THE SYNTHESIS OF GREEN SILVER NANOPARTICLE CHROMOLAENA ODOROTA LEAF EXTRACT

by

NUR SHAHERAH WAJIHAH BINTI PUASA

J20A0577

A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY

UNIVERSITI MALAYSIA KELANTAN

2024

DECLARATION

I declare that this thesis entitled "The synthesis of green silver nanoparticles *Chromolaena odorota*" is the result of my own research except as cited in the references.

Signature : Shaherahwajihah

Student's Name : Nur Shaherah Wajihah binti Puasa

Date :

Verified by:

Signature :

Supervisor's Name : Dr Wan Suriyani Faliq Adeeba binti Wan Ibrahim

Stamp :

Date :

ACKNOWLEDGEMENT

At the very beginning I would like to express my deepest gratitude to almighty Allah for giving me the strength and the composure to complete the research successfully. During the period of my research, I have received generous help from many quarters, which I like to put on record here with deep gratitude and magnificent pleasure.

First, I would like to express my appreciation to my supervisor, Dr. Wan Suriyani Faliq Adeeba Wan Ibhrahim for her guidance and constant support to complete my thesis with her patience and knowledge. Her vision, sincerity, motivation, and insistence helped me to be enthusiastic about completing my research. Without her kind direction and proper guidance this thesis would not have been a little success.

I would like to thank for our lab assistant, who always help with laboratory equipment and technical support during my FYP thesis. It would not be possible to conduct this research without his assistance.

Finally, my deepest gratitude to my beloved father Mr. Puasa bin Pandin, my beloved mother Mrs. Dyg Rokiah binti Damit and to my dearest siblings for their prayer and encouragement. Special thanks also to all my lovely friends for their support and help especially at times when things were going tough. Also do not forget to those who indirectly contributed to this research, your kindness means a lot for me. This accomplishment would not have been possible enough without them. Thank you.

Sintesis Hijau Nanozarah Perak daripada Ekstrak Daun Kapal Terbang

ABSTRAK

Kajian ini adalah untuk sintesis hijau nanopartikel perak (AgNPs) menggunakan ekstrak daun kapal terbang dengan menggunakan tiga pelarut berbeza iaitu metanol, etil asetat dan heksana sebagai agen penurunan dan penstabil. Pengekstrakan dilakukan menggunakan kaedah Soxhlet. Pendekatan mesra alam ini menawarkan keberkesanan kos dan mengelakkan bahan kimia berbahaya berbanding kaedah konvensional. Teknik pencirian mengesahkan pembentukan AgNP, dengan pemerhatian visual mencadangkan sintesis yang berjaya dalam masa 24 jam. Spektroskopi UV-Vis memberikan bukti lanjut berdasarkan resonans plasmon permukaan, manakala analisis FTIR mengenal pasti kumpulan berfungsi yang berpotensi terlibat dalam bioreduksi dan penstabilan. Elektron yang kaya dengan atom oksigen dalam ikatan rangkap C=O boleh mendermakan elektron kepada ion perak. Spektrum FTIR ekstrak daun C. odorota dan AgNP yang disintesis telah dibandingkan. Walau bagaimanapun, pengoptimuman proses sintesis, menggunakan pelarut yang berbeza berpotensi meningkatkan aktiviti perencatan AgNPs. Walau bagaimanapun, pengoptimuman proses sintesis, menggunakan pelarut yang berbeza berpotensi meningkatkan aktiviti perencatan AgNPs. Tambahan pula mikroskop stereoskopik telah digunakan untuk memerhati sel daun kapal terbang. Penyelidikan ini membuka jalan untuk penerokaan lanjut AgNP yang disintesis hijau daripada daun kapal terbang. Memahami pembentukan, ciri dan potensinya untuk pengoptimuman boleh membuka jalan untuk aplikasinya dalam pelbagai bidang, dan pembangunan bahan nano.

Kata kunci: Daun kapal terbang, Nanopartikel perak, Metanol, Atil asetat, Heksana

The Green Synthesis of Silver Nanoparticles from Chromolaena Leaf Extract

ABSTRACT

This study is to synthesis the green synthesis of silver nanoparticles (AgNPs) using *Chromolaena odorota* leaf extract by using three different solvent which is methanol, ethyl acetate and hexane as a reducing and stabilizing agent. The extraction is done using Soxhlet method. This eco-friendly approach offers cost-effectiveness and avoids harmful chemicals compared to conventional methods. Characterization techniques confirmed the formation of AgNPs, with visual observation suggesting successful synthesis within 24 hours. UV-Vis spectroscopy provided further evidence based on surface plasmon resonance, while FTIR analysis identified functional groups potentially involved in bioreduction and stabilization. The electron that rich oxygen atom in the C=O double bond can donate an electron to a silver ion. The FTIR spectra of leaf extract *of C. odorota* and the synthesized AgNPs were compared. However, the optimization of the synthesis process, using different solvent potentially enhance the inhibitory activity of AgNPs. Furthermore, stereoscopic microscope has been used to observe the *C. odorota* leaf cell. This research paves the way for further exploration of green-synthesized AgNPs from *C. odorota* leaves. Understanding their formation, characteristics, and potential for optimization can pave the way for their application in various fields, and nanomaterial development.

Keywords: *Chromolaena Odorota*, Silver nanoparticles, Methanol, Ethyl acetate, Hexane

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS

C.odorota	*Chromolaena Odorota*
FTIR	Fourier transform infrared spectroscopy
Uv Vis	ultraviolet-visible
XRD	X-ray diffraction
NPs	Nanoparticles
AgNPs	Silver Nanoparticles
$AgNO3$	silver nitrate
Ag	silver
SPR	surface plasmon resonance

LIST OF SYMBOLS

%	Percentages
Cm	Centimetre
m	Meter
Ml	Millilitre
Kg	kilogram
G	Gram
Nm	nanometer
Mm	Millimolar
RPM	Revolutions per minute
Mm	Micrometre
±	Plus-minus

CHAPTER 1

INTRODUCTION

1.1 Background of study

The process of transforming matter at a scale like the atomic level to produce new materials, structures, and devices is known as nanotechnology. The method improves science in many different sectors, including manufacturing, consumer goods, energy, and medicine (Logothetidis, 2011). Nanotechnology is the design of structures, devices, and systems. Between 1 and 100 nanometers are the size range of nanomaterials. At this scale, materials begin to show different characteristics, influencing physical, chemical, and biological behaviour. The research, development, and use of these features is critical to the advancement of new technologies (Whatmore, 2006).

To create NPs, a variety of techniques have been used, including chemical reduction, electrochemistry, photochemistry, and physical procedures. The nanomaterials sector currently uses green synthesis to create NPs. By mixing metal ions with organic component such vitamins, carbohydrates, the plant extracts, microorganisms and biodegradable polymers, green synthesis creates nanoparticles (NPs). Plant extracts can be used as a system stabiliser as well as a lowering agent (Ghorbani et al., 2011).

The plants extract can reduce and capping the agents for nanoparticles synthesis, are more advantageous than other biological processes. Thus, eliminate the elaborated process of culturing and maintaining the cell, and scale up for the large-scale nanoparticle synthesis. Moreover, plant mediated nanoparticles synthesis is preferred because it is cost effective, environmentally friendly, a single step method for biosynthesis process and safe for the usage of human therapeutic. All different parts of plant such as leaf, fruit peels, root has been used for the synthesis of silver, gold, titanium, platinum nanoparticles (Gopinath et al., 2012).

In this experiment, the *C.odorota*, are also known as *siam weed, christmas bush, eupatorium odorota* and so on. *C.odorota* is a flowering plant in the sunflower family name Asteraceae (Mabberley, 1997). South America is where it first came from but as become a widespread invasive species through the subtropical regions around the world, including sections of Africa, Asia, and the Pacific Islands. The plant is a shrubby perennial that can reach a height of 3 m and has hairy stems and leaves. The leaves are aromatic when crushed and have serrated edges. The shrub produces clusters of little white or lavender blooms (Vaisakh & Pandey, 2012).

1.2 Problem statement

The goal of this experiment is to create a process that is affordable, environmentally friendly, and sustainable for producing green silver nanoparticles. Toxic chemicals and high-energy inputs are used in the manufacture of silver nanoparticles utilising chemical and physical processes, which are not environmentally friendly and may cause negative impacts on human health. The challenge is to create a dependable and effective process for synthesising green silver nanoparticles from a leaf extract of *C. odorata* that can be used for several purposes such as biomedicine, nanoelectronics, and environmental remediation.

1.3 Expected outputs

To evaluate the biocompatibility of the synthesized green silver nanoparticles using in vitro cell culture models. The results will provide valuable information on the safety and toxicity of the nanoparticles, which is crucial for their biomedical applications and as wastewater treatment. The Synthesis of green silver nanoparticles which is the primary output of this study is the successful production of green silver nanoparticles using leaf extract of *C. odorata* as a reducing and stabilizing agent. The nanoparticles will be characterized using various analytical techniques, including UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), stereoscopic microscope.

1.4 Objectives

1. To synthesize silver nanoparticles using *C. odorata* leaf extract using different type polarity of solvent which is Hexane. Etyl acetate and Methanol.
2. To characterize the silver nanoparticles using uv-vis spectroscopy, fourier transform infrared spectroscopy (FTIR), stereoscopic microscope.

1.5 Scope of study

The scope of this study is limited to the synthesis of green silver nanoparticles using *C. odorata* leaf extract. Formation of silver nanoparticles will characterize and confirmed by using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), stereoscopic microscope. Besides that, the synthesizing green silver nanoparticles using *C. odorata* leaf extract using different type polarity of solvent which is Hexane, Ethyl acetate and Methanol.

1.6 Significance of study

The findings of this research will assist students in the field of biology in terms of the kind of plant that have the quality of good reducing agents. In which using the green silver nanoparticles that environmentally friendly which highly benefit for various type of field. For example, treatment, diagnosis of medicine, drug delivery and personal health care.

CHAPTER 2

LITERATURE REVIEW

2.1 Chromolaena Odorota

One of the most harmful tropical weeds in the world is *Chromolaena odorata*. It belongs to the tribe *Eupatorieae* of the sunflower family *Asteraceae*. Common names for the plant include *devil weed, Siam weed, French weed,* so on (Anyasi, 2012). The natural habitat of *C. odorota* is in the Americas and widens to Florida (USA) to northern Argentina. *Chromolaena* is a popular weed in tropical and subtropical areas that include west, central, and southern Africa, India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, southern China, Taiwan, and Indonesia (Vaisakh & Pandey, 2012).

C. odorota is widely used in traditional medicine, especially for external illnesses including wounds, skin conditions, inflammation, etc. The leaf extract has anti-inflammatory, antioxidant, a pain reliever antibacterial, cytoprotective, and many other for health purposes significant properties. According to phytochemical studies, plants have many kinds of chemical substances (Gogoi et al., 2020).

C. odorota is a growth rapidly herb with straight, pithy, brittle stems that branch easily, have three-veined, ovate-triangular leaves arranged oppositely, and have a shallow, fibrous root structure. At the tips of the branches, capitula are produced in panicles and are lacking in ray florets. Plants can have flower was white to pale blue or violet colour. The characteristic fruits are black with a whitish pappus. *C. odorata* can reach heights of up to 5-10 m when supported by other vegetation, although it often only reaches heights of 2-3 m in open-land environments. The morphology of *C. odorata* varies significantly within its native range in terms of flower colour, leaf shape and hairiness, crushed leaf scent, and the structure of the plant (Owoyele et al., 2005).

