body's changing needs for reacting to the external and internal environment. On multiple levels, the immune system can be harmed, resulting in suppression or overstimulation. As a result, adequate research into new biological and chemical entities' interactions with the immune system is required before they can be used in biology, medicine, or industry (Ninan *et al.*, 2020).

When designing a nanomaterial for *in vivo* use, three aspects of immunity must be considered. The first is immunotoxicity, which can cause pathological changes in the immune system. The second consideration is immune compatibility, which means that NPs should not obstruct the immune response. Third, rejection or immune destruction, which can result in a defensive immune response that causes nanomaterials to be eliminated (Boraschi *et al.*, 2012).

NPs can enter the organism via the digestive system, airways, and skin inadvertently, or they can be supplied to the body for biomedical reasons (Cupaioli *et al.*, 2014). NPs are likely to come into contact with immune cells inside the body, leading to NP immune system interactions (Smith *et al.*, 2014). Those interactions have the ability to shape the immune system since they can either suppress or activate immune function, causing inflammation, autoimmune illnesses or cancer, or even higher infectious disease exposure (Zolnik *et al.*, 2010).

However, we can design NPs for targeted immune response modification in a few biomedical applications, such as vaccine delivery (Smith *et al.*, 2014). According to various studies, immune organs are the primary sites for nanoparticle deposition following device exposure, and macrophages are very likely to transport nanoparticles to multiple organs. Macrophages are the main cells that infiltrate pathogens and act as the first defense line against them by phagocytosing of the foreign particles. This macrophage strategy can be utilized for actively delivering a drug to the infection site, which reduces drug toxicity (Glass, 2001). Because of their high stability, low toxicity, and hydrophilic-hydrophobic behavior, nano-based drug delivery systems had gotten much interest (Gu *et al.*, 2008).

## **2.4. Stimulation of Immune Response**

Nanoparticles, depending on their chemical and physical features, can induce both adaptive and innate immune responses. Yet, it is still unknown how individual nano-particles are affected.

### **2.4.1. Innate Immune Response Activation**

In the case where NPs enter the body, they can interact with the immune cells and cause an inflammatory response, which is combined with production of signaling molecules (i.e. the cytokines and chemicals) which facilitate communication between coordinate molecular events and immune cells. Positively charged NPs have a stronger inflammatory potential in comparison with the negatively charged or neutral NPs (McNeil and Dobrovolskaia, 2009). This is explained by macrophages that have a negative-charge sialic acid on their surface, which allows them to easily interact with cationic substances. Connective cells' Toll-like receptors (TLR) identify foreign antigens, bind to them, and start the signal transformation and inflammatory response pathway (Dwivedi *et al.*, 2009). In the *in vitro* investigation, Lucarelli *et al.* (2004) discovered enhanced expression of TLR receptors and the production of inflammatory cytokines in human macrophages exposed to non-toxic doses of several NPs (Lucarelli *et al.*, 2004). According to the research, various NPs provoked an inflammatory response in various ways. A few studies, on the other hand, imply that impurities created during NP synthesis are the primary source of inflammation (Pulskamp *et al.*, 2007).

## **2.5. Cancer**

Cancer can be defined as one of the main mortality causes all over the world, and cancer's incidence is increasing. Cancer, also known as a tumor, is a disease that develops in the case when changes in a group of normal cells within the body result in irregular and uncontrolled cell division, as well as malignant behaviors like metastasis and invasion (Argyle & Blacking, 2008). Most cancers form separate clumps, but in leukemia, which is a cancer of the myeloid or lymphoid cells (leukemia), the cancer cells can spread widely throughout the marrow or lymphatic tissues and even spread into the blood (Shilpa *et al.*, 2019).

Tumors are divided to 2 types, which are: benign and malignant. Polyps do not spread to other body parts and are not cancerous. It is made up of cells that are

remarkably comparable with normal cells and grows slowly. It will just become an issue if it grows to the point that it is impossible to remove (Han *et al.*, 2018).

Cancer is a disease where a group of cells ignores physiological basis of cell division and gets out of control. Due to the fact that the cancer cells are self-sufficient, they do not respond to signals which promote the regular cell cycle, leading in uncontrollable transformed cell reproduction and growth (Petrovic, 2016).

This could be fatal if the cancer cells continue to multiply. In reality, 90% of cancer fatalities are caused by cancer cells spreading to other tissues, a process referred to as metastasis. Normal cells grow in a synchronized manner during mitosis, reliant on the availability related to the external growth factors. As a result, the cells stop multiplying when the supply regarding such growth signals is depleted or expires. On the other hand, cancer cells, grow regardless of any factor or signal (Krock *et al.*, 2011).

Initiation, promotion, and progression are the three stages of the transition from normal to cancerous cells. All of such stages include oxidative stress. Different cancer development aspects, which are divided into many categories and include cell reproduction, programmed cell death, tissue invasion, angiogenesis, and metastatic tumor, can be aided by oxidative stress. Antioxidant effects on cancer have been the subject of recent research (Lum *et al.*, 2005).

A normal cell's life cycle is well-programmed; it divides around 50 times prior to dying of programmed cell death and being replaced by a new cell. This is due to the limited effectiveness of DNA replication, which causes telomeres to shorten with repeated replication. On the other hand, cancer cells, contain a high level of telomerase activity, which replaces worn-out telomere ends on a regular basis, permitting unrestrained cell proliferation (Sosa *et al.*, 2013).

Cancer has been considered as a multi-factor disease, which is characterized by complex genome alterations influenced by interactions between the host and the environment (Li *et al.*, 2010).

The dysregulation of different regulatory proteins and the extracellular environment which is of high importance in the development and genesis of cancer can be seen in the microenvironment of benign tumors (Sosa *et al.*, 2013). Preserving proliferative signals, avoiding growth inhibitors, activating invasion and metastases,

enabling immortality repetition, stimulating angiogenesis, and resisting cell death are the six characteristics of cancer (Sosa *et al.*, 2013).

The formation of metastasis and the progression of cancer cells requires the development of enough vasculature to feed the cells with nutrients and oxygen, as well as to eliminate harmful molecules. This stage is known as angiogenesis disease, and there are over 100 different types with various cancer subtypes found within specific organs (Petrovic, 2016).

### **2.5.1. Breast Cancer**

Breast cancer can be defined as the most frequent cancer type in the women worldwide, and it represents the second largest mortality cause in women in developed nations (Breast Cancer in Young Women, 2010). It is responsible for roughly 23% of all gynecological cancers and 14% of cancer mortalities (Jamal *et al.*, 2011). The uncontrolled growth regarding the abnormal cells in milk-producing glands of the breast (lobes) or the small tubes (ducts) that convey milk to the nipples is classified as breast cancer (Buem *et al.*, 1994). The three cell surface-specific receptors most typically utilized for classifying the breast cancer are progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor (HER-2) negative or positive (Scott & Drake, 2019). The most frequent kind of breast cancer is hormone receptor positive breast cancer, which is responsible for 75% of all breast cancer cases (Anderson *et al.*, 2014). Estrogen and progesterone are hormones that cause the growth of this type of cancer, HER2-positive breast cancer is identified by cells that overexpress the HER2/neu protein (Sumbaly, 2015).

Hormone receptor positive breast cancer accounts for 20-30% of all cases. HER2-negative cancers are those that do not overexpress HER2/neu (Harries & Smith, 2002). Age, heredity, personal history of breast cancer, hormonal therapy, exposure to ionizing radiations, alcohol consumption, and genetic predisposition are all known risk factors for breast cancer (Colditz & Bohlke, 2014).

AMJ13 can be defined as a type of human breast cancer cell that has been extensively examined worldwide. AMJ13 is a human breast cancer cell line identified in the malignant breast tissue of 69-year-old woman. Since AMJ13 cell lineages have different ideal characteristics specific to the breast epithelium, they are useful for *in vitro* breast cancer research. AMJ13 cells are used as an *in vivo* model as well as *in*

*in vitro* studies of estrogen response (Soule et al., 1973). AMJ13 cells are also sensitive to cytokeratin, and epithelial-like cells form domes and grow in monolayers if cultured *in vitro*. TNF-alpha factor can also slow tumor growth (Osborne and Schiff, 2011).

## **2.6. Flavonoids**

Flavonoids represent the most common polyphenol in the human diet and can be detected in fruits, vegetables, and plant-based beverages like tea. Its anti-allergic, anti-inflammatory, antiviral, and antibacterial properties have been demonstrated, and it also has cytotoxic antitumor properties and is effective in the treatment of neurodegenerative diseases (Brodowska, 2017). Eating more flavonoids appears to be related to a lower cancer risk, according to epidemiological and animal studies. Flavones such as chrysin, apigenin, scutellarine, and baicalin have lately been discovered to be of high importance in nitrogen fixation and chemical defenses (Huntley, 2009). Flavonoids are free radical scavengers that act as anti-oxidants against the free radicals and COX inhibitors ( Pal *et al.*, 2009).

### **2.6.1. Chrysin**

Chrysin (5,7-dihydroxyflavone) can be defined as one of the bioactive flavonoids derived from plants, propolis, and honey. Chrysin has a typical chemical structure, which consists of two rings joined together and attached to the phenyl ring (Figure 2.3) (Anari *et al.*, 2016). Chrysin was shown to have anti-inflammatory, antidiarrheal, and antioxidant properties (Figure 2.4). In leukemia, thyroid, cervical, prostate, esophageal, colon, and breast cancers, chrysin was reported to be antiproliferative and pro-apoptotic, indicating that it is one of the promising anti-cancer agents (Eatemadi *et al.*, 2016).

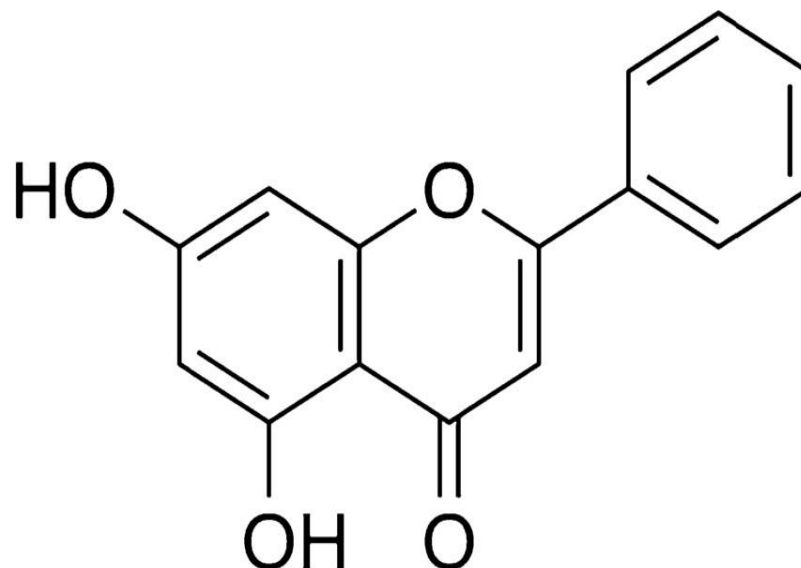


Figure 2.3. Chemical structure of chrysin

Furthermore, some studies have identified the effect of chrysin that inhibits phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway in cancer cells, which may be related to chrysin's antiproliferative activity (Eatemadi *et al.*, 2016). Chrysin has been used for drug loading and release in several studies as an anticancer therapy, however poor water solubility and absorption limited its bioavailability, biocompatibility and bio-medical applications (Mohammedian *et al.*, 2016).

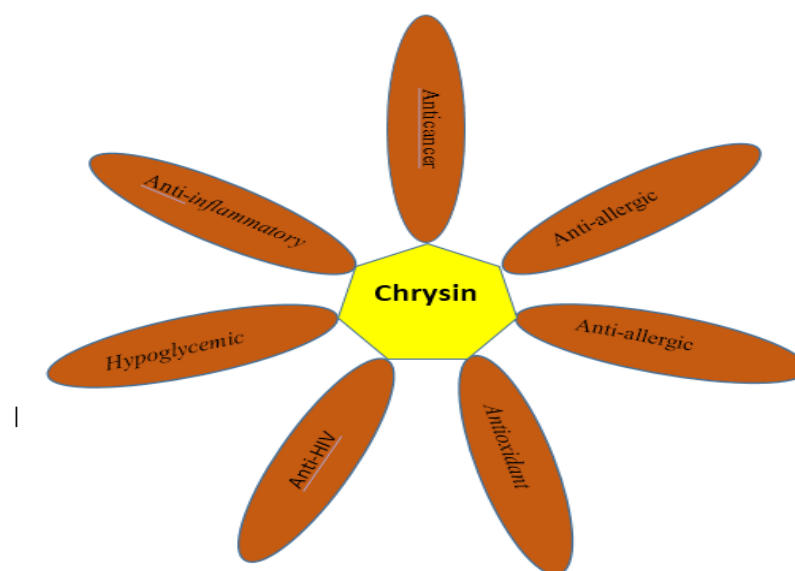


Figure 2.4. Biological activities of Chrysin as complementary medicine (Mohammadian *et al.*, 2016)

A drug delivery monitoring system represents one method of managing drug release for improving drug efficacy. It is most commonly used with low solubility active substances, such as chrysin (Sulaiman *et al.*, 2018). The effects of the chrysin

in the treatments and prevention of different human diseases had been highlighted by using flavonoids as anticancer agents. Interactions between surface atoms and stable bonds in metal nanoparticles are strong influencing factors for optical, stability, biochemical, and electrochemical properties (Teng *et al.*, 2021)

## **2.7. Nanotechnology and Cancer**

Nanotechnology is an interdisciplinary field in which nanoscale devices are designed and manufactured using principles from chemistry, physics, medicine, biology, informatics, and engineering (Sahoo *et al.*, 2007). Nano-technology is defined as measurements of less than one micrometer. However, it usually refers to materials created using bottom-up or top-down geometry and are only a few hundred nanometers in size (Mousa and Bharali, 2011).

The resulting nanomaterials have unique properties that depend on fundamental properties like shape and size, along with practical features provided by surface modifications. Nanotechnology has a potential to change the way tumors and other diseases are diagnosed, treated, and prevented, according to the National Cancer Institute (NCI) (Farokhzad and Langer, 2009). NPs have distinctive physical and chemical features that make them ideal for drug delivery and diagnostic investigations. NPs also offer the highest surface-to-volume ratio, which benefits surface function and a wide spectrum of therapeutic loads. NPs have better access to tumour sites when compared with traditional drug delivery compounds as a result of their nanoscale size and tunable surface characteristics (Banerjee *et al.*, 2016).

According to the WHO's predictions and several research, cancer is one of the most common public health issues all over the globe. Today's cancer treatments include high-doses of nonspecific, highly toxic compounds. Furthermore, because traditional diagnostic tools are unable to distinguish between early-stage and curable cancer, it is too late to prevent the metastasis to the other body organs (Gmeiner and Ghosh, 2014). As a result, the two main issues impeding the effectiveness of cancer treatments are: early cancer detection before it spreads; and certain treatments for cancer cells that do not disturb normal or surrounding tissues, as well as avoiding unnecessary toxicity caused by toxic organic solvents used to dissolve the drug. Nanotechnology brings a fresh perspective to the field of identifying and treating cancer cells at an early stage (Muzammil *et al.*, 2018).

