ANTIBACTERIAL AND DNA CLEAVAGE ACTIVITY OF CADMIUM SULFIDE (CdS) NANOPARTICLES USING *Sesamum radiatum*

By

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Natural Resources Science) with honours

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APPROVAL

"I/ We hereby declare that I/ we have read this thesis and in our opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Applied Science (Natural Resources) with Honors"

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DECLARATION

I declare that this report entitled Antibacterial and DNA cleavage activity of Cadmium Sulfide (CdS) nanoparticles using *Sesamum radiatum* is the result of my own research except as cited in the references. The report has not been accepted for any degree and is not concurrently submitted in the candidature of any other degree.

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ANTIBACTERIAL AND DNA CLEAVAGE ACTIVITY OF CADMIUM SULFIDE (CdS) NANOPARTICLES USING *Sesamum radiatum*.

ABSTRACT

The occurrence of bacterial infection has increased significantly over last few decades. Thus, it has arisen concern of research to produce new effective drugs to control bacterial infection. The new approach based on green synthesized nanoparticles (NPs) will be a turning point for addressing growing global resistance of antibiotics. In this study, the green synthesis approach was used to synthesize Cadmium Sulfide nanoparticles by utilizing *Sesamum radiatum* leaves extract. The prepared Cadmium Sulfide nanoparticles were characterized by UV-Vis spectrophotometer, Fourier Transformation infrared (FTIR), Scanning Electron Microscope (SEM) and Thermal gravimetric analysis (TGA). The UV-Vis spectra of CdSNPs revealed abroad absorption band between 300 nm - 350 nm contributing to the surface Plasmon Vibration of metal nanoparticles. The presence of functional groups of amines, aliphatic amines, alkenes, alkynes, aryl, alcohols, carboxylic acid, alkyl halides, aromatics, nitro group, phenol, ester and anhydrides were confirmed by FTIR analysis. SEM obtained the information regarding to the surface morphology. TGA analysis was carried out to prove that the stability of CdSNPs. The antibacterial activity of CdSNPs against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was carried out by discs diffusion method with increasing concentration (10mg/ml-30mg/ml). The increasing concentration of nanoparticles enchanted inhibition zone. The DNA cleavage activity was performed to visualize the ability of the CdSNPs to cleave pBR322 DNA which indicates that the disappear of band I from the circular plasmid DNA using gel electrophoresis.

ANTI-BAKTERIA DAN AKTIVITI BELAHAN DNA OLEH KADMIUM SULFIDA NANOPARTIKEL MENGGUNAKAN *SESAMUM RADIATUM*

ABSTRAK

Kejadian disebakan oleh infeksi bacteria telah meningkat sejak beberapa abad yang lalu. Oleh itu, ia telah meningkatkan keperihatinan penyelidik untuk menghasilkan dadah yang efektif untuk mengawal infeksi bakteria. Sebuah pendekatan baru berdasarkan sintesis hijau nanopartikel(NPS) telah menjadi titik puncak dalam memperluaskan pada peringkat global untuk kerintangan kepada antibiotik. Dalam kajian ini, pendekatan sintesis hijau telah di gunakan untuk mensintesis kadmium sulfida nanopartikel dengan mengunakan ekstrak daun *Sesamum radiatum.* Nanopartikel kadmium sulfide telah disediakan dicirikan dengan mengunakan UV-Vis spektrofotometer, Transformasi Inframerah Fourier (FTIR), Mikroskop elektron pengimbas (SEM) dan Analisis Termogravimetri (TGA). UV-Vis spektra bagi CdSNPs menunjukan penyerapan lengkungan luas antara 300nm-350nm menyumbang kepada getaran permukaan Plasmon bagi nanopartikel logam. Kehadiran kumpulan amina, amina alifatik, alkena, alkena, aril, alkohol, asid karboksilat, alkil halida, aromatik, kumpulan nitro, fenol, ester dan anhidrida telah disahkan oleh analisis FTIR. SEM memperolehi maklumat morphologi permukaan. Analisis TGA telah dijalankan untuk membuktikan kestabilan CdSNPs. Ujian aktiviti antibakteria nanopartikel kadmium sulfida terhadap *Escherichia coli (E. coli)* dan *Staphylococcus aureus (S. aureus)* telah dijalankan mengunakan kaedah resapan cakera dengan meningkatkan kepekatan nanopartikel (10mg/ml- 30mg/). Peningkatan kepekatan nanopartikel telah mengalakan zon perencatan. Aktiviti belahan DNA telah dijalankan untuk melihat kebolehan CdSNPs untuk membelah pBR322 DNA yang boleh menghilangkan band I dari DNA plasmid bulat dengan menggunakan elektroforesis gel.

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LIST OF ABBREVIATIONS

a.u	Absorbance unit
CdS	Cadmium sulfide
CdSNPs	Cadmium sulfide nanoparticles
DNA	Deoxyribonucleic Acid
FTIR-ATR	Fourier Transmission Infrared- Attenuated Reflectance
MIC	Minimum Inhibition Concentration
MDR	Multi-Drug Resistant
NPs	Nanoparticles
RMP	Revolutions per Minute
Rpm	Rotation per million
SEM	Scanning Electron Microscopy
S. radiatum	*Sesamum radiatum*
TGA	Thermogravimetric Analysis

LIST OF SYMBOLS

θ	Theta
%	Percent
°	Degree
°C	Temperature (degree Celsius)
cm^{-1}	Reciprocal centimeters
eV	Electron-volt
g	Gram
Mm	Millimolar
ml	Milliliter
mm	Millimeter
nm	Nanometer
µg	Microgram
µl	Microliter
$Cd(CH_3CO_2)_2$	Cadmium Acetate
Na_2S	Sodium Sulfide

CHAPTER 1

INTRODUCTION

1.1 General backgrounds

Nanotechnology refers to a technology or technical details that are between 1 to 100 nanometers and has been applied in many industries such as medicine, biotechnology, agriculture, and others. Nanoparticles (NPs) behave as a whole unit with respect to its transport and properties (Hajra et al., 2015). There are three methods that usually applied to synthesize NPs such as physical method (mechanical method, vapor deposition, sputter deposition, ion bean technique and etc.), chemical method (sol-gel method, micro emulsions, hydrothermal synthesis, sonochemical synthesis and etc) and green chemistry (using plant extract, waste or enzyme) (Bensebaa, 2013). This is the common methods that usually use to produce NPs. Among from this method, scientist has chosen green synthesis to produce NPs although physical and chemical methods are most popular compared to biological method. According to Mubayi et al., (2012), green synthesis method is a method that simple, easy, efficient, and eco-friendly compared to another method for production of well characterized NPs (Rai & Rosten, 2013). Besides that, green synthesizes of NPs is cheaper and easier way compared to other method (Polshettiwar & Varma, 2010). Due to this factor, scientist nowadays made a dipper exploration on this method toward the effectiveness in synthesis metal NPs.

The green synthesis of NPs from extraction of plant has been one of the most intensively studied fields that involved the study of newly synthesis of NPs. Various studies have been carried out for the green synthesis of NPs using different organisms such as plant, bacteria, fungi, seaweeds and micro algae (Kuppusamy et al., 2016). Among of these studies, plant is seen as the best choice for bio-synthesis of NPs because it contains hydroxyl (-OH) and carbonyl group(C=O) compound which can act as reducing agent and a stabilizing agent (Cai et al., 2016). To synthesize NPs by using green synthesis, plant parts such as leaves, root, stem flower and fruit or plant extract is required. In this research, the leaves of *Sesamum radiatum* was used as plant extraction to synthesized NPs. *Sesamum* comprises about 20 species, most of which are indigenous to tropical Africa (Vossen et al., 2007). *Sesamum radiatum (S. radiatum*) or black benniseed was used as medical tea that eases childbirth (Orr, 2014). This plant also used as a shampoo and to kill head lice, applied as a treatment for rectal prolapse, to treat metrorrhagia, used as an antidote for scorpion stings and lastly applied externally to treat sprains (Bedigian, 2004).

The number of applications making use of NPs technology is already vast and keeps growing and one of this NPs is Cadmium sulfide nanoparticles (CdSNPs). These CdSNPs belongs to the group of Chalcogenides II-IV group semiconductor nanoparticles and shows size dependent properties due to its very high surface to volume ratio and quantum confinement at nanoscale, which has a narrow band gap of 2.5 eV (Bansal et al., 2012) and has a vital use in biology, medicine, technology, and telecommunication field. Cadmium Sulfide (CS) exists in two types of different polymers which is hexagonal greenockite and cubic hawleyite. The synthesis CdSNPs from plant extraction becomes a choice for further study because the cost of preparation is economically attractive rather than preparation of other nanoparticles such as gold and silver. Basically, the formation

of CdSNPs can be prepared from cadmium (II) salts with sulfide ion. These formations of CS can be produce by using many scientific methods such as Sonochemical (Palanisamy et al., 2015). There is also method that can produce this nano-materials from bulk materials such as solution-based chemistry, mechano-chemical processing, physical and chemical vapor deposition techniques, and etching. CS was used as a pigment in paints as far back as 1819. Synthetic CS pigments are valued for their good thermal stability in many polymers, for example in engineering plastics. By adding selenium, it is possible to obtain colors ranging from a greenish yellow to red violet.

Antibacterial activity has a unique tendency of interaction towards random synthesized drugs that drag attention of clinical researcher. Antibacterial is any materials and event that with the properties of destroying the structure of bacteria and suppress their ability of growth and reproduce. In simple way, this antibacterial agent will destroy the cell wall of bacteria and disrupt their key function to inhibit the growth of bacteria. The antibacterial activity of NPs from plant extract is continuously attracting the attention of clinical researcher because infection caused by bacteria always a tough challenge for them. NPs synthesis from plant extract is best approach in nano-medicine since not produce toxic chemical and also supply natural capping agent for nanoparticles stabilized (Ahmed et al., 2016). In this study, *E. coli* was used to represent gram-negative bacteria (Gupta & Sivakumaran, 2016) while *S. aureus* represent gram-positive bacteria (Okafor et al., 2013) to observe their antibacterial activity against CdSNPs from *S. radiatum*. In this study, CdSNPs was act as antibacterial agents that used to inhibit bacterial growth in the nutrient agar.

Nucleic acids, particularly Deoxyribonucleic acid or DNA are one of the most stable classes of biomolecules. DNA is a thread-like chain of nucleotides that contain the

heredity materials that make up chromosome and gene. These DNA are extremely long double helix (spiral) that used in growth, development, functioning and reproduction of all known living organisms and many viruses (Routh, 2013). DNA has conjugation site which able to bind with another particle such as nanoparticles. DNA has shown specific characteristics in forming bio-conjugates which is able to combine with various type of component (Fisher et al., 2010; Ip et al., 2011; Padmavathy et al., 2010) and this information useful for further research to understanding interaction of DNA and NPs. From previous research, Chen & Wu (2012) has described the mechanisms of binding of nanoparticles with DNA. The mechanisms of DNA bind with nanoparticles has important role in various biomedical applications such as gene expression profiling, disease diagnosis and treatment, drug discovery and forensic analysis which can combat the diseases. To support the statement DNA can bind with the NPs, Qin and Yung (2007) has reported that the gold NPs were successfully interacted with DNA where it can study the DNA properties itself.

In this study, the newly prepared CdSNPs was extracted from *S. radiatum* leaves and the nanoparticles were characterized by using several optical instruments. For the antibacterial activity, two bacteria strains were used which is *E. coli* (gram negative bacteria) and *S. aureus* (gram positive bacteria). Lastly, the ability of CdSNPs to bind with pBR 322 DNA plasmid DNA has been study using gel electrophoresis.