Another benefit of *C. odorota* is phytoremediation. The potential of *C. odorata* in phytoremediation a technique in which plants are employed to absorb and collect contaminants from the soil has been investigated. According to some study, it could be useful in clearing polluted soils of heavy metals. Apart from that, it may be applied to improve soil. The plant's root nodules contain nitrogen-fixing bacteria that may help increase the fertility of the soil. Its biomass can decompose and enrich the soil with organic substances. According to certain research, *C. odorata* extracts may be able to repel insects. This could be helpful in certain medical or agricultural applications.

C. odorota also has several disadvantages, most notably its invasive behaviour and significant health hazards. This is by way of the hazards to health and invasiveness. The fast-growing, dense thickets of outcompeted native *C. odorata* plants inhibit the establishment of native vegetation and lower biodiversity. Ecosystems may be upset, and endangered animals may be put in risk. It also lowers production in agriculture. It competes with crops for sunshine, nutrients, and water in affected regions, resulting in lower yields and financial losses for farmers. In addition, it hinders infrastructure. Its thick growth can obstruct roadways, irrigation systems, and drainage channels, causing floods and causing traffic disruptions. Furthermore, there are health hazards. Pyrrolizidine alkaloids, which are present in *C. odorota,* have the potential to be hazardous to both people and animals in significant doses. Prolonged exposure to these alkaloids has been connected to liver cancer and has the potential to harm the kidneys and liver. Some people may experience allergic responses, including as skin rashes, breathing issues, and eye discomfort, from the pollen and other plant pieces.

The applications of in *C. odorota* leaf extract in various industries. First, corrosion inhibitor. The extract's phytochemicals, like tannins and alkaloids, act as natural corrosion inhibitors for metals like iron, steel, and aluminium. This can be valuable in industries like construction, oil, and gas, and automotive, where corrosion poses a major challenge. Second, green adhesive. The extract's adhesive properties can be harnessed to develop eco-friendly alternatives to synthetic adhesives. This could benefit industries like furniture manufacturing, packaging, and paper production. Third, textile dyeing. The extract contains natural dyes that can be used to colour textiles. This offers a sustainable and vibrant alternative to synthetic dyes, reducing environmental pollution and promoting a circular economy in the textile industry (Gogoi et al., 2020).

Figure 2.1: The leaf and flower morphology of *C. odorota* (Zahara, 2019)

2.2 Nanotechnology

Nanotechnology is the study and manipulation of matter at the nanoscale, where unique phenomena allow for novel applications. The characteristics of matter can differ significantly from those of bulk materials, individual atoms, and molecules at the nanoscale, exhibiting distinctive physical, chemical, and biological characteristics (Nikulainen & Palmberg, 2010). Some nanostructured materials are stronger or have different magnetic characteristics than other forms or sizes of the same material. Others are more adept at transferring electricity or heat. As their size or structure changes, they may become more chemically reactive, reflect more light, or change colour (Fulekar, 2010).

Its benefits are numerous and can have an influence on many facets of our life. Here are a few main advantages. First, there is healthcare, which includes targeted medicine delivery. Drugs may be delivered to damaged cells directly with the help of nanoparticles, which will lessen adverse effects and increase therapy effectiveness. Envision little "drug submarines" traversing your circulation, targeting alone the tumour while sparing healthy cells. Tissue engineering and regeneration are other options. The lack of organ donors may be resolved with the use of nanotechnology in the growth of new tissues and organs for transplantation. This might include repairing injured cartilage, bone, or even complete limbs. Moreover, early diagnosis and identification of illness. Because nano sensors can identify even the smallest changes in the body, illnesses like cancer and Alzheimer's can be diagnosed early, often before symptoms even manifest. Consider them as extremely perceptive bloodhounds that may detect problems before they become major issues.

Nanotechnology can also help the environment. the cleanup of pollutants and the purification of water. Water and soil may be made cleaner and safer by using nanoparticles to eliminate impurities. Imagine cleaning dirty rivers with small sponges to bring them back to their original form. Additionally, renewable energy. We can move away from fossil fuels by using nanotechnology to increase the efficiency of solar panels, wind turbines, and other renewable energy sources. Imagine solar panels that collect twice as much sunlight so that we may use the sunshine in our gardens to power our houses. Next, materials that are strong and lightweight. By using nanomaterials, materials for automobiles, aircraft, and buildings may be made stronger, lighter, and more energy-efficient, which will lower emissions and fuel usage. Envision automobiles composed of incredibly lightweight and durable materials that glide on the road without consuming a lot of fuel. (Logothetidis, 2011)

2.2.1 Nanoparticles

The term nano from Greek terms "nanos" which means small. The nanoparticles sizes are 1 to 1000 nm which are solid colloidal particles made up of macromolecules in which active ingredients like drugs or biological material are dissolved, trapped, encapsulated, or adsorbed (Whatmore, 2006). Nanoparticles has different types based on their shape, size, and properties of material. Nanoparticles has two categories which is organic and inorganic (Khan et al., 2019).

Increased Surface Area is one of the benefits of nanoparticles. Because of their high surface area-to-volume ratio, which increases their reactivity, nanoparticles are a good fit for surface-based and catalytic applications. Moreover, Mechanical properties can be enhanced using nanoparticles. Nanoparticles may be added to materials to greatly increase their mechanical qualities. For instance, the strength, hardness, and durability of polymers or composites can be improved by the addition of nanoparticles. It also improved the optical properties as well. Quantum effects provide for the unique optical features of nanoparticles. They are employed in many different applications, including raising contrast in medical imaging, generating vivid colours in nanocomposite materials, and increasing the efficiency of solar cells.

The disadvantages associated with nanoparticles which is health risks, environmental impact, and ethical considerations. First, health risks. Some nanoparticles, depending on their composition and size, can be toxic to human cells and organs. Inhalation of nanoparticles, for example, can lead to lung damage and inflammation. Certain nanoparticles can potentially damage DNA, raising concerns about cancer development and other genetic abnormalities. Second, environmental impact. Persistence and accumulation. Nanoparticles can be highly persistent in the environment, meaning they don't degrade easily and can accumulate in soil, water, and organisms. This raises concerns about long-term ecological effects. Third, ethical considerations. The rapid development of nanotechnology has outpaced regulations, raising concerns about the safe and ethical use of nanoparticles. The potential benefits of nanotechnology may not be equally distributed, potentially exacerbating existing social and economic inequalities.

2.2.2 Characterization of nanoparticles

The size, shape, surface area, and dispersity of nanoparticles have been identified using UV-visible spectrophotometry, scanning electron stereoscopic microscope, Fourier Transform Infrared Spectroscopy (FTIR), and other techniques. UV-visible spectroscopy has been used by scientists the most frequently identify metal nanoparticles with a size range of 2 to 100 nm. Light with wavelengths between 300 and 800 nm is used to identify metal nanoparticles with a size range of 2 to 100 nm. Silver nanoparticles are often characterised using spectrophotometric adsorption measurements in the 400–450 nm. The FTIR spectroscopy is frequently used to characterise the surface chemistry of nanoparticles.

The development and implementation of nanoparticles are important, which is crucial for a few reasons. First, Comprehending and Forecasting Conduct. Performance is determined by properties. Surface chemistry, size, shape, and composition of nanoparticles all have a fundamental impact on their behaviour, including stability, reactivity, and interactions with other materials. Characterization gives critical data for understanding these features and predicting how nanoparticles will act in different environments. Secondary, ensuring Effectiveness and Safety. The characteristics of nanoparticles can affect their safety and effectiveness. They interact with biological systems in different ways. Characterization aids in the identification of possible dangers, such as toxicity or unwanted immunological responses, which allows for mitigation strategies. Third, regulatory compliance. Meeting high standards. Regulatory agencies have created requirements for nanoparticle usage in a variety of industries. To prove conformity with these criteria and obtain clearance for commercialization, characterization data is essential. Fourth, promoting research and development. New applications result from a deeper comprehension of the characteristics of nanoparticles. The creation of novel nanomaterials with improved functions is guided by characterization data, which also opens the door to innovations in energy, materials science, and health.

2.2.3 Green silver nanoparticles

Green silver nanoparticles are produced using environmentally friendly methods such as plant extracts or natural compounds. The silver nanoparticles production is environmentally friendly and sustainable nanotechnology. In which eliminates the use of toxic chemicals and reduces waste generation (Ghaffari-Moghaddam et al., 2014). Green silver nanoparticles have potential applications in a variety of areas, including medicine, catalysis, and electronics. It has antimicrobial properties, silver nanoparticles which have been used in medicine and so on. The green synthesis techniques could provide safer alternatives to conventional chemical processes (Mohammadlou et al., 2016). Overall, green silver nanoparticles may provide a more ecologically responsible and long-lasting method of producing and using silver nanoparticles. Well, Green nanoparticles have various potential health benefits in medical and biomedical fields (Aboyewa et al., 2021).

An essential step in the biosynthesis process is the creation of metal nanoparticles from live plants, plant extracts, and plant inactivated tissue. Plants have long been recognised to have the ability to lower metal ions in a variety of organs and tissues that are far from the site of ion penetration as well as on their surface (Mohammadlou et al., 2016). Metal ions may be reduced by biomolecules found in plant extracts, such as proteins, enzymes, amino acids, vitamins, polysaccharides, and organic acids like citrates. In this regard, plant extracts are utilised for the bio reduction of metal ions to generate their nanoparticles through the effective development of in vitro techniques in recent years.

Figure 2.2: The size range of AgNPs synthesized (Srikar et al., 2016)

2.3 Green Synthesis Method

Green synthesis is employed to create inorganic nanoparticles by utilising various biologically based systems. The global awareness of adopting "green synthesis method" to limit the production of hazardous waste in the advancement of research and industry is growing because of the rising demand for nanoparticles and nanomaterials (Salem & Fouda, 2021). Green chemistry uses metal nanoparticles having bioreduction capabilities in combination with biomolecules found in plant extracts, such as enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids. Living plants, plant extracts, and inactivated plant tissue are the most common sources of silver nanoparticles (AgNPs) due to the unique diversity of plant kingdom's ability to offer phytochemicals with specific therapeutic qualities (Biswas & Wu, 2005).

The substantial benefits that green synthesis methods offer over conventional synthesis methods have led to their fast adoption in a few scientific domains. This explains why their significance is becoming more widely acknowledged. friendliness to the environment. Green synthesis makes use of naturally produced, biodegradable ingredients and reduces waste creation in contrast to conventional processes, which frequently rely on harsh chemicals and produce hazardous waste. As a result, habitats are protected, and environmental contamination is greatly decreased. Additionally, by using non-toxic materials, this procedure can improve safety and health. Compared to traditional techniques, green synthesis methods often utilise non-toxic or less toxic components, minimising hazards to worker safety and human health during synthesis and possible exposure throughout product usage. Moreover, Improved cost-effectiveness. Decreased Processing Costs. Compared to standard techniques, green methods frequently call for less complex equipment and use less energy, which lowers processing costs and increases accessibility for research and development (Forough & Farhadi, 2010).

Figure 2.3: The green synthesis of A by using leaf AgNPs extracts from the endemic *Buddleja globose* (Carmona et al., 2017).

2.4 Solvent

A solvent is a liquid which other materials dissolve to form a solution. (Burke, 1984) Polar solvents such as water will promote the creation of ions, whereas nonpolar solvents such as hydrocarbons. Solvents can be mostly acidic, mostly basic, both amphoteric and both aprotic, or neither. Alcohols, ketons, esters, ethers, nitrated and halogenated hydrocarbons, as well as aromatic compounds and other hydrocarbons, are examples of organic substances that are employed as solvents. The solvent that been used in this experiment are hexane, ethyl acetate and methanol which has different polarity (Welton & Reichardt, 2011).