Nanoparticles have physical as well as chemical characteristics that make them adequate for use as imaging probes for cancer detection and monitoring. Nanoparticles have tunable surface properties, which means that nanoparticles injectable solutions can be fully functional without the use of the toxic organic solvents to join water-insoluble antitumor agents. Dendrimers, nanocrystals, nanoshells, and quantum dots are examples of nanoparticles that is of high importance in tumor identification and treatment (Cuenca *et al.*, 2006).

### 2.7.1. Nano-particles as Carriers for Drug Delivery

One of the most significant fields, where nano-technology is assisting in advancement of disease treatment, particularly cancer treatment, is drug delivery. Nanoparticles are used as a carrier agent for drugs used in treatment due to their distinctive physical and chemical properties (Fig. 2.5) (Gmeiner and Ghosh, 2014).

The drug might either be covalently attached to nanoparticle surface or dissolved, absorbed, or dispersed through the nanoparticle complex (Kocbek *et al.*, 2007). Targeted drug delivery to a specific part of the body is beneficial for many diseases, including viral infections, cancer, and bacterial infections. Polymers, both natural and synthetic, are used to make nanoparticles (Calixto *et al.*, 2016). In the fields of biotechnology and biomedicine, nanoparticles were studied thoroughly, particularly in systems of drug delivery.

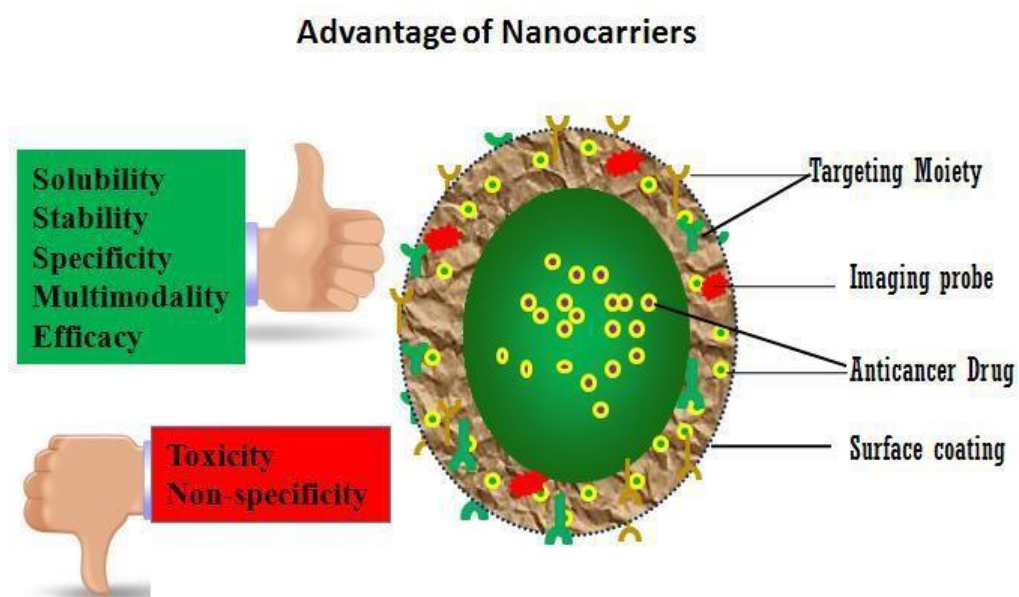


Figure 2.5. Structure of nanoparticle using for drug delivery, with its advantage (Gmeiner and Ghosh, 2014).

Nanoparticles used as drug carriers either adhere to the drug's surface or encapsulate it to prevent it from degrading. The nanomaterials are small but have a large surface area, allowing for high stability, bioavailability, biocompatibility and specificity of cellular interaction. Nanoparticles solve some long-standing issues, especially in biomedical applications including diagnostic imaging and drug transport in physiological contexts. Because nano-sized therapeutic agents have a long turn-around time in the blood-stream, they can reach target tissues more effectively in oncology (Ferrari, 2005).

More specifically, nanoparticles that act as large carriers allow passive targeting of cancer tissues by mimicking tumor biology, such as disrupted blood vessels with high permeability, and reducing the ability of normal tissues to spread. Nanoparticles can easily penetrate malignant cells and malfunctioning lymph vessels within cancer because tumor blood vessels are disrupted (Ding *et al.*, 2013).

Therapeutic agent nanocarriers allow therapeutic agents to be synthesized and retained within cancer cells for extended periods of time. The effect of enhanced penetrability and retention (EPR) refers to the accumulation of nanocarriers within the cancer cell (Le Duc *et al.*, 2014). In order to be efficient in transporting the drug directly to malignant cells in living tissues, the drug nanocomplexes must meet certain criteria, the most important of which are (Thakor and Gambhir, 2013):

- The drug chosen must be able to bind or encapsulate the nanoparticles.
- To allow systemic drug delivery, nano-particle complex must stay stable in serum.
- The NP-drug complex must be transported to malignant cells (either through EPR effect or through receptor-mediated interactions), which reduce any unfavorable side effects of non-targeted delivery.
- The nanoparticle has to be capable of releasing the drug at the cancerous site.
- To allow for harmless degradation, the residual nano-size carrier must be made of a biological or biologically inert material with limited lifetime.

A few of the nanoparticle systems that were employed as drug carriers include mesoporous silica, polymeric, liposomal, solid lipid, and inorganic nanoparticles. Biodegradable polymeric nanoparticles gained a lot of attention as prospective drug

delivery systems recently as a result of their capability in targeting specific tissues and organs and their uses in the controlled release of drugs.

### 3. MATERIALS AND METHOD

#### 3.1. Equipment and Apparatus

There are different equipment and apparatus used in present study and listed in Table3.1.

Table3.1. The equipment and apparatus that have been utilized in present study

<b>Equipment and Apparatuses</b>	<b>Company</b>	<b>Country</b>
Sensitive balance	Ohaus	Germany
CO <sub>2</sub> incubator	Cypress Diagnostics	Belgium
Micro-titer reader	Gennex Lab	US
Laminar flow hood	K & K Scientific Supplier	Korea
Micro-pipette	Cypress Diagnostics	Belgium
Cell culture plates	Santa Cruz Biotechnology	US
Centrifuge	Gemmy industrial CORP	Taiwan
Cooling centrifuge	Sorvall	USA
Camera Digital	Cannon	China
Magnetic stirrer	Hy-hm	Korai
Vortex	Strat scienti fied	UK
Water bath	Menmort	UK
UV-VIS spectrophotometer	Shimadzu-1	Japan
FTIR	Shimadzu	Japan
X-ray diffraction	Shimadzu	Japan
Sonicator	Trade mark	China
FESEM	FEI	Netherlands
Zeta potential	Brook	USA
EDX	TESCAN	(Czech Republic)

### 3.2. Chemicals and Reagents

The different reagents and chemicals that have been used in the present study are shown in Table 3.2.

Table 3.2. The different reagents and chemicals that were used in present study

<b>Chemicals and Reagents</b>	<b>Company</b>	<b>Country</b>
<b>Trypsin/EDTA</b>	Capricorn	Germany
DMSO	Santacruz Biotechnology	USA
RPMI1640	Capricorn	Germany
2,2-Diphenyl-1-Picryl- Hydrazyl (DPPH)	Sigma	Germany
Fetal bovine serum	Capricorn	Germany
HAuCl <sub>4</sub> .3H <sub>2</sub> O	Glentham	UK
Chrysin	Sigma-Aldrich	Germany
3- (Di-methylthiazol-2yl)- 2,5 Di-phenyltetrazolium Bromide (MTT)	Sigma	Germany
Sodium Citrate	US-Biological	USA
NaBH <sub>4</sub>	Sigma	Germany
Nutrient broth	Sigma- Aldrich	USA
Ethanol	BDH	England

### 3.3. Laboratory Experiments

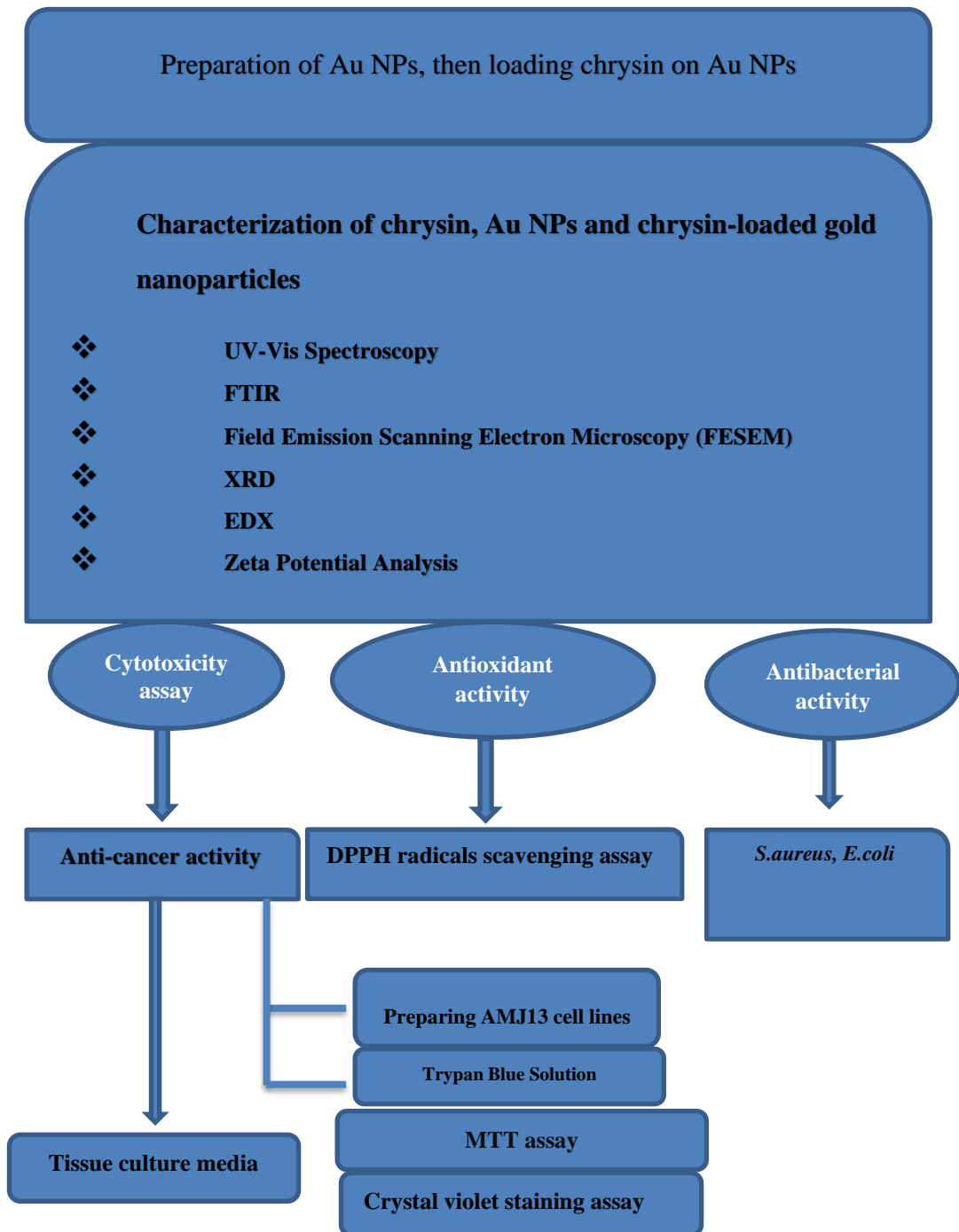


Figure 3.1. Preparation of AuNPs and loading chrysin on AuNPs

### 3.4. Preparation of Gold Nanoparticles

#### 3.4.1. Chemical Synthesis of Gold Nanoparticles

The chemical approach was used to make gold nanoparticles, and the method was done by dissolving 0.1 mg sodium citrate in deionized water at a temperature of 100 °C

for 20 minutes, and then 2 ml of prepared tetrachloroauric acid trihydrate (0.1 ml in 20ml of deionized water) at a 0.3 mM concentration was added and stirred vigorously for 30 min, after which the solution began to turn red. Drops of light, frozen NaBH<sub>4</sub> solution (0.6 ml of ice-cold 0.01 M) were added and stirred until the solution turns dark red. To allow further reaction, the solution is stirred and left at the temperature of the room overnight (Jabir *et al.*, 2019).

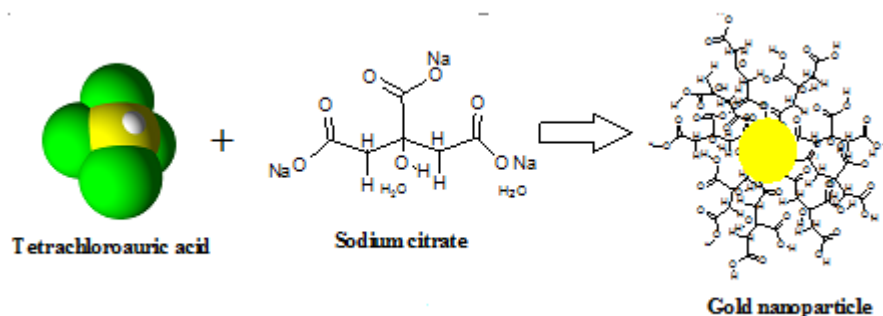


Figure 3.2. Chemical synthesis of Au NPs

### 3.4.2. Loading of Chrysin on Gold Nanoparticles

#### 3.4.2.1. Conjugation of Chrysin with Gold Nanoparticle

To create a nano-conjugated system, the dissolved chrysin (5,7-dihydroxyflavone) was combined with the gold nanoparticles that had previously been prepared (each one ml with 9 ml) followed by a color change to light violet after continuous stirring at room temperature. To remove excess chrysin, an ultrapure centrifuge (13,000 rpm, 30 min) was used. The drug stretch-loaded AuNPs consist of loose granules of light violet color (Jabir *et al.*, 2019).

### 3.4.3. Characterizations of Chrysin(CHR), AuNPs and CHR-AuNPs

#### 3.4.3.1. UV-VIS Spectroscopy Analysis

The absorption spectra of chrysin, gold nano-particles and CHR-AuNPs were measured by UV/Vis spectrophotometer with ranges between 200–800 nm.

#### 3.4.3.2. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR analysis was used for the identification of functional groups that are present in chrysin (CHR), gold nanoparticles, and solution of CHR-AuNPs with wavelengths ranging from 400-4000 cm<sup>-1</sup>.

### **3.4.3.3. XRD Analysis**

XRD measurements had been performed after sample deposition on glass slide. The structure of chrysin, gold nanoparticles and CHR-AuNPs were recorded by XRD that has been equipped with Cu- $\alpha$  radiation at a wave-length ( $\lambda = 0.154056$  nm) generated at 45 kV. With the use of Scherer's formula, the domain size has been evaluated from the width of XRD peaks, assuming they were free of irregular strains:

$$D = K \lambda / \beta \cos \theta$$

In which D represent the crystal particle size, K represents absolute constant (0.94), the X-ray wavelength (0.154056), the width of the XRD peak at half height,  $\theta$  represent the angle of the Bragg diffraction.

### **3.4.3.4. X-ray Spectroscopy With Energy Dispersive (EDX)**

EDX analysis was performed by using TEM. EDX analysis of CHR-AuNPs showed the presence of gold. EDX can be defined as one of the analytical approaches have been utilized for the elemental analyses or the chemical characterization of the CHR-AuNPs.

