GREEN SYNTHESIS OF GOLD NANOPARTICLES USING WAX LEAVED CLIMBER *Cryptolepis buchanani* LATEX AND BIOLOGICAL ACTIVITIES

Mr. Kittiphong Tongsuk

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2018

การสังเคราะห์สีเขียวของอนุภาคทองคำระดับนาโนเมตรด้วยน้ำยางจากเถาเอ็นอ่อน *Cryptolepis buchanani* และฤทธิ์ทางชีวภาพ

นายกิตติพงศ์ ทองสุข

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561

Thesis Title	GREEN SYNTHESIS OF GOLD NANOPARTICLES USING WAX LEAVED CLIMBER *Cryptolepis buchanani* LATEX AND BIOLOGICAL ACTIVITIES
By	Mr. Kittiphong Tongsuk
Field of Study	Chemistry
Thesis Advisor	Professor Nongnuj Muangsin, Ph.D.
Thesis Co Advisor	Professor Chanpen Chanchao, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the Faculty of Science
(Professor POLKIT SANGVANICH, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Vudhichai Parasuk, Ph.D.)

..... Thesis Advisor
(Professor Nongnuj Muangsin, Ph.D.)

..... Thesis Co-Advisor
(Professor Chanpen Chanchao, Ph.D.)

..... Examiner
(Professor Thawatchai Tuntulani, Ph.D.)

..... External Examiner
(Assistant Professor Thitiphan Chimsook, Ph.D.)

กิตติพงศ์ ทองสุข : การสังเคราะห์สีเขียวของอนุภาคทองคำระดับนาโนเมตรด้วยน้ำยางจากเถาเอ็นอ่อน *Cryptolepis buchanani* และฤทธิ์ทางชีวภาพ. (GREEN SYNTHESIS OF GOLD NANOPARTICLES USING WAX LEAVED CLIMBER *Cryptolepis buchanani* LATEX AND BIOLOGICAL ACTIVITIES) อ.ที่ปรึกษาหลัก : ศ. ดร.นงนุช เหมืองสิน, อ.ที่ปรึกษาร่วม : ศ. ดร.จันทร์เพ็ญ จันทร์เจ้า

อนุภาคทองคำระดับนาโนเมตรเป็นอนุภาคนำส่งระดับนาโนเมตรที่กำลังได้รับความสนใจในปัจจุบัน การเตรียมอนุภาคทองคำระดับนาโนเมตรสำหรับการใช้งานทางด้านการแพทย์สามารถเตรียมได้หลายวิธี ได้แก่ วิธีทางกายภาพ วิธีทางเคมี และ การสังเคราะห์สีเขียว อย่างไรก็ตาม การสังเคราะห์ด้วยวิธีทางกายภาพและวิธีทางเคมีอาจทำให้เปลืองพลังงานและอาจก่อให้เกิดความเป็นพิษ ดังนั้นการสังเคราะห์สีเขียวอาจเป็นวิธีที่มีความเหมาะสมมากกว่า ในการสังเคราะห์สีเขียวของอนุภาคทองคำระดับนาโนเมตรอาจใช้องค์ประกอบทางชีวภาพ เช่น fungi bacteria และพืชในการสังเคราะห์ ในงานนี้จึงสนใจการสังเคราะห์สีเขียวของอนุภาคทองคำระดับนาโนเมตรด้วยน้ำยางจากพืชสมุนไพรไทย เถาเอ็นอ่อน (*Cryptolepis buchanani*) ซึ่งองค์ประกอบทางเคมีในน้ำยางจากสมุนไพรนอกจากจะถูกคาดหวังให้มีความสามารถในการอนุภาคทองคำระดับนาเมตรแล้ว ยังถูกคาดหวังให้เป็นสารออกฤทธิ์ทางชีวภาพอีกด้วย การสังเคราะห์สีเขียวของอนุภาคทองคำระดับนาโนเมตรด้วยน้ำยางจากเถาเอ็นอ่อน เริ่มต้นจากการหาสภาวะที่เหมาะสม อนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้ถูกนำไปพิสูจน์เอกลักษณ์ด้วย เทคนิค UV-Vis spectrophotometry Transmission electrons microscope Dynamic light scattering และ Fourier-transform infrared spectroscopy. จากผลการพิสูจน์เอกลักษณ์พบว่าอนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้ดูดกลืนแสงที่ความยาวคลื่น 510 นาโนเมตร ซึ่งสอดคล้องกับภาพถ่ายจาก TEM ที่พบอนุภาคทรงกลมที่มีขนาดประมาณ 5 นาโนเมตร นอกจากนี้อนุภาคทองคำระดับนาโนเมตรที่สังเคราะห์ได้ยังถูกนำมาทดสอบฤทธิ์ทางชีวภาพ ได้แก่ ฤทธิ์การยังยั้งเซลล์มะเร็งและฤทธิ์การยับยั้งการอักเสบอีกด้วย

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2561 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5871910923 : MAJOR CHEMISTRY

KEYWORD: green synthesis, cryptolepis buchanani, gold nanoparticles, anti-inflammatory, anti-cancer

Kittiphong Tongsuk : GREEN SYNTHESIS OF GOLD NANOPARTICLES USING WAX LEAVED CLIMBER *Cryptolepis buchanani* LATEX AND BIOLOGICAL ACTIVITIES. Advisor: Prof. Nongnuj Muangsin, Ph.D. Co-advisor: Prof. Chanpen Chanchao, Ph.D.

Gold nanoparticles (AuNPs) is the one of nanodrug carriers that have been popular in the current trend. To prepare AuNPs for biomedical applications, many techniques have been developed. However, both physical and chemical methods may cause energy consuming and toxic residues so green synthesis would be the most appropriate method for the preparation of AuNPs. The green synthesis of AuNPs could be provided by utilizing of fungi, bacteria and plant. In this work, we focused to use plant latex as reducing and stabilizing agents for the synthesis of AuNPs. The latex was collected from *Cryptolepis buchanani*, a folk medicinal plant in Thailand. By utilizing this plant latex in the synthesis of AuNPs, the latex was not only expected the role in the synthesis of AuNPs but also biological activity. The synthesis of AuNPs by using *C. buchanani* latex was performed by optimizing the reaction parameters. The AuNPs obtained from optimum condition were characterized by UV-Vis spectrophotometry, Transmission electrons microscope, Dynamic light scattering and Fourier-transform infrared spectroscopy. The synthesized AuNPs exhibited the SPR band at 510 nm which also related to the spherical shape of AuNPs with the size around 5 nm. Moreover, the biological activity evaluation of synthesized were also done toward anti-cancer and anti-inflammatory.

Field of Study: Chemistry
Academic Year: 2018

Student's Signature .....
Advisor's Signature .....
Co-advisor's Signature .....

ACKNOWLEDGEMENTS

In the completion of this dissertation, I would like to express my thankfulness for all crucial assistance and contribution. First, I would like to thank Professor Nongnuj Muangsin, my advisor for her guidance as my supervisor. She gave me a lot of opportunity to improve myself. Despite her tight schedule, she had spared her time not only to give constructive advices but also teach me how to write scientific reports which make the dissertation go to a right way. Without her helps and supports, this dissertation would never come so far. Secondly, my gratitude would be express to Professor Chanpen Chanchao who is my co-advisor for her advices in biological parts. With her guidance, I could solve the problems that had happened during my biological experiments. Thirdly, I would like to thank all of the committee for their valuable advices.

My sincere and earnest thankfulness would also express to all of the members in this laboratory including Dr. Urarika Luesakul, Dr. Sakchai Laksee, Dr. Punnida Nonsuwan, Miss Chamaiporn Supachettapun, Miss Kwanruen Chanpeng, Mr.Thanate Tubtimthong, Mr. Nattapong Pangkham and Miss Warinda Marujiwat. Their kindly helps could support me to come across some obstacles. During the discussion with them, they always suggest me the good ideas for my experiment. Thanks to all of them who gave valuable memories at Chulalongkorn University. Finally, I would like to express my thanks to my family for all of their supports and encouragement.

Kittiphong Tongsuk

TABLE OF CONTENTS

Page

List of Figure

Chapter I
INTRODUCTION

1.1 Research background

Currently, nanomaterials have received attention in the medicinal field to improve the maximum activity and reduce side effect of therapeutic agents. The nanoparticles could be obtained from various kinds of material such as dendrimer, polymeric micelles, liposome and noble metal which they were prepared into the size range 1-100 nm. Their diversity allowed the modification for various purposes such as the possibility to monitor diagnose, prevent and treat the disease [1].

Among the nanoparticles, gold nanoparticles (AuNPs), noble metal nanoparticles are one of the best candidates due to their suitable size, high drug loading property and low toxicity [2]. Their surface is readily modifiable due to its labile surface functionality, allowing the surface conjugation of various biomolecules. However, its aggregation during the reduction from its bulk solution could be the crucial challenging which may reduce its efficacy. It is generally known that the particle sizes between 1 to 100 nm are appropriate for cellular uptake [3]. Several methods including physical, chemical and green methods were developed to control the size of AuNPs into 1-100 nm [4]. Turkevich method was known as a traditionally chemical method to synthesize AuNPs which the particle size could be control by vary the concentration of trisodium citrate [5]. However, both chemical and physical methods may cause some toxic residues and energy consuming, respectively. Thus, the green method become more attractive due to easy, low toxic, eco-friendly and cost-effective preparation.

According to the previous reports, the synthesis of AuNPs could be done by using plant extracts. In 2018, Chahardoli *et al.* have been synthesized AuNPs by using *Nigella arvensis* leaf extracts [6]. They suggested that phytoconstituents in plant extract played an important role for the synthesis of AuNPs. The synthesized particles exhibit the spherical shape with the size range of 3-37 nm which were an appropriate size range for cellular uptake. As the synthesis was performed without using any toxic chemical reagent and loading of commercial drug, AuNPs could inhibit the cell

proliferation of cancer cell lines. These evidences suggested that the AuNPs obtained from green method also showed potent property for biomedical applications. A thousand literatures have reported the synthesis of AuNPs using leaves, stems, roots and fruits whereas the using of plant latex for the synthesis of AuNPs was less studied [7-10]. The previous studies were reported the phytoconstituents in plant latex such as proteins, rubber, alkaloids, cardenolides, terpenoids and phenolics [11]. These constituents allow the possibility to use plant latex in the synthesis of AuNPs. A few researchers have been studied the synthesis of AuNPs by using plant latex. For example, Ratul Kumar Das *et al*. have been synthesized AuNPs by the aqueous extract of *Calotropis procera* latex [12]. They found that proteins in the latex could be responsible for the synthesis of AuNPs. The particles showed biocompatibility toward normal cell lines whereas the biological activity have not been reported.