1.2 Problem statement

The outbreak of bacteria which are resistance to antibiotics and able to cause community acquired infection to human and living thing has been a global concern. The incidence of bacterial infection has increased significantly over last few decades. Necrotizing fasciitis, Staph infection, Meningitis, Pneumonia and Tuberculosis is the example of diseases that caused by the bacteria infection. The extended implementation of antibacterial drugs in treating infection has led to resistance among various strains of bacteria. When the bacteria are resistant to several treatments, it can cause a danger to human and environment health. Thus, it has arisen concern of clinical research to produce new effective drugs to control this bacterial infection. The new approach based on green synthesized NPs will be a turning point for address growing global resistance of antibiotics. The study of DNA binding with NPs can be a kick start for further research. Besides, there is no data yet about the study of antibacterial and DNA cleavage activity of CdSNPs by using *S. radiatum*.

1.3 Objective of the Study

The objectives of this study were:

1. To characterize the newly prepared Cadmium Sulfide (CdS) nanoparticles from *Sesamum radiatum* using UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, Scanning Electron Microscope (SEM) and Thermo gravimetric analysis (TGA).

2. To evaluate the antibacterial activity of synthesized Cadmium Sulfide (CdS) nanoparticles against *Esherichia coli* and *Staphylococcus aureus.*

3. To evaluate the DNA cleavage activity of Cadmium sulfide nanoparticles using gel electrophoresis.

1.4 Scopes of study

The scope of this study was focused on antibacterial and DNA cleavage activity of CdSNPs using *S. radiatum*. In this study, CdSNPs was prepared from *S. radiatum* which act a reducing agent and the *S. radiatum* extract has been applied to synthesis of CdSNPs. These NPs were identified and characterized by using some optical instrument such as UV-Visible spectroscopy analysis, Fourier Transform Infrared –Attenuated Total Reflectance (FTIR-ATR), Scanning Electron Microscope (SEM) and Thermo gravimetric analysis (TGA). The antibacterial activity of these synthesized has been tested by agar disc diffusion method and bacteria that used are *E. coli* and *S. aureus*. For DNA cleavage activity, DNA ability to bind with CdSNPs has been analyzes by using gel electrophoresis.

1.5 Significance of study

The significant of this study were to used plant extract from *S. radiatum* for production of CdSNPs since the benefit of this plant was yet to be discovered by the researcher. A research about its potential benefits must carry out to enable its bioactive reductive component inside it can and can be used for prepared environmental NPs with green

synthesized technique and NPs that would be prepared from this plant probably have strong antibacterial properties. CdSNPs synthesized from *S. radiatum* extract and would be tested for antibacterial activity and DNA cleavage activity in the present study. Antibacterial is important for treating bacterial infection agent with is it can treat human and environmental health and can cause damage organ, cell, tissue and nerve. The green approach of synthesized CdSNPs will be a new milestone in treating bacterial infection. The antibacterial and DNA cleavage activity of CdSNPs could be one of the new achieved of nanotechnology. Product related to antibacterial also could be prepared from CdSNPs produced from *S. radiatum*. Lastly, the ability of DNA to bind with NPs can bring a kick start for further study.

CHAPTER 2

LITERATURE REVIEW

2.1 Nanoparticles and synthesis of nanoparticles

Nanoparticles (NPs) are the particle that have a size 1–100 nm in size and have a special place in nanoscience and nanotechnology. The uniqueness of these NPs is not only about their properties resulting from their reduced dimensions, but also because they are promising building blocks for more complex nanostructures which has bring vital benefits to live. NPs can be divided to 4 groups which are: (i) inorganic metals, (ii) inorganic semiconductors, (iii) inorganic insulator including oxides and sulphide and finally (iv) organic and polymers (Bensebaa, 2013). NPs in the nanotechnology are expected to bring enormous advantage to the society in varies field such as drug design and development, water purification, technologies related to information and communication and the synthesis of better, lower weight materials. The uniqueness of NPs such as their unusual and fascinating properties, and applications advantageous over their bulk counterparts has attracted researcher to study about them.

There are two approaches to synthesis the NPs which is bottom up approach and top down approach. For the bottom up approach, the production of NPs is from the arrangement of smaller components into more complex assemblies. It will help by the chemical or physical forces operating at the nano scale to assemble basic units into larger structure. For example, the formation of carbon nanotubes. For the bottom to up approach

it will produce toxic or nontoxic materials depends on how they manufactured. It different with top down approach when NPs is produce by create a smaller device by using larger ones to direct their assembly. The most common top-down approach to fabrication is vapor phase fabrication and liquid phase. It also produces toxic chemical to environment.

Basically, there are two methods to synthesis NPs which is physical and chemical method. In physical method, NPs will produce by two ways which is mechanical (high energy ball milling and melt mixing) and vapor (physical vapor deposition, laser ablation, sputter deposition, electric arc deposition and ion implantation). This method is not environmental friendly because it consumes large amount of energy that increase the surrounding temperature (Iravani et al., 2014). In chemically method, the method that usually used to synthesized NPs is electro deposition, sol–gel process, chemical solution deposition, chemical vapor deposition soft chemical method, Langmuir Blodgett method, catalytic route, hydrolysis, co-precipitation method and lastly wet chemical method. Those methods are not efficient and not environmental friendly. It is because the emission of some gaseous from the process may lead to air pollution.

2.2 Cadmium sulfide nanoparticles

Cadmium sulfide (CS) is a chemical compound that is one of the semiconducting NPs belonging to II-VI group (Sankhla et al., 2015) and known as direct gap semiconductor compound. CS has its own characteristics compared to another chemical compound which is yellow in color and can be a semiconductor of electricity. CS exists in two form of crystal structure which is Greenockite and Hawleyite. But there are easily

confused between these both of crystal structure. Generally, Greenockite is yellowish crystal in the hexagonal system while hawleyite is shiny yellow varnish on siderite.

Table 2. 1: Some basic properties of Cadmium Sulfide

Properties	Value
Physical state and appearance	Solid. (Solid powder.)
Molecular Weight	144.46 g/mole
Color	Yellow or brown
Melting Point	Sublimes. (980°C or 1796°F)
Specific Gravity	4.82 g/cm^3
Solubility	Insoluble in hot and cold water

Source: (AHP Materials, Inc.)

Due to CS uniqueness properties, it has been using in much application especially in the technology. The main applications of CS are as a pigment. CdS nanoparticles also has potential in mesocopic research and development of nano-devices and it has been applying in various fields such as fluorescent probe, biosensors, solar cells, photo-electrolysis and laser light-emitting diodes. These NPs also has reported that have a quite strong and good of antibacterial activity (Malarkodi et al., 2014).

The previous study has shown that only a few researches about green synthesis of CdSNPs using plant have been done. For example, the plant that used from previous study is, Banana peel (Zhou et al., 2014), *Macrotyloma uniflorum* (Lam.) Verdc (Halder, et al., 2015) Papaya peel (Bhuvaneswari & Radjarejesri, 2015) and the latest *N. tabacum* (Borovaya et al., 2016) and *Nigella Sativa L*. (Kumbhakae et al., 2016). In present study, CdSNPs from *S. radiatum* leaf extracts would be synthesized and their antibacterial

activities was study by using *E. coli* and *S. aureus* bacterial strains and also study the DNA cleavage activity of the nanoparticles towards pBR322 DNA.

2.3 Green synthesis of nanoparticles

According to Khani et al (2018), the integration of green chemistry principles of nanotechnology is one of the main subjects in nano-science. Green chemistry is the utilization of a set of principles that reduce or eliminates that use of hazardous substances in the design, manufacture, and application of chemical product (Alfonsi et al., 2008) while green synthesized of NPs is defined as produced metal NPs by green methods with involve plant and microbes in order reduce hazardous substances. Many researchers use biological method or green method compared to another because this method is highly stable and can get a well characterized of NPs. There are several biological methods to synthesis NPs which is i) by using plant extract, ii) using waste materials and lastly iii) use of enzymes and microorganisms. Among these method, using plant extract to synthesis NPs has drag researcher attention because it more advantageous rather than other method. For example, use plant extract to synthesis NPs can reduce cost of micro-organisms isolation and their culture.

Plant-mediated synthesized of NPs is one of green approach that connects nanotechnology with plant. Many of scientific have verified that the plant extract has a promising synthesis of NPs in non-hazardous wall. Plants are used for the synthesized NPs is not only can reduce environmental impact due to the use of harmful chemicals but also can synthesis NPs in larger quantities. In fact, synthesis NPs using plant extract can

reduce metal ion and also become an alternative to produce unique and stable NPs (Iravani, 2011). Plant extract also act both reducing and stabilizing agent during synthesis of NPs.

Plant contains different concentrations and combinations of organic reducing agents. These different sources of concentration and organic agent influence the characteristics of the NPs (Kumar and Yadav, 2009). Typically, there are three kinds of methods use for synthesized of NPs using plant with is intra-cellular (plants), extra-cellular (plant extract) and phytochemical with act as alternative way to substitute conventional physical and chemical procedure. Nowadays, scientist more focus on production of NPs from extra-cellular (plant extract) because it likely can be apply in several commercial applications compared to another method.

2.4 *Sesamum radiatum*

S. radiatum is the flowering plant which a common names benniseed, black benniseed, black sesame and wild benniseed. *S. radiatum* also known as "Bijan", "Gingili" and "lenga" belong to family of Pedaliaceae with a chromosome number 2n=64 (Grubben & Denton, 2004). This plant is of Africa origin at an early date and mainly as weed (Hutchinson & Dalziel, 1963) where is gathered in the wild and use as a potherb. *Sesame* is a flowering plant in the genus *Sesamum* that have approximately 20 species in the flowering plant family. The most common type is *S. angustifolium* (Figure 1), *S. baumii* (Figure 2), *S. calycinum* (Figure 3), *S. radiatum* (Figure 4) and lastly *S. indicum* (Figure 5). Sometimes small horns can be present on fruits of *S. radiatum* and in that case

confusion is possible with *Ceratotheca sesamoides*. In habit *S. radiatum* resembles *S. indicum*, but it can be distinguished by the testa structure (smooth in the latter species). But in this research, it was focused on the use of *S. radiatum* extract for synthesis CdSNPs and test on the antibacterial properties and DNA cleavage activity of CdSNPs.

Figure 2.1: *Sesamum angustifolium*

(Source: Useful Tropical plant- *Sesamum angustifolium*)

Figure 2.2: *Sesamum baumii*

(Source: Flora of Zimbabwe)

Figure 2.3: *Sesamum calycinum*

(Source: Flora of Zimbabwe)

Figure 2.4: *Sesamum radiatum*

(Source: iNaturalist.org)

Figure 2.5: *Sesamum indicum*

(Source: Herbalistics)

S. radiatum is primarily self-pollinated which the flowers open at dawn, after pollination has occurred. However, under extreme conditions some outcrossing may occur. It takes about 6 weeks from anthesis to fruit maturity. The fruits do not open fully; an angular pocket at the base of. This plant is an annual herb growing up to 1.2 to 1.5 meters tall, stem simple or branched, glandular pubescent. The leaves are opposite, or toward the top of the plant, alternately arranged. The leaves are lance-shaped to oval and up to 12 centimeters long. They may be smooth-edged or serrated. Flowers occur singly in the leaf axils. They are pink to purple in color, sometimes white, and somewhat bell-shaped. They measure up to 5 centimeters long. The fruit is a capsule up to 3.5 centimeters long which contains seeds roughly 3 millimeters long.