### **3.4.3.5. Zeta Potential Analysis**

This analysis is used for a measurable index of dispersion stability for CHR, AuNPs and CHR-AuNPs.

### **3.4.3.6. Field Emission Scanning Electron Microscopy (FESEM)**

FESEM analysis has been utilized to analyze the molecule morphology and size of chrysin, AuNPs, and CHR-AuNPs.

## **3.5. Antioxidant Activity**

### **3.5.1. DPPH Radical Scavenging Assay**

#### Materials and Solutions

- Ethanol: Absolute ethanol has been utilized.
- DPPH solution: It has been made by the dissolution of 2.366 mg of 2, 2-diphenyl 1-picrylhydrazyl in 100 mL of the absolute ethanol for the purpose of obtaining 60  $\mu$ M DPPH.
- CHR, AuNPs and CHR-AuNPs solution: The solutions were prepared according to 3.4.1 and 3.4.2 section.

- Ascorbic acid solution: It has been prepared through the dissolution of 1 mg of ascorbic acid in 1mL of the distilled water

The DPPH assay has been utilized in order to assess the antioxidant activity of AuNPs, CHR, and CHR-AuNPs (Joshy et al., 2016). Because of its unpaired electron, the DPPH radical has deep violet color, and its radical scavenging ability could be measured spectrophotometrically as an absorbance loss at 517nm in a case when pale yellow non-radical form is formed. Equal amounts (0.5mL) of DPPH and various concentrations of chrysin (3.1, 6.2, 12 and 25  $\mu\text{g ml}^{-1}$ ), AuNPs, and CHR-AuNPs have been mixed in a cuvette and left to wait for 30 min at the room temperature based on this experiment. The absorbance has been measured in UV/VIS spectrophotometer at 517 nm. As a negative control, only DPPH with ethanol was employed, while ascorbic acid (Vitamin C) has been utilized as positive control. A control solution (DPPH) has also been tested for absorbance. The following formula was used to calculate the sample's percentage inhibition:

$$\text{DPPH Scavenging Activity (\%)} = (A_c - A_s / A_c) \times 100$$

Where  $A_c$  represents the DPPH peak intensity and  $A_s$  is the CHR, AuNPs, and CHR-AuNPs sample solvent peak intensity.

### **3.6. Antibacterial Activity**

With the utilization of the next protocol, the anti-bacterial activity of (AuNPs, chrysin, CHR- AuNPs) has been tested against human pathogens *S. aureus* and *E. coli*. The bacteria were obtained from their stock cultures using a sterile wire loop. To evaluate how chrysin, AuNPs, and CHR-AuNPs effect the bacterial growth curve, we grew the strains on MH agar plates at 37 °C, and fresh culture plates were collected and inoculated with 50 mL of nutrient broth. Bacteria were grown in nutritional broth until they attained an optical density (OD) of 0.10 at 600 nm, which corresponded to a bacterial concentration of  $10^8$ CFU/mL. The bacterial culture (1 mL) was added to the nutrient broth supplemented with NP and incubated at a temperature of 37°C with gentle agitation for 12 hours. Through measuring the OD, a spectrophotometer is utilized for determining the bacterial growth.

### **3.7. Cytotoxic effects of CHR, AuNPs and CHR-AuNPs**

#### **3.7.1. Tissue Culture Media**

##### **3.7.1.1. Roswell Park Memorial Institute (RPMI)-1640 Medium**

To prepare it about 16.4 g of RPMI-1640 powder has been resolved in 800mL of D.W. and the following components have been added and mixed by magnetic stirrer.

- 100 mL of Fetal bovine serum (FBS)
- 0.5 mL of Streptomycin ( $100 \mu\text{g mL}^{-1}$ )
- 0.5 mL of Benzyl penicillin solution ( $100 \mu\text{g mL}^{-1}$ )
- 5-10 mL Sodium bicarbonate (4.4%) to reach final pH of 7.6.

The pH was regulated to 7.2, then, the volume was completed to 1 L and the latter solution was filtrated by 0.22  $\mu\text{m}$  Millipore filter (Sulaiman *et al.*, 2018).

#### **3.7.2. Cancer Cell Line**

##### **3.7.2.1. Maintenance of AMJ13 Cell Line**

The AMJ13 human breast cancer cell lineages were provided by Al-Mustansiriyah University in Baghdad, Iraq. After quick thawing in a temperature of 37 °C water bath, the cells have been incubated for a period of 24 hours at 37°C. The cells were centrifuged at 800 rpm for approximately 10 min before being resuspended in 5ml of the culture medium (RPMI-1640). The medium was then separated and replaced with fresh medium supplemented with 10% FBS.

AMJ13 cells have been suspended in RPMI-1640 medium and cultured in T-50 culture flasks at 37°C in an incubator with 5% CO<sub>2</sub> and 95% air. Once the cultures reached 70-80% confluence, cell sub-strains were cultured and the growth medium has been removed and washed in PBS buffer two more times. Trypsin-EDTA solution was used to separate the cultured cells from the flask surfaces and assemble them. After suspension, trypsin activity was stopped by adding FBS-containing medium, cells were distributed to other flasks, and cultures were subcultured every three days to maintain exponential growth of cell lineages.

### **3.7.3. Viable Cell Counting**

One milligram of trypan blue stain powder was dissolved in 100 mL of PBS and filtered through filter paper. The filtered solution was stored at 4 °C until needed. It was diluted in PBS in a ratio of 1:10 before use.

About 0.2mL of cells were added to 0.2 mL of trypan blue stain, and 1.6 mL of PBS was mixed with the cells and counted using a double ruling counting chamber. The viable cells do not stain and appear glamorous under the inverted microscope while the dead cells stain blue. Then, to count the cells (cells per milliliter), the following equation was used.

$$C = N \times D \times 10^4$$

When C represents the number of the viable cells per ml, N represents the number of the viable cells counted, and D represents the factor of dilution (=10)

### **3.7.4. Anticancer Activity**

#### **3.7.4.1. MTT Assay**

To prepare MTT solution, 0.2 g MTT has been dissolved in 100 ml PBS. Then, the solution has been filtered by a 0.20 µm Millipore filter for removing any blue formazan that had formed in the solution and stored at -20 °C in a sterilized black glass container. Within two weeks of preparing the solution, it should be used.

The colorimetric MTT method was utilized for determining the cancer cells' proliferation. AMJ13 cells have been seeded at a density of  $10^7$  cell/well in RPMI-1640 culture medium supplemented with 10% heat-activated FBS and incubated at 37°C in a 96-well plate with a flat bottom in an incubator with 5% CO<sub>2</sub>. The AMJ13 cells have been exposed to different chrysin concentrations, AuNPs and CHR-AuNPs (6.25, 12.5, 25, 50, and 100 g mL<sup>-1</sup>) for an additional 24 hours after entering the exponential growth phase after 48 hours. After 24 hours, 100 mL of yellow-colored methyl thiazolyl tetrazolium (MTT) solution has been added into every one of the wells. In addition, the microplate has been incubated at a temperature of 37°C for 4 hrs to allow metabolically active cells to reduce MTT. The supernatants were then removed. 100 mL DMSO was added to every one of the wells as a solubilisation solution for dissolving the MTT crystals, and the microplate was incubated for 5 minutes. Using an ELISA reader with a 550 nm

absorbance, the optical density of each well was determined. The next formula was used for calculating the rate of cell: inhibition rate (%) =  $(A - B / A) \times 100$

When A denotes the negative control's optical density and B denotes the samples' optical density.

#### **3.7.4.2. Crystal Violet Staining Assay**

For the preparation of this solution, 5 g of crystal violet dye powder were dissolved in 100 ml of methanol and 50 ml of 40% formaldehyde, distilled water has been added to reach the final 1000 ml volume. The solution has been filtered through filter paper then stored at the room temperature in a dark container (Dobrovolskaia and McNeil, 2009).

The cells were exposed to chrysin, AuNPs, and CHR-AuNPs ( $50 \text{ mg mL}^{-1}$ ). After the consumed medium has been separated and cells have been washed with PBS,  $50 \mu\text{L}$  of crystal violet was added to each well. After 20 minutes, the cells have been carefully washed three times with water, and the wells have been stained with distilled water and left to dry. An inverted microscope with a 40X magnification was used to examine the morphological aspects of cells.

#### **3.8. Statistical Analysis**

The acquired data was evaluated statistically with the use of Graph Pad Prism and an unpaired t-test. The results were provided as mean  $\pm$  standard deviation of triplicate measurements.

## 4. RESULTS AND DISCUSSION

### 4.1 Chemical Fabrication of AuNPs

The AuNPs in this study were synthesized by reducing citrate from gold salts. A solution of tetrachlorine trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) was added dropwise to boiling sodium citrate, and the color began to change from pale yellow to bluish-gray, then to purple, and finally to red after addition of  $\text{NaBH}_4$ . The citrate reduction method for AuNP production is advantageous due to the ease with which AuNPs are dispersed in electrode solvents like the water, due to the negative charge related to citrate ions on surface of AuNPs. The surface-confined form of the plasmonic resonance (SPR) absorber was most likely responsible for the discoloration, which is common in metallic nanoparticles such as gold (Zhong et al., 2015). Because these electrons are very small and do not move freely, the particles interact with light in a different way to spherical particles smaller than 50 nanometers. Small domains of AuNPs with sizes of 20-50 nm are used in cancer therapy to reach cancer cells, allowing them to easily turn on and bind to molecules. Because of their ability to bind to cell membrane, these functional AuNPs may have good drug carrier activity (Christophe Deraedt et al., 2014).

With the existence of  $\text{NaBH}_4$ , thiols on AuNPs have been demonstrated to be adsorbed reductively, allowing the AuNPs to grow. In a nutshell, the unique attribute of such AuNPs is that they are stable for over a month while remaining extremely active and stimulating. The property could be explained by first stabilizing AuNP with  $\text{H}^-$  and/or  $\text{BH}_4^-$ , which had been demonstrated to establish strong bonds with AuNP core, and after that reducing AuNP with  $\text{Cl}^-$ -induced  $\text{HAuCl}_4$ . Electrostatic stability is found only in  $\text{B}(\text{OH})_4^-$  and  $\text{Cl}^-$ . Then there is an AuNP nucleus with sodium cations (Figure 4.1) (Christophe Deraedt *et al.*, 2014).

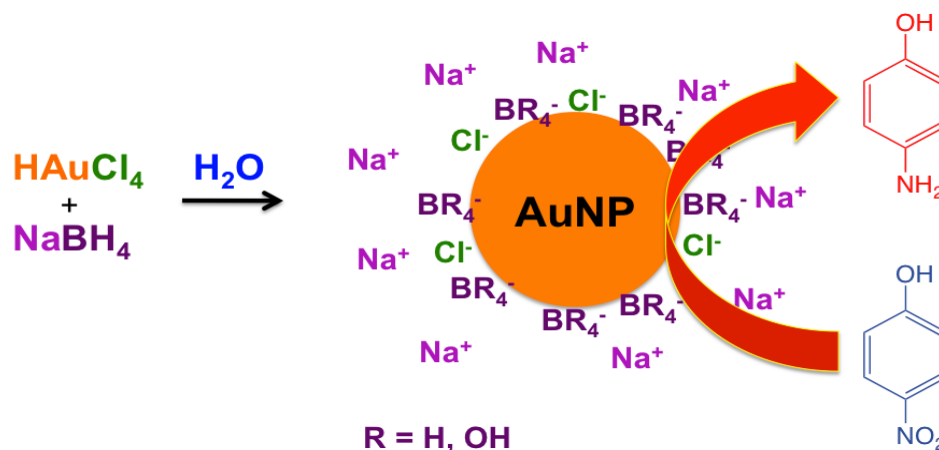


Figure 4.1. Stabilization properties with NaBH<sub>4</sub> and interaction on the surface of AuNPs (Christophe Deraedt et al., 2014).

#### 4.1. Chrysin-Gold Nanoparticle Conjugation

Chrysin has antimicrobial activity and is utilized for treating specific human cancer types as a therapeutic agent (Zhong et al., 2015). When creating the nano-conjugated system, dissolved chrysin was combined with gold nanoparticles overnight, we observed a color change to light violet after continuous stirring at room temperature. To remove excess chrysin, the chrysin (CHR-AuNPs) produced by the process was removed by ultracentrifugation (13,000 rpm, 30 minutes) (Figure 4.2).

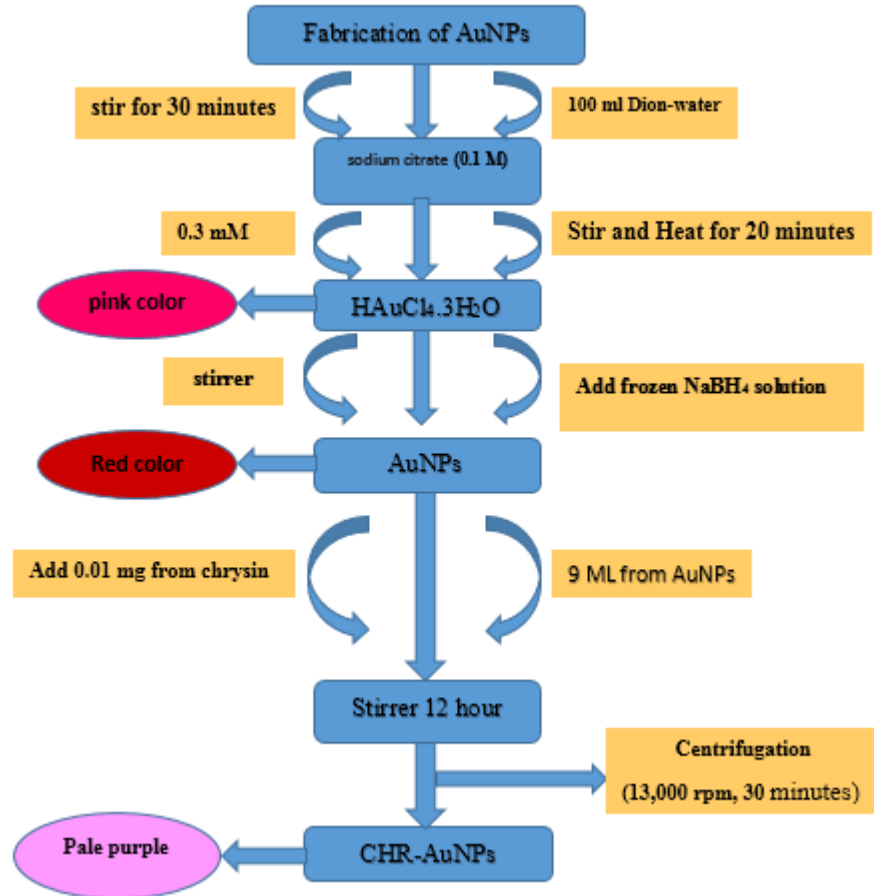


Figure 4.2. Chemical fabrication of gold nanoparticles

The citrate reduction process is favorable since AuNPs are simply dispersed in polar solvents like water, which is a result of the negative charge of citrate ions on AuNPs surface. The emergence of localized surface plasmon resonance absorption bands, which are characteristic of metallic NPs like gold, might cause discoloration. In addition, AuNPs are relatively small particles in which the electrons aren't as free to move as in bulk gold (Zhou *et al.*, 2014). This restriction in motion allows the Au particles to interact differently with the light source. Bulk gold, for instance, appears yellow, whereas AuNPs in colloidal suspension appear blue/purple for larger spherical and nanoscale particles or intense red for smaller spherical particles that are less than 50 nm (Fig. 4.3).