2.5 Rotary evaporator

Rotary evaporators, often known as rotovaps, are equipment used to effectively evaporate solvents. Due to its superior extraction and distillation capabilities, the rotary evaporation process is one of the most common methods for solvent evaporation (Lemaire et al., 1999). In addition to the continuous distillation of volatile solvents, rotary evaporators are also used for concentration, crystallisation, drying, separation, and solvent recovery. They are employed in a variety of disciplines and applications, including the pharmaceutical, chemical, and biotechnology industries (Parliment, 2020).

2.6 Soxhlet

A Soxhlet extractor is a piece of scientific glassware used to extract chemicals from solids. Franz von Soxhlet created it in 1879, and it's still in use today (Raynie, 2019). A round-bottomed flask, a syphon tube, a condenser, and a thimble make up the Soxhlet extractor. The solid substance to be removed is put within a filter paper thimble. Next, the thimble is inserted into the Soxhlet extractor's extraction chamber.

In the flask with a circular bottom, the solvent typically a boiling organic solvent is heated until it boils. As the boiling solvent's vapour ascends the condenser and cools, it condenses back into a liquid. The solid substance within the thimble is subsequently covered in a layer of liquid solvent. As the solvent drips on the solid substance, it dissolves part of the soluble components. The extraction chamber's bottom is where the dissolved material gathers. The syphon tube is activated when the extraction chamber's solvent level exceeds a particular threshold. When the solvent level hits a particular point, the syphon tube is meant to let the solvent return to the flask with a circular bottom. Until all of the required material has been removed from the solid substance, this procedure is performed again. One highly effective method for removing chemicals from solid materials is the Soxhlet extraction. Because the solvent used in the extraction process is continuously recycled, it is also a rather safe method (López-Bascón & De Castro, 2020)

The advantages of Soxhlet extract use. Firstly, high efficacy. When it comes to extracting chemicals from solid materials, the Soxhlet extractor is incredibly effective. This is a result of the solvent's continuous recycling and interaction with the solid substance. Secondly, Safety. Because the solvent in the Soxhlet extractor is continuously recycled, it is a rather safe method of extracting chemicals. This lowers the possibility of encountering dangerous solvents. Third, it is easy to use. Utilising the Soxhlet extractor is quite simple. It may be kept running unattended for extended periods of time after it is set up (De Castro & Priego-Capote, 2010)

The disadvantages associated with Soxhlet extractor use. The duration of the Soxhlet extraction procedure varies based on the material being extracted and the intended yield. A solvent is also needed for the Soxhlet extraction process, which can be costly and dangerous.

Furthermore, not all materials lend themselves to the Soxhlet extraction. The heated solvent may cause harm to certain materials (De Castro & Garcıa-Ayuso, 1998).

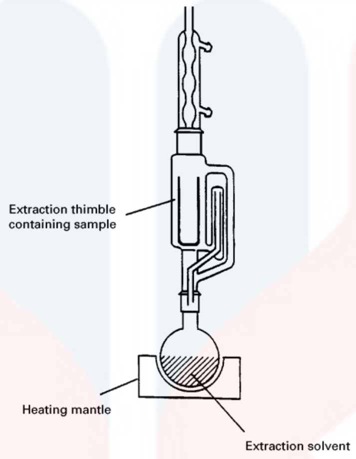


Figure 2.6: The Soxhlet extraction apparatus (De Castro & Priego-Capote, 2010)

2.7 UV-Vis Spectroscopy

UV-Vis Spectroscopy are Spectrophotometry quantitative method to calculate the light of molecule that absorbs. It done by comparing the amount of light that passes through a sample through the light that goes to te reference sample. This method is applicable to a widarious of sample types, including solid, glass. liquids and thin films (Perkampus, 2013).

The function of UV-Vis Spectroscopy. UV-Vis spectroscopy measures the absorption of light in the ultraviolet and visible regions of the electromagnetic spectrum. It provides information about the electronic transitions that occur in molecules. Besides that, it also been used for quantitative analysis of the concentration of a substance in a sample. The absorbance of light is directly proportional to the concentration of the absorbing species. UV-Vis spectra also help identify compounds based on their characteristic absorption patterns. Different molecules absorb light at different wavelengths, allowing for compound identification. (Rajendrachari et al., 2013)

The advantages of UV-Vis Spectroscopy are rapid analysis which UV-Vis spectroscopy provides quick and efficient analysis, making it suitable for high-throughput applications. Besides that, side applicability. UV-Vis spectroscopy is applicable to a broad range of compounds, including organic and inorganic molecules, as long as they absorb in the

UV or visible regions. Furthermore, Sensitivity. UV-Vis is a sensitive technique, capable of detecting low concentrations of analytes in a sample (Bratovčić et al., 2009).

While UV-Vis spectroscopy is a potent and commonly used analytical method, it does have significant limits and disadvantages. The following are some of the main disadvantages are Insufficient Structural Data. While precise structural information about the molecule may not be obtained, it can reveal information on electronic transitions. Its ability to provide details on the spatial arrangement of atoms is very limited (Akash et al., 2020).

2.8 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a strong analytical method that employs infrared light to identify and characterise materials' chemical compositions. Besides that, FTIR spectroscopy is a technique used to obtain the absorption or emission infrared spectrum of a solid, liquid, or gasprovides a unique "fingerprint" of organic, inorganic, and polymeric materials by measuring how much infrared light a sample absorbs at various wavelengths (Mohamed et al., 2017).

FTIR is extremely sensitive and can identify small amounts of substance. This makes it useful in situations with limited sample sizes or where great sensitivity is required. FTIR also enables quick data gathering across a broad spectral range. Compared to conventional dispersive infrared spectroscopy, the method's use of interferometry allows for the simultaneous collection of all wavelengths, which speeds up the collecting of data.

The identification of functional groups. Functional groups in both organic and inorganic substances can be found using FTIR (Prati et al., 2010). Different chemical bonds absorb infrared light at distinct frequencies, enabling the identification of certain functional groups. FTIR is also commonly used for quality control and process monitoring in sectors such as medicines, food, and polymer manufacture. It provides for real-time analysis to ensure product uniformity and quality.

2.9 Stereoscopic Microscope

An optical microscope that shows the specimen in three dimensions is called a stereoscopic microscope, often referred to as a stereo microscope. A stereoscopic microscope creates a three-dimensional image using two distinct optical channels and two eyepieces which is binocular vision, as opposed to a compound microscope which employs a single objective lens (Yamamoto & Sano, 2002).

The particulars of the nanoparticles, their size, and their preparation or arrangement on a substrate will all influence the observations made while examining silver nanoparticles using a stereoscopic microscope (Vazquez-Muñoz et al., 2014). First, the size and form are possible observations. For analysing the general size and form of particles, stereoscopic microscopes are helpful. You could see if the silver nanoparticles have a uniform or a variable shape, as well as their size distribution. Next, grouping together. Silver nanoparticles may have an aggregating tendency, depending on the circumstances and technique of synthesis. The kind and degree of particle clustering may be determined with the use of a stereoscopic microscope. Third, consider the surface morphology. Using a stereoscopic microscope, one may see how the substrate and nanoparticles' surfaces are shaped. It is possible to notice characteristics such as abnormalities, textures, or rough surfaces. Fourth, 3D structure. A three-dimensional vision is very well-provided by stereoscopic microscopes. Understanding the nanoparticles' three-dimensional structure can be helped by this, especially if they are organised in intricate or layered patterns (Schreier et al., 2004).

CHAPTER 3

MATERIALS AND METHODS

Figure 3: The *C. odorota* leaf

3.1 Sample Collection

C. odorata leaves was taken from Taman Pinggiran, University Malaysia Kelantan in Jeli. The fresh leaves were separate and properly washed with distilled water to reduce dust particles. After that, the leaves was air dried at room temperature for a week. To avoid contamination, the dried leaves were pulverised into powder and stored in a dry, airtight container. The powder that will create can be use for further analysis. This method was done according to the method described by Ang hui Yi (2018), with some modification.

3.2 Laboratory Preparation of Extracts from Plants

In this experiment, there will be 3 different solvent that will be use which is Hexane, Ethyl Acetate and Methanol. An electronic weighing balance will be used to weigh 30 g of *C.*

odorata leaf powder. After that, packed in a muslin cloth and place in the soxhlet thimble. 200 mL each of the solvent in 3 different round bottom flasks of 500 ml capacity. The upper part of thimble must connect to a condenser. The apparatus will be kept on a hot mantle and heated at boiling point of each solvent. The process of extraction will take about 8 hours, The extract will be collected and concentrate by using rotary evaporator. The final extract will dry in the fume hood and get dry paste leaf extract. This method was done according to the method described by Ang hui Yi (2018), with some modification.

3.3 Green Synthesis of AgNPs from the Extract

Synthesis of *C. odorota* AgNP have done according to the method described by Ang hui Yi (2018), with some modification. For the green synthesis of silver nanoparticles, an equal amount of 5 % *C. odorota* leaf extract, 0.042 g of 0.1mM silver nitrate, ($AgNO_3$) solution and 250 ml of distilled water was mixed using magnetic stirrer inside the 500 ml beaker on the hot plate. After it mixed well, let it cool. To keep the mixture from being directly exposed to light, the mixture was kept in a 250 ml amber laboratory bottle. Room temperature was maintained for the combination. The combination was incubated for 24 hours in the dark and in the room temperature. The UV-Vis spectroscopy reading was taken while the colour shift being noticed. The combination was incubate for 24 hours in the dark and in the room temperature.

3.4 AgNPs Characterization

3.4.1 Fourier Transform InfraRed (FTIR) Spectrum

The functional groups that are resulting in the production of silver nanoparticles may be found using the Fourier Transform InfraRed Spectrophotometer (FTIR), which will also be utilised for recording the FTIR spectrum. These improve the reduction and stabilize the silver nanoparticles. Thermo Scientific TM Nicolet TM iS T10 FT-IR Spectrometer, USA, was used for the FTIR analysis. A dried powder sample was examined in the 500 to 4,000 cm wavelength range. Next, the mixture will be centrifuge at 6,000 rpm for 25 m following incubation. Silver nanoparticles, or AgNPs, were the pellet that was formed. The particle was three times resuspended in ethanol before being allowed to dry at room temperature. There will be 6 sample to be analysis in liquid form in which 3 sample for the *C. odorota* leaf extract and 3 sample of *C. odorota* AgNPs with different solvent leaf extract was analysis. The method was done according to the method described by Ang hui Yi (2018), with some modification.

3.4.2 UV Visible Spectroscopy

The absorption spectra of the bio-reduction plant extract silver nanoparticles were monitored in the visible band at 200-800 nm using 1 mL of 3 sample of *C. odorota* AgNPs with different solvent leaf extract was analysis. As the blank, 1 mL of distilled water was used. UV-Vis spectroscopy will use to monitor the bio-reduction of nanoparticles during 24 hours of incubation. The wavelength represented on the x-axis of a graph with absorbance on the y-axis. Spectrophotometry is a quantitative measurement of a material's absorption, transmission, or reflection as a function of wavelength. Although it is referred to as UV-Vis, the wavelength range usually used spans from 190 nm up to 1,100 nm in the near infrared. The amount or concentration of a known chemical substance can be easily ascertained using a spectrophotometer and absorption measurements. In which, by counting the number of photons light intensity that arrive at the detector. This method was done according to the method described by Ang hui Yi (2018), with some modification.