Cryptolepis buchanani (also called Thao En On) is a climbing tree which is widely used as folk medicinal plant in Thailand [13, 14]. Its stem has been utilized for the treatment of muscle and joint pain. The methanolic extract of the stem showed analgesic and anti-inflammatory activities while the aqueous extract from leaf showed an anti-dermatophyte activity [15, 16]. Various phytocomponents could be also extracted from *C. buchanani* including tannins, alkaloid, saponin, flavonoid, and phenolics [17]. As it was one part of the same plant, its latex could showed the same biological activity. A novel serine protease could be extracted from this latex in 2006 [18]. However, the biological activity of the latex has not been reported yet. Because of the phytocomponent and biological activity found in *C. buchanani* suggested that its latex could be responsible for the synthesis of AuNPs and bioactive compound. Thus, we focused to investigate the synthesis of AuNPs using *C. buchanani* latex and their biological activities.

However, the immiscibility of latex could be the major problem for the using of latex in aqueous solution. A strategy that could overcome these problems is using a surfactant. The adsorption of hydrophobic and hydrophilic parts of a surfactant molecule onto the interface could homogenize latex gum and aqueous solution [19]. Moreover, charged surfactant could be used to stabilize AuNPs due to their charge

repulsions [20]. Therefore, sodium dodecyl sulphate (SDS), an anionic surfactant which commonly uses with natural latex, was one of an interesting choice [21].

In this work, *C. buchanani* latex stock solution was prepared in the presence of SDS. The latex stock solution was used for the synthesis of AuNPs. The reaction parameters including effect of latex concentration, effect of volume of 1%NaOH, effect of reaction temperature and effect of volume of 1%$HAuCl_4$ were investigated toward size and shape of AuNPs. The synthesized latex-AuNPs were also characterized by using UV-Vis spectrophotometry (UV-Vis), Transmission electron microscope (TEM), Dynamic light scattering (DLS), Zeta potential and Fourier-transform infrared spectroscopy. The biological activity of latex-AuNPs was evaluated including anti-cancer and anti-inflammatory.

1.2 Research objective

This research aimed to use *C. buchanani* latex for the green synthesis of AuNPs. The synthesized latex-AuNPs were expected to improve the biological activity of latex such as anti-cancer and anti-inflammatory activities. The objectives of this work were described in detail as follow;

1) To synthesize gold nanoparticles by green method using *C. buchanani* latex.

2) To evaluate the biological activities of synthesized latex-AuNPs.

1.3 The expected beneficial outcomes

To obtain the green synthesis of AuNPs using *C. buchanani* latex.

Chapter II

Literature reviews

2.1 Nanodrug carrier

Nowadays, nanoscience and nanotechnology have attracted attention due to their excellent properties. It is well known that nanoparticles were the object that have size in the range of 1-100 nm. The research in nanotechnology field has brought about the possibility to diagnose and treat the disease. Due to their large surface area, quantum properties and their ability to carry other substances, nanoparticles were interesting to apply for the biomedical applications [22, 23]. The nanoparticles could be prepared from the variety of raw material such as polymer, liposome and inorganic matter.

2.1.1 Polymeric nanoparticles

Polymeric nanoparticles are usually used for nanomedicine applications in the formed of soft materials which have biocompatibility and biodegradability. The colloidal polymeric nanoparticles could be prepared by various methods to improve therapeutic efficiency, drug released controlling and drug loading efficacy [24, 25]. Drugs could be encapsulated in various polymers including poly (**ε**-caprolactone), poly (lactic acid) and N-(2-hydroxypropyl)-methacrylamide copolymer (HPMA), or natural polymer such as chitosan, gelatin, karageenan and dextran. These polymer matrixes were used to encapsulate the drugs in order to control their delivery. The polymeric nanoparticles could control drug release through their surface erosion, swelling and specific response to physical and chemical stimuli of targeted cells. To reduce toxic side effects and enhance anti-cancer activity of doxorubicin, the dextran could be used to conjugate with doxorubicin and then encapsulate in the hydrogel [26]. PLGA was also applied to deliver Tamoxifen which was another type of commercial drug for anti-cancer treatment [27].

2.1.2 Liposome

Liposome have both hydrophobic and hydrophilic in their particles, they could be used to deliver both hydrophobic and hydrophilic drugs. Their particles have made of self-assembly of aqueous phase centered in lipid bilayer which could form small and spherical particles. Their biodegradability and biocompatibility can be tuned for drug delivery system. The conventional liposome could deliver doxorubicin by the encapsulation of the drug in core shell using ammonium sulphate [28]. Many techniques were developed to prolong half-life of liposome in blood stream. For example, polyethylene glycol (PEG) functionalized-liposome could prolong the circulation half-life of the particles up to several hours [29]. Moreover, the conjugation between liposome and target ligand have been used to control drug release into the target cell by using pH-responsive approaches [30, 31].

2.1.3 Inorganic nanoparticles

Nowadays, inorganic materials have attracted attention to develop as a platform for imaging and therapeutic treatment. Their potential advantages such as biocompatibility, bioavailability, large surface area, and high drug loading capacity, have brought about the greater property in drug delivery application compared to those polymeric nanocarriers. According to previous study, calcium phosphate has been used as drug and gene nanocarrier. Calcium ion could form complex with negatively charged therapeutic agents through chelation which could provide the possibility to use this inorganic material for drug delivery system [32, 33]. In addition, superparamagnetic iron oxide nanoparticles are also developed to use for cancer treatment. The particles could reach the target site by apply external magnetic field. The external stimulus was also applied to the nanoparticles which the heating was subsequently generated to provide hyperthermia for cancer treatment [34]. Moreover, noble metal nanoparticles such as gold nanoparticles have been extensively used due to their inertness, readily surface functionalization and thermosensitive property. These advantages have brought about the multifunctional nanoparticles for nanomedicine applications [35].

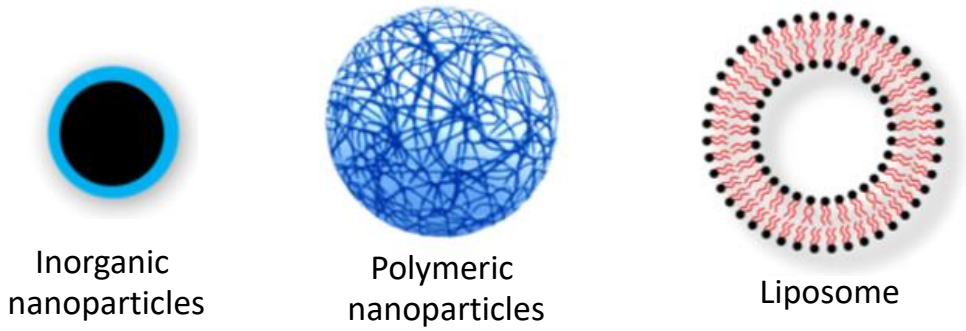


Figure 1 Schematic illustrated different type of nanoparticles [36].

2.2 Gold nanoparticles

Among drug delivery system, gold nanoparticles (AuNPs) have been utilized for the nanomedicine applications due to their biologically inertness so they were considered as the non-toxic platform [37]. AuNPs provided the capability for photothermal therapy due to their unique optical properties which it was strongly depend on their localized surface plasmon resonance (LSPR) [38]. Their surface was also readily to functionalize with various molecules which could vary the advantage for drug delivery system and bio-sensor [39]. Moreover, their suitable size also improved drug loading capacity, cellular uptake and specificity toward cancer cells [40]. Due to their excellent properties, many techniques have been developed to synthesize AuNPs including physical, chemical and green methods.

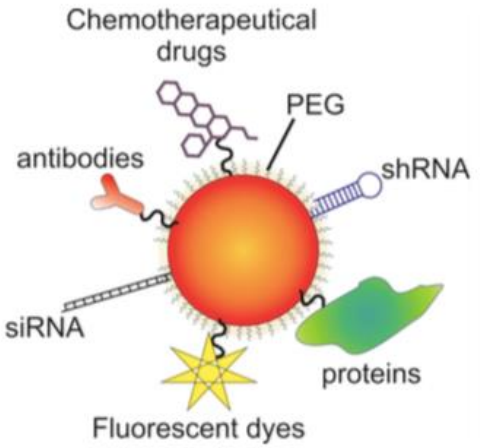


Figure 2 Schematic illustrated AuNPs functionalized with various biomolecules [41]

2.3 Synthesis of AuNPs

2.3.1 Physical method

There are several physical methods using for the synthesis of AuNPs such as the utilizing microwaves, laser ablation and ultrasonic waves [42-44]. As they have rapid heat capacity and high penetrating power, these techniques could provide homogeneous nucleation of metal nanoparticles resulting in the formation of desire size and shape. For example, the using of microwaves in the synthesis of AuNPs were studied by Subrata Kundu et al [42]. They have prepared AuNPs by using 2,7-dihydroxy naphthalene (2,7-DHN) and cationic surfactant as reducing and stabilizing agents, respectively. The synthesis was performed in the environment of microwave heating with the reaction time less than 90 seconds. The results showed that the desired size and shape of AuNPs could be controlled by varying the amount of the reactants. In 2018, Claure N. Lunardi et al. also suggested that AuNPs obtained from microwaves method showed uniform size and shape. The AuNPs were synthesized by using *Euphorbia tirucalli* latex as reducing and stabilizing agents which it was performed in the microwaves heating environment [45]. The results showed that the AuNPs obtained from microwave-assisted *Euphorbia tirucalli* latex have more narrow size distribution compared to those AuNPs synthesized by *Euphorbia tirucalli* latex. These results suggest that this method was a rapid method which could control size and shape of AuNPs effectively. However, it also caused the energy consuming so other techniques should be more appropriate to obtain AuNPs.

2.3.2 Chemical method

Turkevich method are one of the traditionally chemical methods to synthesize AuNPs. The AuNPs were synthesized by using trisodium citrate which could responsible for the reduction of Au^{3+} and stabilization of Au^{0} [46]. The size of AuNPs could be controlled by varying the molar ratio of trisodium citrate. Paul K. Ngumbi et al have studied the effect of trisodium citrate concentration toward size of AuNPs [47]. The results showed that higher concentration of trisodium citrate could provide the AuNPs in smaller size. Not only trisodium citrate could be used for the synthesis of AuNPs but

also other chemical reagents such as sodium borohydride, hydroquinone and aspartate [48-52]. In 1996, the fabrication of amine-capped gold nanocrystal has been studied [48]. The reaction was performed by mixing $HAuCl_4$ and dodecylamine and oleylamine, stabilizing agents. The resultant color was suddenly changed to purple color after adding of $NaBH_4$. This visual observation suggested that $NaBH_4$ play an important role in the synthesis of AuNPs. According to the previous reports, chemical method could be used to control size and shape in a similar manner to physical method. However, the chemical may cause some toxic residues adsorbed on AuNPs surface which was not suitable for medical applications. Thus, green synthesis seems to be the most applicable method to obtain AuNPs for biomedical applications.