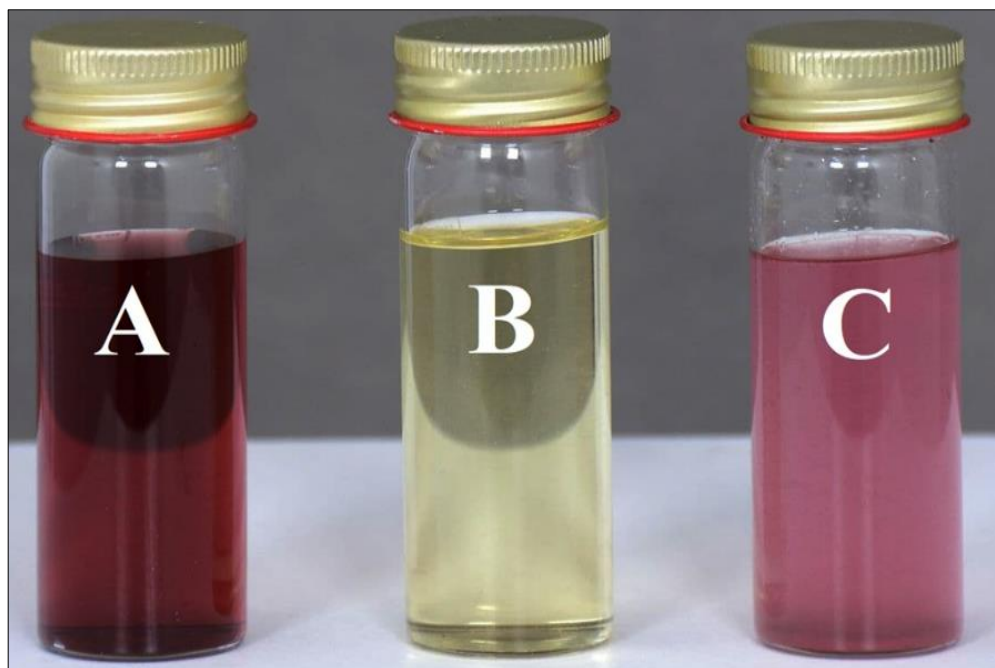


Figure 4.3. Chrysin-gold nanoparticle conjugation. (A) AuNPs solution (B) Chrysin and (C) CHR-AuNPs

## 4.2. Characterizations of CHR, AuNPs and CHR-AuNPs

### 4.2.1. UV-VIS Spectrum Analysis

The optical absorption analysis has been determined by UV-visible spectroscopy for confirming the formation and binding of AuNPs on CHR. The maximum absorption spectra of AuNPs revealed at 520 nm is shown in the Figure 4.4 due to the small and spherical nature of nanoparticles as well as movement of electrons on AuNP, this was obtained at very high wavelength. On the other hand, CHR was a yellow visible solution indicate the lower wavelength shown at 268 nm. The maximum absorption wavelength of CHR-AuNPs revealed that they were significantly interaction of CHR-AuNP nanocomposite which indicates the shifting of peaks to higher wavelength from 520 to 560 nm.

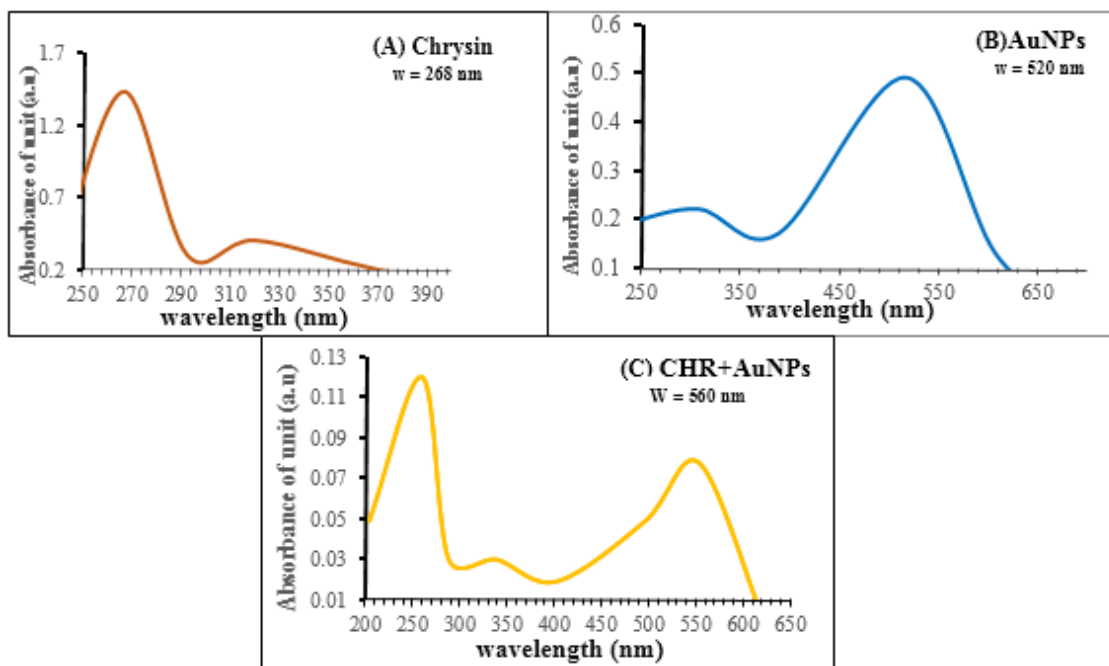


Figure 4.4. UV- VIS spectroscopy analysis (A)AuNPs (B)Chrysin and (C) CHR-AuNPs

The result shows that drug molecules can be blended on the AuNP's surfaces and change their optical properties (Figure 4.4). This is due to phenomenon of clay surface plasmon resonance (SPR) in precious metals that occurs as a result of the vibration of both electrons in metallic NPs in time with the light wave. Several factors, including particle morphology and aggregation, determine the maximum absorption peak, as well as the width and location of the absorbent tape. Among the particles, it is the buffer environment, and often the secondary metabolites that are accountable for the result of combining motion and vice versa (Shamaila et al., 2016).

#### 4.2.2. FTIR Analysis

FTIR can be defined as one of the most widely utilized spectroscopic methods for determining functional groups in materials. It is a useful and effective tool for obtaining fingerprint information on the structural description and chemical composition of materials.

The FTIR was utilized to analyze the prepared CHR and AuNPs nanocomposites, the FTIR spectra of CHR, CHR-AuNPs, and Au NPs have been detected in spectral range 3600-400  $\text{cm}^{-1}$ . The OH-homologous stretching vibration frequency of the AuNPs was found to be 3,419.79  $\text{cm}^{-1}$  in FTIR spectra, indicating the presence of alcoholic group. At 2,987.74  $\text{cm}^{-1}$ , 2931.80  $\text{cm}^{-1}$  the presence of C-H bond was detected which was due to

vibrating stretching of alkaline series found in the composites materials. However, presence of the (C = O) bond has been detected at  $1583.56\text{ cm}^{-1}$ , the bands at  $1398.39\text{ cm}^{-1}$  belong to the (C-C-C) stretching and vibrating methyl group. In addition, the wave number  $1259.52\text{ cm}^{-1}$  ( $1200\text{-}1400\text{cm}^{-1}$ ) corresponds to multiple aromatic C = C, while the wave number  $1078.21\text{ cm}^{-1}$  indicates the C-O carboxyl group (Jiang et al., 2007).

The wave number  $3012.81\text{ cm}^{-1}$  O-H stretching vibration is indicated in the FTIR spectra of the CHR absorption band. The C-H extension of CH is represented by the bands at  $2,929.87\text{ cm}^{-1}$ ,  $2713.84\text{ cm}^{-1}$  and  $2630.99\text{ cm}^{-1}$ . Peaks at  $1653\text{ cm}^{-1}$ ,  $1612.49\text{ cm}^{-1}$  are due to C =O stretch, whereas those at  $1575.84$  and  $1355.96\text{ cm}^{-1}$  are resulted from C-C-C stretching and bending in C-CO-C of aromatic group while absorption at  $1165\text{ cm}^{-1}$ - $1099.43\text{ cm}^{-1}$  are due to C-O stretch (Elsabahy & Wooley, 2013). The spectra of CHR-AuNPs showed several interactions that occurred between groups. The wave number at  $3448.72\text{ cm}^{-1}$  belongs to the vibrationally extended group hydroxyl (O-H). The alkane is reflected in the band at  $2935.66\text{ cm}^{-1}$  and  $2960.73\text{ cm}^{-1}$ . The spectral bands at  $1587.42$ ,  $1463.97$  and  $1390.68\text{ cm}^{-1}$  are attributed to aromatic C=C stretch and aromatic C-O stretch at  $1276.88\text{ cm}^{-1}$ .

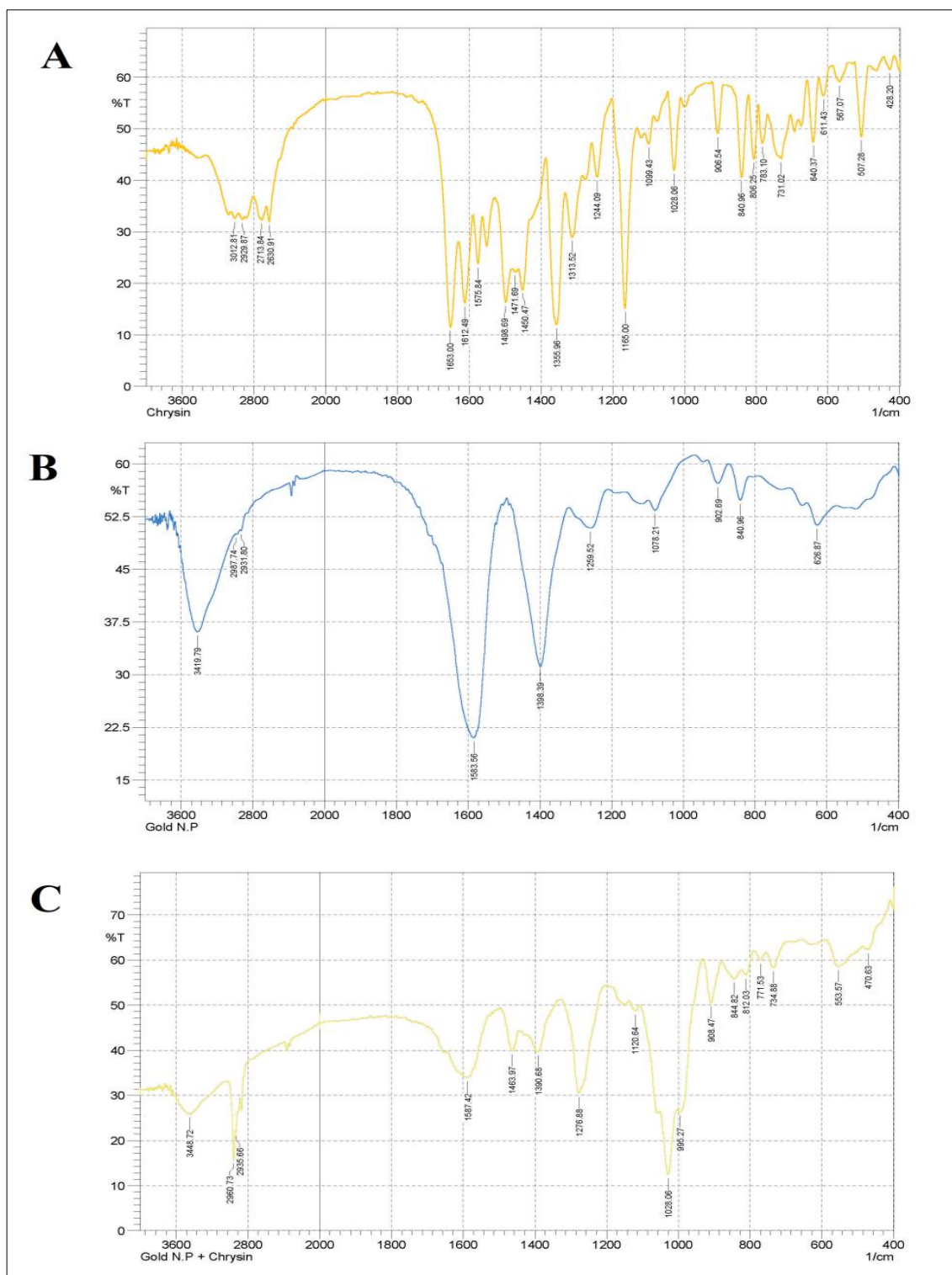


Figure 4.5. FTIR analysis of (A) Chrysin (B) AuNPs (C) CHR-AuNPs

### 4.2.3. X-Ray Diffraction Analysis (XRD)

The majority of polymer materials are not fully crystal since the parts of the chains do not have an order to arrangement of their chains. As a result, such polymers have two phases: amorphous and crystalline (Pierini *et al.*, 2017). Furthermore, the crystalline structures

of CHR, AuNPs and CHR-AuNPs crystal was investigated using X-ray diffractometer and the results is revealed in Figure 4.5. The AuNPs' diffraction peaks are visible at  $2\Theta = 21.77^\circ, 30.56^\circ, 38.12^\circ, 44.18^\circ$  and  $56.35^\circ$  as seen in Figure 4.6 (B). While chrysin crystal structure showed in Figure 4.6 (A) revealed a strong peaks at  $2\Theta = 7.18^\circ, 12.60^\circ, 14.92^\circ, 17.75^\circ, 24.97^\circ, 27.68^\circ$ , indicating that it has a more crystalline structure, additionally several peaks were observed when CHR was incorporated into AuNPs. The peaks found at  $23.5^\circ, 27.55^\circ, 38.15^\circ, 44.01^\circ$ , and  $57.4^\circ$  indicate that the drug is dispersed molecularly or in an amorphous state, and that allows easy drug molecules' diffusion through polymer matrix, which results in sustained drug release from nanoparticles. Since the CHR coupling interaction had no effect on the size of the mineral core, the change in nanoparticle size and the observed difference could only be attributed to the organic envelope of nanoparticle swelling (Zabielska-Koczywaś et al., 2018).