3.4.3 Stereoscopic Microscope

The stereo microscope is the analyst's initial tool, allowing for a fast analysis of the sample to determine what is going. The sample was prepare in a liquid sample. If the concentration is too high, this can include diluting the sample or adding contrast agents if the sample is not visible. Place a tiny quantity of liquid on slide. The analysis was done using a microscope with a 100x objective lens. It may be compact and lightweight or bigger and equipped with extra functions like the ability to zoom in (McConnell et al., 2016).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Yield of *C. odorota* crude extract

Table 4.1: Total yield (%) of *C. odorota* crude extracts

Method	Extraction Solvent	Weight of dried sample (g)	Weight of crude extract (g)	yield (%)
Soxhlet	Methanol	30	3.35	11.17
	Ethyl Acetate	30	2.08	6.93
	Hexane	30	0.8	2.67

Based on table 4.1, methanol yields the highest amount of crude extract, which is 3.35 g from 30g of dried sample, corresponding to a yield of 11.17%. The second highest is ethyl acetate yield of 6.937% and the least total yield is hexane 2.977%. This indicates that methanol was the most efficient solvent for extracting the desired compounds from *C. odorata* leaves in this experiment. The yield of the crude extract depends on several factors, including the polarity of the solvent, the extraction time and temperature, and the specific compounds of interest. Methanol is a polar solvent that can effectively extract a wide range of compounds from plant materials. Ethyl acetate is also a polar solvent, but it is less effective than methanol in this case. Hexane is a non-polar solvent that is not very effective in extracting polar compounds from plant materials. This explains the low yield obtained with hexane. Based on previous study also indicated that the water and methanol solvents showed significantly higher yields compared with the other extraction solvents for the plant leaf and stem, but not the root. The lowest percentage yield was obtained from C. odorata root extracted with hexane solvent. Furthermore, hexane solvent gave a significantly lower percentage yield for all plant parts compared with water, ethanol and methanol solvents (Thophon et al., 2016).

4.2 Synthesis of Silver Nanoparticles (AgNPs)

In figure 4.2, shows that sample A, B and C that the silver nanoparticles were successfully synthesised by green synthesis using *C. odorota* leaf extract this study. Based on visual detection, the leaves extract of *C. odorota* changed from pale green to dark greenish brown after combined with silver nitrate ($AgNO_3$) solution and incubated. Within an hour, the mixture's colour changed, indicating the formation of silver nanoparticles. This method was done according to the method described by Ang hui Yi (2018), with some modification.

The reduction and participation principles were used to create silver nanoparticles. In other words, the colour changes resulted from the reduction of silver ions (Ag+) from silver nitrate into silver atoms (Ag). This is because *C. odorota* leaf extracts contain reducing or stabilising agents. Colour changes can be affected by factors such as incubation duration and concentration of leaf extracts. In addition, surface plasmon resonance (SPR) stimulation affects the intensity of colour changes (McConnell et al., 2016).

Several research have suggested that *C. odorota* possesses anti-diabetic, antibacterial, and anti-inflammatory properties. The bioactive substances present in *C. odorota* are thought to be essential to produce silver nanoparticles. Using a UV-Vis spectroscopy, more proof was obtained on the synthesis of silver nanoparticles from *C. odorota* extracts (Buszewski et al., 2020).

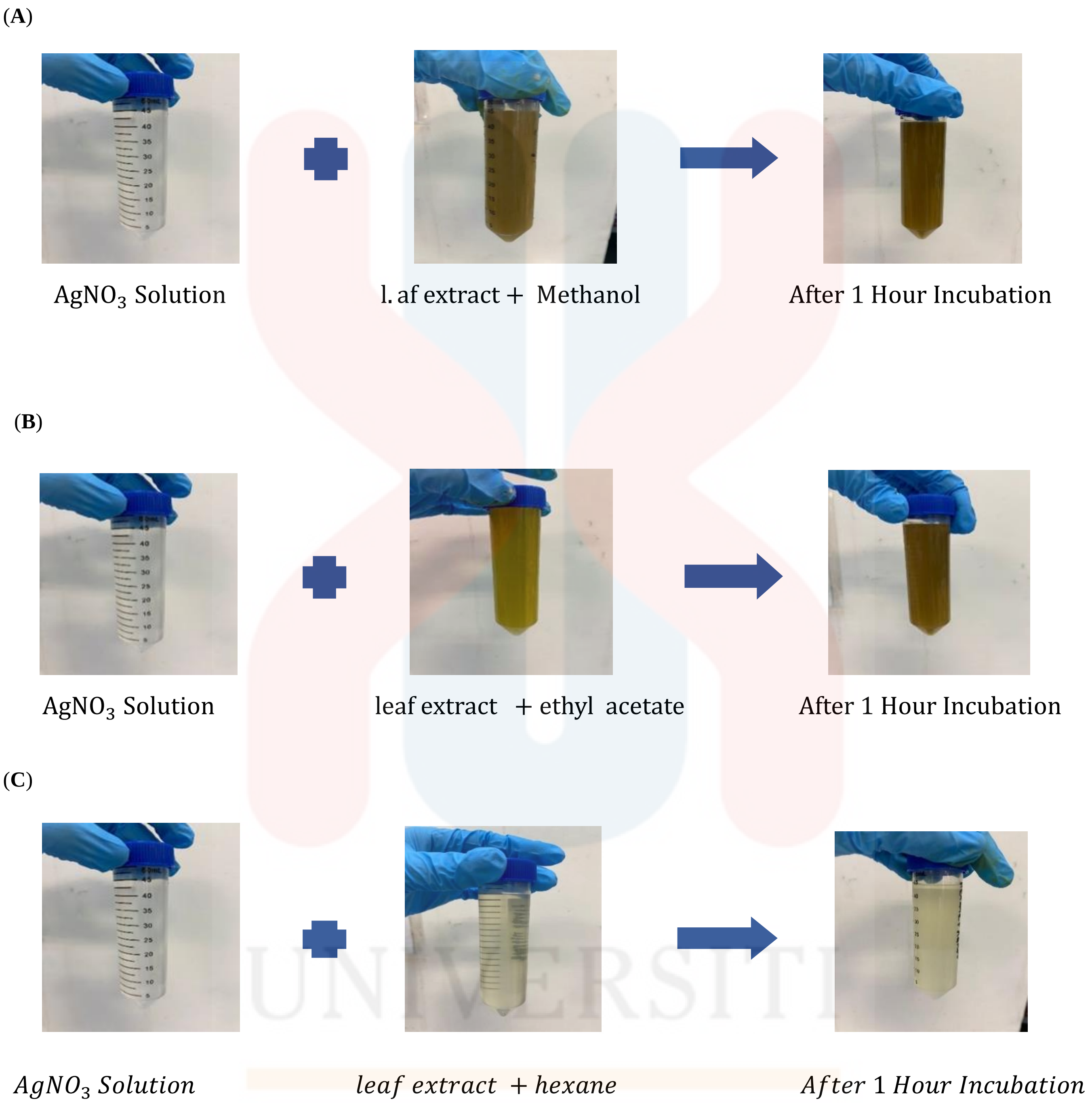


Figure 4.2: The synthesis of AgNPs (**A**) AgNPs *C. odorota* methanol extract (**B**) AgNPs *C. odorota* ethyl acetate extract (**C**) AgNPs *C. odorota* hexane extract

4.3 Characterization of Silver Nanoparticles

4.3.1 UV-Vis Spectroscopy Analysis

In this research, UV-Vis spectroscopy was used to determine the production and stability of silver nanoparticles (AgNPs) based on their size range (Bhui et al., 2009). The UV-Vis spectrum graph was created using silver nanoparticles in 3 different solvents. The wavelength peak and absorbance values were taken after a 24-hour incubation period. In figure 8, shows between the A and B analysis graph are almost the same. Thus, shows the result of the synthesized of silver nanoparticles are successfully. The red line presents for methanol, the blue purple line represents for ethyl acetate and the blue line represent for hexane.

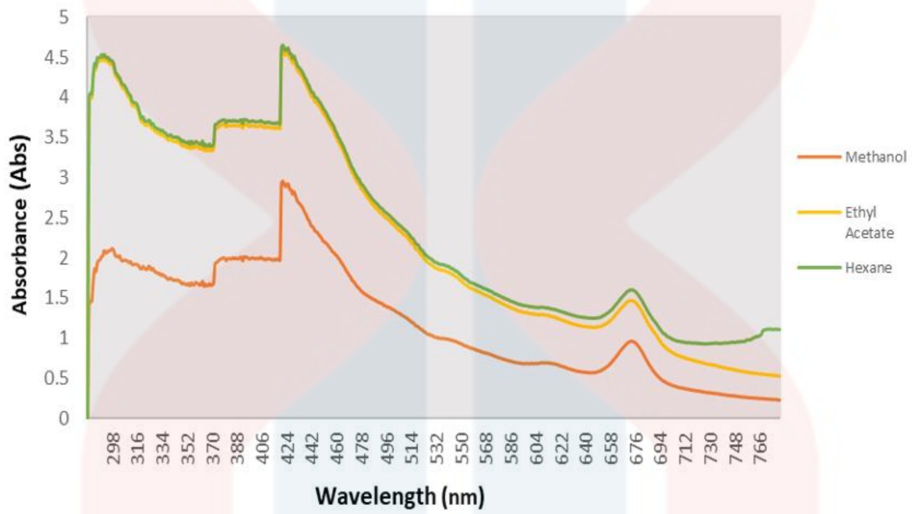
The UV-Vis spectra can provide valuable information on the shape, size, and distribution of nanoparticles based on Surface Plasmon Resonance (SPR) bands. For instance, the appearance of the Ag peak at a shorter wavelength in the UV-Vis spectra reveals the small size of AgNPs that were formed, while a longer wavelength indicates bigger AgNPs (Behzadi et al., 2015).

The *C. odorota* leaf extract demonstrated that SPR vibrations at a wavelength of 400 nm to 500 nm contribute to the reduction of silver nitrate in the sample solution when it was treated with a solution of silver nitrate ($AgNO_3$). When visible light was applied to nanoparticles, the aggregate vibrations of electrons caused SPR to be excited. It serves as the benchmark measurement for materials that are adsorbed onto metal nanoparticle surfaces. After a 24-hour incubation period, a longitudinal Plasmon vibration was seen at 423 nm, as illustrated in Figure 3. AgNPs typically displayed a maximum wavelength in the 400 mm to 550 mm range. (Bhui et al., 2009)

Furthermore, after 24 hours of incubation, a sharp and narrow absorption peak was found at nm. The presence of organic molecules that may have disrupted the bio reduction pathways by reacting with Ag+ ions may have contributed to the weak absorption peak that was detected (Bhui et al., 2009). The impact of the incubation period on the production of silver nanoparticles was validated by the observations made on the UV-Vis spectra. The impact of the incubation duration on the production of silver nanoparticles was established. The AgNO combinations containing *C. odorota* leaf extract were left to react for 24 hours.

The results indicated that the absorbance spectra and colour intensity increased with reaction time due to the decrease of AgNPs. Meanwhile, the stability of AgNPs was accomplished without the detection of precipitate after the incubation process was completed, and the colour stopped changing (Ali et al., 2016).

