GREEN SYNTHESIS OF GOLD NANOPARTICLES USING NATAL LILY *Crinum moorei* EXTRACT AND BIOLOGICAL ACTIVITIES

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การสังเคราะห์สีเขียวของอนุภาคทองคำระดับนาโนเมตรด้วยสารสกัดจากว่านมหาบัว *Crinum moorei* และฤทธิ์ทางชีวภาพ

น.ส.วรินดา มารุจิวัฒน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Miss Warinda Marujiwat
Field of Study	Chemistry
Thesis Advisor	Professor Nongnuj Muangsin, Ph.D.

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ว่านมหาบัวเป็นสมุนไพรทางการแพทย์ที่มีฤทธิ์ทางยามากมาย เช่น มีส่วนประกอบที่มีฤทธิ์การต้านมะเร็ง และฤทธิ์การต้านการอักเสบ อนุภาคทองคำระดับนาโนเมตร (AuNPs) เป็นตัวนำส่งยาที่มีฤทธิ์ต้านมะเร็งที่น่าสนใจ โดยอนุภาคทองคำระดับนาโนเมตรสามารถแก้ปัญหาต่างๆ ในยาต้านมะเร็งเพราะมีสมบัติที่เข้ากับสิ่งมีชีวิตได้ดี ไม่เป็นพิษต่อเซลล์ และมีขนาดที่เหมาะสมสำหรับการนำส่งสู่เซลล์เป้าหมาย ถึงแม้ว่าแต่เดิมได้มีการใช้ตัว reducing agent ชนิดต่างๆ ในการสังเคราะห์อนุภาคทองคำระดับนาโนเมตร ซึ่งนำไปสู้การปนเปื้อนของสารเคมีบนพื้นผิวของอนุภาคทองคำระดับนาโนเมตร และเกิดข้อจำกัดทางการใช้งานทางด้านการแพทย์หรือมีกระบวนการสังเคราะห์ที่ซับซ้อนและใช้เวลานาน ดังนั้น สารสกัดจากพืชจึงได้รับความสนใจเพื่อที่จะนำมาสังเคราะห์อนุภาคทองคำระดับนาโนเมตร รวมถึงเป็นยาที่มีสารออกฤทธิ์ชีวภาพได้ถึงสองอย่าง ใน ในงานวิจัยนี้ อนุภาคทองคำระดับนาโนเมตรได้ถูกสังเคราะห์โดยใช้สารสกัดจากว่านมหาบัวที่เป็นได้ทั้งตัว reducing และ stabilizing agent ในคราวเดียวกัน โดยอนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้จะถูกนำมาทดสอบฤทธิ์ทางชีวภาพ นอกจากนี้อนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้จะถูกพิสูจน์เอกลักษณ์ด้วย Ultraviolet–visible spectroscopy Fourier-transform infrared spectroscopy Dynamic light scattering และ Transmission electron microscopy จากผลการทดลองของ Ultraviolet–visible spectroscopy พบว่าอนุภาคทองคำระดับนาโนเมตร เกิดสัญญาณของ surface plasmon resonance band (SPR) ที่ความยาวคลื่น 530 นาโนเมตร และรูปผลการทดลองของ TEM พบว่าอนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้มีรูปทรงกลม และมีขนาดอนุภาคส่วนมากในช่วงขนาด 7-9 นาโนเมตร นอกจากนี้อนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้จะถูกนำมาทอสอบฤทธิ์ทางชีวภาพ เช่น การทดสอบฤทธิ์การต้านมะเร็งและการทดสอบฤทธิ์การต้านการอักเสบ

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

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Warinda Marujiwat : GREEN SYNTHESIS OF GOLD NANOPARTICLES USING NATAL LILY *Crinum moorei* EXTRACT AND BIOLOGICAL ACTIVITIES. Advisor: Prof. Nongnuj Muangsin, Ph.D.

Crinum moorei as medical herb has different pharmacological activities such as anti-cancer and anti-inflammatory properties. Gold nanoparticle (AuNP) is an interesting anti-cancer drug carrier which can solve anti-cancer drug's problems with good biocompatibility, non-toxicity and suitable size for the intracellular uptake to treat to the target cells. From the beginning, other reducing agents were used to synthesize AuNPs. However, they lead to the contamination of chemicals on AuNP's surfaces which limits the medical application or long process and time consuming. Therefore, plant extracts were studied to make a remarkable dual function: the synthesized AuNPs agent and drug agent. In this research, AuNPs were prepared by using *Crinum moorei* extract as a reducing and stabilizing agent in one-pot synthesis, and their biological activities were evaluated. The synthesized AuNPs, $HAuCl_4$ and *Crinum moorei* extract was optimized and characterized by Ultraviolet–visible spectroscopy, Fourier-transform infrared spectroscopy, Dynamic light scattering and Transmission electron microscopy. The results from UV-Vis spectroscopy show surface plasmon resonance band (SPR) around 530 nm and TEM image show spherical shape with the particle size 7-9 nm. Furthermore, the synthesized AuNPs were also evaluated their anti-cancer and anti-inflammatory activities.

Field of Study: Chemistry Student's Signature .....

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Warinda Marujiwat

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CHAPTER I
INTRODUCTION

1.1 Research background

Nano drug carriers such as polymeric nanoparticles, iron oxide nanoparticles and metal nanoparticles have been studied and developed to specifically deliver drug to the target cells [2]. One of the most interesting nano drug carrier nowadays is metal nanoparticle, because of its tunable synthesis and modification, moreover, the metal nanoparticle is easy to follow up after treat which is the advantage in medical treatment.

In recent times, gold nanoparticles (AuNPs) are the most outstanding metal nanoparticles with 1 to 100 nm. This size is similar to the biomolecules, such as nucleotides or proteins which can pass through the cancer cell by passive targeting process which taking advantage of the enhanced permeability and retention effect (EPR) [3]. Moreover, gold nanoparticles have suitable size, low toxicity, high drug loading capacity, and uncomplicated synthesis method [4]. There are a few popular methods to synthesize gold nanoparticles, for example, chemical method, physical method and biosynthesis method (Green synthesis). However, the classical chemical and physical methods may lead to the environmental contaminations and damaging of normal cell due to the large amount of hazardous chemical product and impurity product of produced nanomaterials, respectively. The green synthesis uses plant extract as both reducing agent and stabilizer.

Crinum moorei has been known as traditional medical herb and widely distributed in Thailand. Its chemical components in the extract have phytochemical compounds, containing hydroxy and carbonyl group that may have possibility to

reduce Au^{3+} ($HAuCl_4$) to Au^0 (gold nanoparticle), indicating that *Crinum moorei* extract can act as reducing agent. Moreover, Makarov and co-worker found that the sugar in plants extract can also remain the shape of metal nanoparticle, because aldose and ketose sugar can act both as stabilizing agent [5]. For anti-cancer property, Fawole and co-workers found that *Crinum moorei* can be used as anti-inflammatory agent, because *Crinum moorei* extract can inhibit against cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes. These compounds lead the main symptom of inflammatory that may cause cancers. They also found that *Crinum moorei* has good antioxidant activity [6]. From these investigations, *Crinum moorei* will be good alternative agent to use as both reducing agent, anti-cancer drug, and anti-inflammatory drug.

The chemotherapy patient must suffer the side-effects of anti-cancer drug since the anti-cancer drug has disadvantages such as non-specific to target cells. This problem causes the damaging of normal cells. Fortunately, nano drug carriers' advantages such as non-toxicity, suitable size, and their good properties in biological system are the factors to overcome anti-cancer drug's issue. This research focused on synthesis of AuNPs, using *Crinum moorei* extract as a dual-action reducing and stabilizing agent in one-pot synthesis which was characterized by ultraviolet–visible spectroscopy, Fourier-transform infrared spectroscopy, dynamic light scattering and transmission electron microscopy. The synthesized AuNPs were also evaluated anti-cancer and anti-inflammatory activities comparing with traditional drug.

1.2 Research objectives

The objective of this research is to use *Crinum moorei* extract as reducing and stabilizing agent for synthesis of gold nanoparticles which is green synthesis to use as drug carriers. Moreover, the synthesized gold nanoparticles with extract will be applied for testing the anti-inflammatory activity by nitric oxide assay and anti-cancer activity by MTT assay. The details of these research were described as following;

1. To prepare gold nanoparticles by *Crinum moorei* extract
2. To evaluate in vitro biological activities of synthesized gold nanoparticles

1.3 The expected beneficial outcomes

Green synthesis of gold nanoparticles reduced from *Crinum moorei* extract will be obtained.

CHAPTER II

Literature review

2.1 Nanoparticles

Currently, nanoparticles (NPs) has gained many attentions and been a popular technology which be used in many applications of sciences. Nanoparticles is considered the object which has diameter in the range of 1 to 100 nanometers in size with various shapes and elements. Their properties lead to the attractive phenomena such as a higher surface area to the volume, increased reactivity in a chemical process, etc. [7],[8]. The nanoparticles research and application are increasing year by year in many fields, for example, in medicine, manufacturing and materials, environment, energy and electronics [9],[10].

2.1.1 Types of nanoparticles

Nanoparticles can be categorized in different ways, for example, using their material-base, which is categorized in three types [7]. First, an organic-base nanoparticles or polymers are biodegradable and non-toxic such as dendrimers, micelles, liposomes and ferritin, etc. The organic nanoparticles are most applied in the biomedical field because they can be injected into some parts of the body effectively. Second is a carbon-based nanoparticle which completely has carbon as the component in their structure [11]. The nano in size of fullerenes, carbon nano tubes (CNT), graphene, carbon nanofibers and carbon black can be classified in carbon-based nanoparticle. Finally, an inorganic nanoparticle which is the particle that made up of metal and metal oxide components. The metal and metal oxide nanoparticles mostly can be prepared from the metals elements and metal oxide

compounds such as aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), zinc (Zn), iron oxide (Fe_2O_3) and aluminium oxide (Al_2O_3) [7],[12],[13]. These nanoparticles have special properties such 1-100 nm in sizes, high surface area to volume ratio, pore size, surface charge and surface charge density, easy detection by color, high reactivity and sensitivity. However, they can be categorized in widely ways using size, morphology, functionality, and chemical properties as shown in Figure 2.1 [14].

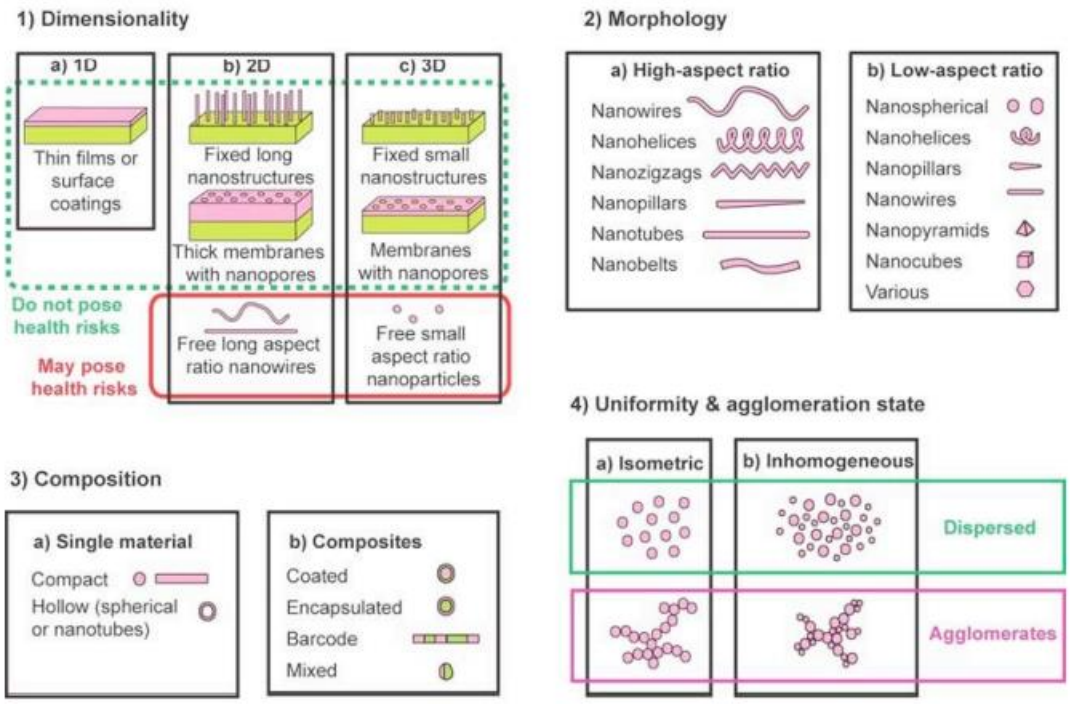

Figure 2. 1 Classification of nanomaterials from 1) dimensions, 2) morphology, 3) composition, 4) uniformity and agglomeration state [13]

2.1.2 Nanoparticles in applications

Nanoparticles can be applied for various industries such as in electricity, food, and medical applications. Here, the interesting applications of nanoparticle are illustrated.

1) Food application

For food applications, nanoparticles are promised to the cultivation, producing, packaging and processing technology, the diversity of these nanoparticles are used as both inert particles, emulsion, and nanocapsules [15]. In 2017, Sorrentino et al. studied the morphology and size of halloysite nanotubes to develop and produce the higher resisted packaging material with low cost [16]. In 2007, Rhin and Ng found that natural biopolymer and silicate can be prepared as the effective nanocomposites. It can improve properties of food packaging, which indicated the huge potential for food industry [17].

2) Medical application and drug delivery

Nanoparticles are used for medical application aids in these days for increasing the efficiency of drug as drug delivery and follow up of diseases. For example, the invention from gold nanoparticles can make gene sequencing less difficult [9]. In 2019, Wang et al. used quantum dots for molecular imaging and tracing of stem cells to control the regulation of proliferation and differentiation of stem cells [18]. Nonsuwan et al. also tried to design selenium nanoparticles as a soft template for the higher anti-cancer drug loading capacity [19]. Also, the work from Luesakul et al.

that prepared pH-responsive SeNPs as nanocarriers for delivery of anti-cancer drug to decrease the problem such as drug-resistant cancer cells [20].

Nowadays, gold nanoparticles are the most impressive noble metal nanoparticle which can be applied for many medical applications [21],[22],[23],[24].