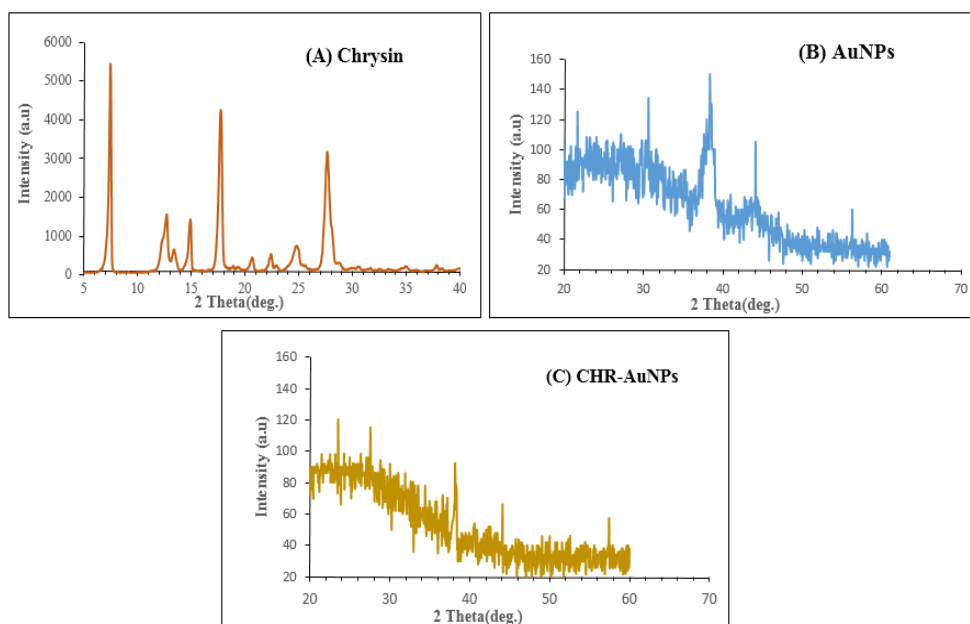


Figure 4.6. X-ray diffraction analysis (A) CHR (B) AuNPs (C) CHR-AuNPs.

#### 4.2.4. X-Ray Spectroscopy with Energy Dispersive (EDX)

The elemental analysis and chemical characterization of the prepared samples was investigated using an EDX spectrometer. The EDX analysis provides qualitative and quantitative information on elements Au and oxygen forming CHR-AuNPs nanocomposites. Its ability to characterize mainly comes from the fact that each element has its own composition. The atomic structure allows for a certain set of electromagnetic emission spectral peaks. In addition, the percentage of carbon and oxygen composition in drug-loaded AuNPs was high, which confirmed that chrysin was loaded on AuNPs. Due

to the high carbon content and low oxygen content of the sample, it revealed a variety of physical and chemical properties of AuNP as a biological binding agent (Chen *et al.*, 2015).

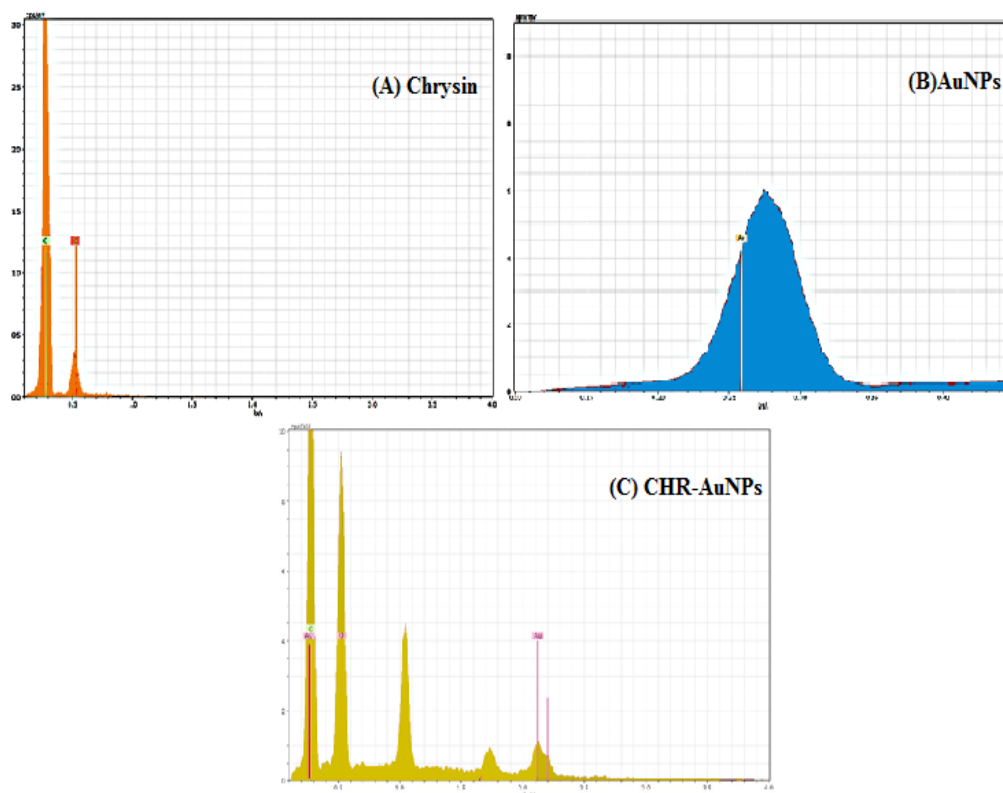


Figure 4.7. Analysis of (A) CHR (B) AuNPs and (C) CHR-AuNPs. X-ray (EDX) analysis

#### 4.2.5. The Zeta Potential Analysis

Amongst the most important factors determining the rate of drug uptake into NPs are particle surface charge and drug affinity for NPs, as well as loading efficiency. The zeta potential is an important physico-chemical indicator of nanoscale stability (Pongsuchart *et al.*, 2012). Extreme positive or negative values result in greater driving repulsive forces, whereas the repulsive forces between particles of similar charges prevent particles from accumulating. Potential values greater than 30 mV ensure good stability in general, while potential values greater than 40 mV ensure excellent stability. A 20 mV value indicates stability at small ranges, while values between 5 mV and 5 mV show rapid assembly (Honary *et al.*, 2010). The zeta analysis of chrysin, AuNPs and CHR-AuNPs was performed. The chrysin solution showed an average zeta potential of -163.23 mV while chemically synthesized AuNPs displayed zeta potential value of -31.54 mV. On the

other hand, CHR-AuNPs exhibited zeta potential of -48.92 mV, which indicates that the chrysin-loaded gold nanoparticles remained stable in solution (Figure 4.8).

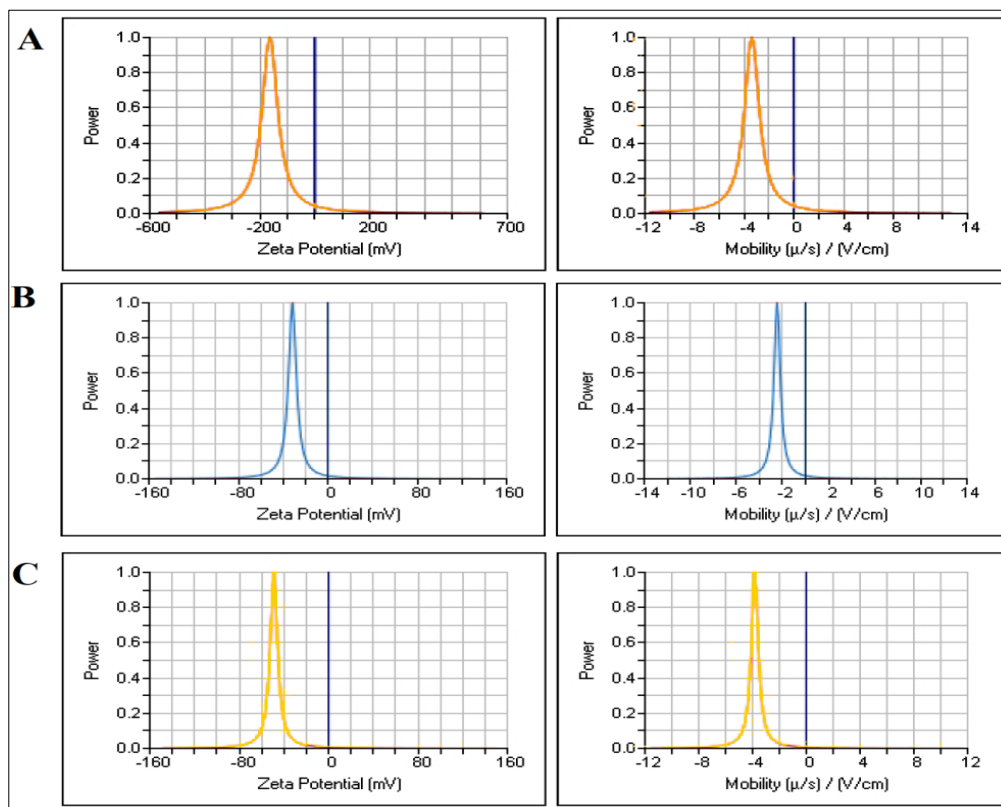


Figure 4.8. Analysis of the zeta potential of (A) CHR (B) AuNPs and (C) CHR-AuNPs

#### 4.2.6. FE -SEM

The morphologies of prepared nanocomposites were investigated and the results have been shown in Fig. 4.9 with the corresponding size distribution. AuNPs are spherical structures with a smooth structure and good separation, with an average size of 17.37 nm at 25.90 nm. The majority of the particles had a peculiar distribution, with no clusters and clear homogeneity, which is attributable to the decrease of AuNPs citrate in polar solvents. Citrate ions can give AuNPs negative charges on their surfaces, making them easier to disperse (Agunloye, 2018).

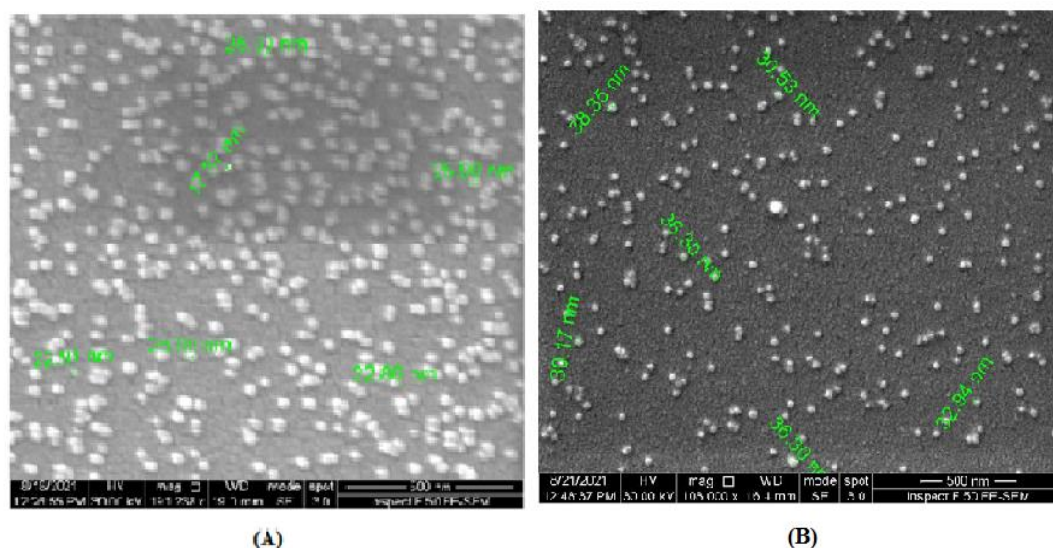


Figure 4.9. FESEM image of (A) AuNPs (B) CHR-AuNPs

In Figure 4.9(B), the gold nanoparticles appeared in a spherical shape and increased in size compared to the AuNPs in Figure 4.9(A), which indicates that chrysin binds to the synthesized gold nanoparticles resulting in an increase in their surface area.

#### 4.3. Antioxidant Activity Assay

The free radical scavenging activity results have been revealed in Figure 4.10 which shows the antioxidant activities of CHR, AuNPs and CHR-AuNPs, which were monitored using four different concentrations. The data show that the oxidation concentrations differed between CHR, AuNPs and CHR-AuNPs, wherein the oxidation concentrations of chrysin were 3.1, 6.2, 12 and 25  $\mu\text{g/ml}$ . The concentration was read and the results showed that chrysin significantly reduced DPPH levels in concentration-dependent manner with 25  $\mu\text{g ml}^{-1}$  significantly better than other concentrations, showed an antioxidant activity of 52.1%. Compared with the activity of AuNPs, CHR-AuNPs have higher ability to reduce DPPH radicals than chrysin. The antioxidant activities of AuNPs and CHR-AuNPs (25  $\mu\text{g/ml}$ ) were calculated as 74.89% and 91.33%, respectively. This could be attributed to the fact that the biological activity of CHR-AuNPs improved by the increase of the specific surface area due to the increased surface energy and catalytic reaction. Significantly, antioxidants might donate electrons to reactive free radicals and convert them to more stable, non-reactive species (Sulaiman, 2016).

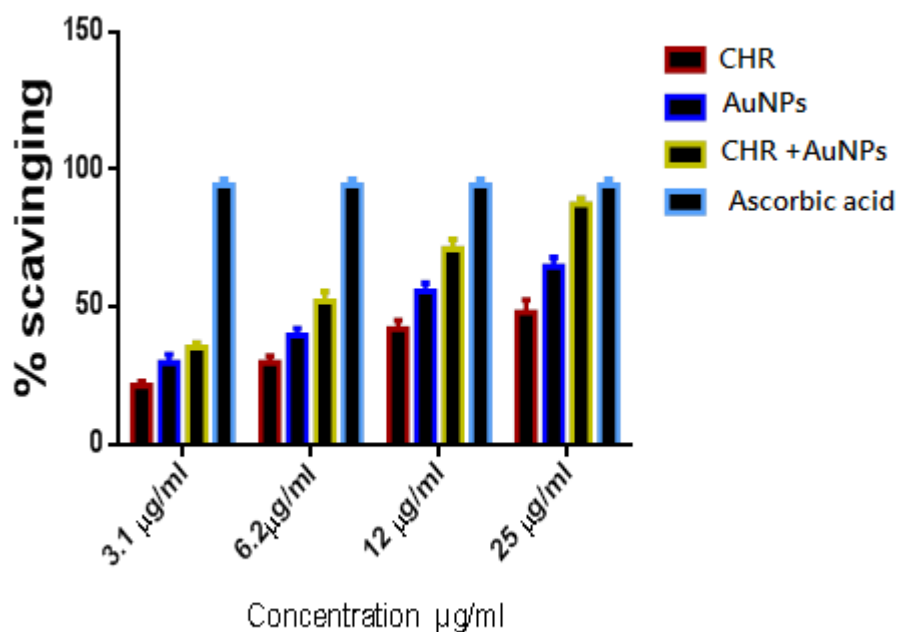


Figure 4.10. Chrysin, AuNPs and CHR-AuNPs increase scavenging of free radicals

#### 4.4. Cytotoxic Effects of Chrysin, AuNPs and CHR-AuNPs

##### 4.4.1. Viability and Cytotoxicity Assay

One of the most common colorimetric assays for assessing cytotoxicity or cell viability is methylthiazolotetrazolium bromide (MTT) (3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide) assay (Daoudi *et al.*, 2013). The MTT assay clearly explains the cellular response to a toxin, and synthesized CHR-AuNPs have higher anticancer activity against AMJ13 cells than chrysin or AuNPs in this study. When compared to chrysin or AuNPs, there was dose-dependent cytotoxicity. Comparing CHR-AuNPs, AuNPs, and chrysin, CHR-AuNPs have the most cytotoxic effect (Figure 4.11).