(A)\



(B)

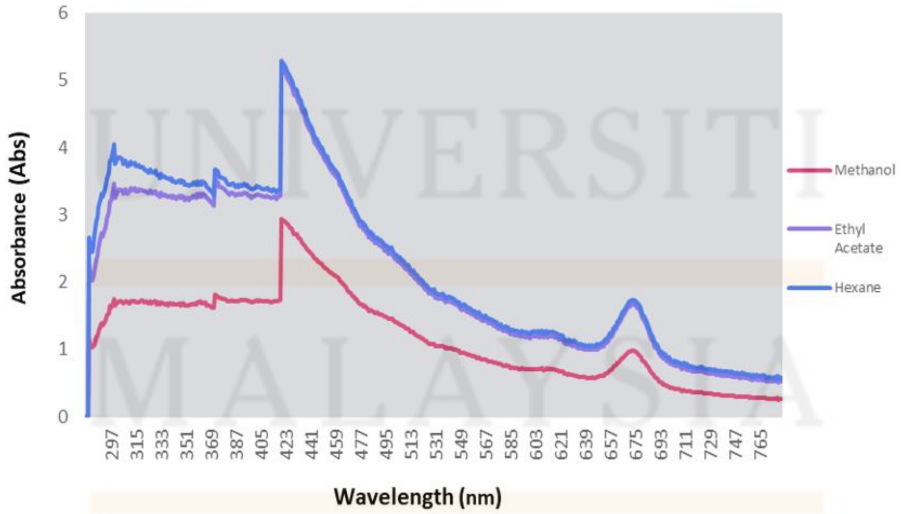


Figure 4.3: Uv - Vis spectra of *C. odorota* with the different solvent analysis solvent analysis (**A**) first analysis (**B**) second analysis

4.3.2 Stereoscopic Microscope

The samples have been observed under a stereoscopic microscope at 100x objective lens. Based on figure 4.4, we can see the image of stereoscopic microscope of *C. odorota* AgNPs leaf extract. Image A shows the result of images sample of leaf extract of *C.odorota* AgNPs that mixed with methanol. It shows that clearly the image of the plant cell of *C.odorota* than image B which sample of leaf extract of *C.odorota* AgNPs that mixed with ethyl acetate. Thus, Methanol is a highly polar solvent with a very high polarity index which is 5.1 (Gupta et al., 1997). It readily dissolves polar molecules due to its strong permanent dipole moment and ability to form hydrogen bonds. Meanwhile, Ethyl acetate is a moderately polar solvent with a medium polarity index which is 4.4. It can dissolve a wider range of solutes compared to hexane and methanol. Furthermore, the image C are images sample of leaf extract of *C. odorota* green silver nanoparticle mixed with hexane are less can be seen than image A and B. This is because hexane is a nonpolar solvent with a very low polarity index which is 0.1 This is a nonpolar solvent with a very low polarity index. It has minimal ability to interact with polar molecules due to its lack of permanent dipole moment and hydrogen bonding potential. (Wakeel et al., 2019) Beside that, it does not dissolve in water.

Silver nanoparticles (AgNPs) cannot directly be seen with their naked eye, including under a stereoscopic microscope, regardless of their color. While some metal nanoparticles can exhibit specific colors due to their surface plasmon resonance phenomenon, this usually requires specialized techniques like UV-Vis spectroscopy or electron microscopy (Walekar et al., 2014). This is because the average size range of silver nanoparticles is 1–100 nanometers (nm). Any optical microscope, even stereoscopic microscopes, whose resolution limit is typically approximately 0.5-1 micrometre (µm), is considerably too narrow to resolve these minuscule dimensions (Mudanyali et al., 2013). Apart from that, individual AgNPs are colourless and transparent. Even while bigger AgNP aggregates can occasionally show colour because of light scattering, they are still too tiny to see clearly under a stereoscopic microscope. It is also possible that the concentration in the leaf extract is quite low, which would make them even more difficult to see with the naked eye (Gao et al., 2014).

In conclusion, the shape that has been observed is varies in shape as much as spherical, triangular, and decahedral. There are many factors affecting the shape of the *C.*

odorota AgNPs leaf extract including solvent polarity, extract concentration, contact time, pH, and silver salt concentration (Hulkoti & Taranath, 2015).

(A)

(B)

(C)

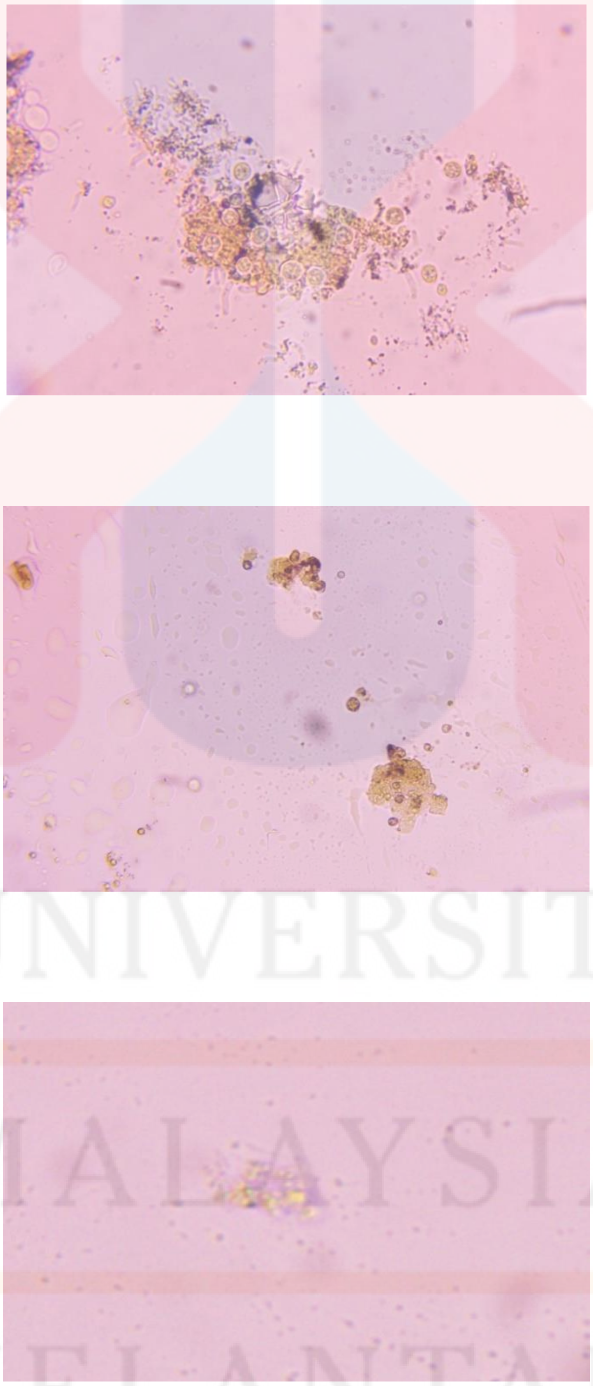


Figure 4.4: The image of streoscopic microscope of AgNPs *C. odorota* extract (**A**) AgNPs *C. odorota* methanol extract (**B**) AgNPs *C. odorota* ethyl acetate extract (**C**) AgNPs *C. odorota* hexane extract

4.3.3 FTIR Analysis

FTIR analysis were carried out to characterize the possible biomolecules responsible for the reduction of Ag+ ions and the stabilization of the bioreduced silver nanoparticles to prevent agglomeration of the nanoparticles and their capping in the aqueous medium (Ashokkumar & Ramaswamy, 2014). In which that, the electron that rich oxygen atom in the C=O double bond can donate an electron to a silver ion. The FTIR spectra of leaf extract *of C. odorota* and the synthesized AgNPs were compared. The synthesised of *AgNPs C. odorota hexane extract* and *C. odorota* hexane extract in liquid were analysed. Based on the table 1, the IR spectrum suggests that the molecule contains the following functional groups, alcohol or carboxylic acid, aliphatic hydrocarbons, carbonyl, alcohol or ether and aromatic rings in *C. odorota* extract.

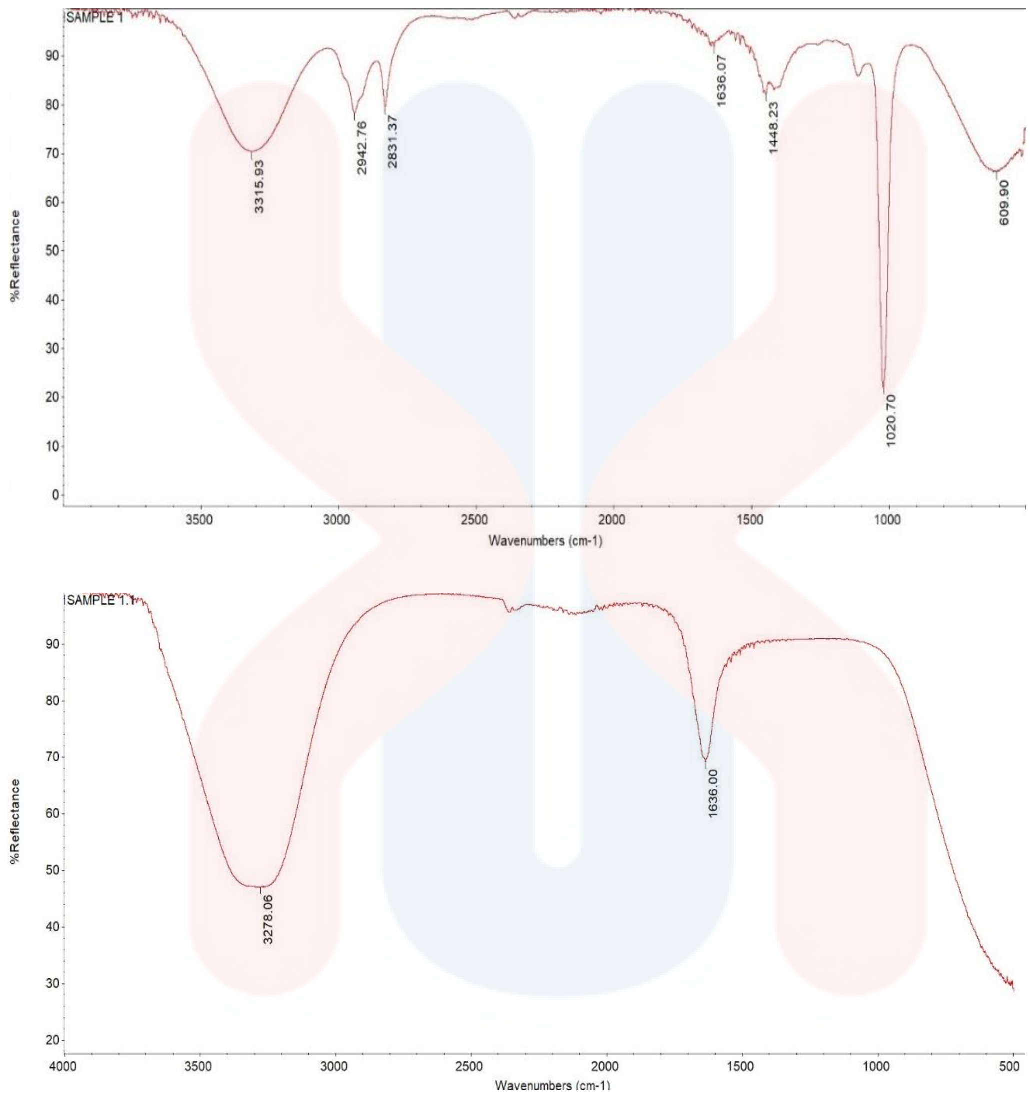
Based on Figure 4.5 A, the peak at $3315.93\ cm^{-1}$ is due to O-H stretching vibrations, which suggests that the molecule contains an alcohol or carboxylic acid functional group. The peaks at $12942.76\ cm^{-1}$ and $2831.37\ cm^{-1}$ are due to C-H stretching vibrations, which suggests that the molecule contains aliphatic hydrocarbons. The peak at $1448.23\ cm^{-1}$ is due to C-H bending vibrations, which is consistent with the presence of aliphatic hydrocarbons. The peak at $1020.70\ cm^{-1}$ is due to C-O stretching vibrations, which suggests that the molecule contains an alcohol or ether functional group. The peak at $609.90\ cm^{-1}$ is due to bending vibrations of aromatic C-H bonds (Hari & Nair, 2018).

Meanwhile, in Figure 4.5 B shows that the wavenumber 3278.06 cm-1 falls within the range for N-H stretching vibrations. These functional groups are commonly found in many organic molecules, including amides, amines, and carboxylic acids. Based on Table 4.6, shows the specific molecule that gives rise to the spectrum in the image cannot be determined without further information. However, the presence of the N-H functional groups suggests that the molecule is likely to be an organic compound (Mallikarjuna et al., 2011)

As we can see that, the graph A and B in Figure 4.5 shows differences in the presence of silver nanoparticles in the AgNP extract. In the previous studies have confirmed the fact that the carbonyl group from amino acid residues and proteins has the stronger ability to bind metal, indicating that the proteins could possibly form a layer covering the metal nanoparticles to prevent agglomeration and thereby stabilize the medium (Prabu & Johnson, 2015).