S. radiatum is adapted to a wide range of habitats, but is most common in savanna. It occupies open localities where few other herbaceous plants grow. It occurs on nutritionally poor sites, growing in gravelly, sandy and rocky localities. It is also a weed and occurs in formerly cultivated fields. It tolerates heat and drought well and continues growth and flowering during the dry season the fruit retains some seeds.

Fresh leaves of *S. radiatum* are known as a leafy vegetable and has several medicinal and cosmetic uses. For example, fresh leaf use to induce labor, decoction of leaves and roots effective against chicken pox and measles and used as hair shampoo for Taenia capitis (Quattrocchi, 2012). Roots of *S. radiatum* also benefits for irregular menstrual cycle. Besides, macerated fresh leafy stems drunk can used as antidote for scorpion stings and applied externally to treat sprains. Nigeria and other tropical area uses leaves of *S. radiatum* for the treatment of catarrh, eye pain, bruises, and erupted skin lesions (Shittu et al., 2009). In Africa, people at their country use leaves and shoots of this

plant as vegetable. These leaves are believing that contain useful amount of nutrient such as amino acid (Kubmarawa, et al., 2008).

In this recent year, many research has been done about these plant tree. For example, structural characterization and rheological properties of a polysaccharide from sesame leaves (*Sesamum radiatum* Schumach. & Thonn) (Nep et al., 2016), In vitro antibacterial effects of *Crateva adansonii*, *Vernonia amygdalina* and *Sesamum radiatum* used for the treatment of infectious diarrhoeas in Benin (Agbankpé et al., 2016), Hydro-alcoholic media effects on theophylline release from *Sesamum* polysaccharide gum matrices (Nep et al., 2017), and the latest is Identification and pathogenicity of *Fusarium* species associated with sesame (*Sesamum indicum L*.) seeds from the Punjab, Pakistan (Nayyar et al., 2018). However, the research about the synthesis of nanoparticles, antibacterial activity and DNA cleavage is not done yet by using *S. radiatum.*

2.5 Antibacterial activity

Antibacterial activity is known as the any activity which can inhibits the increase in bacteria population or growth either chemically, physically or biologically. This term of antibacterial often used synonymously with term antibiotics. Antibiotics are the antimicrobial drug that produces by using organisms such as plant. Selman Waksman (1942) was the first person that used term of antibiotics (Parker, 2013). The use of NPs in medical field as antibacterial agent can exhibit the bacteria growth because metal NPs was proven to destroy the cell wall of the bacteria and disrupt the Adenosine Triphosphate

(ATP) production and cleave the Deoxyribonucleic Acid (DNA) structure (Mirzajani et al., 2013).

Microorganisms have become resistance to drug and continue to plague human being in response to the development of antimicrobial agent (Saga & Yamaguchi, 2009). According to this problem, it has become major global challenges to the clinical researcher to overcome this problem due to resistance of bacteria to any drug. The use of nanostructured is important to combat bacteria as this NPs can behave optically and electrically. II-IV semiconductor NPs such as CS has unique optical and electrical properties with can exhibit antibacterial property. To study this antibacterial activity, disc diffusion method and Broth dilution method are often used for antibacterial determination (Jorgensen & Turnidge, 2015).

2.6 DNA cleavage activity

Recently, many studies have been conducted regarding the synthesis and the use of metal NPs especially in the medical and nutritional field. Metal NPs has a various shape and sized has been synthesized and has been explored for their applications in various fields of life sciences. Their use in the medical and nutritional field has bring a new system which allow targeted delivery of substances and improved permeability and retention (Bogunia and Sugisaka, 2002). Due the uniqueness of these NPs, researcher is focus on improvising the efficiency of drug delivery without be degraded in the body biological system by using strong NPs conjugated (Florence, 2004; Hoffart et al., 2006). Basically, the basic conjugate system of this NPs is the ability of this nanomaterials to conjugate

with biomolecules such as DNA and protein which can increase efficiency of drugs against pathogen to combat disease.

DNA based nanotechnology, especially the study of interaction of DNA and NPs is one of the most intensively study that have taken a big step in many fields such as medicine, biochemistry, analytical chemistry, bio-sensing and other (An & Jin, 2012). The interaction between NPs and DNA bring a vital role and information in genoticity of NP, and it is imperative to characterize the nano/DNA interaction (Capco & Chen, n.d). When the NPs is bind with DNA, the conjugate will form from the interaction of DNA with NPs where these conjugated has been identified to be useful in the many studies of biological system in the future.

The study of interaction between DNA and NPs has focused into the physical parameter that affect the DNA NPs interaction due to DNA that know as unique polymer with extremely high charge density and huge bending stiffness. Besides, when study the interaction between DNA and NPs, it also focused on it stability as a conjugated. This is vital thing to be observed because the stability of the conjugated might affect its ability to carry out its designated task as a conjugated in a system. Many study of the interaction of DNA with NPs showed that many negatively charged biomolecules like nucleotides double strand DNA, nuclei acid and vitamins when hybridized with nanoparticle yield special features like stability against physiochemical and biological degradation and targeted delivery (Jin-Ho et al., 2008).

According Qin and Yung (2007), the detection and quantification of DNA with NPs is an example of DNA and NPs interaction to study about DNA properties was observed that

the precise quantification of single stranded DNA because of the formation of defined NPs-DNA conjugate in the presence of target DNA. This study is not only the interaction of DNA and NPs, but it also can overcome the limitation before in DNA or NPs synthesized and its yields conjugated that are very accurately defined (Christopher et al., 2015).

Through reviewing the previous study, there are an advantage of the interaction between NPs and DNA for the science study especially in the medical field. There are also clear that there is no study has been done by using CdSNPs with *S. radiatum*. There is also no study about capability of this NPs from this plant extract to interact with DNA and how it could yield any important features if available which is important for further research if available. This gap from previous research was try to fulfill by green synthesized of novel CdSNPs from extraction of *S. radiatum* and characterized the NPs and then study about their antibacterial activity by using gram positive and gram negative bacteria and then interaction the NPs with DNA and study the cleavage ability of the DNA strands and the reaction of resulting of the DNA's binding site with NPs which is less clarified in previous research. Through this study, a CdSNPs from *S. radiatum* extraction has been done and these CdSNPs has been used to study the antibacterial activity and DNA cleavage activity using *S. radiatum.*

CHAPTER 3

MATERIAL AND METHOD

3.1 Chemical

Cadmium acetate ($Cd(CH_3CO_2)_2$) from Sigma Aldrich, Malaysia, Sodium Sulfide(Na_2S) from Bendosen, Germany, ethanol from HmbG, Germany, Amoxicillin, Nutrient Agar and Nutrient Broth powder from Oxoid Ltd, England, Agarose gel 1%, EDTA, TAE buffer solution, pBR322 DNA, 6X loading dye, red safe, 1kb DNA ladder and Deionized water (dH_2O),

3.2 Instrument

UV-Visible spectroscopy from (Thermo Fisher Scientific model 4001/1), Fourier Transform Infrared –Attenuated Total Reflectance (FTIR-ATR) Spectroscopy from (Thermo scientific with model number Iz10), Thermo Gravimetric Analysis (TGA) from Mettler Toledo, Scanning Electron Microscopy (SEM), orbital shaker, blender, gel electrophoresis, incubator, hot plate, oven, and autoclave machine.

3.3 Materials and apparatus

Magnetic stirrer, micropipette, tip, disc, conical flask, beaker, falcon tube, loop, cuvette, bunsen burner, spatula, cotton swab, filter funnel, gauze, petri dish, glass rod, serial dilution bottle and filter paper.

3.4 Collection of plant leaves

Fresh leaves of *Sesamum radiatum* (plant sample) were collected in the area around Blok A (near TNB) of Universiti Malaysia Kelantan, Jeli campus. Once the plant sample was collected, the leaves were cleaned by using tap water and rinsed with deionized water to clean all the attached materials. The plant sample was oven dried with the temperature between 40^0C-50^0C. After the plant sample was dried, the plant sample was blander to make it powdered. After that, the powder was sieved to get very fine particles of uniform size.

3.5 Preparation of *Sesamum radiatum* extract

The *Sesamum radiatum* leaves extract was prepared by mixing 10 g of the *S. radiatum* leaves extract with 100ml of deionized water. The resulting mixture was boiled in a water bath at 60^0C for 30 minutes. At the same time, the resulting mixture was kept on stirring at 500rpm to 600rpm with magnetic stirrer. Then, the resulting solution was left for 1 hour for stable and the sticky solution was filtered with gauze. The resulting sticky mixture was dissolved again in 100ml of deionized water and placed in water bath again to reduce stickiness of the *S. radiatum* plant extract. Lastly, the resulting mixture was filtered with filter paper and then the solution was stored in 4°C for further usage.

3.6 Synthesis of Cadmium Sulfide nanoparticles (CdsNPs)

The Cadmium Sulfide nanoparticles was prepared with slight modification according to the method published by (Prasad et al., 2014). 10mM aqueous solution of

Cadmium acetate (Cd (CH_3CO_2)$_2$) and 10mM aqueous solution of sodium sulfide (Na_2S) was prepared and used for synthesis of Cadmium Sulfide nanoparticles. 10 ml of plant extract was added into 100ml of Cadmium Acetate solution for reduction into Cd^{2+} ions and 100ml of Sodium Sulfide solution was added drop by drop into the resulting mixture to allows the Cd^{2+} ions gained with S^{2-} for synthesis CdsNPs. At the same time, kept the magnetic stirrer stirring. After that, the resulting mixture was placed in precise rotatory shaker for 24 hours at 200 rmp, 30^0C. After 24 hours of monitoring, 5ml aliquot of the mixture was sampled. The formation of CdSNPs pellet was centrifuge at 13000rpm for 30 minutes to get more CdSNPs pellet. After that, it was washed with distilled water three times to remove bonded organic matter on the CdSNPs (Rao & Pennathur, 2017) and the pellet was dried in desiccator.

3.7 Characterization of Cadmium Sulfide nanoparticles (CdsNPs)

The characterization of CdsNPs was made by using UV-Visible spectroscopy, Fourier Transform Infrared –Attenuated Total Reflectance (FTIR-ATR) Spectroscopy, Scanning Electron microscopy (SEM) and Thermo gravimetric analysis (TGA). The Cadmium Sulfide was characterized in a UV-Visible spectroscopy and the range for the sample analysis is between 300nm - 800nm. FTIR was used to analyze the functional biomolecules associated with CdsNPs. This FTIR spectra was run in the absorption range between 500cm^{-1} - 4000cm^{-1}. The SEM was used to determine external morphology, crystalline structure of CdsNPs. Thin films of CdsNPs were set on a carbon-coated copper grid. The mercury lamp was used to dry the films of SEM. Thermo gravimetric analyses (TGA) is a method of thermal analysis in which the mass of a sample is measured over time as the temperature changes. It has been done by take 32.2 mg samples of Cadmium

Sulfide nanoparticles on a platinum pan under nitrogen atmosphere. Experiments was performed at a heating rate of 10 ^{0}C/min from 50^{0}C until 700 ^{0}C. These analyses was done in gas flow at 90ml/min.