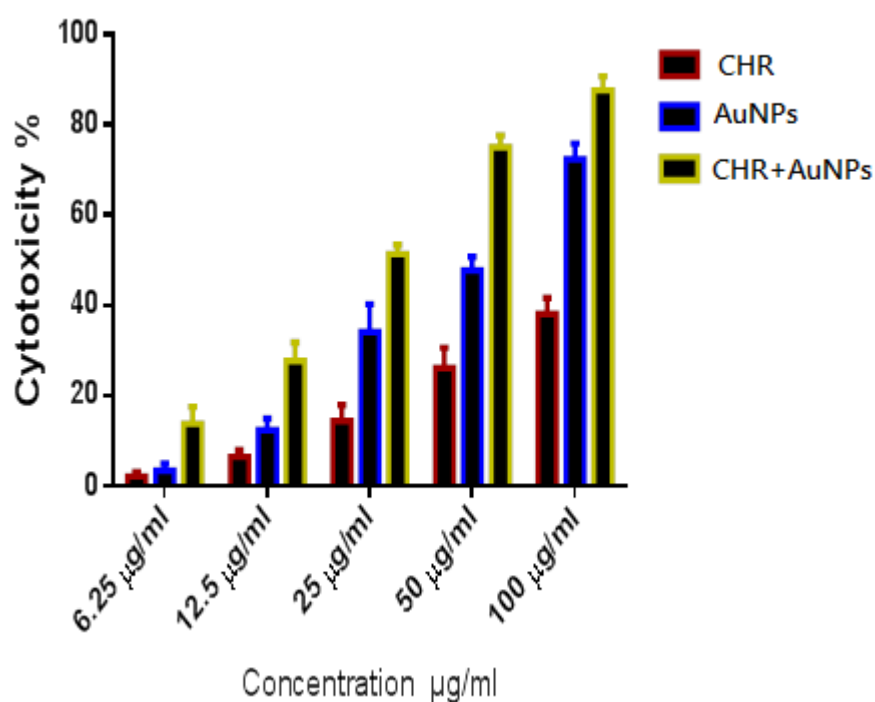


Figure 4.11. Cytotoxic effect of Chrysin, AuNPs and CHR- AuNPs in AMJ13 cells

At 100 µg/ml CHR-AuNPs concentration, the observed highest cytotoxicity was 89% while at 6.25 µg/ml the highest cytotoxicity observed was 17% compared to the control group. AuNPs treatment was 73.3% effective, whereas chrysin treatment was 41.5 % effective at the concentrations of 100µg/ml and 6.25µg/ml, respectively. CHR-AuNPs had a much stronger cytotoxic effect than free chrysin, indicating that chrysin's anticancer efficacy has improved since it was functionalized with Au NPs. It is worth noting that concentration of synthetic CHR-AuNPs used in this study has been significantly lower in comparison to that of chrysin to observe significant level of cytotoxicity. With increasing NP concentrations, cell viability decreases, indicating that more NPs can accumulate inside cells, which causes increased stress and eventually cell death. The biokinetics and toxicity of nanoparticles are influenced by their shape, size, surface area, and surface actuation (Nel *et al.*, 2006).

In a recent research, it was shown that the synthesis of chrysin nanoparticles inhibited cell proliferation and growth in breast cancer cells in concentration-dependent manner (Sulaiman *et al.*, 2018). The data of this work could show CHR-AuNPs cell death by necrosis or programmed cell death. A study is needed on the mechanism by which CHR-AuNPs lead to cell death in cancer cells.

#### 4.4.2. Crystal Violet Staining Method

A crystal violet assay was utilized for confirming the cytotoxic effect of CHR-AuNPs ( $IC_{50}$  concentration) on AMJ13 cells. The results showed that chrysin alone was less cytotoxic to AMJ13 cells and was less effective than AuNPs at the tested concentration. CHR-AuNPs, on the other hand, were found to be significantly more toxic to AMJ13 cells. CHR-AuNPs caused morphological changes such as cell shape changes, treated cells appearing clumped with few cell extensions, and cell communication inhibition, which were not seen in untreated cells (Figure 4.12). As a result of their small size, NPs can penetrate cells more effectively than microparticles, making large particle administration easier.

Nanoparticles, because of their small size, have the ability to penetrate cells more effectively when compared to the microparticles, which simplifies the administration of large quantities of the drug for better performance. Gold nanoparticles, in particular, have biological properties such as antioxidant, anti-inflammatory, and anti-inflammatory for cancer. AuNPs are also good candidates for drug carriers due to their bio-compatibility and lack of toxicity (Ho *et al.*, 2017). Other research has found that chrysin has a cytotoxic effects on many human cancer cell lines, which include cervical, laryngeal, liver and breast cancer cells (Veeramuthu *et al.*, 2017).

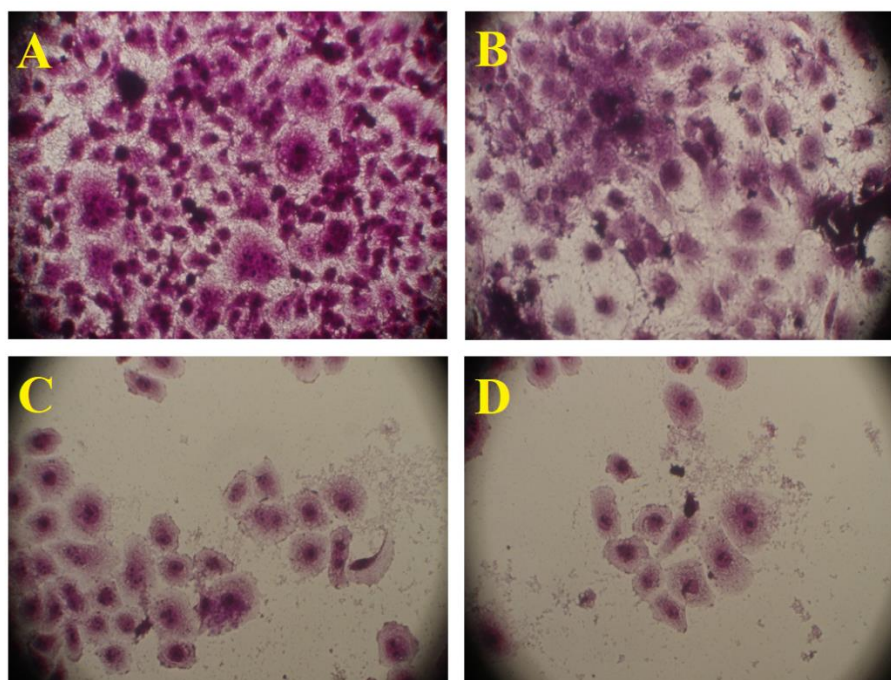


Figure 4.12. Microscopic images of the AMJ13 cell line stained with crystal violet dye after treatment with various concentration levels of CHR, AuNPs, and CHR-AuNPs: (A)

non-treated control cells (B) CHR-treated cells (C) AuNPs-treated cells (D) CHR-AuNPs-treated cells.

#### 4.5. Antibacterial Activity

##### 4.5.1. Antibacterial Activity Assay Against *Staphylococcus aureus*

The antimicrobial activity of CHR, AuNPs, and CHR-AuNPs against *S. aureus* at five different concentrations was analysed. After treatment with different concentrations (31.25, 62.50, 125, 250  $\mu\text{g/ml}$ ), antibacterial effects differed between CHR, AuNPs and CHR-AuNPs, induced by chrysin and gold nanoparticles, and CHR-AuNPs against *Staphylococcus aureus*. CHR-AuNPs had the most effective blocking and killing than AuNPs, followed by CHR. At a dose of 250  $\mu\text{g/ml}$  of CHR-AuNPs, the strongest inhibition was observed at 0.23% uptake, while cells were affected by up to 0.18% AuNPs at the same dose (Fig 4.13). While the inhibition rate of chrysin was 0.04%. However, they showed that CHR-AuNPs had the highest antibacterial inhibition rate, much higher than gold nanoparticles and chrysin, indicating that chrysin improves the antibacterial inhibition efficacy when activated with AuNPs.

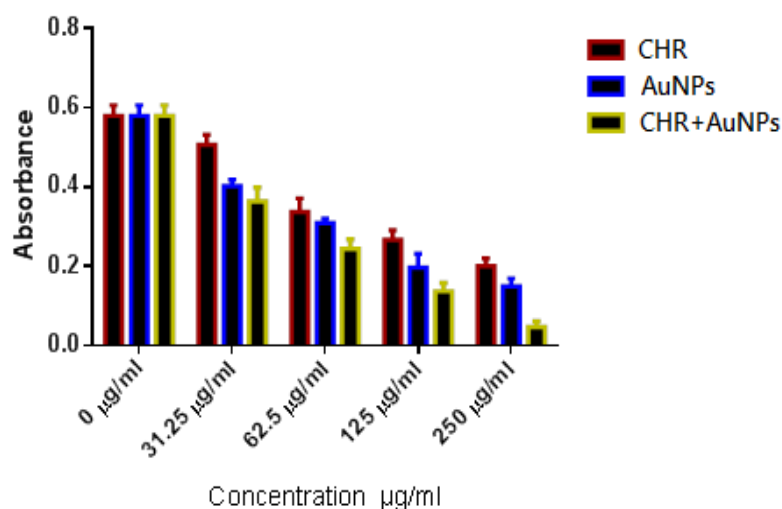


Figure 4.13. Antibacterial activity CHR, AuNPs and CHR-AuNPs against *S. aureus*

#### 4.5.2. Antibacterial Activity Assay Against *E. coli*

The antibacterial activity of CHR, AuNPs and CHR-AuNPs against *E. coli* was measured at five different concentrations. The results showed that the antibacterial activities differed between CHR, AuNPs and CHR-AuNPs after treatment with different concentrations (31.25, 62.50, 125, 250 $\mu\text{g/ml}$ ). CHR-AuNPs were the most effective in inhibiting growth of *E. coli*.

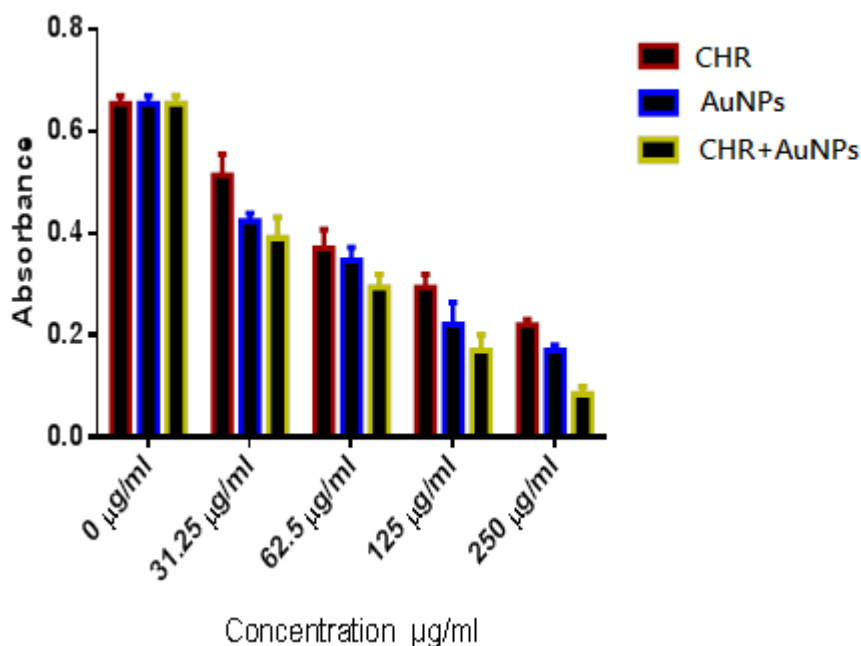


Figure 4.14. Antibacterial activity CHR, AuNPs and CHR-AuNPs against *E. coli*

At a dose of 250  $\mu\text{g/ml}$  CHR-AuNPs, The strongest inhibition has been observed at 0.1% uptake, while cells were affected when up to 0.2% AuNPs were used at the same dose. The inhibitory effect of chrysin treatment was 0.24%. They did, however, show that CHR-AuNPs have the highest antibacterial inhibition rate, much higher than gold nanoparticles and chrysin, indicating that chrysin improves antibacterial inhibition efficacy when activated with AuNPs.

Along with the foregoing, the uncoated free AuNPs exhibited moderate antibacterial activity, whereas the flavonoids have exhibited only weak inhibitory effect against Gram-negative bacteria. Since several flavonoids had previously demonstrated very excellent potential against Gram-positive strains, the outer membrane may act as a shield for Gram-negative bacteria against this natural product type (Su *et al.*, 2020). The presence of flavonoids on AuNPs surface may enhance their ability to inhibit Gram-

negative bacteria. In order to show their antibacterial characteristics, we created Chrysin-coated gold nano-conjugates. The gold nanoparticle coated with chrysin is the most powerful in terms of its effectiveness, as it is able to penetrate *E. coli* cell wall. Through two suggested processes, such nanoconjugates have been capable of combating the Gram-negative bacteria:

- Increasing the rigidity of the bacterial membrane, and thus decreasing its functionality
- Targeting the bacterial unit.

Alteration of cytoplasmic membrane function, inhibition of DNA synthesis, reduced cell binding and biofilm formation, inhibition of energy metabolism, alteration of membrane permeability, inhibition of purines on the cell membrane, and damage to the cytoplasm are some of the proposed antibacterial mechanisms of flavonoids (Renzetti *et al.*, 2020).

From the above results, we note that the inhibition rate of *E. coli* (Gram-negative) is higher than that of *S. aureus* (Gram-positive). This is because Gram-positive bacteria have a much higher cell wall thickness compared to the Gram-negative, and contain a polypeptidoglycan layer, while Gram-negative bacteria have a thin peptidoglycan layer (Gordon *e tal.*, 2011).

## 5. CONCLUSION

In the present work, CHR-AuNPs were prepared using a simple and efficient method that involved several physical and chemical processes. The study found that CHR-AuNPs have high cytotoxicity and induce apoptosis in human breast cancer cells. CHR-AuNPs have the potential to be used as an effective agent in clinical cancer treatment strategies. Moreover, CHR-AuNPs were found to have an important role in inhibiting bacterial growth. The rate of Gram-negative inhibition was higher, because Gram-positive bacteria have a cell wall thickness much higher than that of Gram-negative bacteria. The Gram-positive bacteria contain a thick polypeptidoglycan layer while the Gram-negative bacteria have a thin peptidoglycan layer. Therefore, we note the difference in the ratio of inhibition. Also, it was found that CHR-AuNPs act as concentration-dependent antioxidants, and this can be attributed to the fact that the biological activity of CHR-AuNPs can be increased by increasing the specific surface area due to the increased surface energy and catalytic reaction. Remarkably, antioxidants may donate electrons to reactive free radicals and convert them to more stable, non-reactive species.

In conclusion, the potential of chrysin-loaded gold nanoparticles for drug delivery applications was investigated. Because of this study, the as-prepared particles can be used in multi-drug nano-formulas including antimicrobial, antioxidant and anticancer drugs.