2.2 Gold nanoparticle

Among of all the metal nanoparticles, gold nanoparticles are chosen because they have scale from 1 to 100 nm which are comparable to the size of biomolecules like nucleotides or proteins that can pass through the target cell as shown in Figure 2.2 [25]. Recently, gold nanoparticles are the attractive production to solve drug solubility and specificity to target cells because of their properties such as suitable size to interact with biomolecule, low toxicity and high drug loading capacity [4]

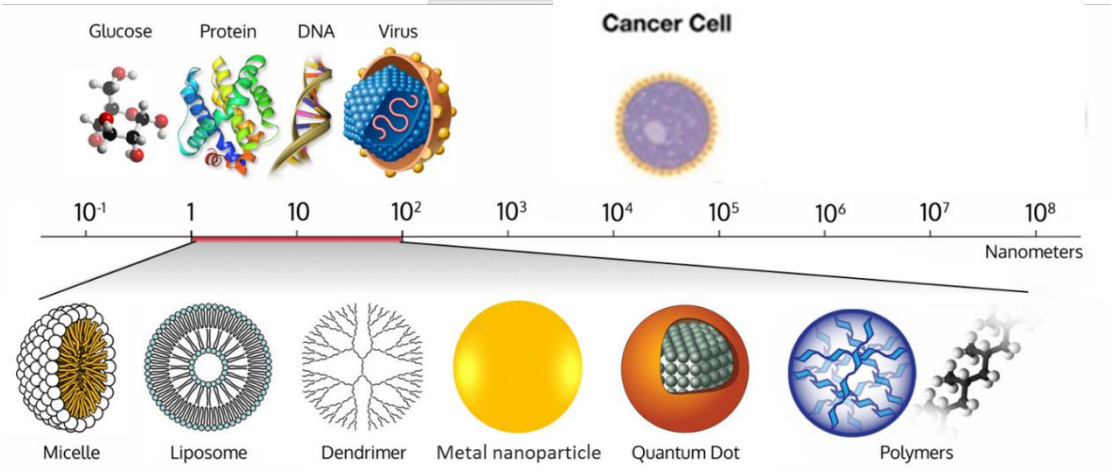

Figure 2. 2 Size scale for nanoparticles (e.g. micelle, liposomes, dendrimers, metal nanoparticle, quantum dots, and polymer) compared to other biomolecules and cancer cells (top) [1]

There are a few ways to synthesize gold nanoparticles known as chemical method, physical method and biosynthesis method or known as green synthesis. However, the classical chemical and physical methods may cause environmental contaminations and damage human normal cell due to the large amount of hazardous chemical product and impurity product of resulting nanomaterials. Thus, green synthesis is an alternative method to reduce the chemical problem that can be harmful to the environment and living organisms [26],[27].

2.2.1 Green synthesis method for gold nanoparticles

During these past years, gold nanoparticles have been used to transform gold ions (Au^{3+} from $HAuCl_4$ precursor) into gold nanoparticles (Au^0) by many biological systems, such as bacteria [28], fungi [29], and plant extract [30],[31],[32],[33],[34]. From the fact that plant extract has various metabolites which is able to reduce gold ions into gold nanoparticles, it was considered as an effective reducing agent that can reduce time consuming and long processing step. For example, Rajagopal et al. successfully optimized the facile, novel and rapid green protocol to synthesize gold nanoparticles by using *Costus pictus* extract as a reducing and capping agent [35]. As mentioned above, plants extract has various plant metabolites, including polytotal phenolic compounds, sugars, and alkaloids. These components play an important role in the bioreduction to produce nanoparticles. Makarov et al. demonstrated the main reducing components in plants extract of metal ions as shown in Figure 2.3 [5], the red highlight on the structure showed the main reducing functional group. Thus, the plant extract will be used to synthesize gold nanoparticles as the green synthesis method.

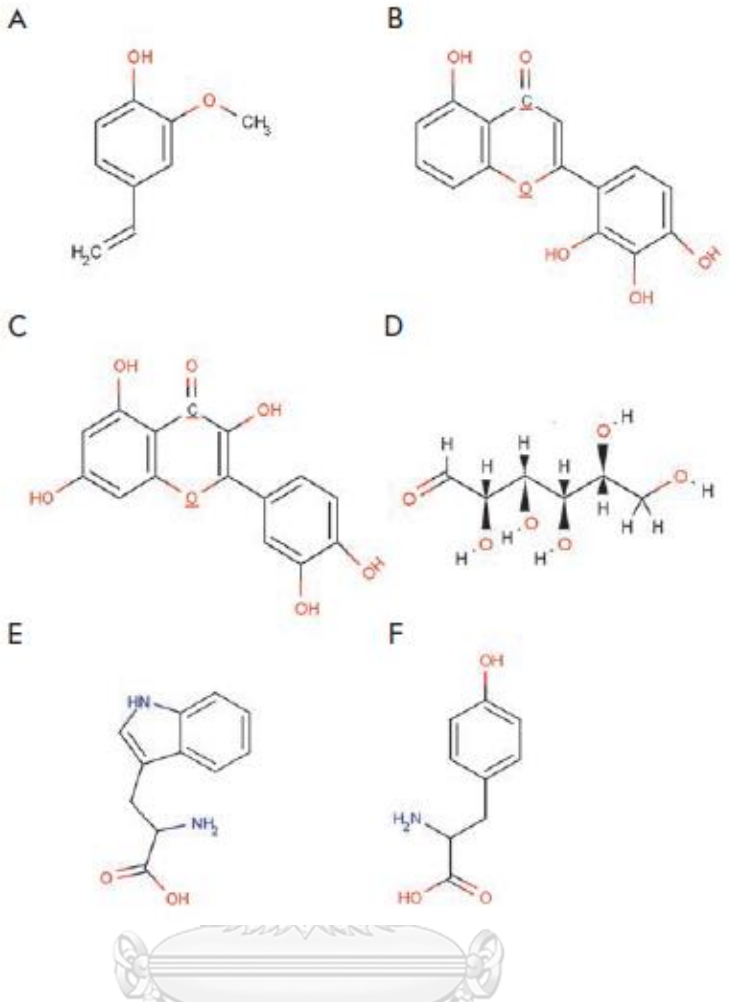

Figure 2. 3 The main reducing components in plants extract A-F [5]

2.3 Crinum moorei

Figure 2. 4 The *Crinum moorei* bulb

Crinum moorei (Figure 2.4) has been known as traditional medical herb and widely distributed in Thailand. Its chemical components in the extract have total phenolic compounds, containing hydroxy and carbonyl group (shown in Figure 2.5) that can reduce Au^{3+} to Au^{0}, that means *Crinum moorei* extract can act as reducing agent. Moreover, Makarov and co-worker found that the sugar in plants extract can also maintain the shape of metal nanoparticle, because aldose and ketose sugar can act both as reducing agent and antioxidants agent [36]. Moreover, Fawole and co-workers found that *Crinum moorei* can be used as anti-inflammatory agent, because *Crinum moorei* extract can inhibit against cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes. These compounds lead to produce the main symptom of inflammatory that may cause cancers. They also found that *Crinum moorei* has good antioxidant activity [33].

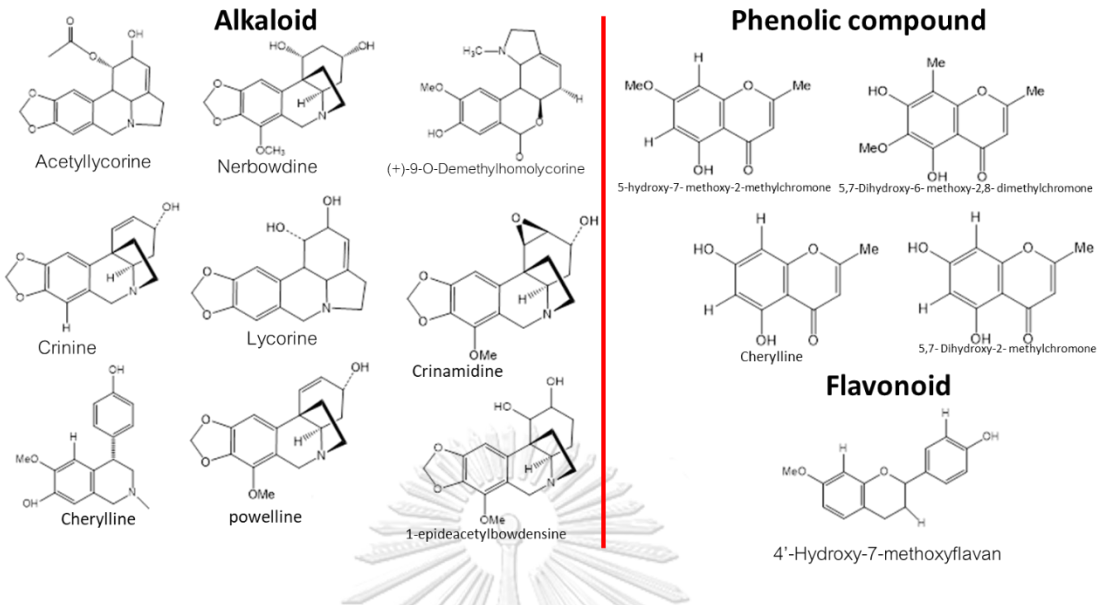

Figure 2. 5 Chemical components found in *Crinum moorei* extract. [5],[37-40]

From these investigations, *Crinum moorei* will be good alternative agent to use as both reducing and stabilizing agent, it also can be a good candidate to u'se as a natural anti-cancer and anti-inflammatory drugs. The proposed green synthesis of AuNPs using *Crinum moorei* is shown in Figure 2.6.

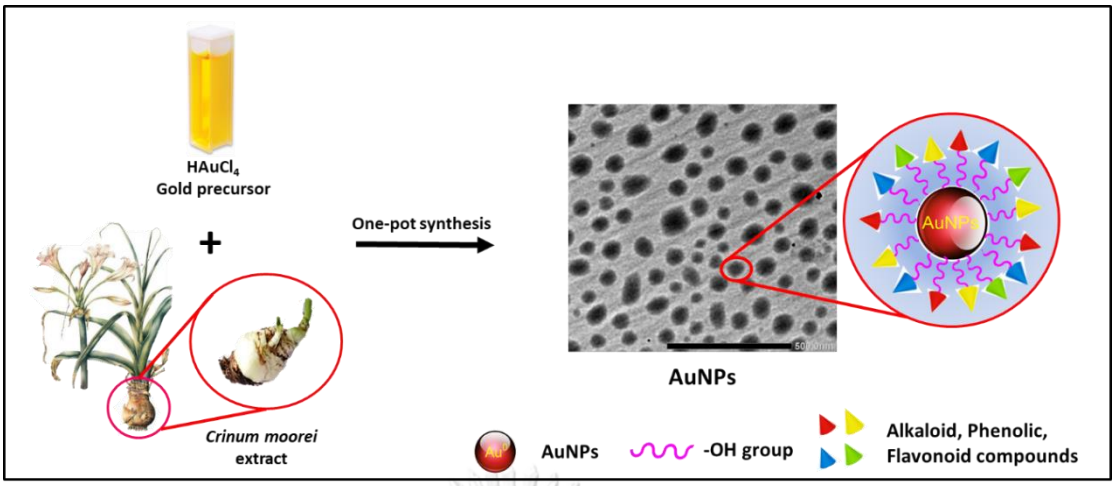

Figure 2. 6 The proposed synthesis of gold nanoparticles using *Crinum moorei* extract.

2.4 The control of the size and morphology of gold nanoparticles

Since, the size and morphology of gold nanoparticles is the key to control their function and application. Thus, the gold nanoparticles must be optimized to gain the right size and morphology for drug delivery. In this study, the nano size with spherical morphology is demanded.

Gold nanoparticles can be synthesized in various shape such as nanorods, nanowires, nanotubes, nanobelts, sphere, stars, pentagons, squares/rectangles, dimpled nanoplates, hexagons, truncated triangles, gold nanotadpoles, gold nanodumbbells (AuNDs), branched AuNPs such as nanopods, nanostars and gold nanodendrites [41]. In the different reaction conditions, including pH, volume of reducing agent, volume of gold precursor, period of the synthesis and temperature are able to change gold nanoparticles size and morphology a shown in Figure 2.7.

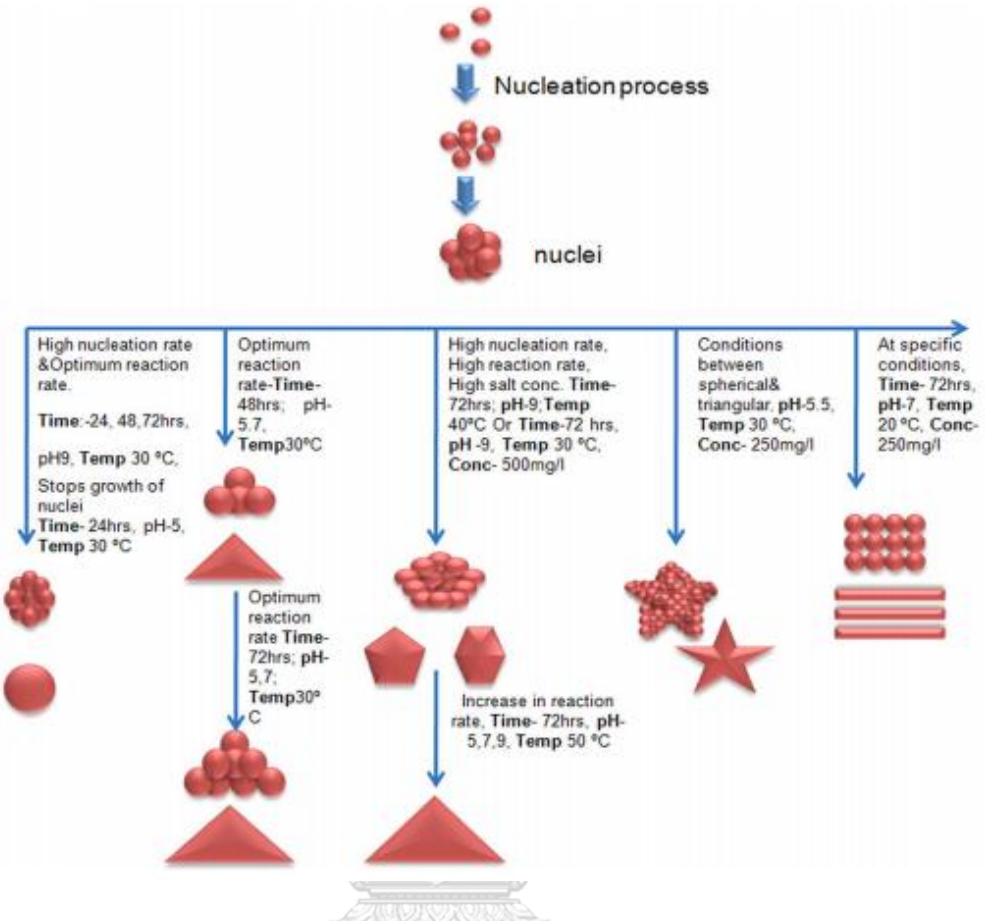

Figure 2. 7 Diagram demonstrating the mechanism of formation of AuNPs in different sizes and morphology under different conditions. [42]

Earlier researches studied about the effect of the concentration of plants extract to size of AuNPs. Das et al. [43] and Song et al. [4] found that the increasing of excess extract (reducing agents), lead to the increasing in particle size. The explanation of these experiment is with the higher concentration of reducing agents, the faster reaction occurs. Therefore, the size of nanoparticles increased.