3.8 Antibacterial test of Cadmium Sulfide nanoparticles

3.8.1 Bacterial Pathogens growth

Escherichia coli and *Staphylococcus aureus* bacteria strain were used in this study and these bacteria were obtain from the stock of Faculty of Earth Science Universiti Malaysia Kelantan. The test bacteria were grown on the nutrient agar plate at the temperature of 37ºC for 24 hours. A single colony of bacteria that have same morphological type were pick and culture with streak plate method on nutrient agar dish. After that, the nutrient agar dish was incubated again at 37^0 C for 24 hours in the incubator.

3.8.2 Preparation of antibacterial agent

Preparation of antibacterial agent was carried out according followed work done by (Shivashankarappa et al.,2015) and (Singh et al., 2014) with slight modification. For the antibacterial test, Cadmium Sulfide nanoparticles was dissolve deionized water to prepare stocks of solution of CdSNPs. 10mg/ml, 15mg/ml, 20mg/ml, 25mg/ml and 30mg/ml of cadmium sulfide nanoparticles were dissolved in deionized water to find which concentration of antibacterial agent were performed well in antibacterial test.

3.8.3 Antibacterial Test using Disc diffusion method

For antibacterial test, work was done by (Yadav et al., 2016) and (Sign et al., 2014) with some modification. Agar medium was prepared by pour 2ml of broth culture into serial bottle and after that a single colony of bacteria with common morphological type were selected from agar plate culture and inoculate in broth culture. The fluid culture was incubated for 24 hours at 37^0C until it reaches turbidity of the 0.5 McFarland standards. After that, the bacteria agent was picked using micropipette and transfer it into the nutrient agar. Cotton swab was used to spread the bacteria in the nutrient agar to make sure the bacteria were distributed well in the agar. Standard antibacterial drug Amoxicillin (15mg/ml) and deionized water used as a positive (+ve) and negative (-ve) control of this study. After that, a sterile disc were used for different solvents of antibacterial agent prepared on the surface of the agar. The sterile micropipette was used to fill the each antibacterial agent onto the disc surface. For proper diffusion of the antibacterial agent, the plate were left for 10 minute. After that, the agar plate were incubated for 24 hours at 37^0C. After 24 hours, the inhibition zones of bacteria were measured by using vernier caliper.

3.8.4 Statistical Analysis for Antibacterial activity

All data were expresses as Mean±SD (Standard deviation) of three separate experiment by using excel.

3.9 DNA Cleavage activity of Cadmium Sulfide nanoparticles

3.9.1 Preparation of stock solution

1 mg of CdSNPs was weighed and then was dissolved in 1ml of deionized water and has been used as the sample during run the gel electrophoresis. The solution was mix well to make sure the nanoparticles was dissolved completely in the water. By using 0.5 µl -10 µl micropipette, 1 µl of CdSNPs and 1 µl pBR322 DNA was take and mixed well. After that, 3 µl of loading dye was take and mixed well in the mixture.

3.9.2 Preparation for buffer and gel electrophoresis

The cleavage experiment of pBR322 DNA was carried out using gel agarose electrophoresis according by method Dhanaraj & Nair (2008) with some modification. To prepare 1% of agarose gel, 0.5g agarose was diluted in 50ml of 1x TAE buffer solution and boiled in the conical flask. The solution was heated to dissolved agarose completely. Let the solution to cool in the room temperature and 5 µl of red safe was added to the solution and mixed well. The warmed agarose was poured and clamped immediately with comb to form sample well. After 30-40 minute, the comb was removed slowly to avoid damaged of well. The gel was mounted into electrophoretic tank and electrophoretic buffer were added to cover the gel. The sample were loaded into the well of the submerged gel using micropipette. The electrophoresis was carried out at 60 V for 60 minutes. After that, the gel was taken out from the buffer and was photographed under UV transilluminator and documented.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Synthesis of Cadmium Sulfide Nanoparticles

In this study, the green synthesis of Cadmium sulfide nanoparticles using *Sesamum radiatum* plant extract was carried out. The changed of colour from colorless mixture to yellow were observed when 10mM Cadmium Acetate solution with *S. radiatum* plant extract was added with 10mM Sodium Sulfide drop by drop for the synthesized of Cadmium Sulfide nanoparticles. The synthesis of Cadmium Sulfide nanoparticles leading changed in colour can be attributed to surface plasmon resonance of CdSNPs (Ali et al., 2012). The formation of coalescent orange-yellow clusters at the bottom of the tube indicated the formation of nanoparticles. A similar result also reported by another researcher (Cho, 2008). The changed from colorless mixture to yellow colour indicates that the Cd^{2+} ions gained with S^{2-} for synthesis CdsNPs. The change of colour also prove that the plant extract not just act as reduction agent but also the stabilizing agent during the formation of NPs (Marquis et al., 2016).

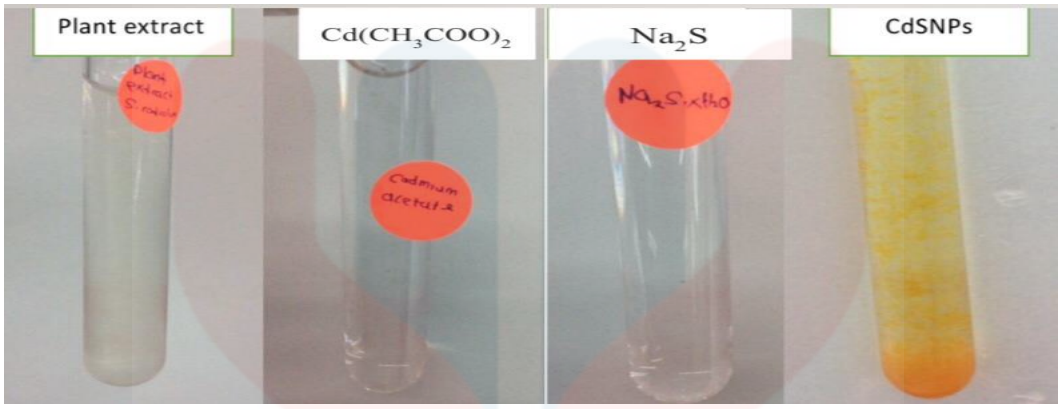

Figure 4.1: The colour changes from colourless to yellowish cluster of synthesized Cadmium Sulfide nanoparticles

4.2 Characterization of Cadmium sulfide nanoparticles

4.2.1 UV- visible spectra of Cadmium Sulfide nanoparticles

The visual study of CdSNPs production from the *Sesamum radiatum* plant extract was confirmed by UV-Visible spectroscopy from Thermo Fisher Scientific model 4001/1 by recording the absorbance from 300nm -800 nm. Uv-visible spectrophotometer was used to characterize the excitation spectra of CdSNPs. In this method, result revealed that the extraction of *S. radiatum* leaves extract, CdSNPs were synthesis at different time interval which is within 2, 4, 6, 8 and 10 hours incubation period. The band of UV- Vis spectra was shown in Figure 4.2. From the graph, it shown that strong absorption peaks from 300 nm - 400 nm were exist during the synthesized of CdSNPs. The broad peaks formed correspond to the surface plasmon resonance which indicated that the presence of stable nanoparticles and this band is shifted at range 400nm - 450nm. By looking the figure

below, it shown that the synthesized of Cadmium Sulfide nanoparticles was complete after 6 hours of incubation period.

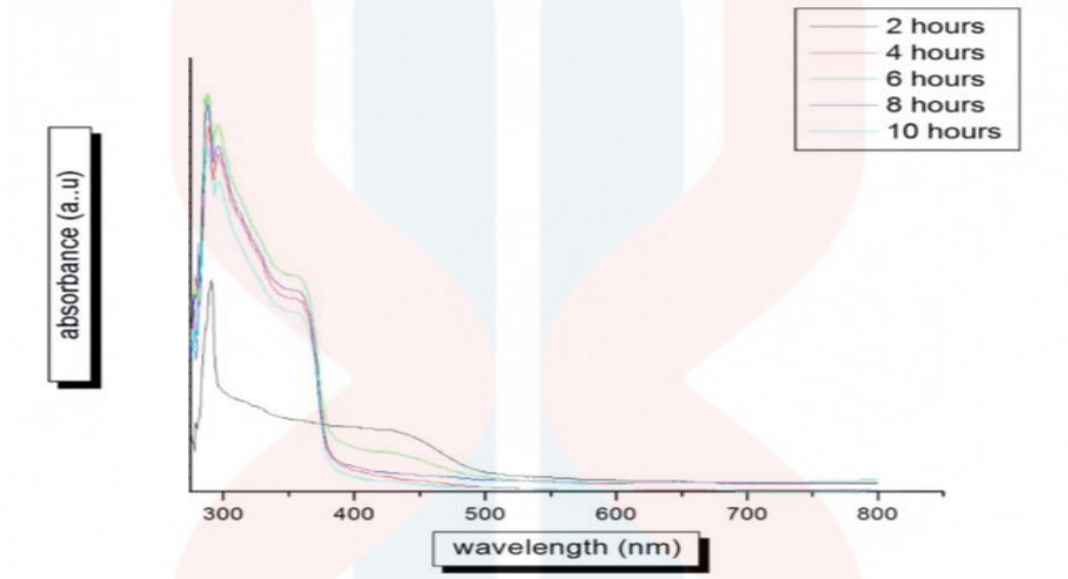

Figure 4.2: UV- Vis spectrum of synthesized Cadmium Sulfide nanoparticles at different time interval of incubation period.

4.2.2 FTIR analysis of Cadmium Sulfide nanoparticles

FTIR analysis is used to study the purify and also the composition of the synthesized product. In this study, FTIR analysis were used to determine the functional groups and types of bonds present in the sample. In FTIR analysis is used to analyze the major compound that responsible to the reduction of Cadmium ion and Sulfide ion to form Cadmium Sulfide nanoparticles from *Sesamum radiatum* plant extract. In this study, FTIR analysis was carried out from 500cm^{-1} to 4000cm^{-1}. In Figure 4.3, it showed the spectrum of plant extract and the synthesized CdSNPs.