## REFERENCES

- Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S., & Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties*, 20 (8), 1689–1699.
- Abou El-Nour, K. M. M., Eftaiha, A., Al-Warthan, A., and Ammar, R. A. A. (2010). Synthesis and applications of silver nanoparticles. *Arabian Journal of Chemistry*, 3 (3), 135–140.
- Agunloye, E. G. (2018). Mathematical modelling of the formation of gold nanoparticles via the citrate synthesis method. September.
- Anari, E., Akbarzadeh, A., and Zarghami, N. (2016). Chrysin-loaded PLGA-PEG nanoparticles designed for enhanced effect on the breast cancer cell line. *Artificial Cells, Nanomedicine and Biotechnology*, 44 (6), 1410–1416.
- Anderson, W. F., Rosenberg, P. S., Prat, A., Perou, C. M., and Sherman, M. E. (2014). How many etiological subtypes of breast cancer: Two, three, four, or more? *Journal of the National Cancer Institute*, 106 (8), 1–11.
- Argyle, D. J., and Blacking, T. (2008). From viruses to cancer stem cells: Dissecting the pathways to malignancy. *Veterinary Journal*, 177 (3), 311–323.
- Arora, S., Rajwade, J. M., and Paknikar, K. M. (2012). Nanotoxicology and in vitro studies: The need of the hour. *Toxicology and Applied Pharmacology*, 258 (2), 151–165.
- Athar, M., and Das, A. J. (2014). Therapeutic nanoparticles: State-of-the-art of nanomedicine. *Advanced Materials Reviews*, 1 (1), 25–37.
- Aw-Yong, P. Y., Sasmita, A. O., Gan, P. H., Mak, S. T., and Ling, A. P. K. (2018). Nanoparticles as Carriers of Phytochemicals: Recent Applications Against Lung Cancer. *International Journal of Research in Biomedicine and Biotechnology*, 7 (1), 1–11.
- Banerjee, A., Qi, J., Gogoi, R., Wong, J., and Mitragotri, S. (2016). Role of nanoparticle size, shape and surface chemistry in oral drug delivery. *Journal of Controlled Release*, 238, 176–185.
- Bartneck, M., Keul, H. A., Gabriele, Z. K., and Groll, J. (2010). Phagocytosis independent extracellular nanoparticle clearance by human immune cells. *Nano Letters*, 10 (1), 59–64. <https://doi.org/10.1021/nl902830x>
- Bhateria, R., and Singh, R. (2019). A review on nanotechnological application of magnetic iron oxides for heavy metal removal. *Journal of Water Process Engineering*, 31 (October 2018), 100845.
- Biffi, S., Voltan, R., Rampazzo, E., Prodi, L., Zauli, G., and Secchiero, P. (2015). Applications of nanoparticles in cancer medicine and beyond: Optical and multimodal in vivo imaging, tissue targeting and drug delivery. *Expert Opinion on Drug Delivery*, 12 (12), 1837–1849.
- Biswas, A. K., Islam, M. R., Choudhury, Z. S., Mostafa, A., and Kadir, M. F. (2014). Nanotechnology based approaches in cancer therapeutics. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 5 (4).

- Boldeiu, A., Simion, M., Mihalache, I., Radoi, A., Banu, M., Varasteanu, P., Nadejde, P., Vasile, E., Acasandrei, A., Popescu, R. C., Savu, D., and Kusko, M. (2019). Comparative analysis of honey and citrate stabilized gold nanoparticles: In vitro interaction with proteins and toxicity studies. *Journal of Photochemistry and Photobiology B: Biology*, *197*, 111519.
- Boraschi, D., Costantino, L., and Italiani, P. (2012). Interaction of nanoparticles with immunocompetent cells: Nanosafety considerations. *Nanomedicine*, *7* (1), 121–131.
- Breast cancer in young women*. (2010). 10549.
- Brodowska, K. M. (2017). Natural flavonoids: classification, potential role, and application of flavonoid analogues. *European Journal of Biological Research*, *7* (2), 108–123.
- Buem, S., Xozen, M., and Ph, D. (1994). *What Is the Etiology of Human Brain Tumors ? A Report on the First Lebow Conference*. 709–713.
- Calixto, G. M. F., Bernegossi, J., De Freitas, L. M., Fontana, C. R., Chorilli, M., and Grumezescu, A. M. (2016). Nanotechnology-based drug delivery systems for photodynamic therapy of cancer: A review. *Molecules*, *21* (3), 1–18.
- Chatterjee, S. J., Datar, R., Youssefzadeh, D., George, B., Goebell, P. J., Stein, J. P., Young, L., Shi, S. R., Gee, C., Groshen, S., Skinner, D. G., and Cote, R. J. (2004). Combined effects of p53, p21, and pRb expression in the progression of bladder transitional cell carcinoma. *Journal of Clinical Oncology*, *22* (6), 1007–1013.
- Chen, B. X., Wilson, S. R., Das, M., Coughlin, D. J., and Erlanger, B. F. (1998). Antigenicity of fullerenes: Antibodies specific for fullerenes and their characteristics. *Proceedings of the National Academy of Sciences of the United States of America*, *95* (18), 10809–10813.
- Chen, G. L., Fan, M. X., Wu, J. L., Li, N., and Guo, M. Q. (2019). Antioxidant and anti-inflammatory properties of flavonoids from lotus plumule. *Food Chemistry*, *277*, 706–712.
- Chen, Z., D'Alfonso, A. J., Weyland, M., Taplin, D. J., Allen, L. J., and Findlay, S. D. (2015). Energy dispersive X-ray analysis on an absolute scale in scanning transmission electron microscopy. *Ultramicroscopy*, *157*, 21–26.
- Chithrakumar, T., Thangamani, M., and Pon miruthu vasini, S. (2021). Exploring nanotechnology for diagnostic, therapy and medicine. *IOP Conference Series: Materials Science and Engineering*, *1091* (1), 012062.
- Colditz, G. A., and Bohlke, K. (2014). Priorities for the primary prevention of breast cancer. *CA: A Cancer Journal for Clinicians*, *64* (3), 186–194. <https://doi.org/10.3322/caac.21225>
- Cuenca, A. G., Jiang, H., Hochwald, S. N., Delano, M., Cance, W. G., and Grobmyer, S. R. (2006). Emerging implications of nanotechnology on cancer diagnostics and therapeutics. *Cancer*, *107* (3), 459–466.
- Cupaioli, F. A., Zucca, F. A., Boraschi, D., and Zecca, L. (2014). Engineered nanoparticles. How brain friendly is this new guest? *Progress in Neurobiology*, *119–120*, 20–38. <http://dx.doi.org/10.1016/j.pneurobio.2014.05.002>

- Danaei, G., Vander Hoorn, S., Lopez, A. D., Murray, C. J. L., and Ezzati, M. (2005). Causes of cancer in the world: Comparative risk assessment of nine behavioural and environmental risk factors. *Lancet*, 366 (9499), 1784–1793.
- Daoudi, A., Aarab, L., and Abdel-Sattar, E. (2013). Screening of immunomodulatory activity of total and protein extracts of some Moroccan medicinal plants. *Toxicology and Industrial Health*, 29 (3), 245–253.
- De Matteis, V., Cascione, M., Rizzello, L., Manno, D. E., Di Guglielmo, C., and Rinaldi, R. (2021). Synergistic effect induced by gold nanoparticles with polyphenols shell during thermal therapy: Macrophage inflammatory response and cancer cell death assessment. *Cancers*, 13 (14).
- Deraedt, C., Salmon, L., Gatard, S., Ciganda, R., Hernandez, R., Ruiz, J., and Astruc, D. (2014). Sodium borohydride stabilizes very active gold nanoparticle catalysts. *Chemical Communications*, 50(91), 14194-14196.
- Ding, Y., Li, S., and Nie, G. (2013). Nanotechnological strategies for therapeutic targeting of tumor vasculature. *Nanomedicine*, 8 (7), 1209–1222.
- Dobrovolskaia, M. A., and McNeil, S. E. (2009). Immunological properties of engineered nanomaterials. *Nanoscience and Technology: A Collection of Reviews from Nature Journals*, 278–287.
- Dreaden, E. C., Alkilany, A. M., Huang, X., Murphy, C. J., and El-Sayed, M. A. (2012). The golden age: Gold nanoparticles for biomedicine. *Chemical Society Reviews*, 41 (7), 2740–2779.
- Dudzinski, S. O. (2020). *th*, 2020.
- Dwivedi, P. D., Misra, A., Shanker, R., and Das, M. (2009). Are nanomaterials a threat to the immune system? *Nanotoxicology*, 3 (1), 19–26.
- Eatemadi, A., Daraee, H., Aiyelabegan, H. T., Negahdari, B., Rajeian, B., and Zarghami, N. (2016). Synthesis and Characterization of Chrysin-loaded PCL-PEG-PCL nanoparticle and its effect on breast cancer cell line. *Biomedicine and Pharmacotherapy*, 84, 1915–1922.
- Elsabahy, M., and Wooley, K. L. (2013). Cytokines as biomarkers of nanoparticle immunotoxicity. *Chemical Society Reviews*, 42 (12), 5552–5576.
- Farokhzad, O. C., and Langer, R. (2009). Impact of nanotechnology on drug delivery. *ACS Nano*, 3 (1), 16–20.
- Fattovich, G., Stroffolini, T., Zagni, I., and Donato, F. (2004). Hepatocellular carcinoma in cirrhosis: Incidence and risk factors. *Gastroenterology*, 127 (5 SUPPL.), 35–50.
- Feng, B., Matsui, H., and Tomizawa, K. (2011). Nanoparticle-based Drug Delivery Systems for Solid Brain Tumors. *Current Nanoscience*, 7 (1), 47–54.
- Ferrari, M. (2005). Cancer nanotechnology: Opportunities and challenges. *Nature Reviews Cancer*, 5 (3), 161–171.
- Glass, C. K. (2001). Potential roles of the peroxisome proliferator-activated receptor- $\gamma$  in macrophage biology and atherosclerosis. *Journal of Endocrinology*, 169 (3), 461–464.

- Gmeiner, W. H., and Ghosh, S. (2014). Nanotechnology for cancer treatment. *Nanotechnology Reviews*, 3 (2), 111–122.
- Gordon, T.; Perlstein, B.; Houbara, O.; Felner, I.; Banin, E.; Margel, S. Synthesis and characterization of zinc/iron oxide composite nanoparticles and their antibacterial properties. *Colloids Surfaces A Physicochem. Eng. Asp.* **2011**, 374, 1–8.
- Gu, F., Zhang, L., Teply, B. A., Mann, N., Wang, A., Radovic-Moreno, A. F., Langer, R., and Farokhzad, O. C. (2008). Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proceedings of the National Academy of Sciences of the United States of America*, 105 (7), 2586–2591.
- Han, S. S., Kim, M. S., Lim, W., Park, G. H., Park, I., and Chang, S. E. (2018). Classification of the clinical images for benign and malignant cutaneous tumors using a deep learning algorithm. *The Journal of Investigative Dermatology*. <https://doi.org/10.1016/j.jid.2018.01.028>
- Harries, M., and Smith, I. (2002). The development and clinical use of trastuzumab (Herceptin). *Endocrine-Related Cancer*, 9 (2), 75–85.
- Hassan, M., and Research Online, G. (2017). *The Role of Human Papillomaviruses in the Aetiopathogenesis of Head and Neck Cancer in South Asia, and Approaches to Treatment* Author. <http://hdl.handle.net/10072/366967>
- Heiligtag, F. J., and Niederberger, M. (2013). The fascinating world of nanoparticle research. *Materials Today*, 16 (7–8), 262–271.
- Ho, B. N., Pfeffer, C. M., and Singh, A. T. K. (2017). Update on nanotechnology-based drug delivery systems in cancer treatment. *Anticancer Research*, 37 (11), 5975–5981.
- Honary, S., Jahanshahi, M., Golbayani, R., Ebrahimi, R., and Ghajar, K. (2010). Doxorubicin-loaded albumin nanoparticles: Formulation and characterization. *Journal of Nanoscience and Nanotechnology*, 10 (11), 7752–7757.
- Huntley, A. L. (2009). The health benefits of berry flavonoids for menopausal women: Cardiovascular disease, cancer and cognition. *Maturitas*, 63 (4), 297–301.
- In, L. B., Pregnant, H. H., In, W., and State, E. D. O. (2018). *Healthy Hiv-Seronegative Pregnant Women In Ekpoma, Edo State*,. 7 (1), 18–27.
- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. (2005). ICH Harmonised Tripartite Guideline S8: Immunotoxicity Studies for Human Pharmaceuticals. *Ich*, September. <http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html> *j.cell.2011.02.013.pdf*. (n.d.).
- Jabir, M. S., Taha, A. A., Sahib, U. I., Taqi, Z. J., Al-Shammari, A. M., and Salman, A. S. (2019). Novel of nano delivery system for Linalool loaded on gold nanoparticles conjugated with CALNN peptide for application in drug uptake and induction of cell death on breast cancer cell line. *Materials Science and Engineering C*, 94, 949–964.
- Jain, S., Hirst, D. G., and O’Sullivan, J. M. (2012). Gold nanoparticles as novel agents for cancer therapy. *British Journal of Radiology*, 85 (1010), 101–113.
- Jemal, A., Bray, F., and Ferlay, J. (2011). *Global Cancer Statistics*. 61 (2), 69–90.

- Jiang, G., Wang, L., and Chen, W. (2007). Studies on the preparation and characterization of gold nanoparticles protected by dendrons. *Materials Letters*, 61 (1), 278–283.
- Josh, K. S., Sharma, C. P., Kalarikkal, N., Sandeep, K., Thomas, S., and Pothan, L. A. (2016). Evaluation of in-vitro cytotoxicity and cellular uptake efficiency of zidovudine-loaded solid lipid nanoparticles modified with Aloe Vera in glioma cells. *Materials Science and Engineering C*, 66, 40–50.
- Juárez-Maldonado, A., Ortega-Ortíz, H., Morales-Díaz, A. B., González-Morales, S., Morelos-Moreno, Á., Cabrera-De la Fuente, M., Sandoval-Rangel, A., Cadenas-Pliego, G., and Benavides-Mendoza, A. (2019). Nanoparticles and nanomaterials as plant biostimulants. *International Journal of Molecular Sciences*, 20 (1), 1–19.
- Khan, A. K., Rashid, R., Murtaza, G., and Zahra, A. (2014). Gold nanoparticles: Synthesis and applications in drug delivery. *Tropical Journal of Pharmaceutical Research*, 13 (7), 1169–1177. <https://doi.org/10.4314/tjpr.v13i7.23>
- Kocbek, P., Obermajer, N., Cegnar, M., Kos, J., and Kristl, J. (2007). Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody. *Journal of Controlled Release*, 120 (1–2), 18–26.
- Kong, F. Y., Zhang, J. W., Li, R. F., Wang, Z. X., Wang, W. J., and Wang, W. (2017). Unique roles of gold nanoparticles in drug delivery, targeting and imaging applications. *Molecules*, 22 (9).
- Krishnan, U., Barbara, S., Arifullah, M., Noori Abdul-nabi, N., and Antonella, P. (2018). Relevance of Nanotechnology in Food Processing Industries. *International Journal of Agriculture Sciences* 9107, 10 (7), 5730–5733.
- Krock, B. L., Skuli, N., and Simon, M. C. (2011). *Hypoxia-Induced Angiogenesis : Good and Evil*. 1117–1133.
- Le Duc, G., Roux, S., Paruta-Tuarez, A., Dufort, S., Brauer, E., Marais, A., Truillet, C., Sancey, L., Perriat, P., Lux, F., and Tillement, O. (2014). Advantages of gadolinium based ultrasmall nanoparticles vs molecular gadolinium chelates for radiotherapy guided by MRI for glioma treatment. *Cancer Nanotechnology*, 5 (1), 1–14.
- Lee, K. X., Shameli, K., Yew, Y. P., Teow, S. Y., Jahangirian, H., Rafiee-Moghaddam, R., and Webster, T. J. (2020). Recent developments in the facile bio-synthesis of gold nanoparticles (AuNPs) and their biomedical applications. *International Journal of Nanomedicine*, 15, 275–300.
- Lee, A. V., Oesterreich, S., and Davidson, N. E. (2015). MCF-7 Cells - Changing the Course of Breast Cancer Research and Care for 45 Years. *Journal of the National Cancer Institute*, 107 (7), 1–4.
- Levine, A. J., Hu, W., and Feng, Z. (2008). Tumor Suppressor Genes. *The Molecular Basis of Cancer*, 31–38.
- Li, N., Zhao, P., and Astruc, D. (2014). Anisotropic gold nanoparticles: Synthesis, properties, applications, and toxicity. *Angewandte Chemie - International Edition*, 53 (7), 1756–1789.
- Li, Xiangqian, Xu, H., Chen, Z. S., and Chen, G. (2011). Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*, 2011.