The pH effect is also important. It can control the oxidation state and reducing power of the reducing agents present in plant extracts. From the previous studies, Armendariz et al. [44] and Sneha et al. [45] showed that the particle size is decreased with the increasing of pH and the tetrahedral, hexagonal, decahedral and rod-shaped nanoparticles occurs at the low pH. Every methodology involves 2 steps for growing gold nanoparticles. First step is nucleation and the second step are crystal growth [46],[47]. The step of gold nanoparticles growth is shown in Figure 2.8. These can be described that at the lower pH, there are repulsions between negative charge of $AuCl_4^-$ ions and the smaller nuclei resulting in larger colloids gold [48]. The reduction of the carboxylic group in plant extract results in uncontrollable nucleation of gold seeds, then the larger formation of shape is collected [44]. They reported that when the pH reached the pH around 9.0, the most of prepared AuNPs consisted of spheres. At the higher pH, Cl^- ions in $AuCl_4^-$ can be substituted by hydroxy groups of free plant extract, leading to the repulsion between the two negative charges of free plant extract and gold ions.

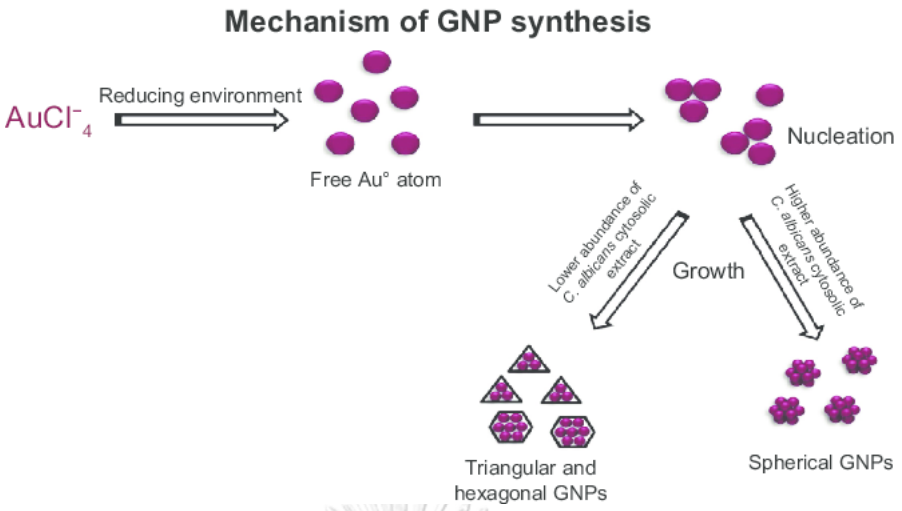

Figure 2. 8 Diagram of gold nanoparticles growth in different sizes and morphology under different conditions by plant extract. [49]

In the previous study of gold precursor ($HAuCl_4$) effect, the smaller particles can be obtained from the lower concentration of gold precursor, compared to the particles formed at the higher concentration. While the higher concentration of gold precursor can react with the free plant extract concentration better, leads to capping and stabilizing action of reducing agents [43], leading to the random collisions and fusion of nuclei to be formed larger nanoparticles.

This research will focus on synthesis of AuNPs, using *Crinum moorei* extract as a dual-action reducing and stabilizing agent in a one-pot synthesis, and their biological evaluation will be also presented. The details of the experiment are described in Chapter 3.

CHAPTER III

MATERIALS AND METHODS

In this chapter all of materials and instruments were described, the gold synthesized methods and biological activities tests are described. The briefly procedures in this study are illustrated in Figure 3.1

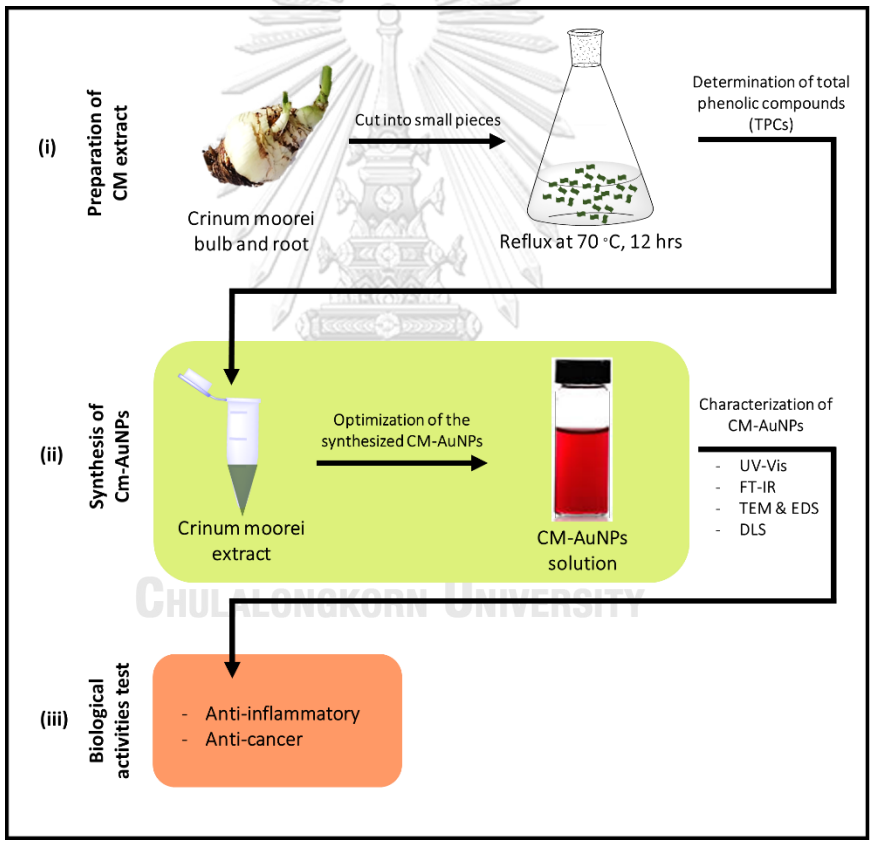

Figure 3. 1 The briefly procedures for the whole experiments of this thesis; (i) Preparation of Crinum moorei extract, (ii) Synthesis of CM-AuNPs from CM extract and (iii) Biological activity tests

3.1 Materials

3.1.1 Crinum moorei

Fresh bulb of *Crinum moorei* (60 g) was obtained from Bangkok, Thailand.

3.1.2 Chemicals

- Methanol (AR grade and purified according to standard procedures)
- Hydrochloric acid (RCI Labscan Limited, Thailand)
- Nitric acid (RCI Labscan Limited, Thailand)
- Sulfuric acid acid (RCI Labscan Limited, Thailand)
- Sodium hydroxide (Merck, Germany)
- Sodium carbonate (Merck, Germany)
- Folin-Ciocalteu reagent (Sigma-Aldrich, USA)
- Garlic acid (Merck, Germany)
- Doxorubicin hydrochloride (DOXs) (Packbuy M&C Co., Ltd., Beijing)
- 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye (Sigma-Aldrich, USA)

3.1.3 Instruments

All the instruments used in this study are listed in Table 3.1.

Table 3. 1 List of the used instrument

Instruments	Model	Company
Ultraviolet–visible spectrometer	HP-8453	Agilent
Fourier-transform infrared spectrometer	Nicolet 6700	Thermo Scientific
Transmission electron microscopy	JEM2001	JEOL
Zeta potential analyzer	S4700	Malvern instrument Ltd.

3.2 Preparation of *Crinum moorei* extract and determination of the phenolic compound

Bulb and root of *Crinum moorei* were cut into small pieces and dried at room temperature. Then, 500 mL methanol was used to extract 70 g of dried *Crinum moorei* by refluxing at 70 ℃ for 12 hours. The extract was filtered through filter paper and concentrated using rotary evaporator at 70 ℃. The crude extract was kept in refrigerator.

The total phenolic compound in *Crinum moorei* extract was determined using the Folin-Ciocalteu method followed by Quy et al [50], with minor modification. The *Crinum moorei* extract was dissolved in deionized water as 5% (w/v) concentration. Calibration curve was expressed as milligrams of gallic acid equivalent per grams *Crinum moorei* extract. Garlic acid (0-25 μg/mL) concentration was used to plot calibration curve. 1.6 mL of 5% (w/v) extract and gallic acid were mixed with 0.2 mL

Folin–Ciocalteu reagent (5-fold diluted with deionized water) and stood for 3 minutes, then followed by adding 2 mL of 10% (w/v) sodium carbonate. The mixture was stood for further 30 minutes and measured at 760 nm.

3.3 Synthesis of gold nanoparticle

The synthesis of AuNPs was carried out by mixing chloroauric acid ($HAuCl_4$) solution with *Crinum moorei* extract (CM) and sodium hydroxide. The 1 mL total volume is diluted by deionized water in this experiment. Mixed solution was stirred and heated for 30 minutes. Then, $HClO_4$ solution was introduced into the mixed solution, the final solution was continuously stirred and heated for 30 minutes.

3.3.1 Effect of *Crinum moorei* extract volume

The synthesized conditions were evaluated at 60 ℃ by using 20 µL of 50 mM $HAuCl_4$, 15 µL of 5% (w/v) sodium hydroxide solution. The volume of 5% (w/v) *Crinum moorei* extract is presented in Table 3.2.

Table 3. 2 The synthesized gold nanoparticles by various volume of 5% (W/V) *Crinum moorei* extract.

Volume of CM (μM)	Phenolic compound (μg/mL)	Volume of $HAuCl_4$ (μL)	Volume of NaOH (μL)
10	383.0	20	15
20	766.0	20	15
30	1,149.0	20	15
40	1,532.0	20	15

Table 3. 3 Total phenolic compound content in each of the synthesized CM-AuNPs by various volume of 5% (W/V) Crinum moorei extract (CM).

Volume of CM (μM)	Phenolic compound (μg/g CM)	Volume of $HAuCl_4$ (μL)	Volume of NaOH (μL)
10	383.0	20	15
20	766.0	20	15
30	1,149.0	20	15
40	1,532.0	20	15
50	1,915.0	20	15

3.3.2 Effect of sodium hydroxide solution volume

The synthesized gold nanoparticles conditions at 60 °C by using 20 µL of 5% (w/v) *Crinum moorei* extract, 20 µL of 50 mM $HAuCl_4$ and various volume of 5% (w/v) NaOH solution such as 0, 15, 30, 45, and 60 µL.

3.3.3 Effect of chloroauric acid solution volume

The synthesized gold nanoparticles conditions at 60 °C by using 20 µL of 5% (w/v) *Crinum moorei* extract, 15 µL of 5% (w/v) NaOH solution and various volume of 50 mM $HAuCl_4$ such as 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µL.

3.3.4 Effect of temperature for synthesis of gold nanoparticles

The synthesized gold nanoparticles conditions used 20 µL of 5% (w/v) *Crinum moorei* extract, 15 µL of 5% (w/v) NaOH solution and 60 µL of 50 mM $HAuCl_4$ solution at various temperature (40-80 °C).

3.4 Characterization of gold nanoparticle

The prepared AuNPs were characterized with Ultraviolet–visible spectroscopy to confirm surface plasmon resonance property (SPR) of AuNPs around 520-550 nm. The AuNPs sample and Crinum moorei extract were dropped and ground with KBr, then analyzed by using a Fourier-transform infrared spectroscopy in the range of 400-4000 cm^{-1} to confirm formation between AuNPs sample and *Crinum moorei* extract. The morphological of the prepared AuNPs were illustrated by transmission electron microscopy equipped with an energy-dispersive X-ray spectrometer and dynamic light scattering.

3.5 Biological activities test

3.5.1 Anti-inflammatory activity

3.5.1.1 Cell culture

Murine Macrophage Leukemia cancer cells (RAW 264.7) were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University. And RAW 264.7 cells were cultured in DMEM which has 4.5 g/L glucose, with sodium pyruvate at 38°C in an incubator. The experiments performed and repeated three times independently.

3.5.1.2 Calibration curve of nitrite standard

Sodium nitrite which is 0.69 mg/ml stock solution was prepared by dissolving sodium nitrite ($NaNO_2$) in deionized water. After that, the stock solution was prepared: 0, 0.5, 1, 2, 5, 10, 20, 40, 60 and 80 µM in triplicate down the 96-well plate (100 µL /well). Then Griess reagent was added 100 µL into diluted solutions and incubated at room temperature for 15 minutes in dark environment. The absorbance was measured at 540 nm by ELISA reader. The calibration curve of nitrite standard was shown in Figure 3.2.