2.3.3 Green method

According to the drawback of by physical and chemical method including energy consuming and toxicity, the green method could be considered as easy, eco-friendly, cost effective and low toxic method. There are many ways to synthesize AuNPs by green synthesis method such as the utilizing of fungi, bacteria and plant. Among these materials, using plant are the most applicable method because its extracts could be prepared more easily compared to those fungi and bacteria. In 2013, Sujitha *et al* have studied the using of *Citrus fruits* including *Citrus limon*, *Citrus reticulata* and *Citrus sinensis* aqueous extract for the synthesis of AuNPs [53]. The results showed that the synthesized AuNPs have size the range of 32-56 nm which was the appropriate range for biomedical applications. The effect of aqueous extract concentration was also investigated which it was indicated the decreasing of AuNPs size at higher concentration. This result indicated the important role of phytoconstituents in aqueous extract of the plant which it mainly consisted of citric acid and ascorbic acid.

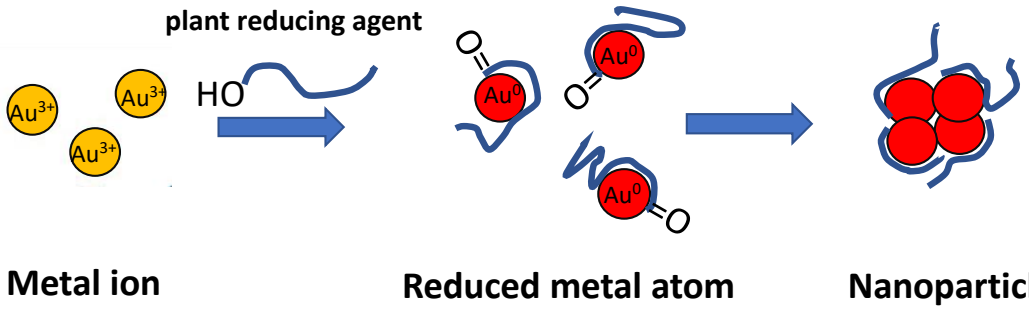


Figure 3 Schematic illustrated the reduction of Au^{3+} by hydroxy groups to form AuNPs [54]

In 2015, the AuNPs synthesized by using the aqueous extract of *Stevia rebaudiana* leaf was reported [55]. The synthesized particles exhibited spherical shape with the size ranging from 5 to 20 nm. The FTIR results showed the shifting of C-N peak indicating the binding of N atom on AuNPs surface. These results suggested that protein, one of phytoconstituents in plant extract, could act as a capping agent to prevent the aggregation of AuNPs. In addition, the role of phenolic compound in plant extract was studied in the synthesis of AuNPs by Chahardoli et al. [56]. They have synthesized AuNPs using *Nigella arvensis* leaf extract which they could obtain spherical nanoparticles in the size range 3-37 nm. The results showed that after the synthesis of AuNPs, the decreasing in the total phenolic content was observed suggesting an important role of OH group in phenolic compound for the reduction of Au^{3+} in synthesis of AuNPs.

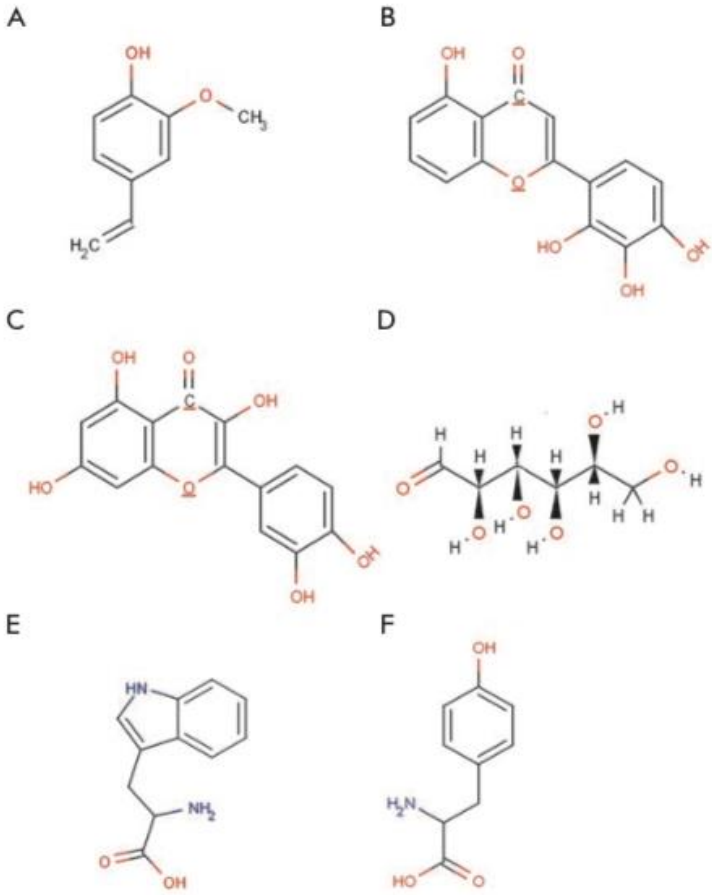


Figure 4 Chemical structures of reducing agents in plant extract [54]

Many researches have published the study of synthesis of AuNPs using root, leaf, stem and fruits extract whereas the using of plant latex for the synthesis of AuNPs are scarce. The phytoconstituents of plant latex basically consist of proteins, rubber, alkaloids, cardenolides, terpenoids and phenolics which suggested the ability to use for the preparation of AuNPs. There are a few reports of using plant latex to fabricate noble metal nanoparticles. For example, Bar *et al.* have synthesized silver nanoparticles by using the latex of *Jatropha curcas* [57]. The synthesized AuNPs showed the spherical shape with the size of 20-30 nm in diameter. The results suggested that the proteins in the latex could play an important role for both reducing and stabilizing agents. These evidences suggest us the possibility to apply plant latex for the synthesis of AuNPs.

2.4 *Cryptolepis buchanani*

Figure 5 The *Cryptolepis buchanan*i young stem

Cryptolepis buchanani (also called Thao En On) is a climbing tree which has been widely used as a folk medicinal plant in Thailand. Its stem has been utilized for the treatment of muscle and joint pain [13, 14]. In India, the latex of this plant has been applied on wounds, boils and sores. The past literature reported the phytochemical constituents of the stem, which consist of flavonoid, alkaloid and saponin (Figure 7). The methanolic extract of the stem showed analgesic and anti-inflammatory activities while the aqueous extract from leaf contained various phytochemical components including tannins, alkaloid, saponin and flavonoid [17]. Some of them have shown an anti-dermatophyte activity [15]. In addition, the latex of *C. buchanani* was reported to consist of a novel glycosylated serine protease [18]. This type of protease is commonly used in haemostasis and wound healing [58]. According to the application mentioned, we expect that the latex of *C. buchanani* can act as anti-inflammatory and anti-cancer agents. Thus, we are focusing on the investigation of applying *C. buchanani* latex in the synthesis of AuNPs.

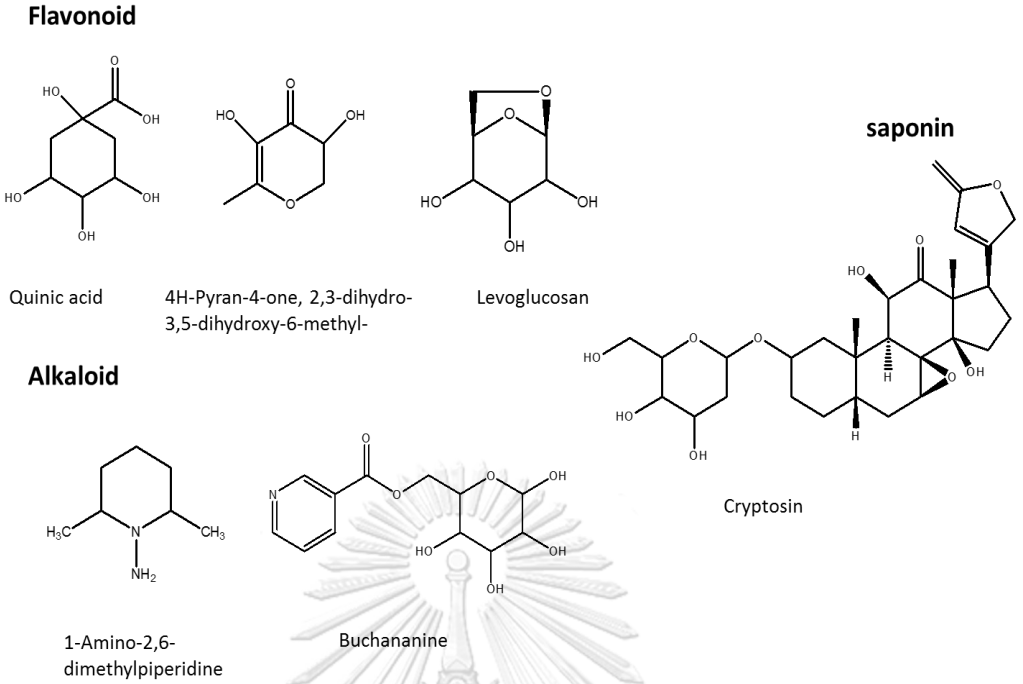


Figure 6 The phytochemical constituents found in *Cryptolepis buchanani [59-61]*.

2.5 Sodium dodecyl sulphate

The coagulation of latex particles in the aqueous medium was the major drawback of using plant latex. This problem could overcome by using a surfactant. The adsorption of hydrophobic and hydrophilic parts of a surfactant molecule onto the interface could homogenize latex gum and aqueous solution [19]. Sodium dodecyl sulphate is an ionic surfactant which commonly use with natural latex [21]. Its anionic charged sulphate head group could provide the colloidal stability of hydrophobic particles through the electrostatic repulsion. Moreover, the previous study was reported that SDS also stabilized AuNPs to prevent the self-agglomeration due to their charge repulsions [21]. Therefore, sodium dodecyl sulphate (SDS), an anionic surfactant which commonly uses with natural latex, was one of the interesting choices.

In this work, the preparation of *C. buchanani* latex solution in the presence of SDS was done to obtain the homogeneous latex stock solution. The latex stock solution was then used in the synthesis of AuNPs. The synthesized AuNPs were characterized by many techniques which will be discussed in the next chapter.

Chapter III

MATERIALS AND METHODS

Materials and methods used in the synthesis of AuNPs and their biological activities evaluation are described in this chapter. The experiments in this work are performed as shown in Figure 3.1.