These nanoparticles exhibit a characteristic surface plasmon resonance (SPR) band in the ultraviolet-visible (UV-Vis) range, typically around 400 to 450 nm (Behzadi et al., 2015). This band, absent in the leaf extract, contributes significantly to the overall shape and intensity of the AgNP extract graph. Besides that, Functional Group Interactions. Silver nanoparticles can interact with various functional groups present in the leaf extract, such as proteins, carbohydrates, and phenolic compounds (Ajitha et al., 2015). These interactions can lead to shifts in peak positions (Faghihzadeh et al., 2016).

The interactions also might affect the intensity of certain peaks, potentially enhancing or masking them. In some cases, new peaks can appear due to nanoparticle-functional group interactions do not present in the original leaf extract (Ovais et al., 2016). Furthermore, aggregation and morphology size, shape, and aggregation state of silver nanoparticles can influence the intensity and characteristics of the SPR band. Differences in these properties between different samples can lead to variations in the figure 4.5 A and B. The solvent used for extraction and the concentration of both extracts also can affect peak intensity and potentially introduce minor spectral differences. (Oirere et al., 2015).



(A)

(B)

Figure 4.5: FTIR spectrum analysis(**A**) AgNPs *C. odorota* hexane extract (**B**) *C. odorota* hexane extract

Table 4.5: FTIR spectrum of AgNPs C. odorota hexane extract wavelength and functional group analysis

Wavenumber (cm^{-1})	**Functional group**
3315.93	O-H stretching (alcohol or carboxylic acid)
2942.76	C-H stretching (aliphatic hydrocarbons)
2831.37	C-H stretching (aliphatic hydrocarbons)
1448.23	C-H bending (aliphatic hydrocarbons)
1020.7	C-O stretching (alcohol or ether)
609.9	Bending vibrations of aromatic C-H bonds

Table 4.6: FTIR spectrum of *C. odorota* hexane extract wavelength and functional group analysis

Wavenumber (cm^{-1})	**Functional group**
3278.06	N-H stretching (amines)

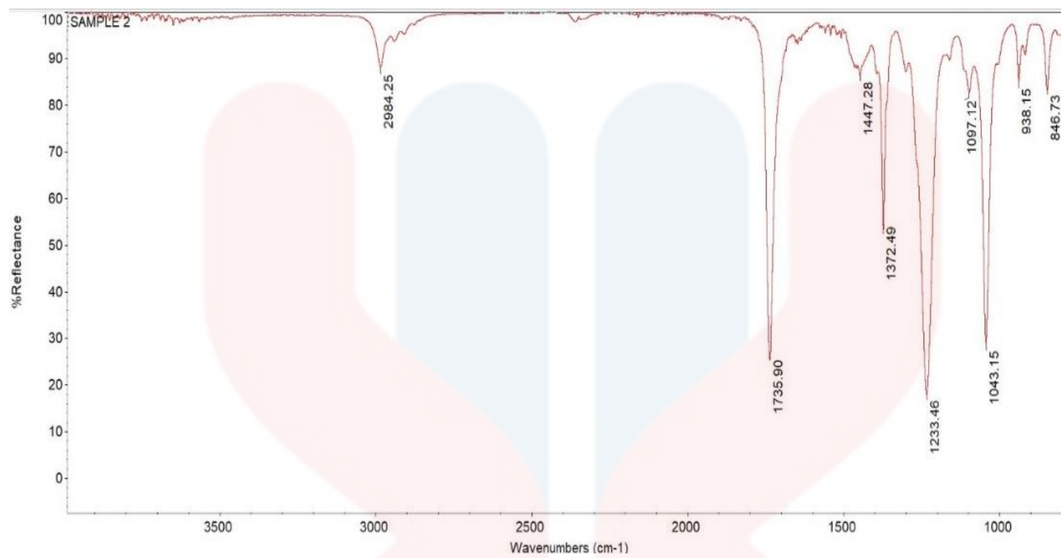
The spectrum appears to show the infrared (IR) absorption spectrum of a solid or liquid sample (Sodha et al., 2015). The wavenumbers are displayed on the x-axis, ranging from 4000 to 400 400 cm $^{-1}$. The y-axis shows the percentage of infrared light reflected by the sample at each wavenumber. Based on the table 4.7, the presence of these peaks, the molecule that produced the spectrum is likely to contain the following functional groups which are broad, alcohol or phenol, ester, ketone, alkane, methyl, ester, and ether.

Based on the graph in Figure 4.6 A, The IR spectrum shows several peaks that are characteristic of different functional groups. The peaks at 1233.46 cm^{-1} and 1097.12 cm^{-1} are also due to C-O stretching vibrations in the ester group (Lommerse et al., 1997). The peaks at 2984.25 cm^{-1} is due to C-H stretching vibrations in aliphatic groups. The peaks at 1447.28 cm^{-1} and 1372.49 cm^{-1} are due to C-H bending vibrations (Margoshes & Fassel, 1955). The peaks at 1043.15 cm^{-1} and 846.73 cm^{-1} are due to C-C skeletal vibrations (Stefanović et al., 2005). The weak peak at 938.15 cm^{-1} could be due to C=C stretching vibration of an alkene group, but it is also possible that it is due to an overtone or combination band of other functional groups. There are three different alkene structural isomers that consist of a C=C bond with two hydrogens and two R-groups attached. In a vinylidine molecule the two hydrogens are attached to one carbon while the R-groups are on the other carbon. The other two types of C=C bonds with two hydrogens are called cis and trans (Smith, 2016).

As we can see that, Figure 4.6 B, shows the FTIR spectrum of AgNPs of leaf extract of *C. odorota* and Ethyl Acetate. The IR spectrum shows several peaks that are characteristic of different functional groups. The strong peak at 3274.69 cm^{-1} is due to the stretching vibration of the N-H bond in a primary amine (Cooper et al., 2011). The peak at 2359.52 cm^{-1} is due to the stretching vibration of the C≡N bond in a nitrile group (Downs & Tyler, 2014). The peak at 1540.75 cm^{-1} is due to the bending vibration of the N-H bond in a primary amine. (Sheny et al., 2011)

The two samples are likely to have different compositions. Silver nanoparticles interact with components of the AgNPs *C. odorota extract*, while the leaf extract itself includes a variety of phytochemicals. These variations can produce separate peaks in the spectra. In the *C. odorota* AgNPs case, silver nanoparticles can interact with various functional groups in the leaf extract, leading to altered peak intensities or even new peaks due to the formation of new bonds or coordination complexes (Sheny et al., 2011).

(A)



(B)

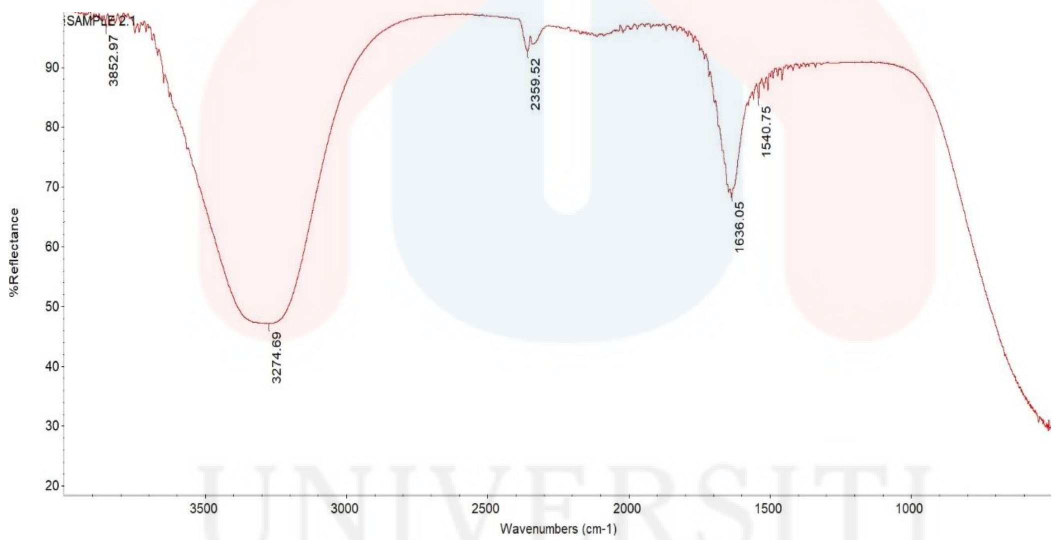


Figure 4.6: FTIR spectrum analysis **(A**) AgNPs *C. odorota* ethyl acetate extract (**B***) C. odorota* ethyl acetate extract

Table 4.7: FTIR spectrum of AgNPs *C. odorota* ethyl acetate extract wavelength and functional group analysis

Wavelength (cm^{-1})	Functional group
2984.25	C-H stretching
1447.28	C-H bending (methyl)
1372.49	C-H stretching
1233.46	C-O stretching (ester)
1097.12	C-O stretching (alcohol)
1043.15	C-C stretching
938.15	O-H bending (alcohol)
846.73	C-C stretching

Table 4.8: FTIR spectrum of leaf extract of *C. odorota* ethyl acetate extract wavelength and functional group analysis

Wavenumber (cm^{-1})	Functional Group
3274.69	N-H
2359.52	C≡N
1540.75	N-H

Based on Figure 4.7 A, the peak at $3316.73\ cm^{-1}$ is due to O-H stretching vibrations, which suggests that the molecule contains an alcohol or carboxylic acid functional group. The peaks at $2942.19\ cm^{-1}$ and $2831.48\ cm^{-1}$ are due to C-H stretching vibrations, which suggests that the molecule contains aliphatic hydrocarbons. The peak at $1635\ cm^{-1}$ is due to C=O stretching vibrations, which suggests that the molecule contains a carbonyl group (C=O). The peak at $1448.19\ cm^{-1}$ is due to C-H bending vibrations, which is consistent with the presence of aliphatic hydrocarbons. The peak at $1020.73\ cm^{-1}$ is due to C-O stretching vibrations, which suggests that the molecule contains an alcohol or ether functional group. The peak at $606.64\ cm^{-1}$ is due to bending vibrations of aromatic C-H bonds. Overall, the IR spectrum suggests that the molecule contains the following functional groups, alcohol or carboxylic acid, aliphatic hydrocarbons, carbonyl, alcohol or ether and aromatic rings.

As we can see in figure 4.7 b, the peak at $3500\ cm^{-1}$ is due to O-H stretching vibrations, which suggests that the molecule contains an alcohol or carboxylic acid functional group. The peak at $3277.39\ cm^{-1}$ is due to N-H stretching vibrations, which suggests that the molecule contains an amine functional group. The peak at $2359.13\ cm^{-1}$ is due to CO2 stretching vibrations, which suggests that the molecule contains a carboxylic acid functional group. Thus, the IR spectrum suggests that the molecule contains the following functional groups which is alcohol or carboxylic acid, Amine, carboxylic acid.

The results of this FTIR analysis verify that the carbonyl groups in amino acid residues have a strong binding affinity for silver nanoparticles (Proniewicz et al., 2021). These groups may also function as reducing and stabilising agents to prevent agglomeration, which keeps the silver nanoparticles stable in the medium (Restrepo & Villa, 2021). It is commonly recognised that proteins may attach to silver nanoparticles via free amine groups or cysteine residues, and that the protein that is surface attached stabilises throughout synthesis (Saware & Venkataraman, 2014).