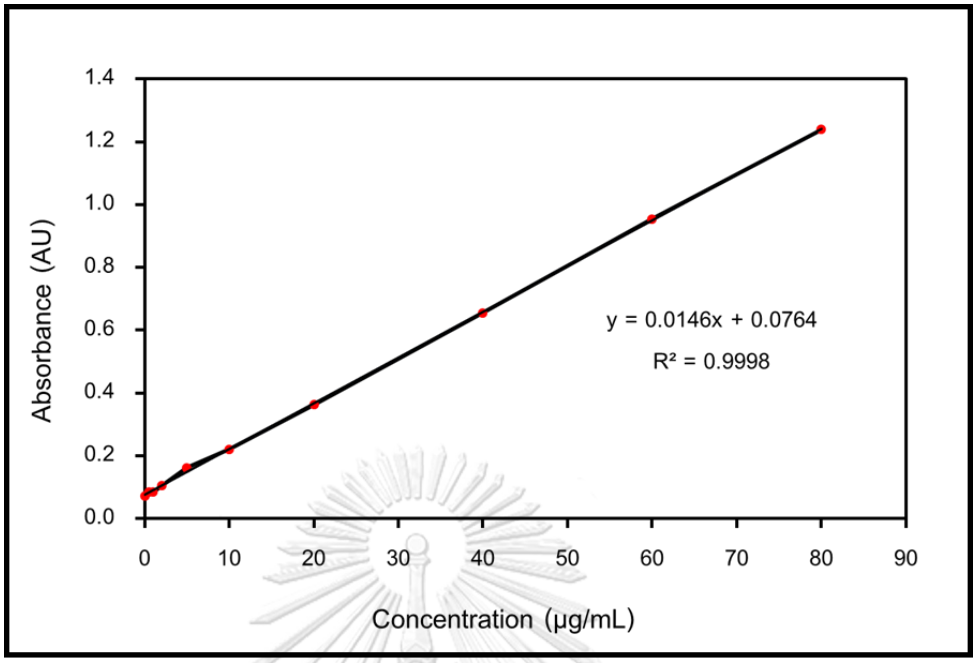

Figure 3. 2 The calibration curve of nitrite standard

3.5.1.3 NO determination

The RAW 264.7 cells were seeded in 96-well plate at a density of 1.44×105 cells/well (8×105 cells/mL) at 180 µL of Dulbecco's modified Eagle's medium (DMEM) and incubated for 24 hours. In the next step, the pre-incubated RAW 264.7 cells with the DMEM medium was removed 10 µL and taken the place of fresh DMEM medium at various concentrations of 0-100 µL. Then, the 96-well plate with RAW 264.7 cells was incubated. Lipopolysaccharide (LPS) 500 µg/mL was added about 2 µg/mL to activate NO production for 24 hours. The 100 µL of supernatant was kept and transferred into new 96-wells plates, then mixed with 100 µL of Griess reagent. Further, the mixture was incubated at room temperature for 15 minutes in dark condition. The absorbance was measured at 540 nm by ELISA reader. In every experiment, the fresh culture medium was used as a blank. The NO inhibitory activity

can be calculated the concentration of nitrite by comparison to calibration curve of sodium nitrite standard.

3.5.2 Anti-cancer activity

3.5.2.1 Cell culture

The all cancer cells were grown in MEM medium supplemented with 10% (v/v) of fetal bovine serum (FCS). For the cultured cell lines were incubated in normal atmosphere of 5% CO_2 atmosphere at 37°C and after that the cultured cell was sub-cultured by trypsin enzyme every 72 hours.

3.5.2.2 Cytotoxicity by MTT assay

Cell viability was evaluated by MTT assays which demonstrate by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), measuring with MTT colorimetric method. The cancer cells were cultured in 96-well plate at a density of 5×10^3 cells/well for 24 hours. Furthermore, the supernatant was removed 20 µL and treated with various concentrations of *Crinum moorei* extract at 0.5-5% of *Crinum moorei* extract for 2 days. After that, the media was incubated with 10 µL of MTT (5 mg/mL) for 4 hours at 37°C. Therefore, the supernatants were removed, the dark violet crystals were dissolved in 150 µL of DMSO and absorbance was measured at 540 nm by ELISA reader. The percentage of dead cells was determined relative to the control group. The data that were collected in triplicates and calculated as following equations

$$\% \text{ Cell viability} = \frac{\text{Absorbance of treated cell}}{\text{Absorbance of untreated cell}} \times 100 \quad \text{.....(1)}$$

3.6 Cellular uptake test

3.6.1 Cell culture

The all cancer cells were grown in MEM medium supplemented with 10% (v/v) of fetal bovine serum (FCS). For the cultured cell lines were incubated in normal atmosphere of 5% CO_2 atmosphere at 37°C and after that the cultured cell was sub-cultured by trypsin enzyme every 72 hours. After treated CM-AuNPs samples, the excess AuNPs were extensive washed with cold PBS solution immediately to be used for digesting in next step.

3.6.2 Cellular uptake test by Inductively coupled plasma spectrometer (ICP)

All cell samples were digested by concentrated aqua regia for ICP measurements, which followed the previously researches [51, 52]. After this digestion step, the concentrated solution samples were diluted to the desired concentration by 1% nitric acid. The known-concentration of gold solution was used as calibration curve to calculate the concentration of CM-AuNPs cellular uptake to cancer cells.

CHAPTER IV

RESULTS AND DISCUSSION

Recently, gold nanoparticles (AuNPs) are the most impressive noble metal nanoparticle which can be applied for many medical applications. Among of all the nanoparticles, gold nanoparticles are chosen because they have the suitable properties such as suitable size to interact with biomolecules, non-toxicity and high drug loading capacity. Furthermore, they can be used as a drug carrier to solve the drug solubility and specificity to target cells by using the enhanced permeability retention (EPR) effect to carry drug into the target cells. In the previous reports, AuNPs were synthesized by many difficult methods, those methods have expensive cost, long process, and use of toxic reducing agents which not suitable for drug carrier application. However, the green synthesis is the key to decrease all the problems, there have been a lot of reports of the green synthesis which is an eco-friendly and fast synthesis (one-pot synthesis) of AuNPs using plant extract as a reducing and stabilizing agent without any toxic reducing reagents. Since, *Crinum moorei* has been known as traditional medical herb and widely distributed in Thailand and its chemical components in the extract have total phenolic compounds, containing hydroxy and carbonyl group that could reduce Au^{3+} to Au^{0}. Therefore, we focus on the development of the synthesis of AuNPs using *Crinum moorei* extract as a dual functional, reducing and stabilizing agent, for drug carrier systems in order to enhance their biological activities such as anti-cancer and anti-

inflammatory and reduce the side effects to normal cell. In this chapter, the results and discussion are presented.

4.1 Determination of the phenolic compound

Total phenolic compounds are components in plant extract. It was expected that it will be able to reduce gold ion (Au^{3+}) form into gold nanoparticles (Au^{0}) form, since its structure contains hydroxyl groups. Moreover, previous study reported that it has medical bioactivity such as anti-inflammatory and antioxidant [35,39]. Thus, the Folin-Ciocalteau method was used to determine the amount of total phenolic compounds in *Crinum moorei* extract. The standard gallic acid calibration curve (Figure 4.1) was used to calculate the total phenolic compounds. The results (Table 4.1) showed that there are 766.0 μg/g CM of total phenolic compounds in the 20 μL of 5% (w/v) *Crinum moorei* extract at the same volume of the synthesized gold nanoparticles. The total phenolic compounds in the synthesized CM-AuNPs are 477.5 μg/g CM. The amount of the total phenolic compounds was reduced from 755.0 to 477.5 μg/g CM, implied that the total phenolic compounds can reduced the Au^{3+} to Au^{0}. This can confirm that total phenolic compounds were used in the process to synthesize gold nanoparticles.

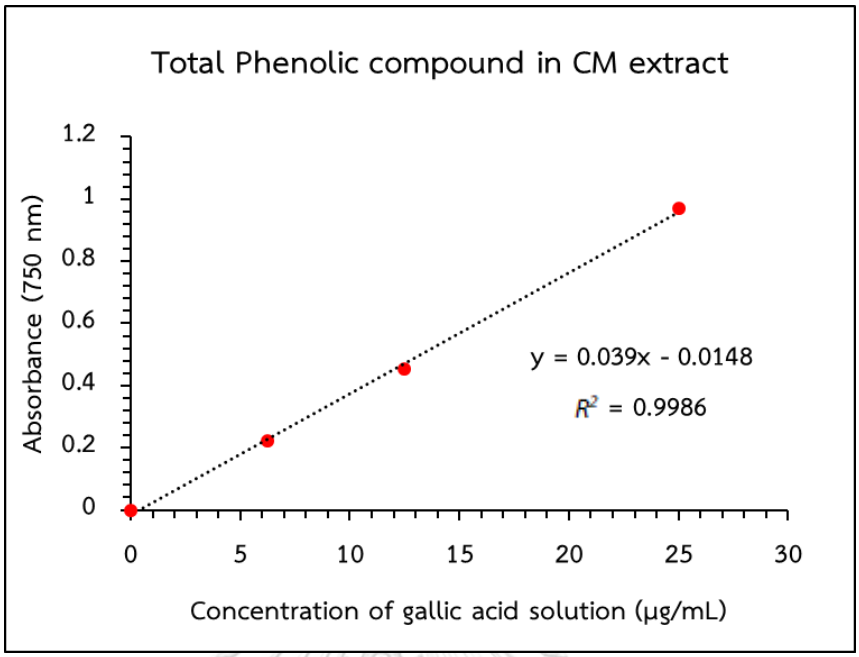

Figure 4. 1 Standard curve of gallic acid against absorbance from Folin-Ciocalteau method [50].

Table 4. 1 The amount of total phenolic compounds in *Crinum moorei* extract and synthesized CM-AuNPs solution

Compound	Phenolic compounds (µg/g CM)
Crinum moorei extract	766.0
CM-AuNPs	477.5

4.2 Synthesis of gold nanoparticle

This experiment focuses on synthesizing the appropriate size and shape of CM-AuNPs which should have scale from 1 to 100 nm, which are comparable to the size of biomolecules that can pass through the target cancer cells. Fortunately, CM-AuNPs can be monitored easily by observing color of the prepared gold solution which will be in red wine color solution (λ_{max} around 500 to 530 nm) [53], this is an characteristic of gold nanoparticle solution with the particle sizes around 5-20 nm. The more purple color shade solution means the bigger gold nanoparticles which called aggregation process, according to Mie theory [54]. This phenomenal of CM-AuNPs can be explained by surface plasmon resonance band (SPR) which not only found in gold nanoparticles but also in the other metals such as silver [55] and selenium [19],[20] nanoparticles. Thus, the optimum conditions of the synthesized CM-AuNPs will be studied including to the effect of (i) *Crinum moorei* extract [4],[43], (ii) sodium hydroxide solution [44],[45],[46],[47], (iii) chloroauric acid solution [43], and (iv) temperature.

4.2.1 Effect of *Crinum moorei* extract

Since the *Crinum moorei extract* contains reducing agents such as the total phenolic compounds, thus the appropriate volume of the extract is necessary to verified. Herein, the picture and UV-Vis spectra of the synthesized CM-AuNPs samples was shown in Figures 4.2 and 4.3, 10 µL *Crinum moorei* extracts condition gave light pink color which implied that there are insufficient of reducing agents to reduce Au^{3+} to Au^{0} nanoparticles and small amount of gold nanoparticles were synthesized at the amount of 10 µL *Crinum moorei* extracts solution.

In contrast of the condition at 30-50 µL *Crinum moorei* extracts gave dark purple, there is more number of phenolic compounds as a reducing agent that can more reduce Au^{3+} in solution to Au^{0} in this solution. Later, the reduced gold nanoparticles can aggregate and form as the bigger particles, suggesting that the stability of gold nanoparticles is reduced, probably due to no stabilizer in this synthesis. From the results, we suggest that the stabilizer should be added to prevent aggregation of gold nanoparticles because only the phenolic compounds and *Crinum moorei* extracts cannot stabilize gold nanoparticles. Thus, the aggregation occurred in the high volume of *Crinum moorei* extracts conditions (The amount of total phenolic compounds in each condition is shown in Table 3.3).

The synthesized CM-AuNPs at 20 µL *Crinum moorei* extracts condition gave a perfect red wine color. In addition, the photo as well as corresponding to UV-vis spectra. The solution of the condition at 20 µL *Crinum moorei* extract appeared the highest peak intensity at around 530 nm and it showed red wine color, this can be indicated more narrow size of gold nanoparticles than the other conditions. Thus, the condition at 20 µL of 5% (w/v) *Crinum moorei* extracts will be used as optimized condition to find optimized sodium hydroxide solution volume in the next section.

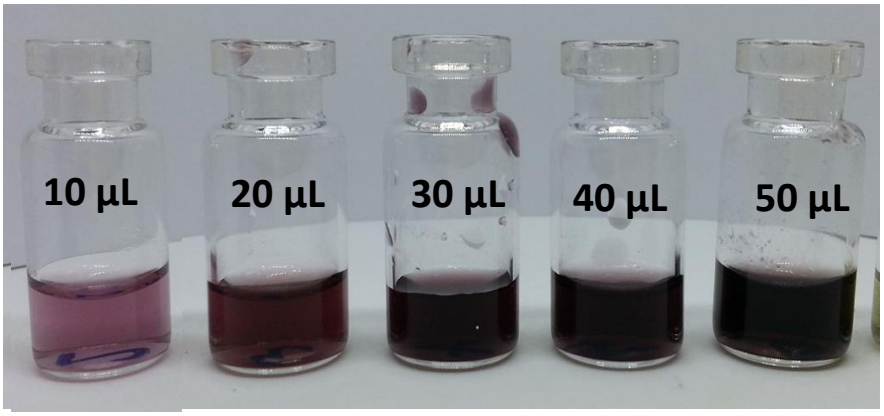

Figure 4. 2 The picture of synthesized CM-AuNPs prepared from solution of 10 to 50 µL of 5% (W/V) *Crinum moorei* extracts.