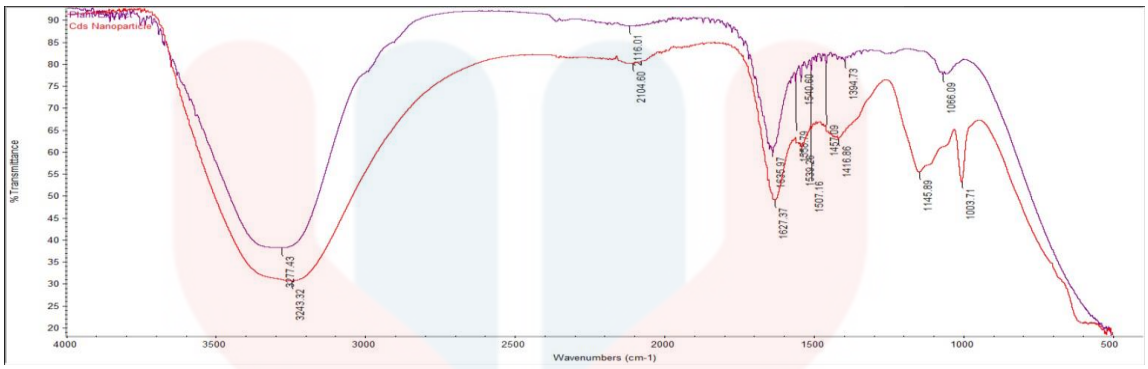

Figure 4.3: FT-IR spectrum of CdSNPs (blue curve) and *Sesamum radiatum* plant extract (red curve)

The bands between CdSNPs and *Sesamum radiatum* plant extract showed slightly different between peaks. For the plant extract, the bands appeared is 3277.43 cm^{-1}, 2116.01cm^{-1}, 1635.97cm^{-1}, 1558.79 cm^{-1}, 1540.60 cm^{-1} , 1507.16 cm^{-1} , 1457.09 cm^{-1}, 1394.73 cm^{-1} and 1066.09 cm^{-1} while for the bands for synthesized CdSNPs located at 3243.32cm^{-1}, 2104.60 cm^{-1}, 1627.37cm^{-1}, 1539.26 cm^{-1}, 1416.86 cm^{-1}, 1145.89cm^{-1} and 1003.71cm^{-1} respectively which represent the diverse functional groups of the adsorbed biomolecules on the surface of the CdS nanoparticles. The peaks found in this research was identify and compare with IR table to estimate the functional group that involved in the synthesized of CdSNPs. The shift occurs from 3277.43 cm^{-1} of the plant extract to the 3243.22 cm^{-1} of CdSNPs was occur due the presence of O-H stretches, and N-H stretch that come from functional group of alcohol, phenols, carboxylic acid, and amines. It indicates that the reduction of Cd^{2+} and S^{2-} ion due the bond that present in alcohol or phenols, carboxylic and amines groups. The peak of plant extract located at 2116.01cm^{-1} were shift to 2104.60 cm^{-1} of CdSNPs can be assigned as the involvement of -C≡C- stretching vibration presence of alkynes functional group in the organic mixture.At the peak 1635.97cm^{-1} of plant extract was shift to 1627.37 cm^{-1} of CdSNPs and the functional

and the molecular motion that involves is the double bond of C=C stretch and triple bond of C≡C from alkenes and N-H bend from amines group. This reduction was occurred which involves amines and alkenes group in the organic mixture. The absorption peak located at 1558.79 cm^{-1} ,1540.60 cm^{-1} ,1507.16 cm^{-1} for *S. radiatum* and peak 1539.26 cm^{-1} of CdSNPs can be assigned as the involvement of various functional group. At adsorption peak located at 1558.79 cm^{-1} , 1540.60 cm^{-1} and 1539.26 cm^{-1} can be assigned as the involvement of single bend of N-H from amines group and -NO_2 (aliphatic) from nitro group. For peak located at 1507.16 cm^{-1} it was due to N-O asymmetric stretching vibration presence of Nitro compound and single bond C-C stretching Vibration presence of aromatics functional group. The shift occurs from 1457.09 cm^{-1} of plant extract to 1416.86 cm^{-1} of CdSNPs is assigned as the involvement of single bond -C-C- stretching vibration in the presence of aromatic functional group during the synthesized of CdSNPs. This is due the adsorption peak located wave range of 1500 cm^{-1} -1400 cm^{-1} range from IR has a single bond C-C stretching vibration that exist in aromatic functional group. The shifts these peak to 1145.89 cm^{-1} of CdSNPs can be assigned to the involvement of single bond C-O stretch (alcohols), -C-O-C- stretch (aryl), C-O stretch(anhydrides), C-N stretch (aliphatic amines), and lastly -C-F- stretch (alkyl halides) in organic mixture. For the *S. radiatum* plant extract, the absorption peak located at 1394.73 cm^{-1} can be assigned as the involvement C-H bond and C-F stretch from alkenes alkenes and alkyl halides functional group that exist naturally in the plant. Lastly, the shift that occur at 1066.09 cm^{-1} of plant extract to 1003.71 cm^{-1} of Cadmium Sulfide nanoparticles can be assigned that the presence of several bond which is -C-O-, -C-O-C, and -C-F-. The functional group that involves in the shift of peak from plant extract to CdSNPs is alcohol that wave range from

1260 cm^{-1}-1000 cm^{-1} ethers wave range at 1300 cm^{-1} -1000 cm^{-1}, anhydrides that wave range from 1300 cm^{-1} -900 cm^{-1} and lastly alkyl halides at wave range 1400 cm^{-1} - 1000 cm^{-1}. The variations in the peak positions and the shifts of peak form plant extract to CdSNPs from the Figure 4.3 can be assigned that the involvement of some metabolites compound such as tannins, flavonoids alkaloids, and carotenoids which are rich in *S. radiatum* plant extract and these metabolites compound was act as reducing agent in the formation of the CdS nanoparticles.

In this study, FTIR peak was given an information about amount of bond that exist in the CdSNPs and plant sample. The different peak between the peaks of plant extract with cadmium sulfide nanoparticles in the Figure 4.3 shown the different functional group that exist naturally in plant sample or occur after the synthesized of CdSNPs where the plant extract is act as stabilizing agent during the formation of Cadmium Sulfide nanoparticles in the organic mixture. It also can be concluded that the formation of cadmium sulfide nanoparticles from *S. radiatum* extract capped by proteins and mineral which contain functional groups of amines, aliphatic amines, alkenes, alkynes, aryl, alcohols, carboxylic acid, alkyl halides, aromatics, nitro group, phenol, ester and anhydrides

4.2.3 Scanning electron microscopy(SEM) analysis

In this study, the morphology, shape and size of the synthesized Cadmium Sulfide nanoparticles using *Sesamum radiatum* was obtained by using Scanning Electron

microscopy (SEM). Figure 4.4 and Figure 4.5 show the image of CdSNPs and *S. radiatum* that was analyzed using SEM in various magnification scale. From Figure 4.4, SEM image of Cadmium sulfide NPs was seen under the SEM with magnification x30, x250, x500 and x1500 respectively while for plant sample it was captured with magnification x30, x250, x500, and x1500 magnification. From the SEM that taken by using SEM, it was clear that the image of CdSNPs are observed to be agglomerated in the certain part maybe due to high pH during the synthesized this nanoparticles in this study.

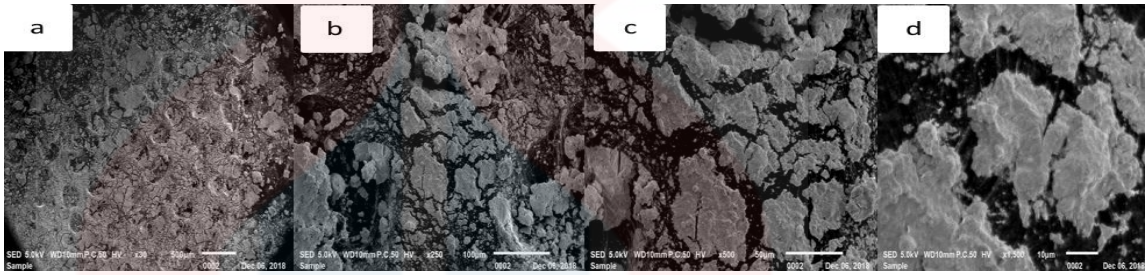

Figure 4.4: SEM image of CdSNPs nanoparticles under magnification (a) x30 ,(b) x250, (c)x500 and (d) x1500.

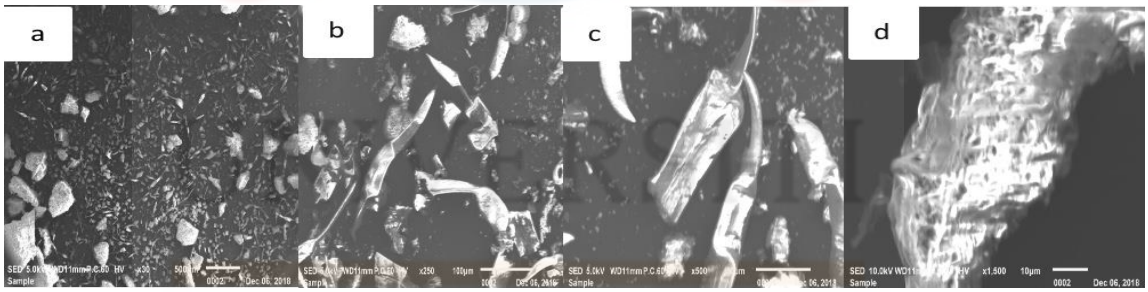

Figure 4.5: SEM image of *Sesamum radiatum* plant powder magnification (a) x30, (b) x250, (c) x500 and (d) x1500.

From the Figure 4.4, SEM image of different magnification from this CdSNPs showed amorphous mass with very fine agglomeration particle structure. A decrease in porosity was observed indicating reduction of particle size. Definite particle shape was

not visible in this study due to some or more a fine amorphous powder that contain in the sample holder during the analysis.

The different shapes of Cadmium Sulfide NPS structure from the Figure 4.4 may happen due to availability of different quantities and different type of biomolecule that may exist naturally on the plant sample. This situation can be related from FTIR result which the different peak and shift that was found during the FTIR analysis. From the Figure 4.5, it shows different shape of S. *radiatum* plant powder which can cause the different shape of nanoparticles. Different size and shape and size of plant materials also contain different quantities and biomolecule that exist in plant.

The formation of Cadmium sulfide nanoparticles that have been analyzed in SEM image was an outcome from the interaction and hydrogen bonding between the bio organic molecules bound to the Cadmium Sulfide nanoparticles. The larger image that capture from SEM image might be cause by aggregation of smaller ones or residue of plant and also due to the SEM measurement.

4.2.4 Energy Dispersive X-ray Spectroscopy (EDS)

Energy Dispersive X-ray Spectroscopy (EDS) pattern of Cadmium sulfide nanoparticles were depicted in Figure 4.6 and 4.7 while for *Sesamum radiatum* was depicted in Figure 4.8 and 4.9. In this study, EDS analysis was used in different point of CdSNPs and plant powder. Table 4.1 and 4.2 show the element composition of Cadmium sulfide nanoparticles while table 4.3 and 4.4 show the element composition that exist naturally in the plant sample.

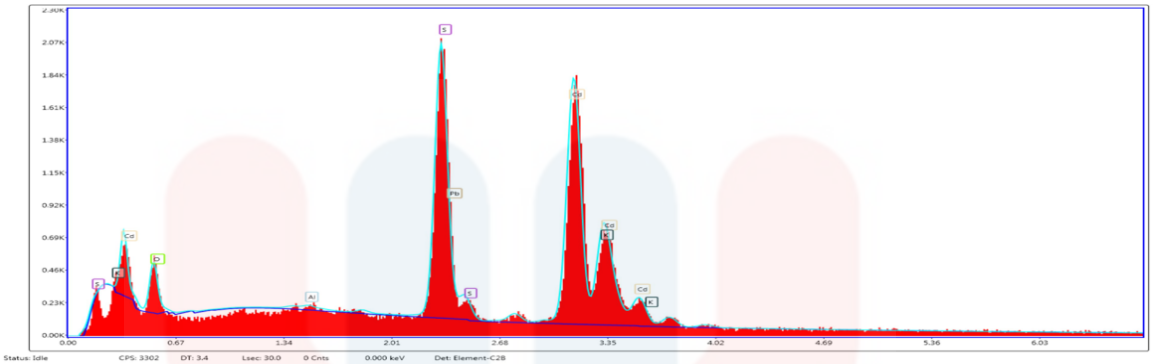

Figure 4.6: EDS pattern of Cadmium Sulfide nanoparticles (Point 1)

Table 4.1: EDS data of Cadmium Sulfide nanoparticles (Point 1)

Element	Weight%	Atomic %
O	2.58	10.88
Al	0.07	0.17
S	20.91	43.98
Pb	3.29	1.07
Cd	73.16	43.90