Thus, the application of nanoparticlcles is also determined by their chemical stability in the absence of deterioration, such as partal oxidation. We may infer from the FTIR data that some of the biological components in the lichen extract created a potent capping agent on the nanoparticles, which enabled to stabilise them. (Khandel et al., 2018)

(A)

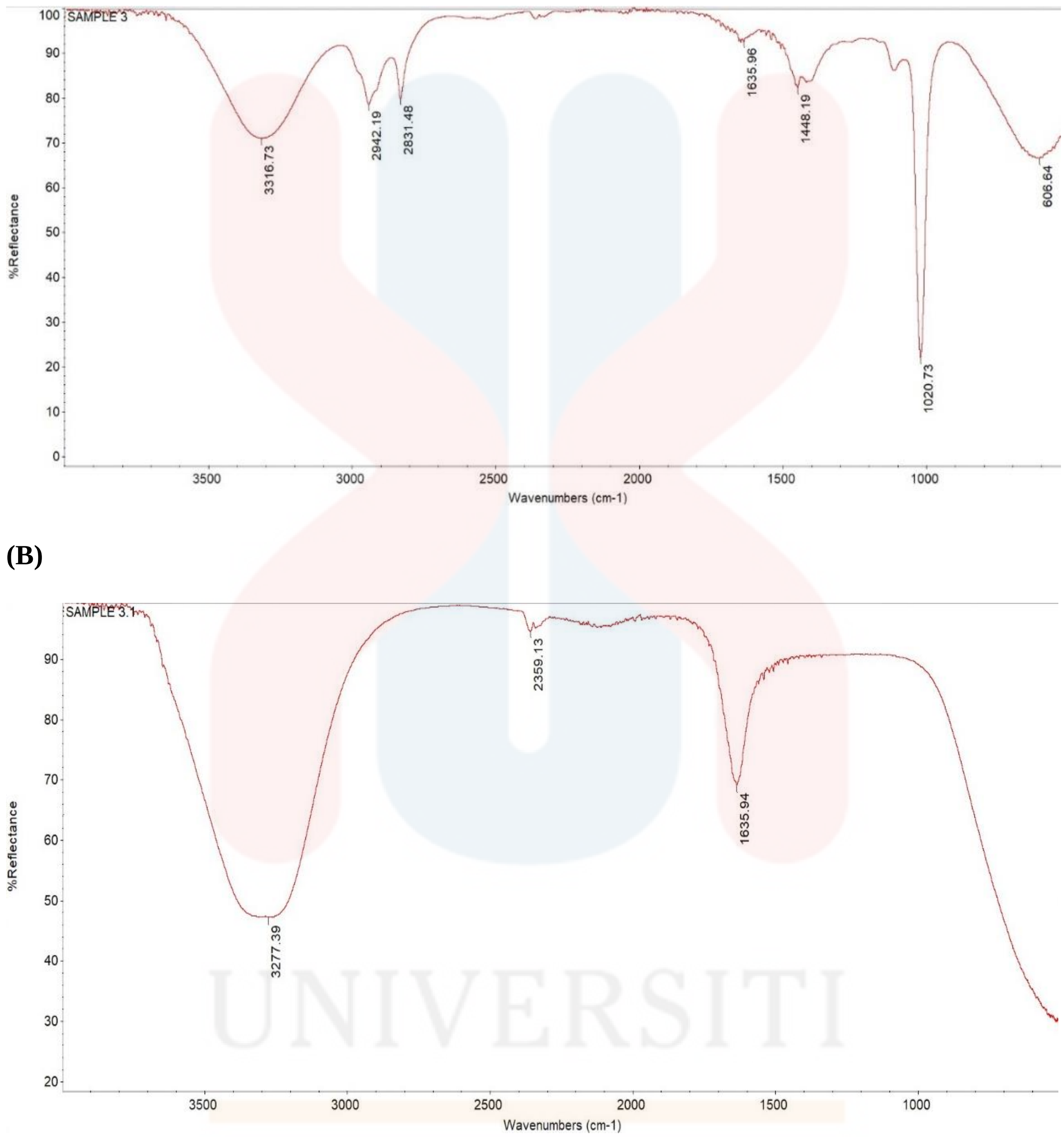


Figure 4.7: FTIR spectrum analysis (**A**) AgNPs *C. odorota* methanol extract (**B**) *C. odorota* methanol extract

Table 4.9: FTIR spectrum of AgNPs *C. odorota* methanol extract wavelength and functional group analysis

Wavenumber (cm^{-1})	**Functional group**
3316.73	O-H stretching (alcohol or carboxylic acid)
2942.19	C-H stretching (aliphatic hydrocarbons)
2831.48	C-H stretching (aliphatic hydrocarbons)
1635	C=O stretching (carbonyl)
1448.19	C-H bending (aliphatic hydrocarbons)
1020.73	C-O stretching (alcohol or ether)
606.64	Bending vibrations of aromatic C-H bonds

Table 5: FTIR spectrum of C. odorota methanol extract wavelength and functional group analysis

Wavenumber (cm^{-1})	**Functional group**
3277.39	N-H stretching (amines)
2359.13	CO2 stretching (carboxylic acid)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the synthesis of AgNPs *C. odorota* extract are using different type polarity of solvent which are methanol, ethyl acetate. The characterize of the silver nanoparticles are using uv-vis spectroscopy, fourier transform infrared spectroscopy (FTIR), stereoscopic microscope in reseaching the most effective solvent for reducing functional groups during silver nanoparticle production was successfully carried. In which determined by the individual functional groups involved as well as the reaction circumstances. various solvents with various polarity, such as hexane, methanol, and ethyl acetate, may have distinct effects on the reduction of functional groups. Methanol can be utilised to reduce the functional groups involved in the manufacture of silver nanoparticles since it is a polar solvent in general. Because methanol is miscible with water, it is frequently used in conjunction with reducing chemicals to reduce silver ions and create silver nanoparticles, such as citrate or sodium borohydride.

5.2 Recommendation

In the present study, synthesis of silver nanoparticles from *C. Odorota* leaf extract by using green synthesis method was successfully carried out. Green synthesis approach is highly promising due to its cost-effective and eco-friendly nature. *C. Odorota* leaf extract has been acted as the reducing agent that contributed to the formation of silver nanoparticles. Meanwhile, more research and study are needed to identify the reasons why the inhibitory effect of synthesized silver nanoparticles is lower than the original leaf extract.

Optimization of the synthesizing process could possibly enhance the inhibitory effect of *C. Odorota* AgNPs. Additionally, a better understanding on the formation of silver nanoparticles from *C. Odorota* could be assist by the help of advance facilities. For example, the characterization of from *C. Odorota* AgNPs could be further studied by determine the particles size by using Particle Size Analyzer and a higher magnification should be applied to obtain a clear image such as SEM. The stereoscopic microscopes provide a three-dimensional picture, they lack the high magnification levels found in compound microscopes or specialised devices such as transmission electron microscopes. Additional methods like spectroscopy, scanning electron microscopy (SEM), or transmission electron microscopy (TEM) may be used for more thorough characterization (Inkson, 2016).

Besides that, the crystalline and the size of the silver nanoparticles can be analyzed by using XRD machine. Examination of Crystal Strain and Size The size and strain of crystallites in a material may be determined using XRD. XRD include that it is a non-destructive method, which means that it does not harm the sample during analysis. This makes it possible to examine priceless or unique samples. XRD is a useful technique for identifying minute differences in materials as it is also extremely sensitive to tiny changes in crystal structure.

For the further analysis, the researcher can optimize synthesis parameters. In which, explore different plant parts. Investigate the use of other parts of *C. odorata* like flowers, stems, or roots for potential higher yields or unique properties. Besides that, modulate reaction conditions. Experiment with variables like temperature, pH, extract concentration, and reaction time to fine-tune nanoparticle size, shape, and stability. Furthetmore, exploring novel applications. Investigate synergistic effects. Combine AgNPs with other natural compounds example essential oils, antibiotics to explore enhanced antibacterial or antifungal activity.

REFRENCES

Aboyewa, J. A., Sibuyi, N. R., Meyer, M., & Oguntibeju, O. O. (2021). Green synthesis of metallic nanoparticles using some selected medicinal plants from southern africa and their biological applications. *Plants*, *10*(9), 1929.

Ajitha, B., Reddy, Y. A. K., & Reddy, P. S. (2015). Green synthesis and characterization of silver nanoparticles using Lantana camara leaf extract. *Materials Science and Engineering: C*, *49*, 373-381.

Akash, M. S. H., Rehman, K., Akash, M. S. H., & Rehman, K. (2020). Ultraviolet-visible (UV-VIS) spectroscopy. *Essentials of pharmaceutical analysis*, 29-56.

Ali, M., Kim, B., Belfield, K. D., Norman, D., Brennan, M., & Ali, G. S. (2016). Green synthesis and characterization of silver nanoparticles using Artemisia absinthium aqueous extract—A comprehensive study. *Materials Science and Engineering: C*, *58*, 359-365.

Anyasi, R. O. (2012). *Bioremediation of polychlorinated biphenyls (PCBs)-contaminated soil by phytoremediation with Chromolaena odorata (L) RM King and Robinson*

Ashokkumar, R., & Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. *International journal of Current Microbiology and applied Sciences*, *3*(1), 395-406.

Behzadi, S., Ghasemi, F., Ghalkhani, M., Ashkarran, A. A., Akbari, S. M., Pakpour, S., Hormozi-Nezhad, M. R., Jamshidi, Z., Mirsadeghi, S., & Dinarvand, R. (2015). Determination of nanoparticles using UV-Vis spectra. *Nanoscale*, *7*(12), 5134-5139.

Bhui, D. K., Bar, H., Sarkar, P., Sahoo, G. P., De, S. P., & Misra, A. (2009). Synthesis and UV–vis spectroscopic study of silver nanoparticles in aqueous SDS solution. *Journal of Molecular Liquids*, *145*(1), 33-37.

Biswas, P., & Wu, C.-Y. (2005). Nanoparticles and the environment. *Journal of the air & waste management association*, *55*(6), 708-746.

Bratovčić, A., Odobašić, A., & Ćatić, S. (2009). The advantages of the use of ion-selective potentiometry in relation to UV/VIS spectroscopy. *Agriculturae Conspectus Scientificus*, *74*(3), 139-142.

Burke, J. (1984). Solubility parameters: theory and application.

Buszewski, B., Plugaru, V. R., Pomastowski, P., & Sidorenco, A. (2020). Silver nanoparticles: synthesis, characteristics, and application. In *Handbook of Research on Emerging Developments and Environmental Impacts of Ecological Chemistry* (pp. 432-449). IGI Global.

Carmona, E. R., Benito, N., Plaza, T., & Recio-Sánchez, G. (2017). Green synthesis of silver nanoparticles by using leaf extracts from the endemic Buddleja globosa hope. *Green Chemistry Letters and Reviews*, *10*(4), 250-256. https://doi.org/10.1080/17518253.2017.1360400

Cooper, J. K., Franco, A. M., Gul, S., Corrado, C., & Zhang, J. Z. (2011). Characterization of primary amine capped CdSe, ZnSe, and ZnS quantum dots by FT-IR: determination of surface bonding interaction and identification of selective desorption. *Langmuir*, *27*(13), 8486-8493.

De Castro, M. L., & Garcıa-Ayuso, L. (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica chimica acta*, *369*(1-2), 1-10.

De Castro, M. L., & Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of chromatography A*, *1217*(16), 2383-2389.

Downs, E. L., & Tyler, D. R. (2014). Nanoparticle catalysts for nitrile hydration. *Coordination Chemistry Reviews*, *280*, 28-37.

Faghihzadeh, F., Anaya, N. M., Schifman, L. A., & Oyanedel-Craver, V. (2016). Fourier transform infrared spectroscopy to assess molecular-level changes in microorganisms exposed to nanoparticles. *Nanotechnology for environmental engineering*, *1*, 1-16.