- Li, Xinyan, Jiang, S., and Tapping, R. I. (2010). Toll-like receptor signaling in cell proliferation and survival. *Cytokine*, 49 (1), 1–9.
- Liu, D., Yang, F., Xiong, F., and Gu, N. (2016). The smart drug delivery system and its clinical potential. *Theranostics*, 6 (9), 1306–1323.
- Liu, Y., Jiao, F., Qiu, Y., Li, W., Qu, Y., Tian, C., Li, Y., Bai, R., Lao, F., Zhao, Y., Chai, Z., and Chen, C. (2009). Immunostimulatory properties and enhanced TNF- $\alpha$  mediated cellular immunity for tumor therapy by C60 (OH)<sub>20</sub> nanoparticles. *Nanotechnology*, 20 (41).
- Loeve, S. (2010). About a definition of nano: How to articulate nano and technology? *Hyle*, 16 (1), 3–18.
- Lucarelli, M., Gatti, A. M., Savarino, G., Quattroni, P., Martinelli, L., Monari, E., and Boraschi, D. (2004). Innate defence functions of macrophages can be biased by nano-sized ceramic and metallic particles. *European Cytokine Network*, 15 (4), 339–346.
- Lum, J. J., Bauer, D. E., Kong, M., Harris, M. H., Li, C., Lindsten, T., and Thompson, C. B. (2005). *of Autophagy and Cell Survival in the Absence of Apoptosis*. 120, 237–248.
- Luo, Y. H., Chang, L. W., and Lin, P. (2015). Metal-Based Nanoparticles and the Immune System: Activation, Inflammation, and Potential Applications. *BioMed Research International*, 2015 (Figure 1).
- M. Satyaprakash, T. N., B. Sadhana, S. S. V., and Padal, S. B. (2017). A Review on Polycyclic Aromatic Hydrocarbons: Their Transport, Fate and Biodegradation in the Environment. *International Journal of Current Microbiology and Applied Sciences*, 6 (4), 1627–1639.
- Merza, K. S., Al-Attabi, H. D., Abbas, Z. M., and Yusr, H. A. (2012). Comparative Study on Methods for Preparation of Gold Nanoparticles. *Green and Sustainable Chemistry*, 02 (01), 26–28.
- Mohammadian, F., Pilehvar-Soltanahmadi, Y., Mofarrah, M., Dastani-Habashi, M., and Zarghami, N. (2016). Down regulation of miR-18a, miR-21 and miR-221 genes in gastric cancer cell line by chrysin-loaded PLGA-PEG nanoparticles. *Artificial Cells, Nanomedicine and Biotechnology*, 44 (8), 1972–1978.
- Moliner, M., Gabay, J. E., Kliewer, C. E., Carr, R. T., Guzman, J., Casty, G. L., Serna, P., and Corma, A. (2016). Reversible Transformation of Pt Nanoparticles into Single Atoms inside High-Silica Chabazite Zeolite. *Journal of the American Chemical Society*, 138 (48), 15743–15750.
- Mousa, S. A., and Bharali, D. J. (2011). Nanotechnology-based detection and targeted therapy in cancer: Nano-bio paradigms and applications. *Cancers*, 3 (3), 2888–2903.
- Muzammil, S., Hayat, S., Fakhar-e-Alam, M., Aslam, B., Siddique, M. H., Nisar, M. A., Saqalein, M., Atif, M., Sarwar, A., Khurshid, A., Amin, N., and Wang, Z. (2018). Nanoantibiotics: Future nanotechnologies to combat antibiotic resistance. *Frontiers in Bioscience - Elite*, 10 (2), 352–374.
- Nanda, H. S. (2016). Preparation and biocompatible surface modification of redox altered cerium oxide nanoparticle promising for nanobiology and medicine. *Bioengineering*, 3 (4).

- Nel, A., Xia, T., Mädler, L., and Li, N. (2006). Toxic potential of materials at the nanolevel. *Science*, 311 (5761), 622–627.
- Ninan, N., Goswami, N., and Vasilev, K. (2020). The impact of engineered silver nanomaterials on the immune system. *Nanomaterials*, 10 (5), 1–26.
- Osborne, C. K., and Schiff, R. (2011). Mechanisms of endocrine resistance in breast cancer. *Annual Review of Medicine*, 62, 233–247.
- Pal, R. S., Ariharasivakumar, G., Girhepunjhe, K., and Upadhay, A. (2009). In-vitro antioxidative activity of phenolic and flavonoid compounds extracted from seeds of *Abrus precatorius*. *Int J Pharm Pharm Sci*, 1(2), 136-40.
- Panahi, Y., Mohammadhosseini, M., Nejati-Koshki, K., Abadi, A. J. N., Moafi, H. F., Akbarzadeh, A., and Farshbaf, M. (2017). Preparation, Surface Properties, and Therapeutic Applications of Gold Nanoparticles in Biomedicine. *Drug Research*, 67 (2), 77–87.
- Pesic, I., Krstic, M., Pavlovic, M., and Ilic, D. (2014). *Original articles Hormone Sensitivity Of Tumor In Women With Breast*. 2 (April 2007), 25–30.
- Petrovic, N. (2016). *Targeting Angiogenesis in Cancer Treatments : Where do we Stand ?* 19 (2), 226–238.
- Pongsuchart, M., Danladkaew, C., Khomvarn, T., and Sereemasapun, A. (2012). Effect of Glutathione-Stabilized Gold Nanoparticles in 3T3 Fibroblast Cell. *2012 International Conference on Clean and Green Energy IPCBEE*, 27, 98–102.
- Prior, I. A., Lewis, P. D., and Mattos, C. (2012). A comprehensive survey of ras mutations in cancer. *Cancer Research*, 72 (10), 2457–2467.
- Pulskamp, K., Diabaté, S., and Krug, H. F. (2007). Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicology Letters*, 168 (1), 58–74.
- Ramos, R. G., and Olden, K. (2008). Gene-environment interactions in the development of complex disease phenotypes. *International Journal of Environmental Research and Public Health*, 5 (1), 4–11.
- Renzetti, A., Betts, J. W., Fukumoto, K., and Rutherford, R. N. (2020). Antibacterial green tea catechins from a molecular perspective: Mechanisms of action and structure–activity relationships. *Food & function*, 11(11), 9370-9396
- Ring, C. J. A. (2002). Cytolytic viruses as potential anti-cancer agents. *Journal of General Virology*, 83 (3), 491–502.
- Rosen, J. E., Chan, L., Shieh, D. Bin, and Gu, F. X. (2012). Iron oxide nanoparticles for targeted cancer imaging and diagnostics. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 8 (3), 275–290.
- Rosière, R., Berghmans, T., de Vuyst, P., Amighi, K., and Wauthoz, N. (2019). The position of inhaled chemotherapy in the care of patients with lung tumors: Clinical feasibility and indications according to recent pharmaceutical progresses. *Cancers*, 11 (3).
- Ruenraroengsak, P., Cook, J. M., and Florence, A. T. (2010). Nanosystem drug targeting: Facing up to complex realities. *Journal of Controlled Release*, 141 (3), 265–276.

- Sahoo, S. K., Parveen, S., and Panda, J. J. (2007). The present and future of nanotechnology in human health care. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 3 (1), 20–31.
- Scott, D. A., and Drake, R. R. (2019). Glycosylation and its implications in breast cancer. *Expert Review of Proteomics*, 16 (8), 665–680.
- Shamaila, S., Zafar, N., Riaz, S., Sharif, R., Nazir, J., and Naseem, S. (2016). Gold nanoparticles: An efficient antimicrobial agent against enteric bacterial human pathogen. *Nanomaterials*, 6 (4), 1–10.
- Shilpa, B. S., Padmamma, S., Kumara, T. S. A., and Walmiki, R. H. (2019). Mapping of scientific articles on Leukemia: A scientometric study. *Library Philosophy and Practice*, 2019.
- Siegel, R. L., Miller, K. D., and Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, 69 (1), 7–34.
- Singh, P., Pandit, S., Mokkapati, V. R. S. S., Garg, A., Ravikumar, V., and Mijakovic, I. (2018). Gold nanoparticles in diagnostics and therapeutics for human cancer. *International journal of molecular sciences*, 19(7), 1979.
- Smith, M. J., Brown, J. M., Zamboni, W. C., and Walker, N. J. (2014). From immunotoxicity to nanotherapy: The effects of nanomaterials on the immune system. *Toxicological Sciences*, 138 (2), 249–255.
- Sosa, V., Moliné, T., Somoza, R., Paciucci, R., Kondoh, H., and Lleonart, M. E. (2013). Oxidative stress and cancer: An overview. *Ageing Research Reviews*, 12 (1), 376–390.
- Soule, H. D., Vazquez, J., Long, A., Albert, S., and Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma<sup>1,2</sup>. *Journal of the National Cancer Institute*, 51 (5), 1409–1416.
- Su, C., Huang, K., Li, H., Lu, Y., and Zheng, D. (2020). *Antibacterial Properties of Functionalized Gold Nanoparticles and Their Application in Oral Biology*. 2020.
- Sulaiman, Ghassan M., Jabir, M. S., and Hameed, A. H. (2018). Nanoscale modification of chrysin for improved of therapeutic efficiency and cytotoxicity. *Artificial Cells, Nanomedicine and Biotechnology*, 46 (sup1), 708–720.
- Sulaiman, Ghassan Mohammad. (2016). Molecular structure and anti-proliferative effect of galangin in HCT-116 cells: In vitro study. *Food Science and Biotechnology*, 25 (1), 247–252.
- Sumbaly, R. (2015). *Diagnosis of Breast Cancer using Decision Tree Data Mining Technique*. March, 15–24.
- Sztandera, K., Gorzkiewicz, M., and Klajnert-Maculewicz, B. (2019). Gold Nanoparticles in Cancer Treatment. *Molecular Pharmaceutics*, 16 (1), 1–23.
- Szunerits, S., and Boukherroub, R. (2016). Antibacterial activity of graphene-based materials. *Journal of Materials Chemistry B*, 4 (43), 6892–6912.
- Taha, S. O. (2013). In Vivo Antimicrobial Activity of Ethanol Extract of Sumac (*Rhus coriaria*) on *Klebsiella pneumoniae*. *British Journal of Pharmacology and Toxicology*, 4 (1), 1–4.

- Tang, S., and Zheng, J. (2018). Antibacterial Activity of Silver Nanoparticles: Structural Effects. *Advanced Healthcare Materials*, 7 (13), 1–10.
- Teng, H., Zheng, Y., Cao, H., Huang, Q., Xiao, J., and Chen, L. (2021). Enhancement of bioavailability and bioactivity of diet-derived flavonoids by application of nanotechnology: a review. *Critical Reviews in Food Science and Nutrition*, 0 (0), 1–16.
- Thakor, A. S., and Gambhir, S. S. (2013). Nanooncology: The future of cancer diagnosis and therapy. *CA: A Cancer Journal for Clinicians*, 63 (6), 395–418.
- Watanabe, M. A. E., Amarante, M. K., Conti, B. J., and Sforcin, J. M. (2011). Cytotoxic constituents of propolis inducing anticancer effects: A review. *Journal of Pharmacy and Pharmacology*, 63 (11), 1378–1386.
- Xia, Y., Zhou, Y., and Tang, Z. (2011). Chiral inorganic nanoparticles: Origin, optical properties and bioapplications. *Nanoscale*, 3 (4), 1374–1382.
- Zabielska-Koczywaś, K., Wojtalewicz, A., Użarowska, E., Klejman, A., Wojtkowska, A., Dolka, I., Wojnicki, M., Sobczak, K., Wójcik, M., Shen, H., Ferrari, M., and Lechowski, R. (2018). Distribution of glutathione-stabilized gold nanoparticles in feline fibrosarcomas and their role as a drug delivery system for doxorubicin—preclinical studies in a murine model. *International Journal of Molecular Sciences*, 19 (4).
- Zhang, Z., and Wu, Y. (2010). Investigation of the NaBH<sub>4</sub>-Induced aggregation of Au nanoparticles. *Langmuir*, 26 (12), 9214–9223.
- Zhong, G., Liu, J., and Liu, X. (2015). A fast colourimetric assay for lead detection using label-free gold nanoparticles (AuNPs). *Micromachines*, 6 (4), 462–472.
- Zhou, Y., Harrison, D. E., Love-Myers, K., Chen, Y., Grider, A., Wickwire, K., Burgess, J. R., Stochelski, M. A., and Pazdro, R. (2014). Genetic analysis of tissue glutathione concentrations and redox balance. *Free Radical Biology and Medicine*, 71, 157–164.
- Zolnik, B. S., González-Fernández, Á., Sadrieh, N., and Dobrovolskaia, M. A. (2010). Minireview: Nanoparticles and the immune system. *Endocrinology*, 151 (2), 458–465.
- Pal, R. S., Ariharasivakumar, G., Girhepunjhe, K., and Upadhyay, A. (2009). In-vitro antioxidative activity of phenolic and flavonoid compounds extracted from seeds of *Abrus precatorius*. *Int J Pharm Pharm Sci*, 1(2), 136-40.
- Veeramuthu, D., Raja, W. R. T., Al-Dhabi, N. A., and Savarimuthu, I. (2017). Flavonoids: anticancer properties. *Flavonoids: From Biosynthesis to Human Health*, 287.

## APPENDIX I

### Mueller–Hinton agar

Beef Extract	2.0g
Casein Hydrolysate	17.5g
Starch	1.5g
Agar	17.0g
Distilled Water	1000ml

### Roswell park memorial institute (RPMI)-1640 medium

RPMI	16.4g
FBS	100 ml
Streptomycin	0.5ml
Benzyl penicillin	0.5ml
Sodium bicarbonate	5-10 ml
Distilled water	800 ml

## CURRICULUM VITAE

Ahmed Jasim . After graduating from High school, he complete BSc Biochemical Technology from the Technology University in Iraq.

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### Publications

- Ahmed Jasim, Hilal Ay. (2022). Gold nanoparticles are loaded with chrysin and used in a successful biocompatible, anticancer, and anti-inflammatory delivery system. 7th International Conference on Engineering and Technology Management (16-17-18 April 2022 Istanbul).
- Ahmed Jasim, Ghassan Sulaiman, Hilal Ay, Salman Mohammed, Hamdoon Mohammed, Majid Jabir, Riaz Kahn. (2022). Pilot trials of the gold nanoparticles conjugated chrysin: A preliminary assessment towards enhancing anti-oxidant, anti-microbial, and cytotoxic activity of a nanoformulated flavonoid. Nanotechnology Reviews (In Press).