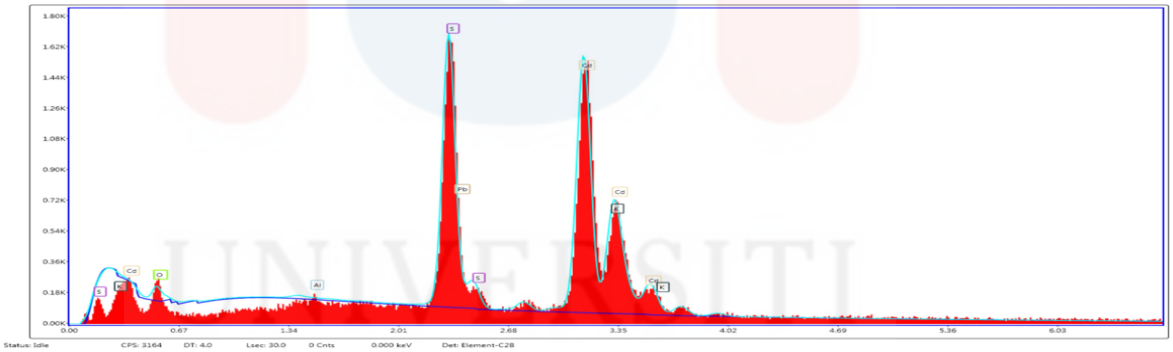

Figure 4.7: EDS pattern of Cadmium Sulfide nanoparticles (Point 2)

Table 4.2: EDS data of Cadmium Sulfide nanoparticles (Point 2)

Element	Weight%	Atomic%
O	0.90	4.08
Al	0.04	0.11
S	19.23	43.52

pB	4.09	1.43
Cd	74.14	47.88
K	1.60	2.97

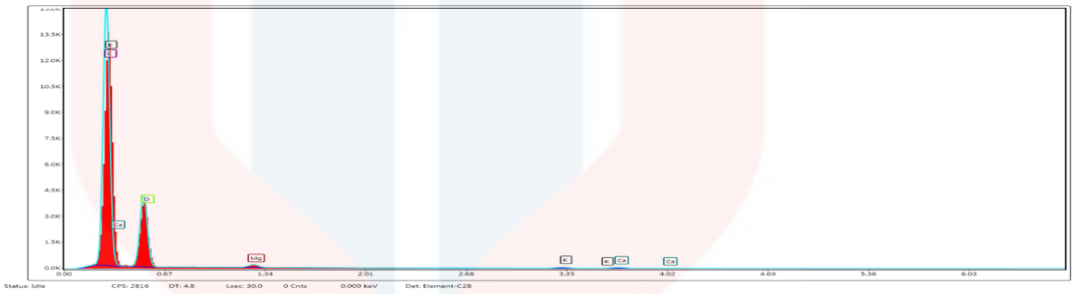

Figure 4.8: EDS pattern of *Sesamum radiatum* (Point 1)

Table 4.3: EDS data of *Sesamum radiatum* (Point 1)

Element	Weight%	Atomic%
C	59.56	67,63
O	35.80	30.52
Mg	1.15	0.65
K	1.49	0.52
Ca	2.00	0.68

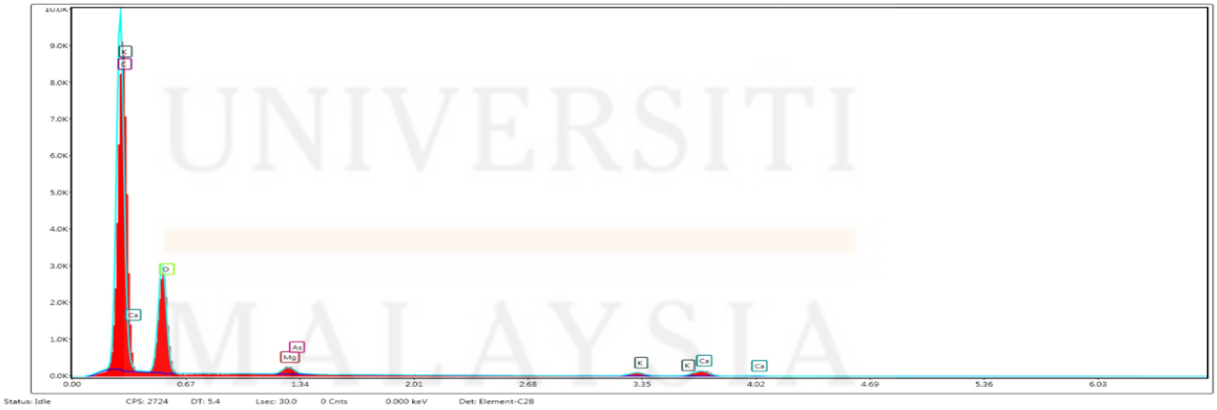

Figure 4.9 : EDS pattern of *Sesamum radiatum* (Point 2)

Table 4.4: EDS data of *Sesamum radiatum* (Point 2)

Element	Weight%	Atomic%
C	50.74	62.00
O	36.15	33.17
Mg	1.21	0.73
As	1.68	0.33
K	3.16	1.19
Ca	7.07	2.56

Figure 4.6 and 4.7 shown a strong signal, approximately at 1.84keV and 1.55keV was found, which is typically absorption of Cadmium atom. The strong signal of Sulfur atom also can be assigned which is shown at Figure 4.6 and Figure 4.7 which is the signal is approximately 2.07keV and 1.62keV. This was confirming the presence of elemental of Cadmium and Sulfur in the sample and it was supporting the hypothesis that Cadmium Acetate was reduced by *S. radiatum* plant extract. Moreover, from the EDS analysis of plant sample, it show the signal of other macronutrient element that exist in plant sample such as Carbon, Oxygen, Magnesium, Potassium and Calcium which these macromolecules was used as the stabilizing agent during synthesis the CdSNPs.

4.2.5 TGA analysis of Cadmium Sulfide nanoparticles

Thermal analysis is a technique to determine the amount of weight change of a material, either as a function of increasing temperature over times, or isothermally as a function of time. The weight change of a material can be used to evaluate thermal stability and material characterization. In this research, TGA was used to identify the stability of the synthesized CdSNPs and the *S. radiatum* when increasing the temperature to know the total residue left which can indicates that the stability of the sample. It also to test whether

the synthesized CdSNPs can maintain until high temperature and pressure. In Figure 4.10 and 4.11, it showed the thermal decomposition of plant extract and the synthesized CdSNPs that have carried out at heating rate 10^0C /min at the temperature 50^0C – 700^0C.

Figure 4.10: Thermal decomposition of CdSNPs at temperature 50^0C – 700^0C.

In the Figure 4.10, it shows the curve of cadmium sulfide nanoparticles in the temperature of 50^0C to 700^0C. From the graph, the sample of cadmium sulfide nanoparticles was start heating from range 56.37^0C - 691.23^0C. It can be assigned that the weight loss of CdSNPs was start at temperature 56.37^0C. From this graft also, the decomposition of CdSNPs was involved two steps which first one the sample was degraded speedily and the last step the plant was decomposed slowly and for last step at temperature 130^0C the sample was degraded slowly. Initially, 32.8mg of sample of Cadmium sulfide nanoparticles was weighted to study the stability of the synthesized nanoparticle. From the graft, it shows that about 52% of weight loss due to decomposition of the nanoparticles. The total residue that left during the decomposition process is about 48 % (15.79g) from 100 % (32.8g) of initial weight. The weight loss during thermal

decomposition analysis is mainly due to loss of absorbed water on their surface material or organic compound that attach to the CdSNPs.

Figure 4.11: Thermal decomposition of *Sesamum radiatum* at temperature 50^0C – 700^0C

Figure 4.11 shown the thermal decomposition of *Sesamum radiatum* at same temperature of nanoparticles. From the figure, the plant sample started decomposed at two step which is at the first step is 59.05^0C to 352.72^0C and for second step is 353.70^0C – 689.13^0C. Initially, the weight of plant sample was 32.10mg (100%) was used to compare with the thermal stability of cadmium sulfide nanoparticles. From the graft, at the temperature 688^0C, the weight loss of sample is about 70% (22.50mg). From the total weight loss of the sample, the residue that left is about 9.6017mg or 30%. The Figure 4.5 also give information about plant sample was start to decompose at two different temperature which 59.05^0C to 352.72^0C and 353.70^0C to 689.13^0C.

From the Figure 4.10 and 4.11, it can be summarized that the thermal stability of CdSNPs was higher than thermal stability of plant sample. The reason to support that, from the Figure 4.10, there was no weighted loss of CdSNPs after the temperature was increased in between 140^0C to 700^0C. Compare to plant sample, it was decomposed at

two different temperature which is at 59.05^{0}C to 352.72^{0}C at the first step and 353.70^{0}C to 689.13^{0}C for the second step. Besides, the lower weight loss of Cadmium sulfide nanoparticles (52%) compare to *S. radiatum* (70%) also can be assigned that the CdSNPs nanoparticles was more stables compare to plant sample.

4.2.6 (DSC) analysis of Cadmium Sulfide nanoparticles

DSC analysis measures the amount of energy absorbed or released by a sample when it was heated or cooled, providing quantitative and qualitative data on endothermic (heat absorption) and exothermic (heat released) processes. It usually studies to structural response of sample materials by using heat. From these study, Differential scanning calorimetry analysis (DSC) has been done to the CdSNPs and *Sesamum radiatum*. The Differential scanning calorimetry (DSC) of Cadmium sulfide nanoparticles was show in Figure 4.12.

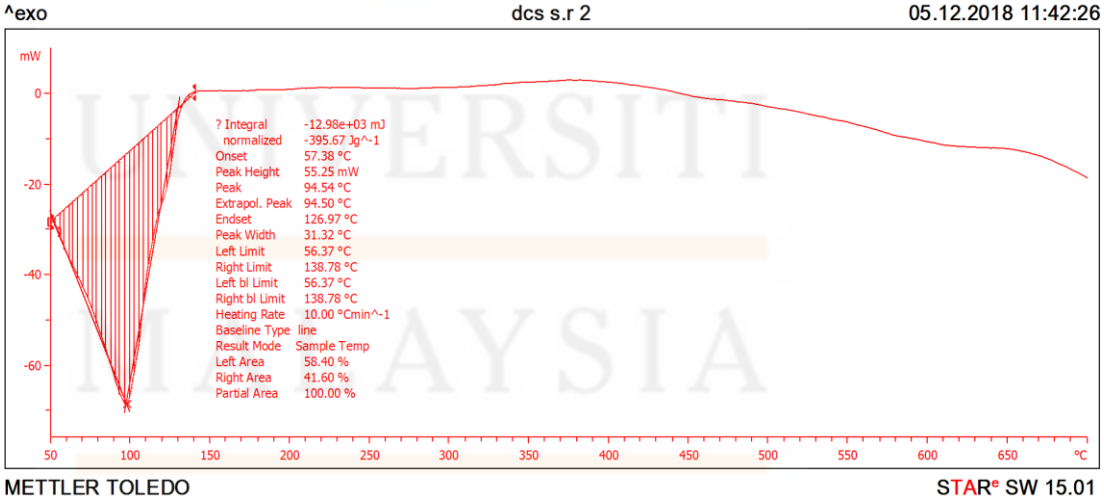

Figure 4.12: Differential scanning calorimetry (DSC) of Cadmium sulfide nanoparticles

From the Figure 4.12, the lower curve of the DSC plot in the Figure 4.6 shows the endotherm which results when the sample was heated at a rate of 10°C/min from 50°C to 140°C in nitrogen gas flowing at a rate of 90ml/min. The temperatures of these transitions are a function of the Cadmium sulfide nanoparticles. The endothermic peaks of CdSNPs at were found 57.38°C (on-set temperatures 57.38°C).