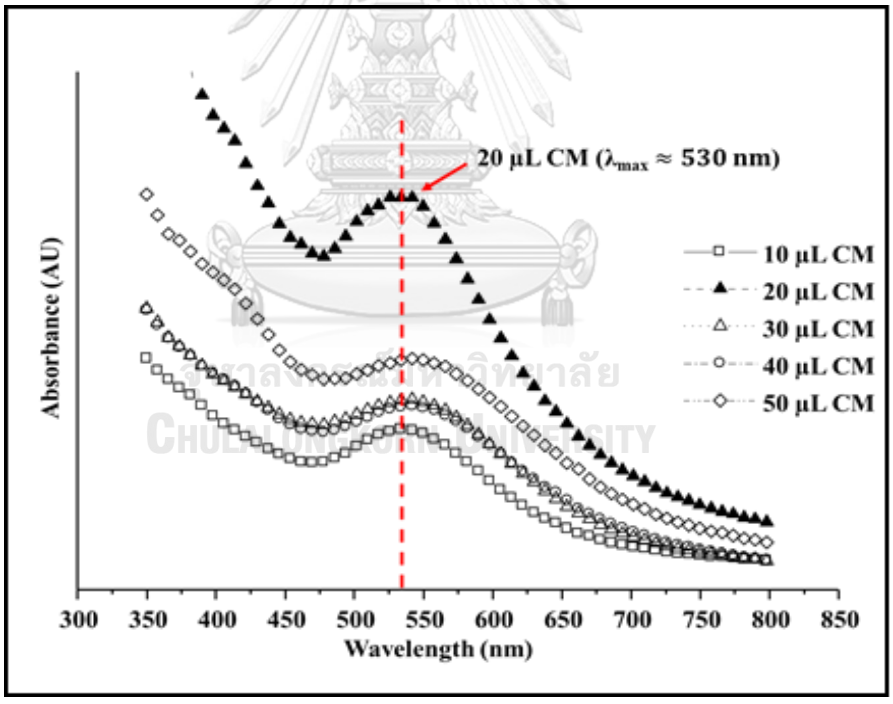

Figure 4. 3 UV-vis spectra of synthesized CM-AuNPs prepared from solution of 10 to 50 µL of 5% (W/V) *Crinum moorei* extracts.

4.2.2 Effect of sodium hydroxide solution

From the study of the *Crinum moorei* extract effect, the condition at 20 µL of 5% (w/v) *Crinum moorei* extracts is fixed as optimized condition. For this section, Sodium hydroxide solution will be studied and find the optimized volume of sodium hydroxide solution. Sodium hydroxide is used as pH controller for the synthesized CM-AuNPs solution, it also helps the polysaccharide or tannin in the extract available for stabilizing AuNPs.

The picture and UV-Vis spectra in Figure 4.4 and 4.5 show the synthesized gold nanoparticles samples. Only the prepared CM-AuNPs which prepared at condition 15 µL of 5% (w/v) NaOH gave red wine color which mean the appropriate size. In the other hand, at 0 µL NaOH gave colorless that mean no CM-AuNPs occur. Since the pH, plays important role in CM-AuNPs synthesis, then the solution pH should appropriate to form CM-AuNPs [56]. Okitsu et al. reported also that the changing of the pH could result (i) the change in redox potential of Au^{3+} ion, (ii) the change in the catalytic activity on surface of gold seed, (iii) the change in reactivity of Au^{3+} on gold seeds, (iv) the shape and size of CM-AuNPs. They suggested that the alkaline solution is the key to control size and shape [40]. Adding NaOH may also help to increase ring opening reaction in *Crinum moorei*'s sugar [57]. This transform sugar can also be the reducing agent. The UV-vis spectra show SPR peak at 530 nm which get along with the picture. Therefore, the appropriate condition to synthesize CM-AuNPs is 20 µL of 5% (w/v) *Crinum moore*i extract, 15 µL of 5% (w/v) NaOH and 20 µL of 50 mM $HAuCl_4$.

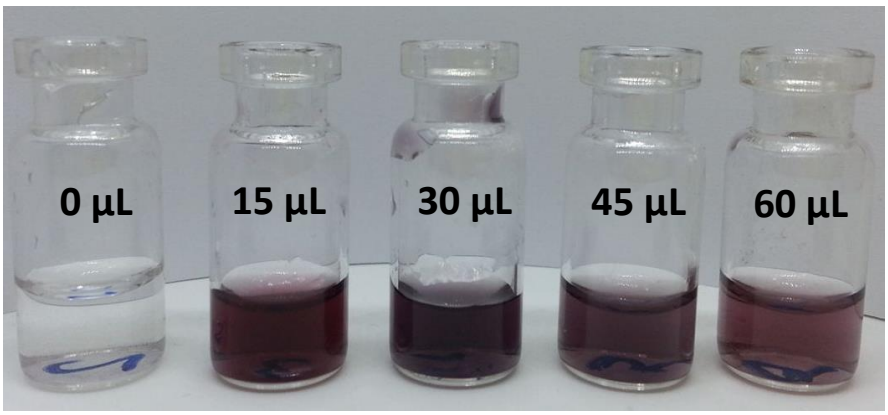

Figure 4. 4 The picture of synthesized CM-AuNPs prepared from 0 to 60 µL of 5% (w/v) sodium hydroxide solution.

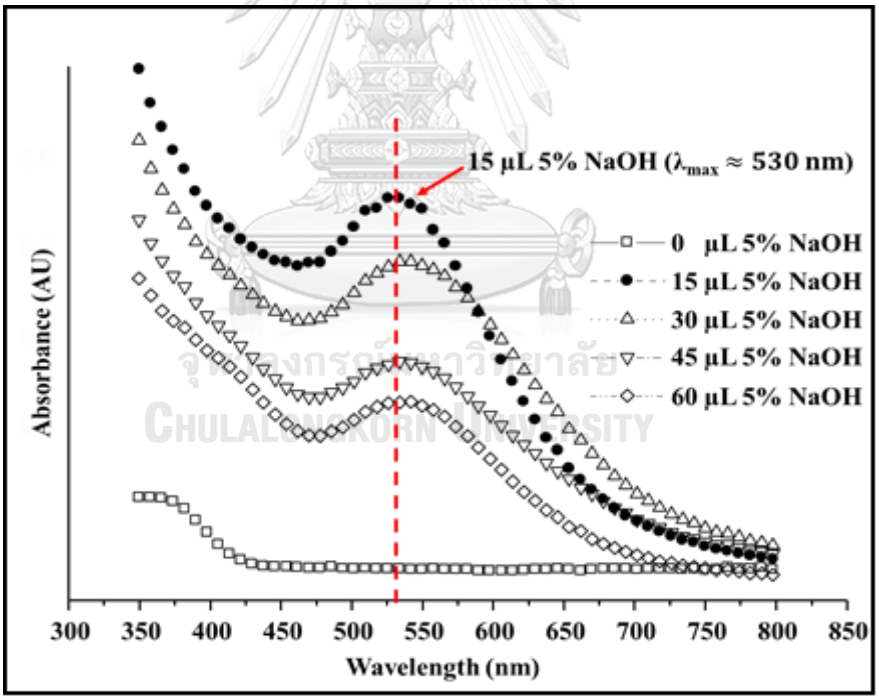

Figure 4. 5 UV-vis spectra of synthesized CM-AuNPs prepared from 0 to 60 µL of 5% (w/v) sodium hydroxide solution.

4.2.3 Effect of chloroauric acid solution

From the Figure 4.6, the picture of synthesized CM-AuNPs showed a good color trend. As increasing the volume of 50 mM chloroauric acid ($HAuCl_4$) solution, the color tended to have a darker red wine shade indicating to the higher number of the appropriate size of CM-AuNPs. However, at high volume (80-100 µL) of $HAuCl_4$ solution, the synthesized CM-AuNPs turns into purple solution which indicating to the aggregation of small CM-AuNPs, especially, the 100 µL of 50 mM $HAuCl_4$ condition which has lighter purple shade solution and a substance precipitated from a solution. These data can be explained that too much amount of $HAuCl_4$ solution causes the aggregation, at the same time too small gold precursor gave low concentration of CM-AuNPs. Although, the condition which used 60 and 70 mM $HAuCl_4$ solutions seem to be the best optimized condition observed by naked eyes, but it cannot be exactly chosen as the most optimized condition. Thus, UV-vis spectra were used to observe the SPR peak. Not only the optimum condition can be identified, but also it can confirm the results that were predicted by the picture. Figure 4.7 shows UV-vis spectra of the condition at 0 µL to 80 mM $HAuCl_4$ solution. The increasing of intensity peak at 525 nm occurred when the volume of gold precursor was increased. Although, the 525 nm peak of the conditions which used 60 and 70 mM $HAuCl_4$ solutions gave almost same intensity. But the condition at 60 µL of 50 mM $HAuCl_4$ solution will be used to find optimized temperature in next experiment, because there is no significant between the intensity of the condition at 60 and 70 µL of 50 mM $HAuCl_4$ solution.

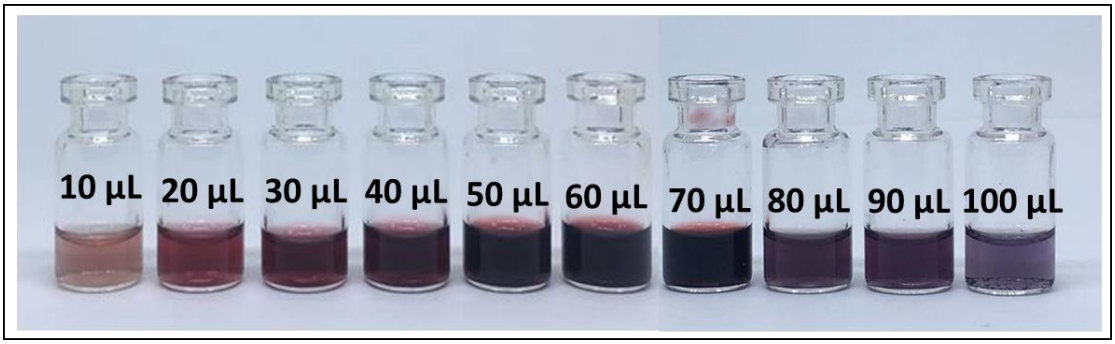

Figure 4. 6 The picture of synthesized CM-AuNPs prepared from 10 to 100 µL of 50 mM chloroauric acid solution

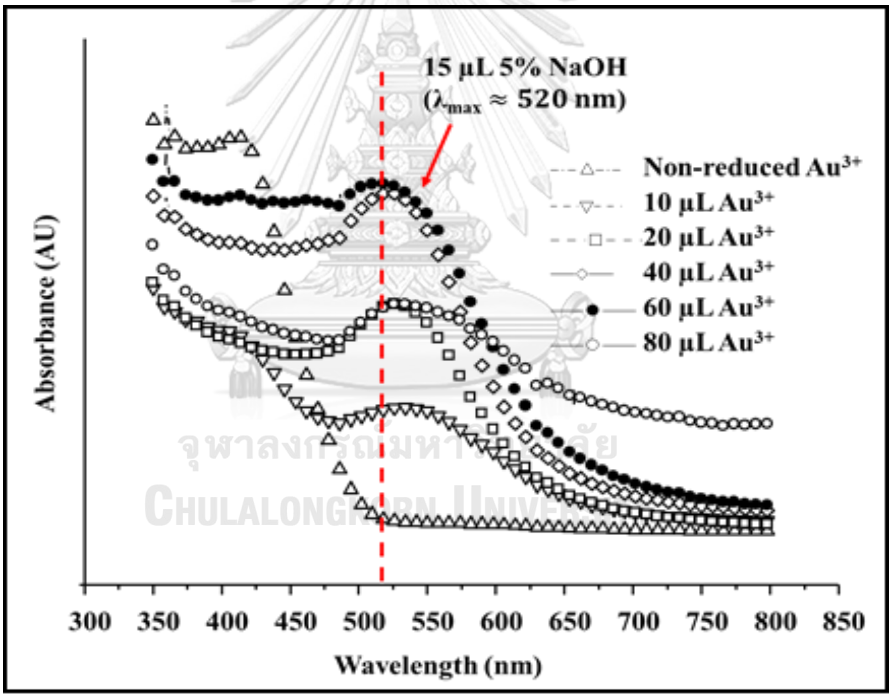

Figure 4. 7 UV-vis spectra of synthesized CM-AuNPs prepared from 0 to 60 µL of chloroauric acid solution.

4.2.4 Effect of temperature

The particle size of gold nanoparticles decreases, when the temperature was increase between 40°C to 80°C and CM-AuNPs clearly increasing productivity with increasing temperature as show in Figure 4.8. Mountrichas et al. confirmed that the higher is temperature reaction, the lower size of CM-AuNPs [57]. They also explained that the smaller CM-AuNPs occur at higher temperature, because at the higher temperature the hydrodynamic radius is higher during the incubation period [58]. UV-vis spectra (Figure 4.9) shows the highest temperature gave the SPR peak around 530 nm. According to the optimization by observing the effect of *Crinum moorei* extract volume, NaOH solution volume and synthesizing temperature, UV-vis spectra and the picture show that the optimized condition is 20 µL of 5% (w/v) *Crinum moorei* extract, 15 µL of 5% (w/v) NaOH and 60 µL of 50 mM $HAuCl_4$ at 80°C.

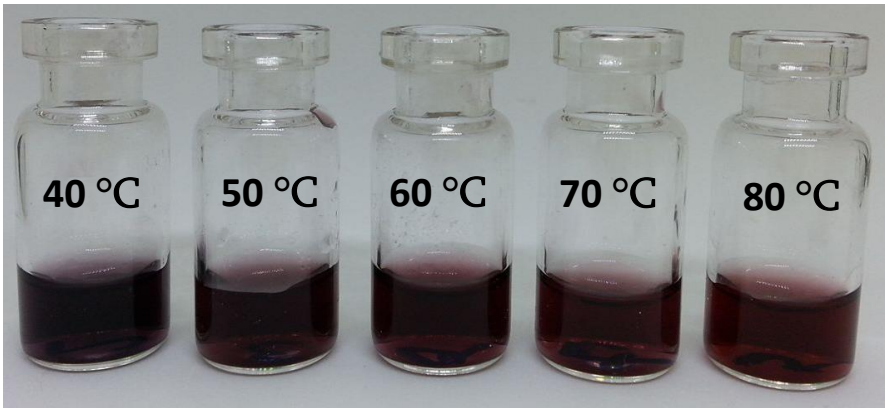

Figure 4. 8 The picture of synthesized CM-AuNPs prepared from temperature between 40°C to 80°C.