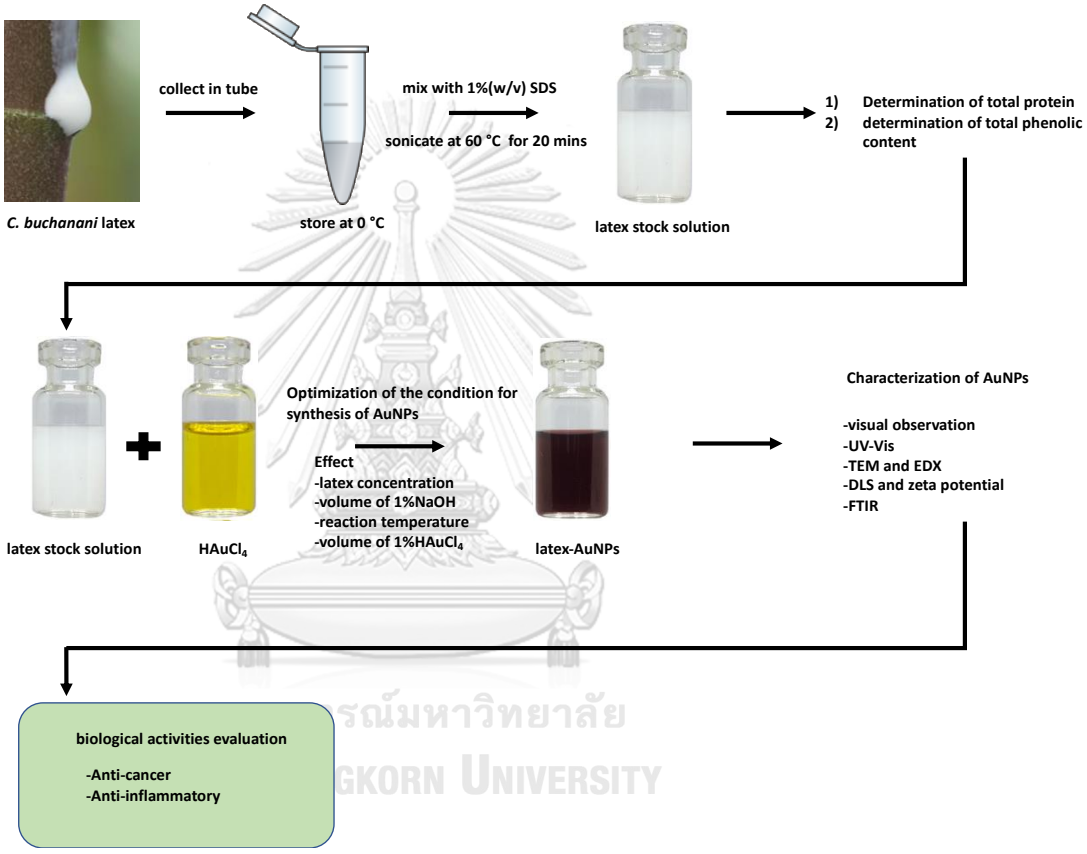


Figure 7 The schematic of the synthesis of latex-AuNPs from *C. buchanani* latex.

3.1 Materials and chemicals

Cryptolepis buchanani latex were collected from Phanomsarakham, Chachoengsao. hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$), Sodium hydroxide (NaOH), Sodium dodecyl sulphate (SDS), Doxorubicin hydrochloride, Dulbecco's Modified Eagle Medium (DMEM), Eagle's Minimum Essential Medium (EMEM), RPMI medium, Trypsin, Fetal calf serum (FCS), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-

diphenyltetrazolium bromide (MTT), lipopolysaccharide (LPS), Griess reagent, KB (cervical cancer cells), SW620 (colon cancer cells), Hep-G2 (liver cancer cells), Wi-38 (human normal lung cells) and RAW 264.7 (murine macrophage cells),

3.2 Methods

3.2.1 Preparation of *C. buchanani* latex stock solution

Milky latex was collected from young climber parts of *C. buchanani*. 3 grams of *C. buchanani* latex were mixed with 0.5%SDS, surfactant solution and made up total volume to 100 mL. The resultant was sonicated at 60°C for 20 minutes. The monodispersed solution of *C. buchanani* latex in 1%SDS was used as *C. buchanani* latex stock solution in further the experiments.

3.2.2 Determination of total protein assay

Total protein content in *C. buchanani* latex was determined by using Folin-lowry method. 0.1 mL of 3% latex stock solution or standard was hydrolyzed with 0.1 mL of 2N NaOH at 100°C for 10 min. The hydrolysate was cooled down to room temperature, added 1 mL of complex-forming reagent* and stand at room temperature for 10 minutes. The mixture was added by Folin-Ciocalteau reagent (1N) 0.1 mL, using the vortex mixer and let it stand at room temperature for 30-60 min. Then, the resultants were measured the absorbance at 750 nm by using UV-Vis spectroscopy and bovine serum 0-2000 µg/mL were used as a standard.

* The complex-forming reagent was freshly prepared by mixing 2%(w/v) Na_2CO_3, 1%(w/v) $CuSO_4 \cdot 5H_2O$ and 2%(w/v) sodium potassium tartrate in the ratio of 100:1:1 (by vol).

3.2.3 Determination of total phenolic assay

Total phenolic content was determined by Folin-ciocalteu method. The calibration curve of gallic acid was done by using the linear range 0-100 µg/mL. 160 µL of the latex sample or gallic acid standard was mixed with 20 µL of 0.4 N Folin-ciocalteu reagent in 96 well plate. After that 20 µL of 10%(w/v) Sodium carbonate was

subsequently added to the mixture. The resultant was allowed to stand for 30 minutes at room temperature and measured the absorbance at 760 nm using a UV–VIS spectrophotometer.

3.2.4 Synthesis of gold nanoparticles

3.2.4.1 Effect of latex concentration

To synthesized AuNPs, various ratio of latex to $HAuCl_4$ including 1:1, 5:1, 10:1, 20:1 and 40:1 were done. The various volume of 3%(w/v) latex stock solution including 2, 7, 67, 133 and 267 µL were mixed with 20 µL of 1%(w/v) $HAuCl_4$ and deionized water. The mixtures were stirred continuously at 60^0C for 30 mins. Subsequently, 60 µL of 1%NaOH was added to provide basic condition. The total volume was controlled at 1000 µL. The resultant solution was stirred continuously at 60^0C for 1 hour to complete the reaction.

3.2.4.2 Effect of pH

To study the effect of NaOH volume in the synthesis of AuNPs, the ratio of latex to $HAuCl_4$ was fixed at 20:1 and various volume of 1%NaOH including 0, 20, 30, 40 and 50 µL were done. 3%(w/v) latex stock solution 133 µL were mixed with 20 µL of 1%(w/v) $HAuCl_4$ and deionized water. The mixtures were stirred continuously at 60^0C for 30 mins and then added 1%NaOH the resultants with the volume 0, 20, 30, 40 and 50 µL the total volume was controlled at 1000 µL. The resultant solutions were stirred continuously at 60^0C for 30 mins to complete the reaction.

3.2.4.3 Effect of reaction temperature

To optimized reaction temperature in the synthesis of AuNPs, the ratio of latex: $HAuCl_4$ at 20:1 and 60 µL of 1%NaOH was used to synthesize AuNPs. The reactions were performed at various reaction temperature including room temperature, 40, 50, 60, 70, 80 and 90^0C. Firstly, 3%(w/v) latex stock solution 133 µL were mixed with 20 µL of 1%(w/v) $HAuCl_4$ and deionized water. The resultants were stirred at given

temperatures for 30 mins and then added 60 µL of 1%NaOH. Finally, the mixtures were stirred continuously for 30 mins.

3.2.4.4 Effect of 1%(w/v) $HAuCl_4$ volume

The volume of 1%(w/v) $HAuCl_4$ was optimized by using various volume of 1%(w/v) $HAuCl_4$ including 20, 60 and 100 µL. The synthesis of AuNPs was performed as same as previous experiment. The latex concentration was kept constant at the optimum condition from the previous experiments whereas pH of the solutions was controlled by using NaOH: $HAuCl_4$.

3.2.5 Characterization of gold nanoparticles

3.2.5.1 UV-Vis

The SPR band of synthesized AuNPs from all conditions were recorded by UV-Visible spectrophotometer (HP-8453, Agilent). Five times dilution sample were recorded the UV-Vis spectra in the range between 200 to 800 nm with the cell width 10 mm.

3.2.5.2 DLS and zeta potential

Size distribution and zeta potential of synthesized AuNPs from optimized condition were obtained from Zetasizer (Zetasizer, MALVERN, Nano ZSP). The size distribution and surface charge of AuNPs were obtained from five times dilution of the resultant.

3.2.5.3 TEM

The morphology of synthesized AuNPs from optimized condition was observed from TEM (TEM, JEOL, JEM2001). To prepare the sample, the synthesized AuNPs without any purification were diluted twenty times and dropped on copper grid. The sample was dried in desiccators overnight. The images were obtained by operating TEM at 200 kV.

3.2.5.4 FTIR

FTIR spectra were obtained in order to evaluate the role of latex-phytochemical compounds in the synthesis of AuNPs. To prepare the sample for FT-IR analysis, the latex and latex-AuNPs were mixed into KBr and the mixture was grounded. The FT-IR spectra were recorded in the region 4000 to 400 cm^{-1} by using Nicolet 6700 FT-IR spectrometer.

3.2.6 Cell cultures

For the cell culture, KB (cervical cancer cells) and Wi-38 (human normal lung cells) were culture in EMEM. SW620 (colon cancer cells) and Hep-G2 (liver cancer cells) were cultured in RPMI and RAW 264.7 (murine macrophage cells) was cultured in DMEM. All of cell lines were cultured in the medium which contained 10% FCS. The cells were incubated at 37°C in 5% carbon dioxide (CO_2) and high moisture atmosphere.

3.2.7 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 cell lines and normal cell line were seeded in 96-well plate at a density of 5×10^3 cells/well with total volume 180 µL and incubated at 37°C in 5% carbon dioxide (CO_2) and high moisture atmosphere for 24 h. After that 20 µL of latex stock solution, SDS, latex-AuNPs and doxorubicin at various concentrations were treated and incubated with the cells were for 72 h. Then, the media was incubated with 10 µL of MTT (5 mg/mL) for 4 h at 37°C. Then, the supernatants were removed, the dark violet crystals were dissolved in 150 µL of DMSO and absorbance was measured at 540 nm by UV-Vis microplate reader. The percentage of inhibition was determined relative to the control group.

$$\%inhibition = 100 - (\frac{Sample - control}{control} \times 100)$$

3.2.8 NO assay

The evaluation of anti-inflammatory activity was done by using NO assay. RAW 264.7 cells in DMEM containing 10% fetal calf serum were plated in 96-well plate with cell density of $8x10^5$ cells/mL. The seeded cells were incubated overnight at 37 °C in 5% carbon dioxide (CO_2) and high moisture atmosphere. After that the cells were treated with 10 µL of LPS (100 ng/mL) and 10 µL of latex and latex-AuNPs at various concentrations. After incubation for 72 h, 100 µL of the supernatants were added to new 96-well plate and mixed with the same volume of Griess reagent. The absorbance of samples was read at 540 nm and nitric oxide concentration were determined from calibration curve using $NaNO_2$ as standard. The NO concentration of treated LPS at each concentration of latex and latex-AuNPs samples were compared with untreated LPS samples. All of treated LPS sample was calculated inhibitory percentage by using the same equation as above. In addition, the percentage of cell viability was also determined by MTT assay.