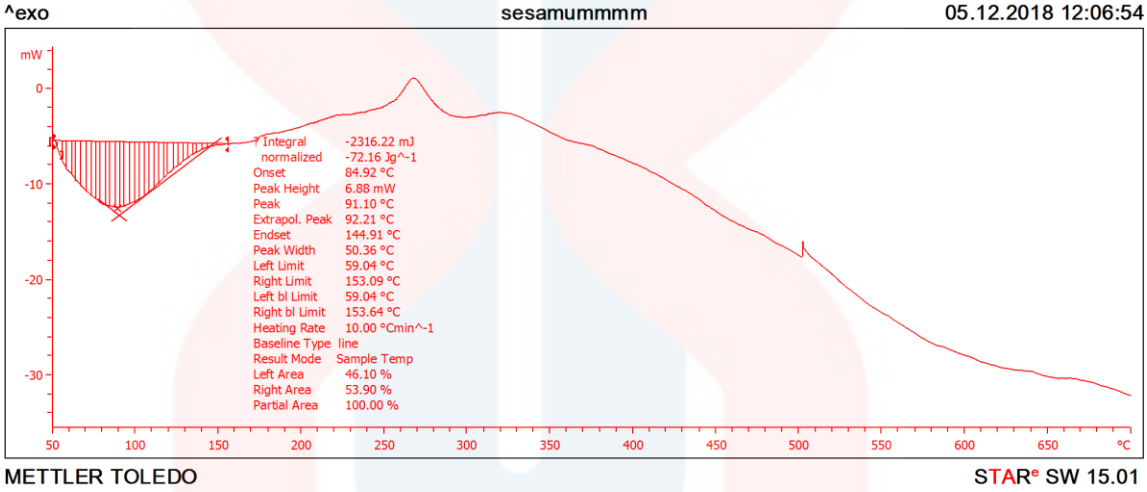

Figure 4.13: Differential scanning calorimetry (DSC) of *Sesamum radiatum*

From the Figure 4.13, DCS thermogram showed recognized endothermic peaks of *S. radiatum* at the temperature 84.92 °C (onset temperature 84.92 °C). From the both figure, the result revealed that CdSNPs was more stable that the raw materials to synthesize to it where CdSNPs endothermic peak shifts to higher value and less energy was released by breaking ionic interaction and during nanoparticles thermal decomposition. Plant sample at Figure 4.13 showed two endothermic peaks while the CdSNPs only showed one peak. Two endothermic peaks of plant materials transform into one revealing the formation of CdSNPs.

4.3 Antibacterial activity of Cadmium Sulfide nanoparticles

The antibacterial activity in this research was conducted to find the minimum inhibition concentration of CdSNPS. Antibacterial activity of synthesized CdSNPs was conducted to observe the capability of the synthesized CdSNPs as an antibacterial agent. Antibacterial activity of Cadmium sulfide nanoparticles was conducted by using environmental and clinically pathogenic bacteria which gram positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*). These antibacterial activities of cadmium sulfide nanoparticles against *S. aureus* and *E. coli* was carried out by using Disc diffusion method (Yadav et al., 2016) and (Sign et al., 2014). The inhibition zone of Multi- Drug Resistance (MDR) bacteria *E. coli* and *S. aureus* were tabulated in Table 4.1 by using disc diffusion method.

Table 4.5: Zone of bacteria inhibition

Bacteria pathogen	**Concentration of Cadmium sulfide nanoparticles mg/ml**							
	Amoxicilin15 mg/ml	Water	Plant extract	10	15	20	25	30
	Zone of inhibition, cm							
E. coli	1.60	0.00	1.00	1.20	1.10	1.20	1.90	1.80
	1.70	0.00	0.90	1.10	1.00	1.30	1.50	1.70
	1.40	0.00	1.10	1.30	1.20	1.40	1.70	1.90
Average	**1.56**	**0.00**	**1.00**	**1.20**	**1.10**	**1.30**	**1.70**	**1.80**
S. aureus	2.10	0.00	1.40	1.40	1.00	1.30	1.50	1.90
	1.80	0.00	1.30	1.30	1.40	1.50	1.80	1.90
	2.00	0.00	1.40	1.30	1.60	1.40	1.80	1.80
Average	**1.97**	**0.00**	**1.36**	**1.33**	**1.33**	**1.40**	**1.70**	**1.86**

Disc diffusion method was a method that easy and flexible to test on antimicrobial activity (Jiang, 2011). These method also are already been use for antibacterial activity of silver nanoparticles -disc diffusion test against *E. coli* (Cunha, et al., 2016), A Study on

the Antibacterial Activity Of Zno Nanoparticles Prepared By Combustion Method against *E. Coli (*Surti, Radha, & Garje, 2013) and Biosynthesis, characterisation and antimicrobial activity of silver and gold nanoparticles by Dolichos biflorus Linn seed extract (Basu, Maji & Ganguly 2016). In the present study, the antibacterial activity of Cadmium sulfide nanoparticle using *Sesamum radiatum* was carry out to find the MIC of antibacterial against bacteria pathogen. Figure 4.4 shown that antibacterial activity of CdSNPs against S. *aureus* and *E. coli* .

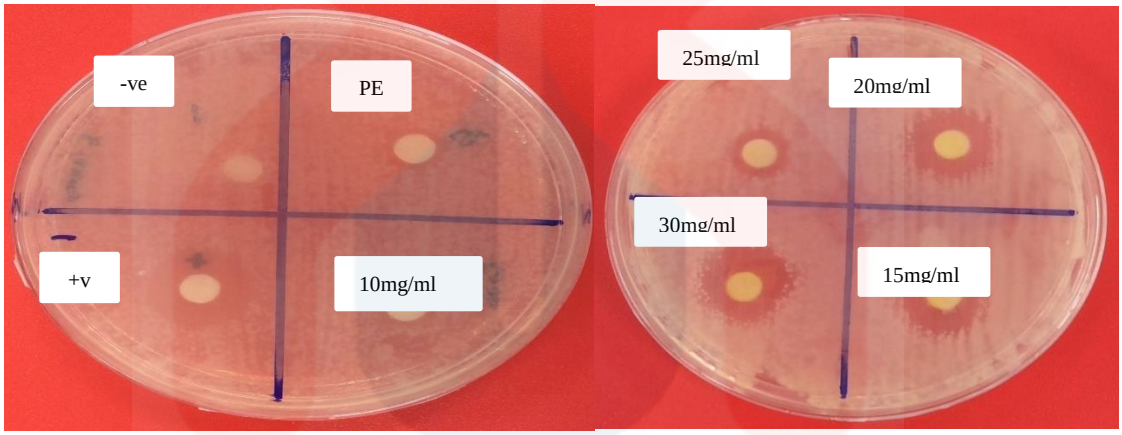

Figure 4.14:Antibacterial activity of cadmium sulfide nanoparticles agaisnt *E. coli.*

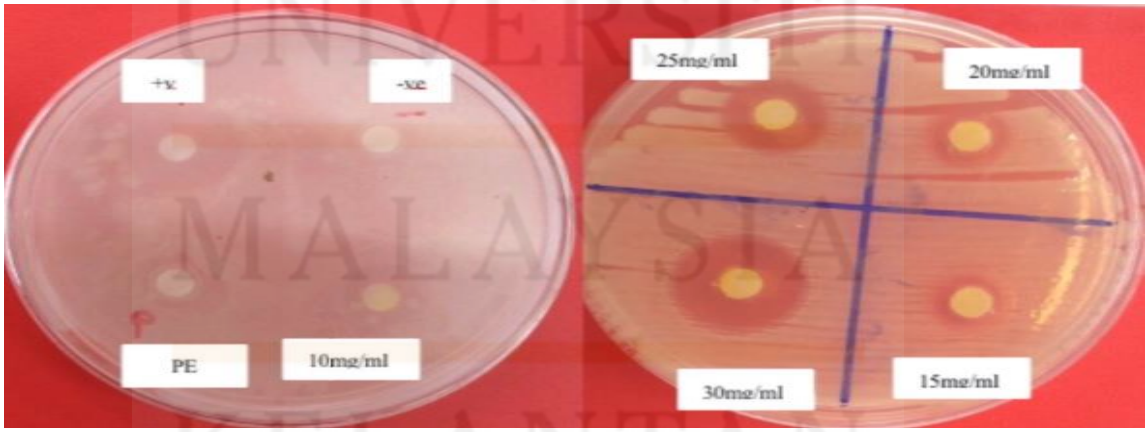

Figure 4.15: Antibacterial activity of cadmium sulfide nanoparticles against *S. aureus*.

The presence of teichoic acids linked to either the peptidoglycan or to the underlying plasma membrane in the gram-positive bacteria and the presence of outer covering of phospholipids and lipopolysaccharides in the gram-negative bacteria and cause negatively charges in both bacteria which attract the positively charges of nanoparticles that cause zone of inhibition was occur. From the result, it indicates that synthesize Cadmium sulfide nanoparticles was effective against gram positive and gram-negative bacteria and the inhibition zone of bacteria was occur due to CdSNPs were positively charges that will attract to negatively charges of microorganisms by electrostatic magnetics and the protein present on the bacteria cell wall.

In this research, *S. radiatum* has shown that antimicrobial properties such as tannins, terpenoids, alkaloids, and flavonoids in this plant extract which can use to inhibit gram positive and gram-negative bacteria. In this experiment, two controls was use which is positive control (amoxicillin) and the negative control (water). The use of positive control in the research is to give a positive result in this experiment while for negative control, it will show a negative result in the result which it can be used to find how effective the Cadmium Sulfide nanoparticles against the both gram positive and gram-negative bacteria. The research was carry out by using different concentration of Cadmium sulfide nanoparticles which are 10mg/ml, 15mg/ml, 20mg/ml,25mg/ml and 30mg/ml against *E. coli* and *S. aureus* and it showed that these nanoparticles has ability to eliminate these resistant bacteria. In this study, it also was found that when the concentration of the nanoparticles was increase, the zone of bacteria inhibition also increases. Figure 4.5 and 4.6 has shown that the highest inhibition zone of both gram positive and gram-positive bacteria is at 30mg/ml followed by 25mg/ml, 20mg/ml,

15mg/ml and lastly 10mg/ml. It also can conclude that the concentration of Cadmium sulfide nanoparticle use on this study is directly proportional to the zone of inhibition of bacteria.

Arakha et al (2015) has reported that the nanoparticles have higher potential to inhibit gram positive bacteria compared to gram negative. In this study, gram positive bacteria, *S. aureus* has shown high inhibition zone (1.33cm, 1.33cm, 1.40cm, 1.70cm and 1.86cm) compare to gram negative bacteria, *E. coli* (1.20cm, 1.10cm, 1.30cm, 1.70cm, and 1.80cm). This is due to the gram-negative bacteria are more resistant rather that gram positive bacteria because gram negative bacteria have outer membrane comprises a complex lipopolysaccharide while the gram-positive bacteria do not contain outer cell membrane layer which make the gram positive bacteria easily to absorbed CdSNPs which act as antibacterial and made it easy to kill. But at the concentration of 25mg/ml of CdSNPs, the zone of inhibition bacteria of *S. aureus* and *E. coli* is same. This error of result due to some mistake during conducting this study. For example, the contaminated of tip micro pipet or the disc was contaminated because it was exposed to the environment.