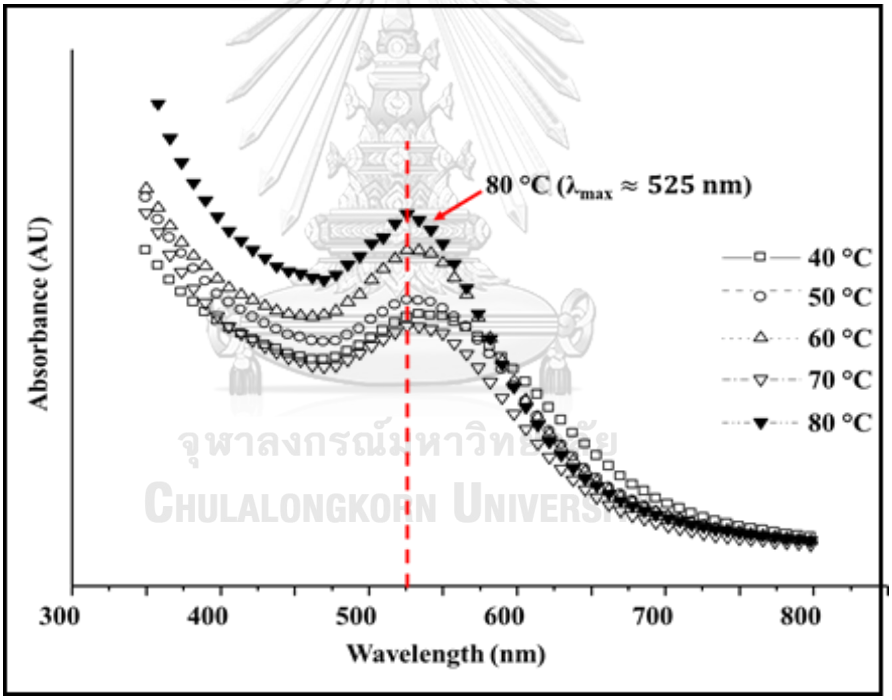

Figure 4. 9 UV-vis spectra of synthesized CM-AuNPs prepared from temperature between 40°C to 80°C.

4.3 Fourier-transform infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy (FT-IR) was used to confirm that the gold ions (Au^{3+}) were reduced by the phenolic compounds as reported previously by the pictures and UV-Vis spectra.

It has been reported that the *Crinum moorei* extract contains total phenolic compounds. FT-IR spectra of *Crinum moorei* extract and the synthesized gold nanoparticles (CM-AuNPs) were illustrated in Figure 4.10. *Crinum moorei* extract (Figure 4.10a) shows the characteristic peaks of hydroxyl (–OH) group (3,413 cm^{-1}) and C–O stretching of primary and secondary alcohol (1,055 cm^{-1}) groups, indicating the present of total phenolic compound in the extract [29],[59]. FT-IR spectrum of CM-AuNPs (Figure 4.10b) shows the different peaks from *Crinum moorei* extract spectrum. The decreased peaks at 3,413 cm^{-1} (–OH group) and 1,055 cm^{-1} (C–O stretching) suggested that –OH groups of the total phenolic compounds in *Crinum moorei* extract decreased. The peak area ratios of C–H stretching (2,900-2,800 cm^{-1}) and –C=O stretching (1,636 cm^{-1}) were assigned as A_1:A_2 and B_1:B_2 for *Crinum moorei* extract and CM-AuNPs, respectively. *Crinum moorei* extract has A_1:A_2 ratio around 1:1, and CM-AuNPs has B_1:B_2 ratio around 1:3, indicating the increasing of –C=O groups. The decreasing of –OH groups in *Crinum moorei* extract and the increasing of –C=O groups in CM-AuNPs, imply that Au^{3+} was reduced to Au^0 by using hydroxyl groups in the total phenolic compounds and they transformed to carbonyl groups as shown in Figure 4.11 [60].

These results corresponding to the total phenolic compounds test in the previous experiment that the total phenolic compounds decreased after the synthesis of CM-AuNPs.

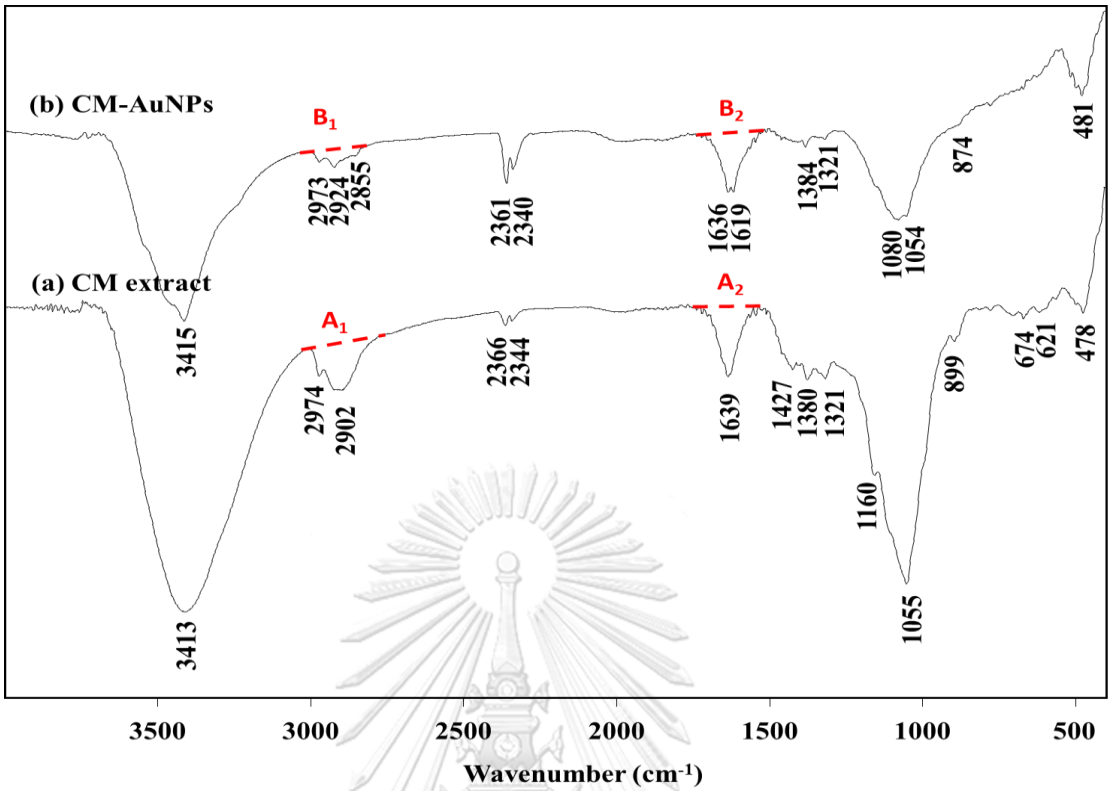

Figure 4. 10 FT-IR spectra of a) *Crinum moorei* extract and b) the synthesized gold nanoparticles (CM-AuNPs) obtained by reaction of 20 µL of 5 %(w/v) *Crinum moorei* extract, 60 µL of 1 mM $HAuCl_4$ solution and 15 µL of 5 %(w/v) NaOH.

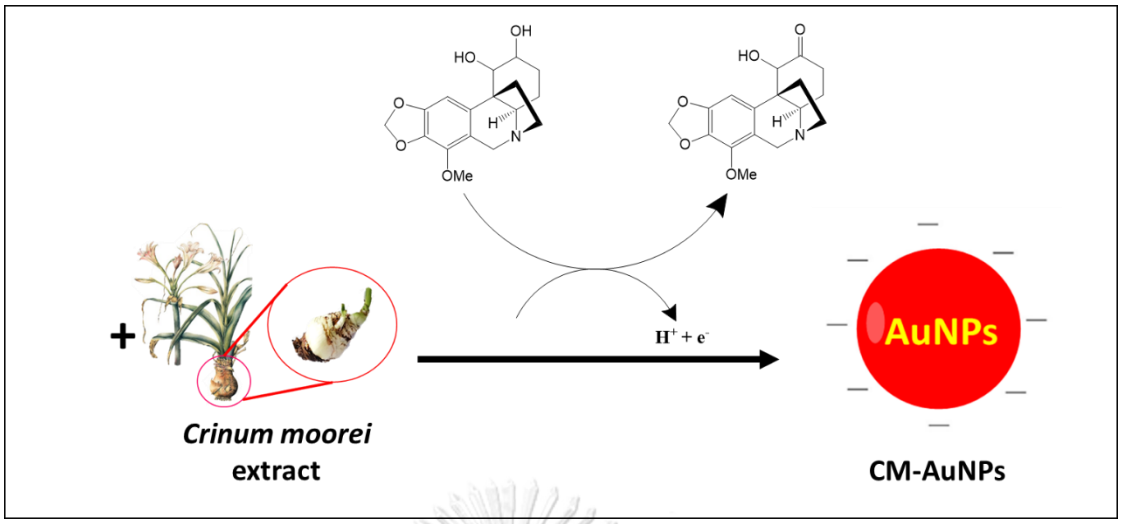

Figure 4. 11 Proposed mechanistic pathways for synthesized CM-AuNPs [58]

4.4 Transmission electron microscopy (TEM) and Energy Dispersive X-ray spectroscopy (EDX)

This section, Transmission electron microscopy (TEM) was used to confirm size and shape of the synthesized CM-AuNPs. The expected size of AuNPs is around 1-20 nm, the spherical in shape for AuNPs can enhance the drug loading capacity for nano drug carrier [61]. Energy Dispersive X-ray spectroscopy (EDX) is able to confirm the particle component and to ensure the existence of gold element.

Figure 4.12a and Figure 4.12b showed TEM images under different magnifications of synthesized CM-AuNPs which has mostly same shapes and sizes of particles. The grey area around black center of CM-AuNPs which suggesting CM-AuNPs were surrounded by phytochemicals and polysaccharides from *Crinum moorei* extract. The detected particles had spherical in shapes. The most of population of synthesized CM-AuNPs is around 7-8 nm which showed in the high narrow histogram (Figure 4.12c) of size distribution in Figure 4.12b. The samples exhibit similar symmetric distribution curves with a very small slow decaying tails towards large particle

diameters, it still shows a good condition of size which is lower than the expectation size (20 nm) that can be allowed to pass through cells as a good drug carrier. Moreover, these curve shapes are similar to those obtained in the UV-Vis part as showed in the previous result and the EDX spectrum (Figure 4.13) could confirm the existing of gold nanoparticles by showing Au peaks. Therefore, it could confirm that the black sphere particles and gray area in TEM image is gold nanoparticles that surrounded by phytochemical compounds from *Crinum moorei* from carbon, nitrogen, and oxygen peak of the EDX spectrum. that the phytochemicals of *Crinum moorei* is able to act as stabilizer for gold nanoparticles.

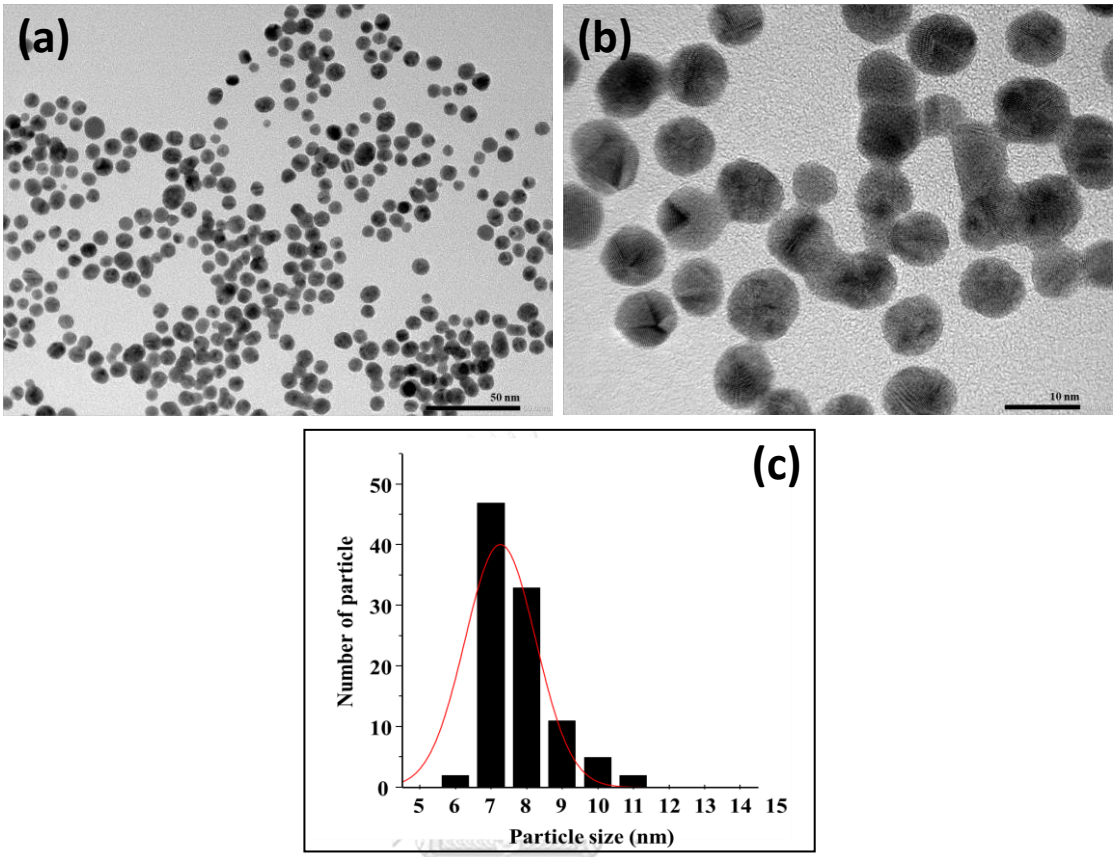

Figure 4. 12 TEM images under different magnifications (a,b) and histogram (c) captured of synthesized gold nanoparticles obtained by reaction of 20 µL of 5 %(w/v) *Crinum moorei* extract, 60 µL of 1mM $HAuCl_4$ solution and 15 µL of 5 %(w/v) NaOH.