Chapter IV
RESULTS AND DISSCUSION

Among drug delivery system, AuNPs are one of the most interesting candidates due to their superior properties including biocompatibility, non-toxicity and labile surface functionality. Additionally, it is possible to control their size in the size range of 1-100 nm which was suitable for cellular up-taking. The AuNPs with suitable size could carry the drug to the target site specifically. Especially, the delivering of anti-cancer agents, the nanoparticles could be selectively up-taken by cancer cells due to EPR effect. However, the most challenging of using AuNPs was their self-aggregation. To control the size of AuNPs, several techniques have been investigated. Generally, trisodium citrate was used as both reducing and stabilizing agent in the preparation of AuNPs [46]. However, the presence of citrate residue in the product showed cytotoxicity against normal cells. Therefore, green synthesis becomes a more attractive method because it is non-toxic and economical processes. The phytochemical constituent in plants such as polysaccharide, polyphenol, terpenoids, amino acid and protein can act as reducing and stabilizing agents and allow the ability in the synthesis of AuNPs. For example, there were reports shown that amine and carboxyl groups of cysteine protease in phytolatex possibly play a crucial role in the synthesis of AuNPs [62].

In this chapter, we are interested in the latex of *Cryptolepis buchanani* which is a folk medicinal plant. Many parts of this plant showed a broad range of biological activity such as anti-inflammatory and anti-dermatophyte activity. Its extract showed a variety of phytochemical constituents including tannins, alkaloid, saponin, flavonoid and novel serine protease. These components could suggest their ability in the synthesis of AuNPs. To synthesize AuNPs by using *C. buchanani* latex, the latex was dissolved in an anionic surfactant, SDS, solution in order to obtain homogeneous latex stock solution. The reaction parameters including latex concentration, volume of 1%NaOH, reaction temperature and volume of 1%$HAuCl_4$ which could affect to AuNPs

morphology were studied. The synthesized latex-AuNPs were characterized by TEM, DLS, zeta potential and FTIR to predict the property of the particles. Finally, their biological activities including anticancer and anti-inflammatory were also investigated.

4.1 Determination of total protein and total phenolic contents

According to the previous studies, there were reports shown that protein and phenolic content play a crucial role in the synthesis of AuNPs as both reducing and stabilizing agents. For example, it has been reported that total proteins with the value of 8.2 mg/ml and total phenolic content with the value of 145 µg/mL could reduce Au^{3+} and stabilize AuNPs. In this work, total protein and total phenolic contents in *C. buchanani* latex were determined using BSA assay and Folin method, respectively. The total protein and total phenolic content of *C. buchanani* latex were shown in Table 1. The results showed that 3%(w/v) latex stock solution, which is the maximum solubility of latex contained 7.2 mg/mL and 151.0 µg/mL of protein and phenolic compound, respectively, which are in the same range as previous reports. Therefore, the prepared latex stock solution was subsequently used for the synthesis of AuNPs.

Table 1 Total proteins and phenolic contents of latex and latex-AuNPs

	Linear equation	R^2	Total contents
protein	y =0.0003x+0.0572	0.9918	7.2 mg/mL
phenolic	y =0.0278x-0.0658	0.9925	151.0 µg/mL

4.2 green synthesis of latex-AuNPs

4.2.1 Optimization of the condition for the synthesis of AuNPs

4.2.1.1 Effect of latex concentrations

The synthesis of AuNPs was investigated by using *C. buchanani* latex. The effect of latex concentrations in the synthesis of AuNPs were studied by varying the ratio of latex to $HAuCl_4$ in the range of 1:1 to 40:1. The reaction temperature was fixed at 60 °C which it could be considered as mild temperature for the synthesis of AuNPs [63]. By controlling concentration of $HAuCl_4$ at 1 mM, the synthesis was performed in a basic condition which are the condition that could facilitate the reduction of Au^{3+} to form AuNPs [64]. After mixing of latex stock solution with $HAuCl_4$ and NaOH, the yellow color of $HAuCl_4$ turned to various colors indicated the reduction of Au^{3+} to Au^0 by phytocomponent in the latex. The resultants in grey, black, violet, red and violet-red colors were obtained after synthesis of AuNPs using ratio of latex to $HAuCl_4$ including 1:1, 5:1, 10:1, 20:1 and 40:1, respectively.

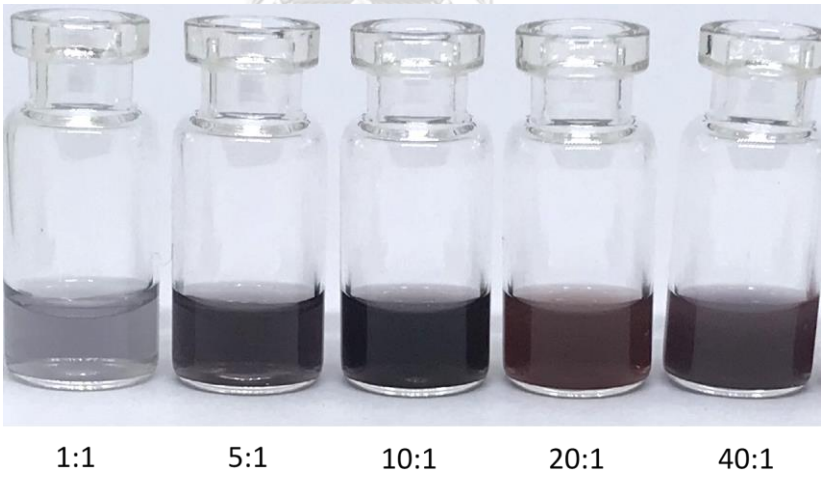


Figure 8 Optical photograph of synthesized latex-AuNPs prepared by using ratio of latex to $HAuCl_4$ at 1:1, 5:1, 10:1, 20:1 and 40:1.

Since, AuNPs were metal in very small size, the electrons on their surface could be oscillated by electromagnetic of an incident light resulting in the oscillation in a specific frequency. This phenomenon was called localize surface plasmon resonance

(LSPR) [65, 66]. The total extinction of incident light after passing through AuNPs was strongly dependent on the adsorption and scattering process. According to Mie theory, the total extinction of the light after pass through AuNPs in size 20 nm was strongly dependent on absorption process whereas AuNPs in larger size, the scattering process become dominated. The domination of light scattering was increased with the size of AuNPs resulting in the adsorption at longer wavelength.

For the biological application, the AuNPs in small size were chosen as a suitable size for cellular uptake. Due to the LSPR effect of AuNPs, the visual observation of AuNPs colloidal solution could be used as preliminary method to evaluate the size and shape of the synthesized particles. As show in Figure 8, the ratio 20:1 provided the ruby-red solution which reflected LSPR of spherical AuNPs in small size around 20 nm. The AuNPs solutions in grey, black, violet and violet-red colors reflected the absorption at longer wavelength which could be considered as AuNPs in larger size [67].

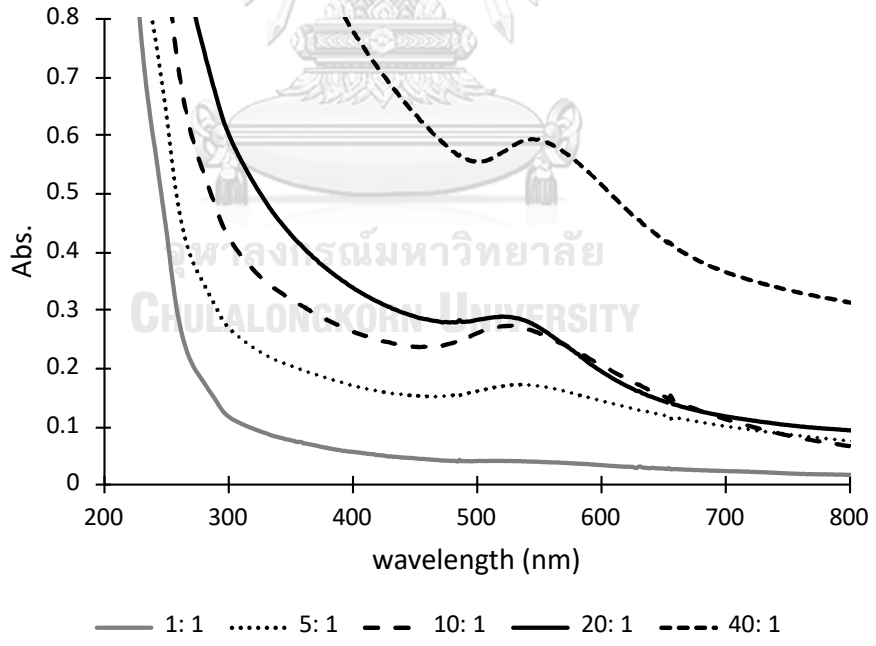


Figure 9 The UV-Vis spectra of synthesized latex-AuNPs prepared by using ratio of latex to $HAuCl_4$ at 1:1, 5:1, 10:1, 20:1 and 40:1.

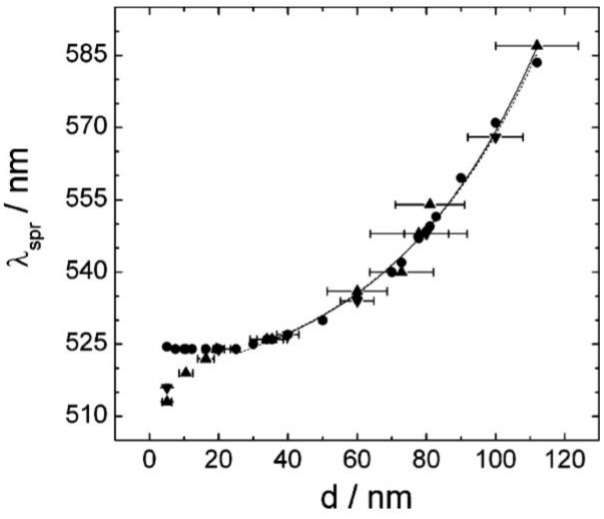


Figure 10 Position of the surface plasmon resonance peak (λ_{spr}) as a function of the particle diameter for GNPs in water: calculated (circles); experimentally measured (downward pointing triangles, commercial GNPs; upward-pointing triangles, inhouse synthesized GNPs). An exponential fit to the theoretical (experimental) data for d > 25 nm is shown as a dotted (dashed) line [68].

To optimize the condition precisely, the UV-Vis spectra of LSPR of AuNPs were obtained in wavelength range 200-800 nm, show in Figure 9. Theoretically, the wavelength at maximum absorbance (λ_{max}) decreased with the increasing of AuNPs size as shown in Figure 10. Thus, in this work, the condition that could provide shortest λ_{max} would be considered as the optimum condition. The optimum condition was subsequently chosen as the condition that could controlled size of AuNPs without aggregation. For the ratio latex:$HAuCl_4$ 1:1 to 20:1, the LSPR peaks of synthesized AuNPs were shifted to lower wavelength with the increasing of latex concentration because the higher latex concentration provided greater reducing and stabilizing efficacy (Figure 11). However, the AuNPs provided from 40:1 of latex: $HAuCl_4$ the peak was shifted to higher wavelength indicating the larger size of AuNPs. This result could be explained by the effect of viscosity toward the reduction efficacy. The increasing in latex concentration not only provided higher amount of reducing residue but also viscosity. Thus, the high viscosity at high latex concentration could lower the reduction efficacy

of $HAuCl_4$ leading to the aggregation of AuNPs. Therefore, the ratio of latex: $HAuCl_4$ at 20:1 which provided the lowest λ_{max}, was chosen as an optimum condition for the green synthesis of AuNPs.