4.4 DNA cleavage of Cadmium Sulfide nanoparticles.

DNA exist in three main form topological which are supercoiled form (Form I) the circular form (Form II) and linear form(Form III) (Lai et al., 2018). Some pure compound or metal compound introduced in gel electrophoresis will cause a cleavage in DNA band from Form 1 (native plasmid DNA) to Form II (Single stranded, nicked circular plasmid DNA) or from Form II to Form III (linear plasmid DNA). In the present

study, the cleavage ability of cadmium sulfide nanoparticles with pBR322 DNA was observed by using gel electrophoresis. From the Figure 4.16, the result show DNA cleavage activity of cadmium sulfide nanoparticles with pBR322 DNA. The DNA cleavage activity was performed by gel electrophoresis.

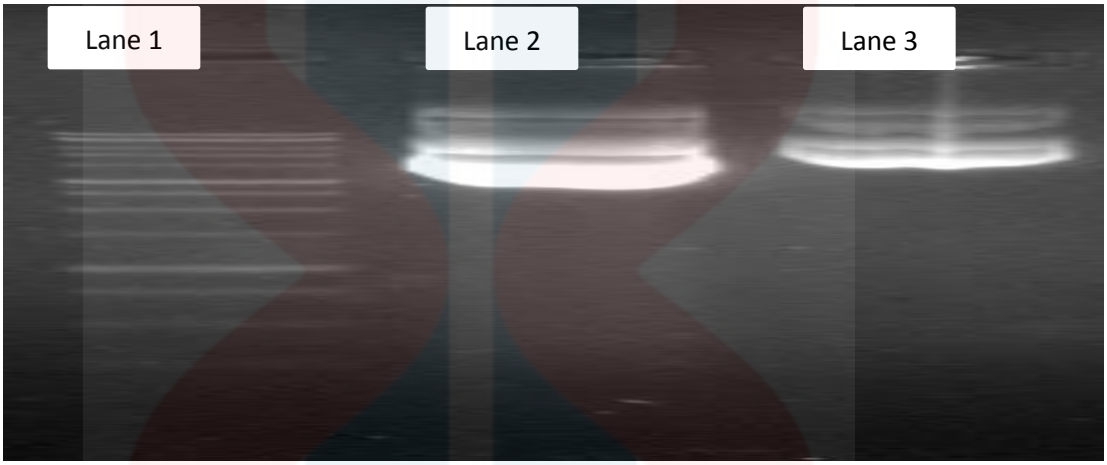

Figure 4.16: DNA cleavage activity of Cadmium sulfide nanoparticles using *Sesamum radiatum*: Lane 1: 2.5 µl DNA ladder + 1.0 µl loading dye, Lane 2: 1 µl pBR322 DNA + 4 µl loading dye, Lane 3: 1 µl pBR322 DNA + 1 µl Cadmium Sulfide nanoparticles + 3 µl loading dye.

From the Figure 4.16, the DNA cleavage activity of Cadmium sulfide nanoparticles was performed at gel electrophoresis at 60 V for 60 minutes. The result was obtained by treating plasmid pBR322 DNA with CdSNPs. From the figure, the gel electrophoresis exhibits the appearance of Form II and Form III before the disappearance of Form I. When the increasing of time taken in the gel electrophoresis, there was the complete removal of Form I. The disappear of band I from the circular plasmid DNA indicates that CdSNPs has strong tendency to cleavage pBR322 DNA in this study. This situation can be indicating that the ability of Cadmium Sulfide nanoparticles to performing complete strand scission: as a consequence which can be used as the therapeutic application because of complete DNA cleavage. It can be assigned that the ability of

Cadmium sulfide nanoparticles from *Sesamum radiatum* can convert the supercoiled DNA to nicked (open circular) form or to linear form. The ability to induce DNA cleavage might be caused by the presence of phenolic and flavoid compound from plant (Gupta, 2011).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this research, CdSNPs were successfully synthesized by using *Sesamum radiatum* leaf extract. The colorless cadmium acetate with plant extract in the beaker is change when Sodium Sulfide is added in the mixture that indicate that Cd^{2+} ions gained with S^{2-} for synthesis CdSNPs. It's also prove that plant extract also can act as stabilizing agent during the formation of cadmium sulfide nanoparticle.

Then, the CdSNPs were characterized by using UV-Vis spectrophotometer, Fourier Transformation infrared (FTIR) and Thermal gravimetric analysis (TGA). From the UV-Vis spectrophotometer, it shown that the synthesized of cadmium sulfide nanoparticles was complete at 6hours of incubation period. The presence of functional groups of amines, aliphatic amines, alkenes, alkynes, aryl, alcohols, carboxylic acid, alkyl halides, aromatics, nitro group, phenol, ester and anhydrides were confirmed by FTIR analysis. From the TGA analysis, the lower weight loss of Cadmium sulfide nanoparticles (52%) compare to *Sesamum radiatum* (70%) also can be conclude that the CdSNPs nanoparticles is more stables compare to plant sample.

Then, the synthesized CdSNPs was used against the multi-drug resistance (MDR) pathogens to identify the minimum concentration inhibition (MIC). In this research, two

strain bacteria *E. coli* and *S. aureus* was used to prove that the synthesize CdSNPs can inhibit the bacteria growth and can replace the use of drug (amoxicillin).

Finally, the ability of Cadmium sulfide nanoparticles to cleave DNA strand of pBR322 DNA has been done to explore the breaks of DNA strand using nanoparticles.

5.2 Recommendation

In this research, it focused on synthesis CdSNPs using *S. radiatum* which is used to study the effectiveness of CdSNPs towards bacteria and study the DNA cleavage of bacteria which study the effectiveness of NPs to denature DNA of bacteria. It is recommended to find the more plant to synthesize CdSNPs since these nanoparticles is more stable and have their advantage compare to another nanoparticle. The uses of different plants may have resulted different characteristics and antimicrobial activity and also DNA cleavage form of bacteria which is can be a kick start for future research in the study of NPs.

It also recommended that to synthesize the nanoparticles with different combination of chemical precursors to find that which chemical is the best to synthesized nanoparticles. And this nanoparticle can be test with some of different bacteria pathogen to find out which bacteria have high inhibition zone when test by the nanoparticles.

Besides that, other recommendation that can be done to improve the study of nanoparticles is to study the photocatalytic activity of CdSNPs by photo-degradation experiment. It is to test whether the synthesized NPs can maintain until the photo degradation cycles.

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APPENDICES A

Table A.1: UV-Vis spectrum

Nm	2hours	4hours	6hours	8hours	10hours
200	0.020	0.073	0.060	0.048	0.065
250	0.067	0.109	0.107	0.097	0.099
300	0.076	0.245	0.263	0.252	0.227
350	0.055	0.147	0.165	0.154	0.137
400	0.049	0.016	0.034	0.019	0.011
450	0.039	0.008	0.024	0.012	0.004
500	0.015	0.003	0.011	0.009	0.003
550	0.011	0.002	0.007	0.008	0.001
600	0.009	0.001	0.007	0.006	0.001
650	0.008	0.001	0.007	0.007	0.002
700	0.006	0.001	0.007	0.007	0.000
750	0.006	0.001	0.007	0.007	0.000
800	0.006	0.001	0.009	0.007	0.001

Table A.2: TGA analysis

Result Mode of Cadmium sulfide nanoparticles		**Result Mode of *Sesamum radiatum***	
°C	mg	°C	mg
56.37	32.79	59.04	32.10
118.65	17.57	121.21	30.28
190.47	17.01	189.82	29.50
261.14	16.97	260.54	25.80
332.58	16.94	331.66	18.21
404.40	16.88	403.13	14.12
476.26	16.82	474.76	11.66
547.90	16.69	546.22	10.74
619.67	16.54	617.62	10.42
691.23	16.25	689.13	10.06

Table A.3: DSC analysis

Result Mode Cadmium sulfide nanoparticles		**Result Mode *Sesamum radiatum***	
°C	mW	°C	mW
56.37	-23.22	59.04	-0.28
118.65	-57.20	121.21	-43.95
190.47	-75.86	189.82	-80.81
261.14	-117.02	260.54	-116.96
332.58	-155.18	331.66	-160.86
404.40	-191.24	403.13	-201.86
476.26	-225.40	474.76	-239.18
547.90	-257.64	546.22	-274.58
619.67	-288.87	617.62	-306.27
691.23	-327.33	689.13	-340.62

Table A.4: Mean and standard deviation

Agent	***E. coli***		***S. aureus***	
	Mean	±SD	Mean	±SD
Amoxicilin	1.56	0.20	1.97	0.22
Water	0.00	0.00	0.00	0.00
Plant extract	1.00	0.08	1.36	0.08
10mg/ml	1.20	0.08	1.33	0.08
15mg/mg	1.10	0.08	1.33	0.43
20mg/ml	1.30	0.08	1.40	0.08
25mg/ml	1.70	0.16	1.70	0.14
30mg/ml	1.80	0.08	1.80	0.13

Table A.5: Final year project planning

FYP I

25th March -25th April 2018	Completing chapter 1,2 and 3
16th April 2018	Submission research proposal
5th July 2018	Submission report FYP I

FYP II

Laboratory work

25th July 2018	Preparation of plant sample
20th August 2018	Synthesized of Cadmium sulfide nanoparticles
17th September 2018	Antibacterial activity of Cadmium Sulfide nanoparticles
25th November 2018	DNA cleavage activity of Cadmium Sulfide nanoparticles

Report writing

30th November 2018	Completing Chapter 4
1st December 2018	Completing Chapter 5
10th December 2018	Submission of Final Draft report

Table A.6: Materials use

Raw materials	Chemical
Sesamum radiatum leaves	Cadmium acetate ($Cd(CH_3CO_2)_2$), Sodium Sulfide(Na_2S), ethanol Amoxicillin, Nutrient Agar and Nutrient Broth powder, Agarose gel 1%,EDTA, TAE buffer solution, pBR322 DNA, 6X loading dye, red safe , ladder

APPENDICES B

Figure B.1: *Sesamum radiatum* powder

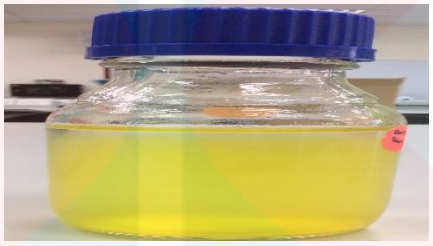

Figure B.2: The synthesized of Cadmium sulfide nanoparticles

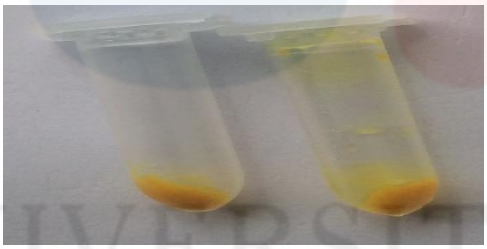

Figure B.3: Cadmium sulfide nanoparticles after centrifuge

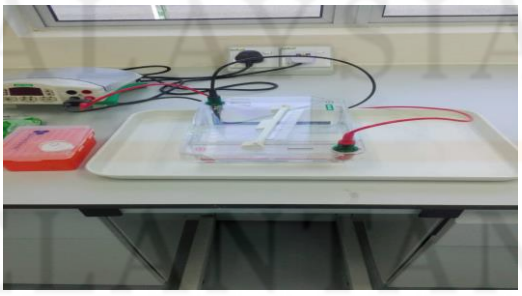

Figure B.4: Agarose and gel electrophoresis