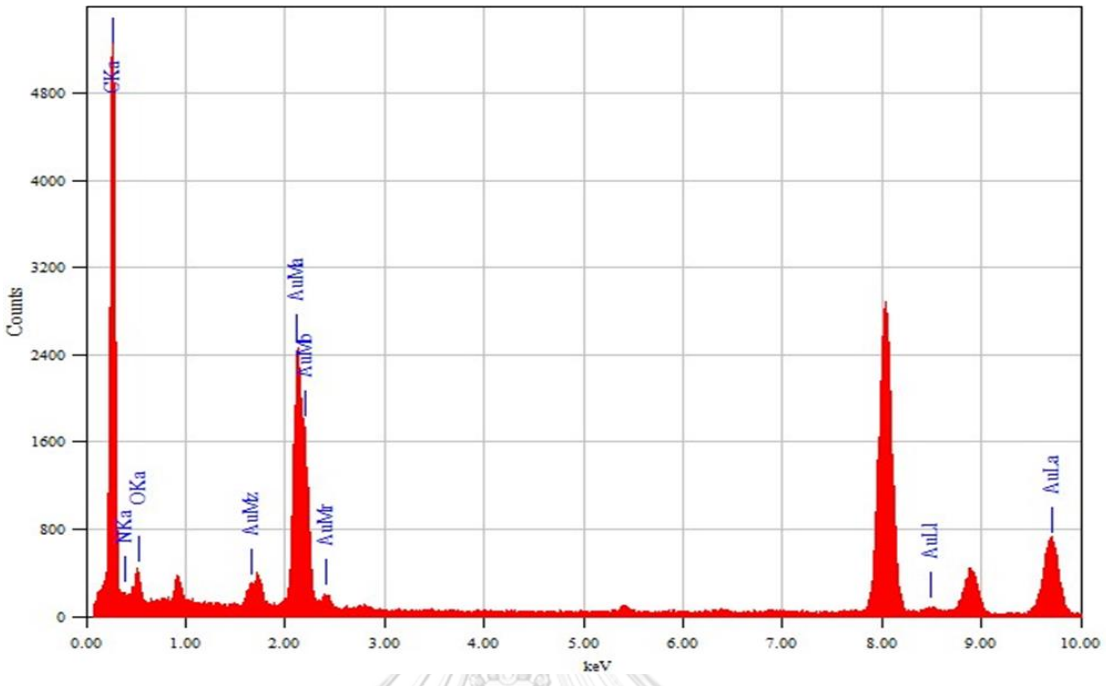

Figure 4. 13 EDX spectrum of synthesized gold nanoparticles obtained by reaction of 20 µL of 5 %(w/v) *Crinum moorei* extract, 60 µL of 1mM $HAuCl_4$ solution and 15 µL of 5 %(w/v) NaOH.

4.5 Dynamic Light Scattering (DLS)

Size distribution (Figure 4.14) of synthesized CM-AuNPs from DLS particle size analysis can be used to confirm TEM result. Dynamic Light Scattering (DLS) was used to measure the particle size of the synthesized CM-AuNPs in the solution, which has similar size to the TEM images. The average size of synthesized gold nanoparticles from DLS is around 16.47 nm with 0.306 PDI, comparing the size between TEM, it has similar size to TEM images (Figure 4.11) which had particle size around 7-8 nm. Due to CM-AuNPs samples from DLS were prepared in solution, while the CM-AuNPs sample from TEM were prepared in dry condition, The sizes were slightly changed.

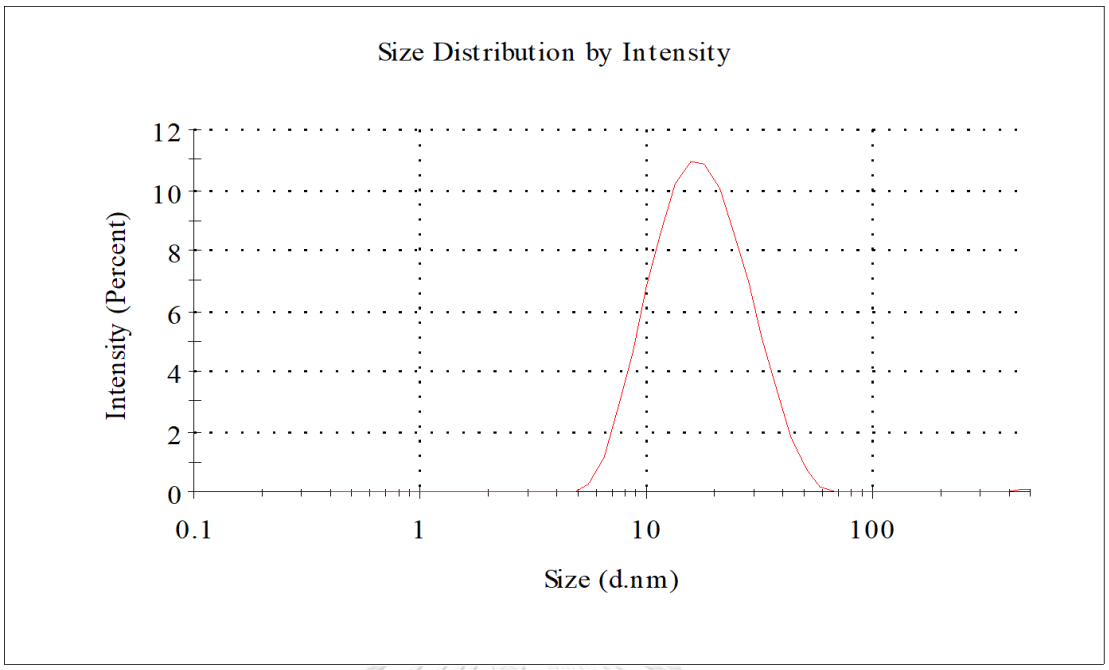

Figure 4. 14 DLS particle size analysis of synthesized gold nanoparticles obtained by reaction of 20 µL of 5 %(w/v) *Crinum moorei* extract, 60 µL of 1mM $HAuCl_4$ solution and 15 µL of 5 %(w/v) NaOH.

In the synthesis and characterization parts, the best condition which was selected by observing the effect of *Crinum moorei* extract volume, NaOH solution volume and synthesizing temperature to synthesized CM-AuNPs is 20 µL of 5% (w/v) *Crinum moorei* extract, 15 µL of 5% (w/v) NaOH and 60 µL of 50 mM $HAuCl_4$ at 80°C. The characterized results show that CM-AuNPs have a suitable size (within 1-100 nm) and shape (sphere) both in dry and wet condition to be used as drug carrier. In the next section, CM-AuNPs will be tested their biological activities such as anti-inflammatory and anti-cancer.

4.6 Biological activities

Because *Crinum moorei* is used as traditional medical herb and its chemical components in the extract which were reported that it has efficiency to be antioxidant and anti-inflammatory agent [35,39] Therefore, *Crinum moorei* will be used not only the reducing and stabilizing agent, but also the anti-cancer and anti-inflammatory drug to reduce the side effects to normal cell comparing to the commercial drug such as doxorubicin hydrochloride (DOXs).

4.6.1 Anti-cancer activity

The synthesized CM-AuNPs was used to evaluate the selectivity to cancer cell. The preliminary screen of cervical cancer cells (KB cell line), colon cancer cells (SW620) and human mouth squamous cell carcinoma cell (CLS-354 cell lines) were tested by used Crinum *moorei* extract to find the most inhibited cell line by 1 to 1000 µg/mL extract.

In this result, the trends of percent cell viability of cancer cell lines were shown in Figure 4.15 to evaluate potential of *Crinum moorei* extract to kill cancer cell and find the most specific cell line for *Crinum moorei* extract. The results show that *Crinum moorei* extract is the most inhibit to KB cell line, comparing to SW-620 and CLS-354 cell lines. The sharp decreasing of cell viability in KB cell is the reason to be pick and tested with CM-AuNPs. Therefore, in the end of this section, KB cell line will be chosen to test cytotoxicity test compare with Wi-38 cell line (normal cell) with the synthesized gold nanoparticle.

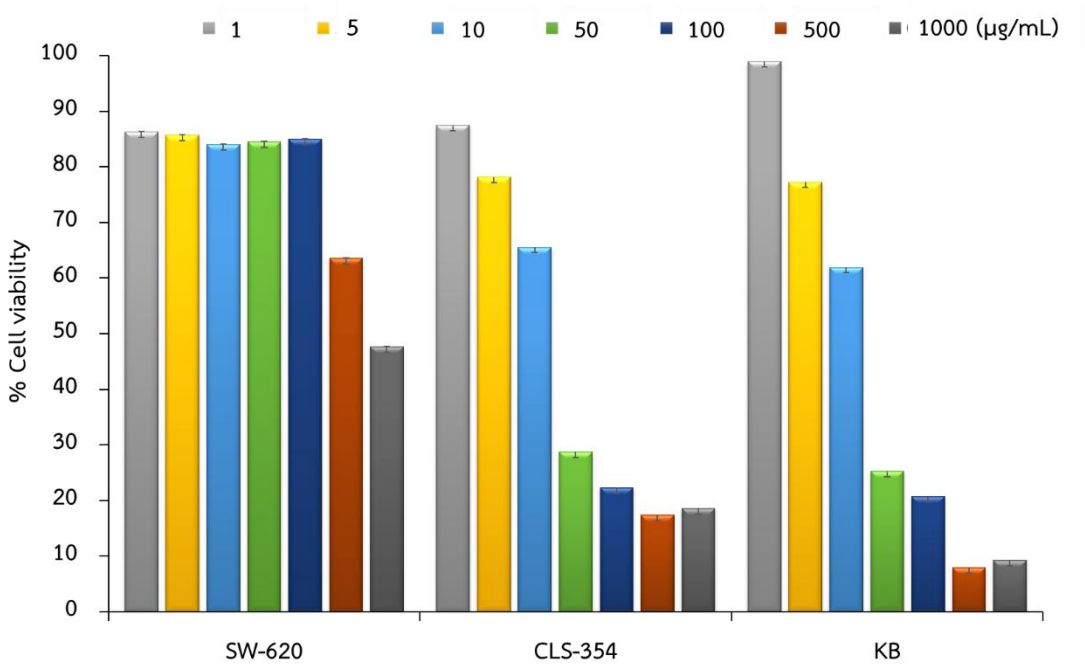

Figure 4. 15 The preliminary percent cell viability screen of SW-620, CLS-354 and KB cell lines treated with 1 to 1000 µg/mL *Crinum moorei* extracts.

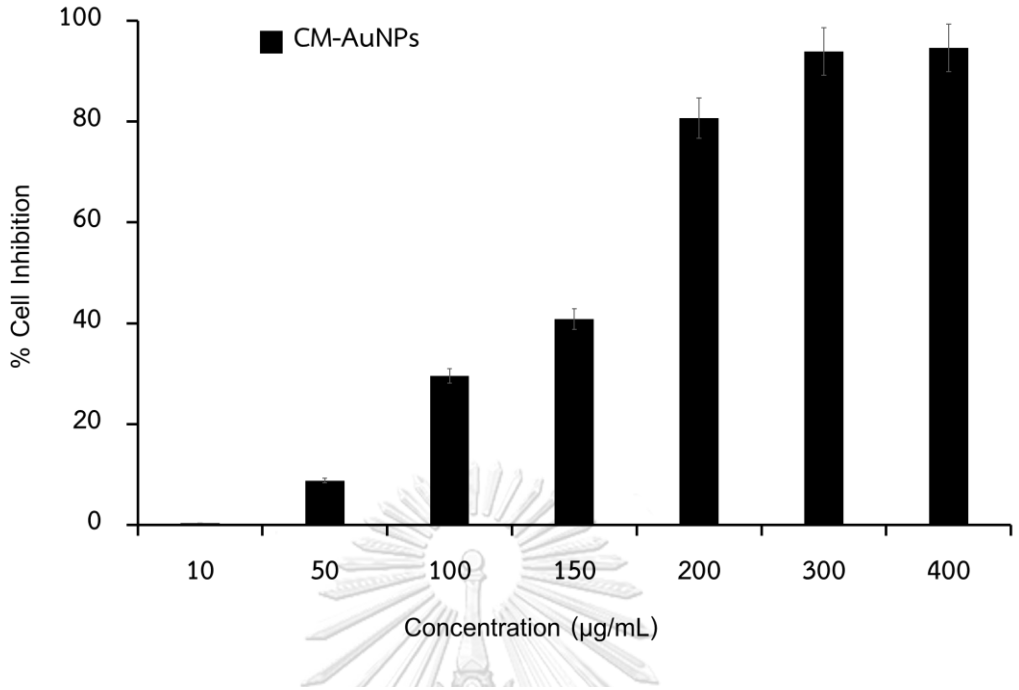

Figure 4. 16 Comparison of percentage inhibition of extract concentration contains in CM-AuNPs in KB cells at different amount of concentrations. Values were derived from the graph of growth inhibition against extract concentration from MTT assay.

Figure 4.16 showed the comparison of percentage inhibition of extract concentration contains in CM-AuNPs vs cell inhibition of KB cells. The curve was used to calculate IC_{50} of CM-AuNPs, the result of calculating IC_{50} is 142.8 µg/mL. To indicate the efficiency of CM-AuNPs and *Crinum moorei* extract on KB cell, Figure 4.17 was used. For KB cells with the same concentrations of CM-AuNPs and *Crinum moorei* extract, both of them can completely inhibit cell growth. However, the inhibitory efficiency of *Crinum moorei* extract is higher than CM-AuNPs. This can be explained that the active functional groups in *Crinum moorei* extract is already used while reducing Au^{3+} which corresponding to the total phenolic compounds test and FT-IR spectra as illustrating in Figure 4.19. Although, the inhibition efficiency of CM-

AuNPs is lower from *Crinum moorei* extract, but the inhibition efficiency from Figure 4.18 showed no significant toxicity to normal cell (Wi-38 cell line). From these results, it can conclude that CM-AuNPs is be able to use as drug carrier to avoid the toxicity to normal cell.