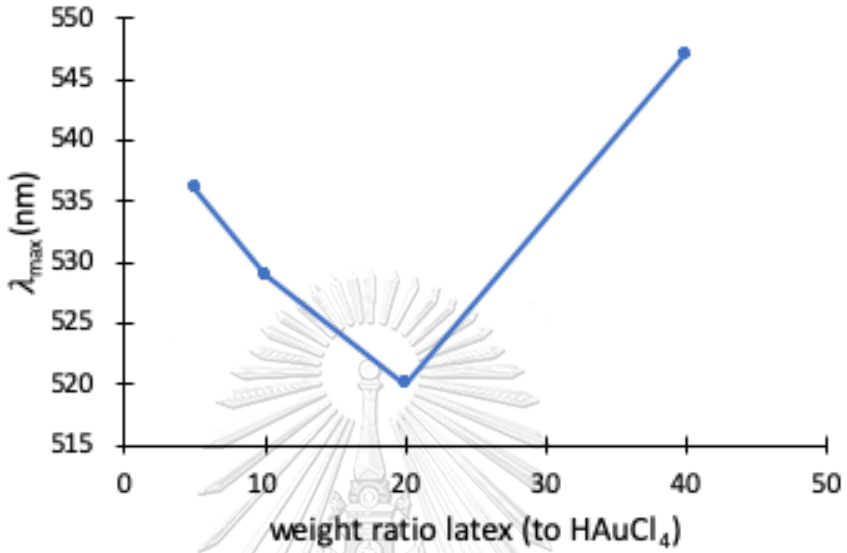


Figure 11 Plot of wavelength at maximum absorbance (λ_{max}) of synthesized latex-AuNPs after synthesis at different ratio of latex to $HAuCl_4$ at 1:1, 5:1, 10:1, 20:1 and 40:1.

4.2.1.2 Effect of pH

The ratio of latex to $HAuCl_4$ at an optimum ratio from previous experiment was chosen to study the effect of volume of 1%NaOH. The green synthesis of AuNPs using latex at the ratio latex: $HAuCl_4$ 20:1 was performed at various volume of 1%NaOH including 0, 20, 30, 40 and 50 µL. After the synthesis the volume of 1%NaOH from 0-30 µL, the synthesized AuNPs exhibited LSPR in violet, violet-red and red color whereas 40 and 50 µL exhibited the same color as 30 µL. The final pH of the resultants was 3, 5, 8, 10 and 12 for the reaction that using of volume of 1%NaOH 0, 20, 30, 40 and 50 µL, respectively. The change in color of AuNPs at final pH from 3-8 could be hypothesized that the higher pH provided smaller size of AuNPs while at pH 8-12, the LSPR of synthesized AuNPs revealed the similar size, Figure 12.

Figure 12 Optical photograph of synthesized latex-AuNPs prepared at various pH including 3, 5, 8, 10 and 12.

The LSPR spectra of AuNPs in the range of 200-800 nm were obtained by UV-Vis spectrophotometry. The results showed that the LSPR bands of synthesized AuNPs were shifted to lower wavelength as the final pH increased up to 8 whereas there was no change in LSPR peaks of AuNPs which provided from pH above 8 (Figure 13). These results could support the hypothesize from visual observation of LSPR of AuNPs. The ability to control the size of AuNPs of each condition was expressed in term of lowest value of wavelength at maximum absorbance (λ_{max}) (Figure 14).

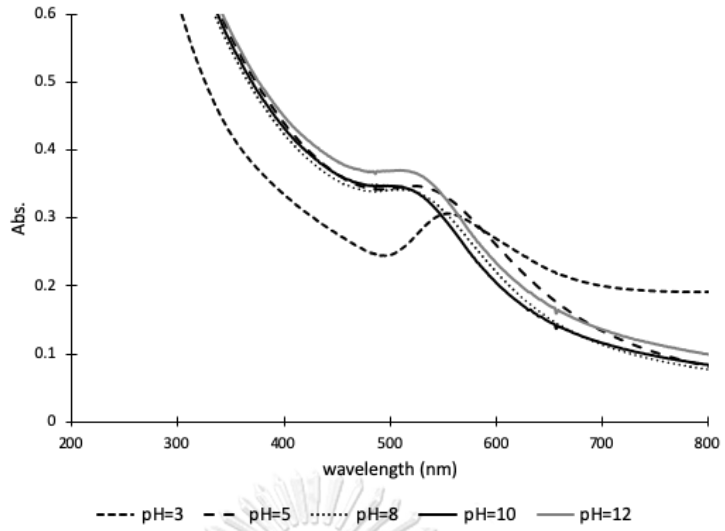


Figure 13 UV-Vis spectra of synthesized latex-AuNPs prepared at various pH including 3, 5, 8, 10 and 12.

The λ_{max} were increased with the final pH including 3, 5, 8 and 10 while the final pH above 10 exhibited the longer wavelength. These results could suggest that the alkaline condition has a significant role to control the size of AuNPs which related to the previous report. In 2019, Zayed et al. have synthesized AuNPs by ethanolic extract of *Ficus retusa*. They suggested results that the increasing in the amount of NaOH could provide the LSPR of AuNPs in smaller size [69]. This could be explained that the OH^- ions in alkaline condition could accelerate electron transferring from phytochemical constituents to Au^{3+}. In that situation the nucleation stage was shorten and the reaction was pushed to growth stage which ensure the formation of AuNPs in small size. Thus, the optimum pH in this experiment would be 10 which was the lowest volume that could provide the shortest λ_{max}.

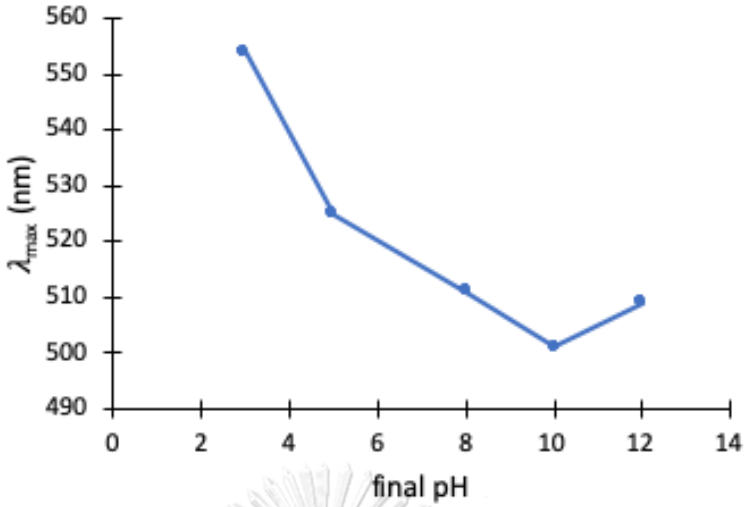


Figure 14 Plot of wavelength at maximum absorbance (λ_{max}) of synthesized latex-AuNPs prepared at various pH including 3, 5, 8, 10 and 12.

4.2.1.3 Effect of reaction temperature

One of the crucial factors that could affect size and shape of AuNPs would be temperature. In this experiment, AuNPs was synthesized by using the ratio of latex: $HAuCl_4$ at 20:1 and 30 µL of 1%NaOH were used to synthesized AuNPs. The reactions were performed at various reaction temperature including room temperature, 40, 50, 60, 70, 80 and 90°C which could provide AuNPs ranging from pale pink to violet color (Figure 15).

T_{room} 40 °C 50 °C 60 °C 70 °C 80 °C 90 °C

Figure 15 Optical photograph of synthesized latex-AuNPs prepared by using reaction temperature at room temperature, 40, 50, 60, 70, 80 and 90°C.

The UV-Vis spectra of synthesized AuNPs were observed LSPR band around 520-557 nm (Figure 16). The results could be divided into two regions. For the first region (room temperature 60 °C), the intensity of LSPR peak was increased as the reaction temperature was increased which the peak was not showed any shifting. The increasing in peak intensity implied the higher amount of AuNPs which could be produced more easily at higher reaction temperature. The second region (above 60°C), the increasing in reaction temperature was not only increased peak intensity but also shifted the peak to longer wavelength which indicated the formation of AuNPs in larger size. The dependence of peak intensity on the reaction temperature could be explained in the same way as previous report. Mingxia Guo *et al.* have studied the effect of reaction temperature in the synthesis of AuNPs by using the aqueous extract of *Eucommia ulmoides* bark [70]. They suggested that the increasing in peak intensity as the reaction temperature was increased that would be due to the increasing in the rate of reaction, which enhance the synthesis of AuNPs. Once the reaction temperature was high enough to complete the reduction, the higher temperature could promote the aggregation of AuNPs [71]. This reason could be used to explain the results in present experiment which the aggregation of AuNPs was observed at reaction temperature above 60°C.

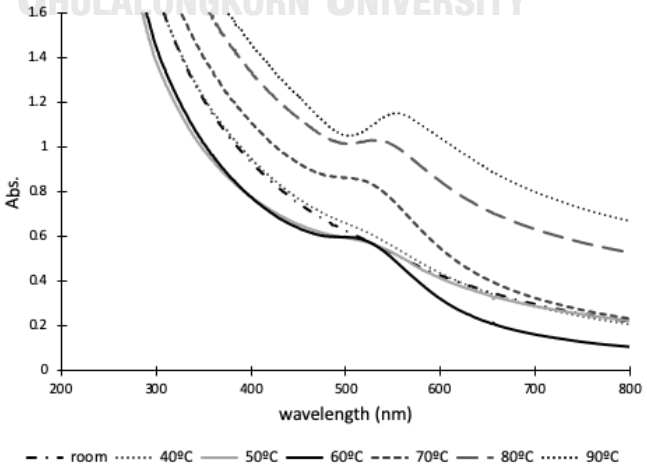


Figure 16 UV-Vis spectra of synthesized latex-AuNPs prepared by using reaction temperature at room temperature, 40, 50, 60, 70, 80 and 90°C.

To choose an optimum reaction temperature, the ability to control size of AuNPs were represented by λ_{max}. (Figure 17) Compare to other conditions, the reaction temperature at 60°C could provide AuNPs in smallest size. Thus, the reaction temperature at 60°C was chosen as the optimum reaction temperature for the synthesis of AuNPs by using *C. buchanani* latex.