Forough, M., & Farhadi, K. (2010). Biological and green synthesis of silver nanoparticles. *Turkish journal of engineering and environmental sciences*, *34*(4), 281-287.

Fulekar, M. (2010). *Nanotechnology: importance and applications*. IK International Pvt Ltd.

Gao, M., Lin, R., Li, L., Jiang, L., Ye, B., He, H., & Qiu, L. (2014). Label-free silver nanoparticles for the naked eye detection of entecavir. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *126*, 178-183.

Ghaffari-Moghaddam, M., Hadi-Dabanlou, R., Khajeh, M., Rakhshanipour, M., & Shameli, K. (2014). Green synthesis of silver nanoparticles using plant extracts. *Korean Journal of Chemical Engineering*, *31*, 548-557.

Ghorbani, H. R., Safekordi, A. A., Attar, H., & Sorkhabadi, S. (2011). Biological and non-biological methods for silver nanoparticles synthesis. *Chemical and Biochemical Engineering Quarterly*, *25*(3), 317-326.

Gogoi, R., Sarma, N., Begum, T., Pandey, S. K., & Lal, M. (2020). North-East Indian Chromolaena odorata (L. King Robinson) aerial part essential oil chemical composition, pharmacological activities-neurodegenerative inhibitory and toxicity study. *Journal of Essential Oil Bearing Plants*, *23*(6), 1173-1191.

Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N. M., Thajuddin, N., & Velusamy, P. (2012). Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: a novel biological approach. *Colloids and surfaces B: biointerfaces*, *96*, 69-74.

Gupta, M. N., Batra, R., Tyagi, R., & Sharma, A. (1997). Polarity index: the guiding solvent parameter for enzyme stability in aqueous-organic cosolvent mixtures. *Biotechnology progress*, *13*(3), 284-288.

Hari, N., & Nair, V. P. (2018). FTIR spectroscopic analysis of leaf extract in hexane in Jasminum azoricum L. *Recent Research in Science and Technology*, *4*(8), 170-172.

Hulkoti, N., & Taranath, T. (2015). Effect of various physico-chemical factors on synthesis of biogenic silver nanoparticles using leaf extract of Cananga odorata (Lam.) Hook. f. & Thomson. and its antibacterial effect. *Int. J. Pharm. Sci. Rev. Res*, *35*(2), 213-218.

Inkson, B. J. (2016). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for materials characterization. In *Materials characterization using nondestructive evaluation (NDE) methods* (pp. 17-43). Elsevier.

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry*, *12*(7), 908-931.

Khandel, P., Kumar Shahi, S., Kanwar, L., Kumar Yadaw, R., & Kumar Soni, D. (2018). Biochemical profiling of microbes inhibiting Silver nanoparticles using symbiotic organisms. *International Journal of Nano Dimension*, *9*(3), 273-285.

Lemaire, C., Plenevaux, A., Aerts, J., Del Fiore, G., Brihaye, C., Le Bars, D., Comar, D., & Luxen, A. (1999). Solid phase extraction—an alternative to the use of rotary evaporators for solvent removal in the rapid formulation of PET radiopharmaceuticals. *Journal of Labelled Compounds and Radiopharmaceuticals: The Official Journal of the International Isotope Society*, *42*(1), 63-75.

Logothetidis, S. (2011). Nanotechnology: Principles and applications. In *Nanostructured materials and their applications* (pp. 1-22). Springer.

Lommerse, J. P., Price, S. L., & Taylor, R. (1997). Hydrogen bonding of carbonyl, ether, and ester oxygen atoms with alkanol hydroxyl groups. *Journal of computational chemistry*, *18*(6), 757-774.

López-Bascón, M., & De Castro, M. L. (2020). Soxhlet extraction. In *Liquid-phase extraction* (pp. 327-354). Elsevier.

Mabberley, D. J. (1997). *The plant-book: a portable dictionary of the vascular plants*. Cambridge university press.

Mallikarjuna, K., Narasimha, G., Dillip, G., Praveen, B., Shreedhar, B., Lakshmi, C. S., Reddy, B., & Raju, B. D. P. (2011). Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization. *Digest Journal of Nanomaterials and Biostructures*, *6*(1), 181-186.

Margoshes, M., & Fassel, V. (1955). The infrared spectra of aromatic compounds: I. The out-of-plane CH bending vibrations in the region 625–900 cm− 1. *Spectrochimica Acta*, *7*, 14-24.

McConnell, G., Trägårdh, J., Amor, R., Dempster, J., Reid, E., & Amos, W. B. (2016). A novel optical microscope for imaging large embryos and tissue volumes with sub-cellular resolution throughout. *Elife*, *5*, e18659.

Mohamed, M. A., Jaafar, J., Ismail, A., Othman, M., & Rahman, M. (2017). Fourier transform infrared (FTIR) spectroscopy. In *Membrane characterization* (pp. 3-29). Elsevier.

Mohammadlou, M., Maghsoudi, H., & Jafarizadeh-Malmiri, H. (2016). A review on green silver nanoparticles based on plants: Synthesis, potential applications and eco-friendly approach. *International Food Research Journal*, *23*(2), 446.

Mudanyali, O., McLeod, E., Luo, W., Greenbaum, A., Coskun, A. F., Hennequin, Y., Allier, C. P., & Ozcan, A. (2013). Wide-field optical detection of nanoparticles using on-chip microscopy and self-assembled nanolenses. *Nature photonics*, *7*(3), 247-254.

Nikulainen, T., & Palmberg, C. (2010). Transferring science-based technologies to industry—Does nanotechnology make a difference? *Technovation*, *30*(1), 3-11.

Oirere, E. K., Anusooriya, P., Raj, C. A., & Gopalakrishnan, V. K. (2015). Phytochemical analysis of N-hexane leaf extract of Alpinia purpurata (Vieill.) K. Schum using Uv-Vis, FTIR and GC-MS. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(8), 387-389.

Ovais, M., Khalil, A. T., Raza, A., Khan, M. A., Ahmad, I., Islam, N. U., Saravanan, M., Ubaid, M. F., Ali, M., & Shinwari, Z. K. (2016). Green synthesis of silver nanoparticles via plant extracts: beginning a new era in cancer theranostics. *Nanomedicine*, *12*(23), 3157-3177.

Owoyele, V. B., Adediji, J. O., & Soladoye, A. O. (2005). Anti-inflammatory activity of aqueous leaf extract of Chromolaena odorata. *Inflammopharmacology*, *13*, 479-484.

Parliment, T. H. (2020). Solvent extraction and distillation techniques. *Techniques for Analyzing*, 1-26.

Perkampus, H.-H. (2013). *UV-VIS Spectroscopy and its Applications*. Springer Science & Business Media.

Prabu, H. J., & Johnson, I. (2015). Plant-mediated biosynthesis and characterization of silver nanoparticles by leaf extracts of Tragia involucrata, Cymbopogon citronella, Solanum verbascifolium and Tylophora ovata. *Karbala International Journal of Modern Science*, *1*(4), 237-246.

Prati, S., Joseph, E., Sciutto, G., & Mazzeo, R. (2010). New advances in the application of FTIR microscopy and spectroscopy for the characterization of artistic materials. *Accounts of chemical research*, *43*(6), 792-801.

Proniewicz, E., Tąta, A., Iłowska, E., & Prahl, A. (2021). Is the Use of Surface-Enhanced Infrared Spectroscopy Justified in the Selection of Peptide Fragments That Play a Role in Substrate–Receptor Interactions? Adsorption of Amino Acids and Neurotransmitters on Colloidal Ag and Au Nanoparticles. *The Journal of Physical Chemistry B*, *125*(9), 2328-2338.

Rajendrachari, S., Swamy, B. K., Reddy, S., & Chaira, D. (2013). Synthesis of silver nanoparticles and their applications. *Anal. Bioanal. Electrochem*, *5*(4), 455-466.

Raynie, D. (2019). Looking at the past to understand the future: Soxhlet extraction. *LCGC North America*, *37*(8), 510–513-510–513.

Restrepo, C. V., & Villa, C. C. (2021). Synthesis of silver nanoparticles, influence of capping agents, and dependence on size and shape: A review. *Environmental Nanotechnology, Monitoring & Management*, *15*, 100428.

Salem, S. S., & Fouda, A. (2021). Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. *Biological trace element research*, *199*, 344-370.

Saware, K., & Venkataraman, A. (2014). Biosynthesis and characterization of stable silver nanoparticles using Ficus religiosa leaf extract: a mechanism perspective. *Journal of Cluster Science*, *25*, 1157-1171.

Schreier, H. W., Garcia, D., & Sutton, M. A. (2004). Advances in light microscope stereo vision. *Experimental mechanics*, *44*, 278-288.

Sheny, D., Mathew, J., & Philip, D. (2011). Phytosynthesis of Au, Ag and Au–Ag bimetallic nanoparticles using aqueous extract and dried leaf of Anacardium occidentale. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *79*(1), 254-262.

Smith, B. (2016). The infrared spectroscopy of Alkenes. *Spectroscopy*, *31*(11), 28–34-28–34.

Sodha, K. H., Jadav, J. K., Gajera, H. P., & Rathod, K. J. (2015). Characterization of silver nanoparticles synthesized by different chemical reduction methods. *Int J Pharma Bio Sci*, *6*, 199-208.

Srikar, S. K., Giri, D. D., Pal, D. B., Mishra, P. K., & Upadhyay, S. N. (2016). Green synthesis of silver nanoparticles: a review. *Green and Sustainable Chemistry*, *6*(1), 34-56.

Stefanović, I., Kovačević, E., Berndt, J., Pendleton, Y., & Winter, J. (2005). Hydrocarbon nanoparticles as a diffuse ISM analogue: morphology and infrared absorption in the 2000–500 cm− 1 region. *Plasma physics and controlled fusion*, *47*(5A), A179.

Thophon, S. H. S., Waranusantigul, P., Kangwanrangsan, N., & Krajangsang, S. (2016). Antimicrobial activity of Chromolaena odorata extracts against bacterial human skin infections. *Modern Applied Science*, *10*(2).

Vaisakh, M., & Pandey, A. (2012). The invasive weed with healing properties: A review on Chromolaena odorata. *International journal of Pharmaceutical sciences and research*, *3*(1), 80.

Vazquez-Muñoz, R., Avalos-Borja, M., & Castro-Longoria, E. (2014). Ultrastructural analysis of Candida albicans when exposed to silver nanoparticles. *PloS one*, *9*(10), e108876.

Wakeel, A., Jan, S. A., Ullah, I., Shinwari, Z. K., & Xu, M. (2019). Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. *PeerJ*, *7*, e7857.

Walekar, L. S., Kondekar, U. R., Gore, A. H., Pawar, S. P., Sudarsan, V., Anbhule, P. V., Patil, S. R., & Kolekar, G. B. (2014). Ultrasensitive, highly selective and naked eye colorimetric recognition of D-penicillamine in aqueous media by CTAB capped AgNPs: applications to pharmaceutical and biomedical analysis. *Rsc Advances*, *4*(102), 58481-58488.

Welton, T., & Reichardt, C. (2011). *Solvents and solvent effects in organic chemistry*. John Wiley & Sons.

Whatmore, R. W. (2006). Nanotechnology—what is it? Should we be worried? *Occupational Medicine*, *56*(5), 295-299.

Yamamoto, H., & Sano, T. (2002). Study of micromanipulation using stereoscopic microscope. *IEEE Transactions on Instrumentation and Measurement*, *51*(2), 182-187.

Zahara, M. (2019). Description of Chromolaena odorata LRM King and H. Robinson as medicinal plant: A Review. IOP Conference Series: Materials Science and Engineering,