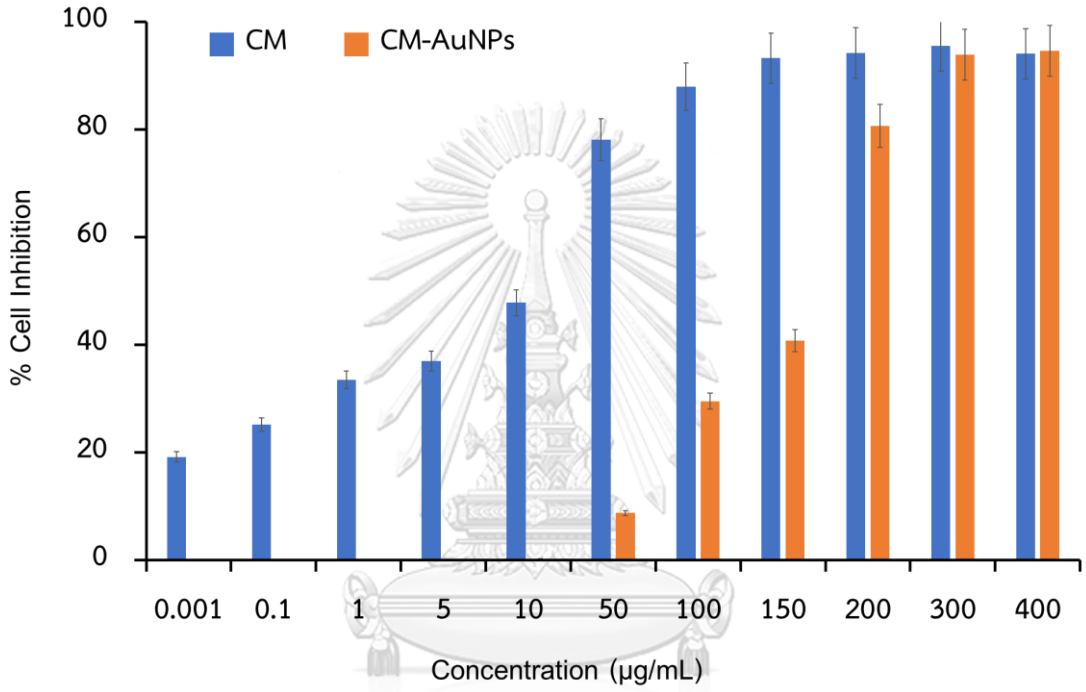

Figure 4. 17 Comparison of percentage inhibition of *Crinum moorei* extract and CM-AuNPs in KB cells at different amount of extract concentration. Values were derived from the graph of growth inhibition against extract concentration from MTT assay.

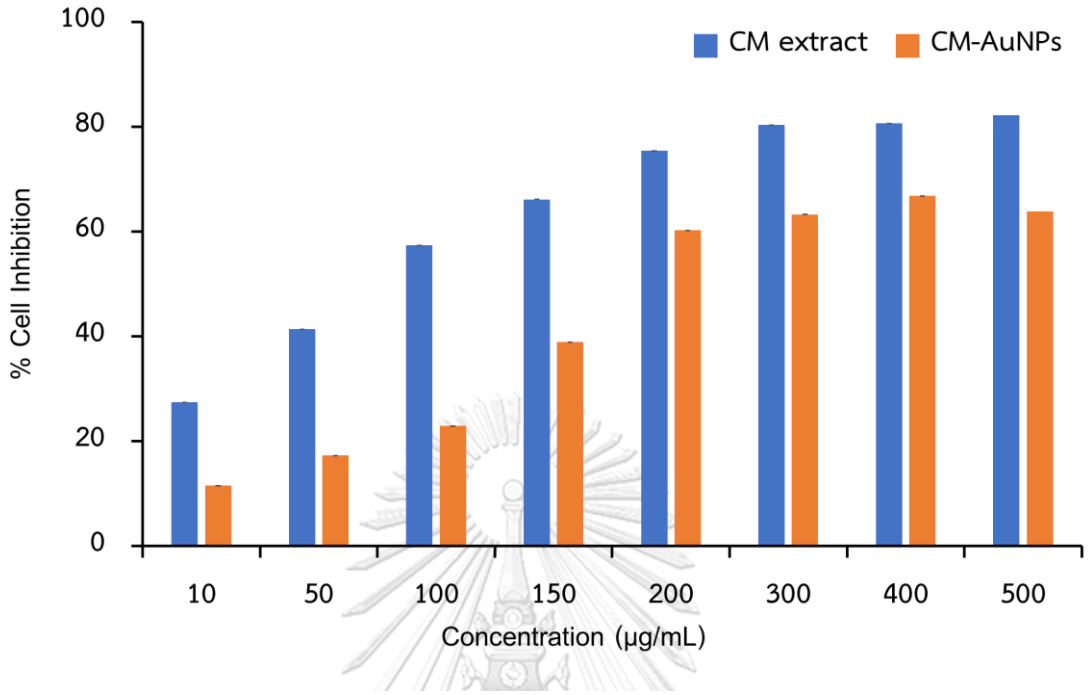

Figure 4. 18 Comparison of Percentage inhibition of *Crinum moorei* extract and CM-AuNPs in Wi-38 cells at different amount of extract concentration. Values were derived from the graph of growth inhibition against extract concentration from MTT assay.

Table 4. 2 In vitro cytotoxicity of each compound against Wi-38 (normal cell) and KB (cancer cells).

Cytotoxicity	Compounds		
IC_{50} (µg/mL)	CM extract	CM-AuNPs	DOXs
Wi-38 cell	78.7	114.9	0.166
KB cell	76.2	142.8	0.357

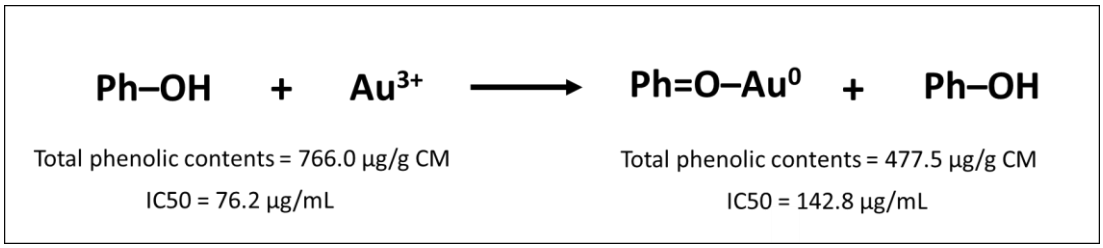

Figure 4. 19 The illustration of the mechanism of total phenolic compounds reduced gold ion into gold nanoparticles

4.6.2 Cellular uptake by Inductively coupled plasma spectrometer (ICP)

The anti-cancer results showed that the CM-AuNPs has low inhibitory efficiency. However, there are two possible ways to explain that the CM-AuNPs has low inhibitory efficiency. First, as explain in previous result that the active functional groups in *Crinum moorei* extract is already used while reducing Au^{3+}. Second, the CM-AuNPs has low ability to pass through cell membrane and release phenolic compounds. For the first reason, it was proved by previous technics. Thus, the cellular uptake test was used to confirm the second hypothesis by using ICP spectrometer.

The CM-AuNPs concentration at 100 µg/mL of *Crinum moorei* extract from figure 4.13, which showed the percentage of cell inhibition around 25%, was selected to use in this experiment. At this concentration, it contains 60 µg/mL of CM-AuNPs, it was considered as the starting concentration. After treating CM-AuNPs into KB cells for 72 hours and measured by ICP spectrometer, the calculate result from gold standard calibration curve (Figure 4.20) showed 1.89±0.01% cellular uptake of CM-AuNPs. Therefore, this result can confirm that the CM-AuNPs has low ability to pass through cell membrane and release phenolic compounds, but only the total phenolic compounds that can pass into cells and damage cancer cell.

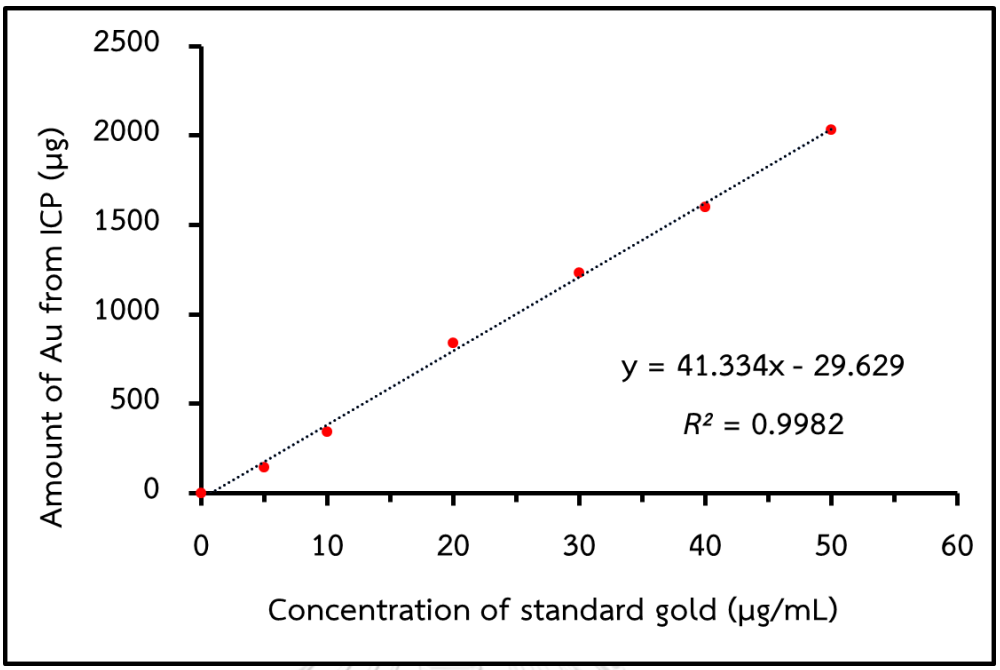

Figure 4. 20 Standard calibration curve of concentration of standard gold against absorbance from ICP spectroscopy

4.6.3 Anti-inflammatory activity

Inflammatory is one of the symptoms that could lead to cancer disease. So, anti-inflammatory activity test is also tested. Figure 4.20 showed the nitrite content, which indicating the inflammatory in cells, after inhibition with various concentration of *Crinum moorei* extract and CM-AuNPs. The higher nitrite content means the higher inflammatory symptom in cell. The results showed that after increasing *Crinum Moorei* extract, the nitrite content decreases. However, after increasing CM-AuNPs concentration, the nitrite content is no significant change. These results associated with the anti-cancer test that the active functional groups in *Crinum moorei* extract is used while reducing Au^{3+}, leads to the lower efficiency of CM-AuNPs compare to Crinum *moorei* extract.

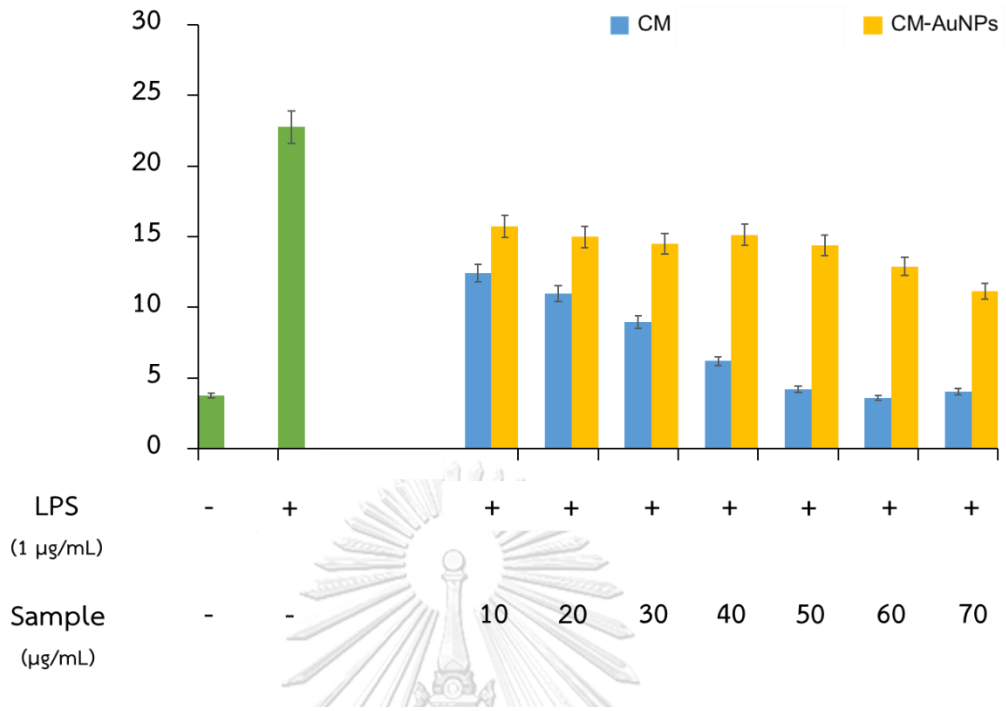

Figure 4. 21 Nitrite content after inhibition with various concentration of *Crinum moorei* extract (CM) and CM-AuNPs

CHAPTER V
CONCLUSION

5.1 Conclusion

The green synthesis of gold nanoparticle was successfully synthesized by using *Crinum moorei* extract which gave spherical in shape with the narrow particle size and the most population particles size is 7-8 nm in this study. This method can be considered as easy, simple, eco-friendly, and effective method to synthesize gold nanoparticles. According to the optimization by observing the effect of *Crinum moorei* extract volume, volume of sodium hydroxide solution, volume of gold precursor solution and synthesizing temperature, the optimized condition is 20 µL of 5% (w/v) *Crinum moorei* extract, 15 µL of 5% (w/v) NaOH and 60 µL of 50 mM $HAuCl_4$ at 80 °C.

The spherical CM-AuNPs was evaluated biological properties, including anti-cancer and anti-inflammatory. The inhibition efficiency of CM-AuNPs to KB cells (cancer cells) was evaluated by MTT assay. MTT assay showed that CM-AuNPs have lower toxicity to cancer cells than *Crinum moorei* extract, but less toxicity to normal cells. This can be explained that the active functional groups in *Crinum moorei* extract is already used while reducing Au^{3+} which corresponding to the total phenolic compounds test and FT-IR spectra. Moreover, the IC_{50} of CM-AuNPs is higher than DOXs (anti-cancer drug), indicating that it has lower toxicity to normal cells than commercial drug. For the inflammatory activity, results associated with the anti-cancer test that the active functional groups in *Crinum moorei* extract is used while reducing Au^{3+}, leads to the lower efficiency of CM-AuNPs compare to *Crinum moorei* extract.

5.2 Suggestion for future work

Drug loading should be done to the synthesized CM-AuNPs, since synthesized CM-AuNPs have lower efficiency to inhibit cancer and inflammatory.

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