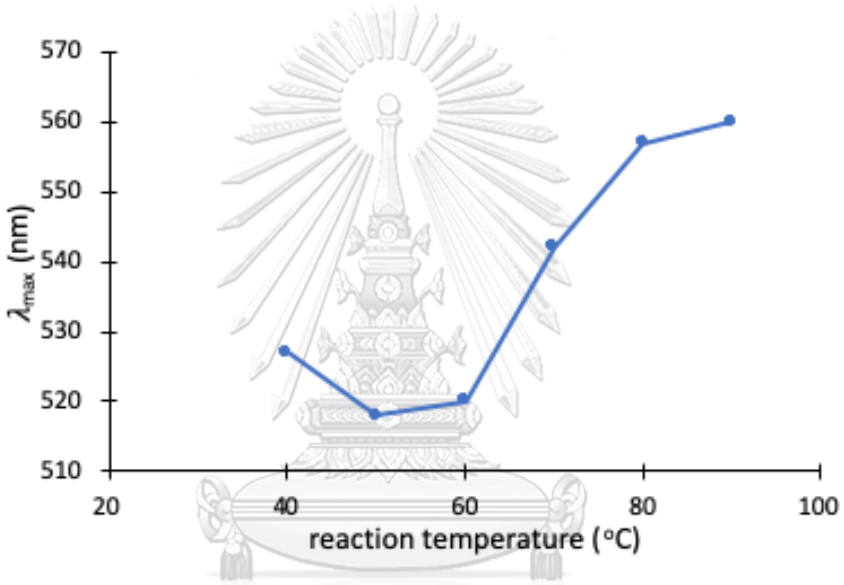


Figure 17 Plot of wavelength at maximum absorbance (λ_{max}) of synthesized latex-AuNPs prepared by using reaction temperature at room temperature, 40, 50, 60, 70, 80 and 90°C.

4.2.1.4 Effect of volume of $HAuCl_4$

According to the optimization from previous experiments, the ratio of latex: $HAuCl_4$ at 20: 1 in the mixture of 30 µL of 1%NaOH under reaction temperature 60 °C was used as an optimum condition for the green synthesis of AuNPs using latex. In this experiment, the effect of $HAuCl_4$ in the synthesis of AuNPs was also investigated by using 20, 40, 60, 80 and 100 µL of 1%$HAuCl_4$. Figure 18 showed the color of AuNPs solution which exhibited the color of red, red, red-purple, purple and black for the using of 1%$HAuCl_4$ at 20, 40, 60, 80 and 100 µL, respectively.

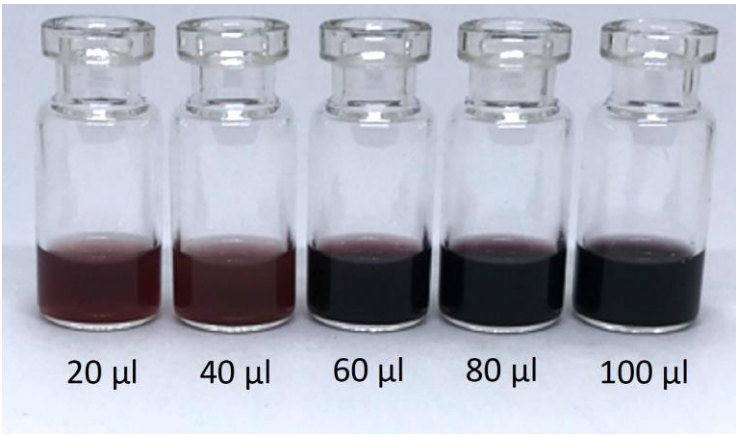


Figure 18 Optical photograph of synthesized latex-AuNPs prepared by using volume of 1%$HAuCl_4$ at 20, 40, 60, 80 and 100 µL.

As the volume of 1%$HAuCl_4$ was increased, the color of resultant solution implied the LSPR effect of spherical AuNPs in larger size. To confirm the visual observation, the UV-Vis spectrophotometry was also investigated Figure 19. The UV-Vis results showed that the volume of 1%$HAuCl_4$ at 20 µL could produce AuNPs which showed the lowest λ_{max} while the higher volume of 1%$HAuCl_4$ volume could shift the peak to higher wavelength Figure 20. In addition, as the volume of 1%$HAuCl_4$ increased to 100 µL, the synthesized AuNPs were observed the peak around 290 nm could increase the intensity at which was the characteristic peak of Au^{3+}. The results showed that the volume of 1%$HAuCl_4$ above 20 µL, the constant concentration of latex could not increase the production of AuNPs. This observation indicated the excess of Au^{3+} at 40 and 100 µL of 1%$HAuCl_4$ which might lead to insufficiency of capping and reducing agents. Therefore, the volume of 1%$HAuCl_4$ at 20 µL was used as the optimum condition for the synthesis of AuNPs by using latex.

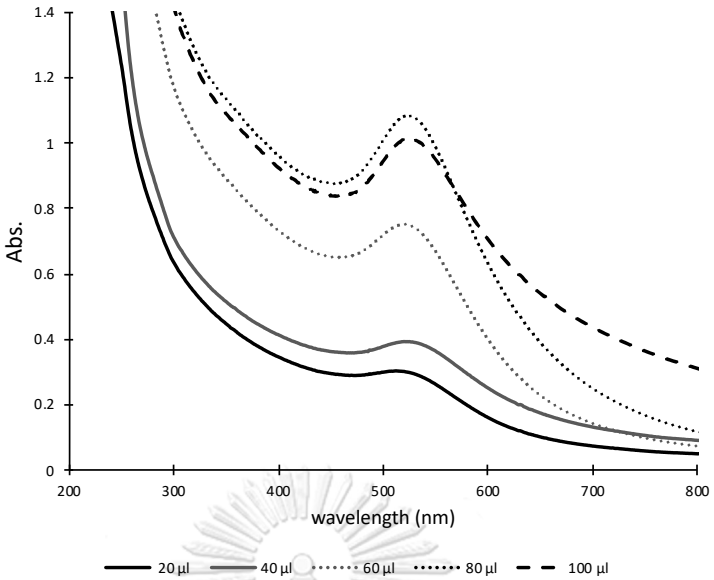


Figure 19 UV-Vis spectra of synthesized latex-AuNPs prepared by using volume of 1%$HAuCl_4$ at 20, 40, 60, 80 and 100 µL.

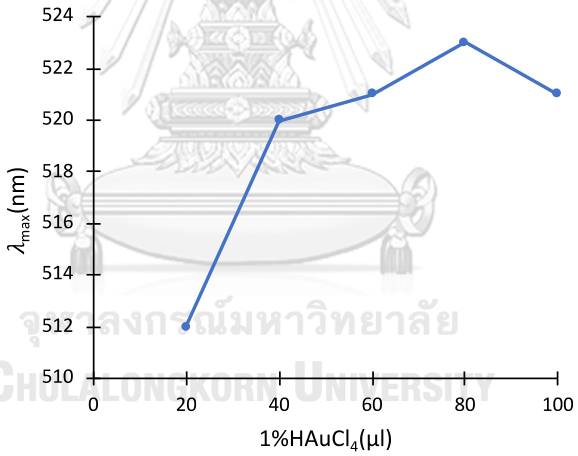


Figure 20 Plot of wavelength at maximum absorbance (λ_{max}) of synthesized latex-AuNPs prepared by using 1%HAuCl4 volume at 20, 40, 60, 80 and 100 µL

The optimization of the condition for the synthesis of AuNPs was done by studying the effect of latex concentration, volume of 1%NaOH, reaction temperature and volume of 1%$HAuCl_4$. In order to choose the optimum condition, the size and shape of latex-AuNPs was represented by the visual observation and UV-Vis spectrum.

From these experiments, we can conclude that the synthesis of AuNPs should be performed by using the ratio of latex:$HAuCl_4$ at 20:1 at the reaction temperature, 60°C. The 1%NaOH with the volume of 30 µL and 1%$HAuCl_4$ with the volume of 20 µL would be the most effective volume to control the particle size.

4.2.2 Characterization of latex-AuNPs

4.2.2.1 TEM image

In this experiment, the AuNPs obtained from optimized condition were characterized by TEM technique which was the most reliable technique to evaluate the size of AuNPs. It is well known that the AuNPs in the size range 1-100 nm are easily taken up by the cells. According to the previous green synthesis of AuNPs by using plant, the obtained AuNPs mostly showed non-uniform shape with the broad size distribution.

a) b)

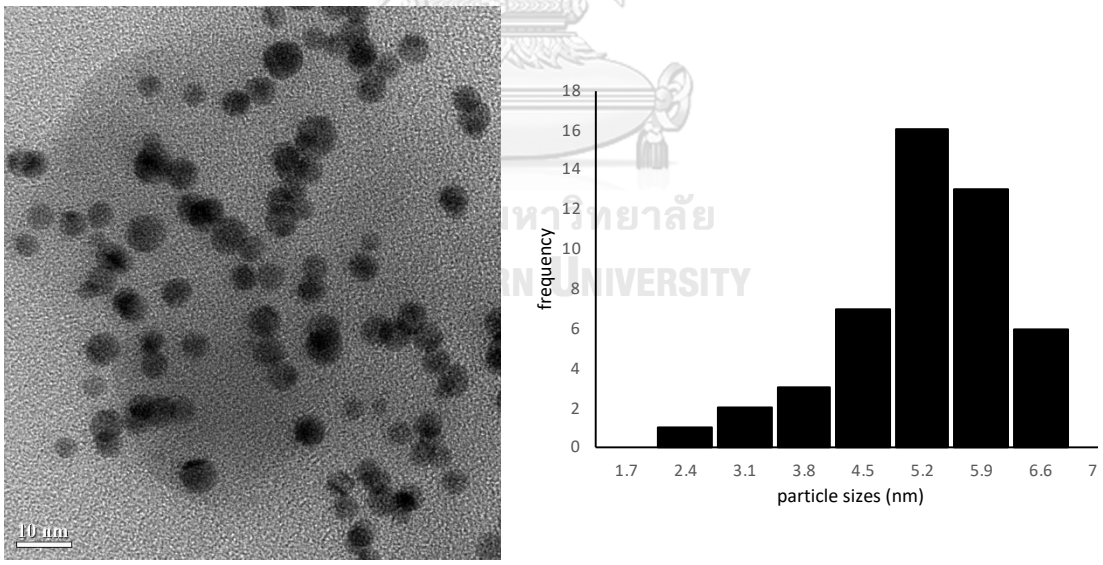


Figure 21 a) TEM image of synthesized latex-AuNPs and b) Size distribution of synthesized latex-AuNPs

In present work, the obtained AuNPs particles showed uniform shape with the narrow range. As shown in Figure 21, the TEM of AuNPs displayed spherical particles

with the size around 5 nm. The grey area around the particles was also observed which might due to the phytochemical component in the latex surrounded on AuNPs. These results could confirm the observation of LSPR of AuNPs at 515 nm in the UV-Vis result which correlated to AuNPs in spherical shape with size 5 nm [47]. Compare to the previous report of green synthesis of AuNPs, our method could control the size and shape of AuNPs into small size with uniform shape. This may due to the high amount of capping agent in latex stock solution such as protein and SDS. The EDX spectrum indicated that the black particles consisted of C, O and Au atoms which could be used to confirm the existing of AuNPs and capping agent on their surface Figure 22.

The using of latex stock solution for the preparation of AuNPs could control the AuNPs into the size range that could be taken up by the cell easily. Thus, the synthesized AuNPs offer the opportunity for using as a drug carrier.

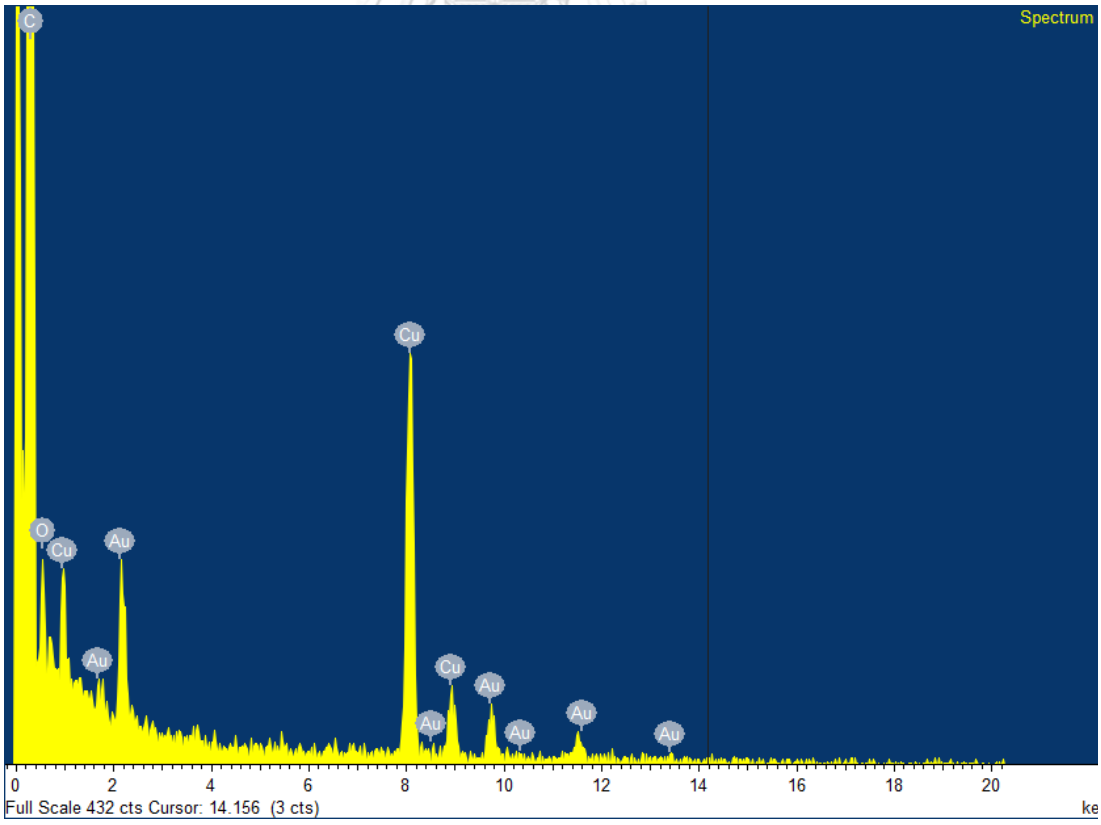


Figure 22 EDX spectrum of synthesized latex-AuNPs

4.2.2.2 DLS analysis

To investigate the stability and monodispersibility of AuNPs in aqueous solution, the hydrodynamic size was obtained from DLS technique. The AuNPs showed the size distribution at 123.4 and 16.48 nm with 0.308 PDI (Figure 23). The size at 123.4 nm (90% intensity) was considered as the dominant size of AuNPs, the particles showed bigger size than the diameter obtained from TEM which may cause by the stabilization of the capping agent on AuNPs surface. These results indicated the swelling of capping agent such as SDS and proteins in the aqueous medium.

In addition, the stability of AuNPs was predicted by zeta potential which could represented the surface charge of AuNPs. The zeta potential of synthesized AuNPs was found at -50 mV which indicated the high stability and negative charge on their surface. These results also imply that the SDS and phytochemical in latex could improve the stability of AuNPs [72].

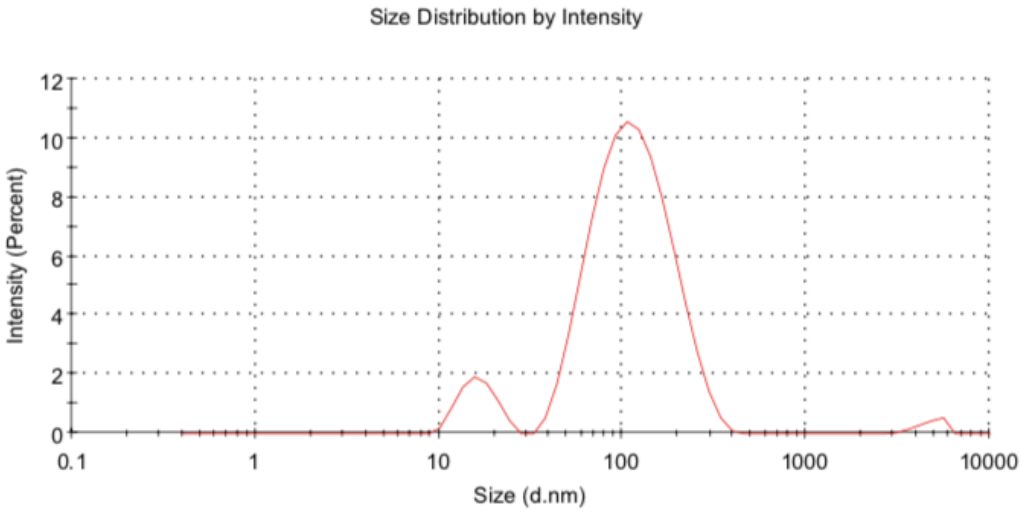


Figure 23 Dynamic light scattering (DLS) analysis

4.2.2.3 FTIR analysis

The role of phytochemical constituents in the synthesis of AuNPs was evaluated by FTIR spectrum (Figure24). The FTIR spectrum of latex were observed the O-H at 3449 cm^{-1} and the wavenumber at 2955, 2920 and 2851 cm^{-1} were assigned as C-H stretching [73, 74]. The wavenumber at 1730, 1647, 1470 and 1385 cm^{-1}

corresponding to C=O stretching of carboxylic group, C-N stretching, C-O stretching of carboxylate and O-H bending, respectively [75-78]. These vibrations were attributed to the phytochemical constituents such as proteins and phenolic compound. The characteristic peaks of SDS were observed at 1223 and 1070 cm^{-1} due to the asymmetric stretching and symmetric stretching of its sulphate head group [79]. The FTIR spectrum of synthesized latex-AuNPs showed slightly different from latex. The FTIR spectrum of latex-AuNPs was observed the addition of new vibration at 1562 cm^{-1} which was assigned as the asymmetric C-O stretching of carboxylate anion [77]. The increasing in peak area of C-O stretching around 1467-1470 cm^{-1} was also observed indicating the increasing in carboxylate moiety. The peak of S=O of SDS was slightly shifted to higher wavenumber indicating the adsorption of sulfate head group on AuNPs surface [20]. The C-N vibration of latex-AuNPs was shifted from the originated latex suggesting the binding of protein on AuNPs surface.

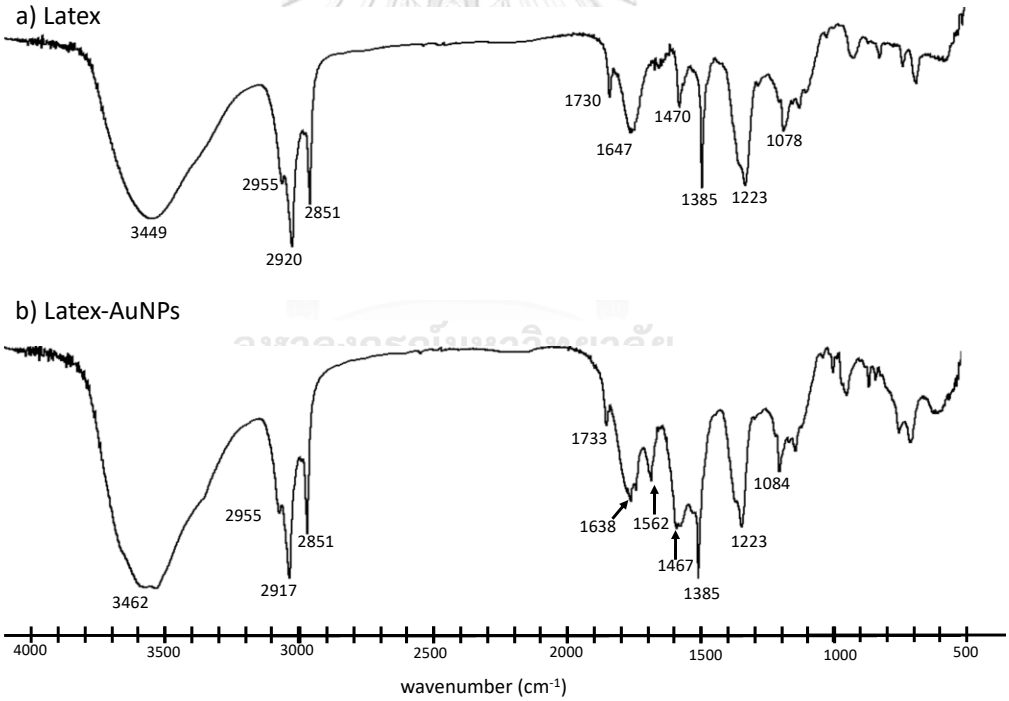


Figure 24 FTIR spectra of a) latex and b) latex-AuNPs

For green synthesis of AuNPs by using plant extract, it is well known that the OH group of phenolic, polyol and glycoside will be used to reduce Au^{3+} to Au^{0} [80].

After the electron transfer from OH to Au^{3+}, the hydroxyl group will be transformed to ketone, aldehyde and carboxylic form as shown in (Figure 25) [77]. In those situations, the FTIR analysis should be observed the decreasing of OH peak and the increasing of C=O peak. Nevertheless, our results were not observed significantly change which it was not in good agreement with the mechanism described above.

To confirm this hypothesis, the total protein and phenolic contents of the latex before and after the synthesis of AuNPs determined in order to investigate the role of protein and phenolic compound in the synthesis of AuNPs. The latex concentration at the optimum condition showed the total phenolic content with the value of 20.1 µg/mL which it was decreased to 2.58 µg/mL after the synthesis of AuNPs whereas the total protein was not significantly changed, Table 2. The results showed that the total phenolic content of latex-AuNPs was decreased from the originated latex significantly which could support the decreasing of OH after the reduction of Au^{3+}(Table2).

Table 2 The total contents of proteins and phenolic compounds presented in latex concentration at optimum condition

compound	latex	Latex-AuNPs
protein	952 µg/mL	927 µg/mL
phenolic	20.1 µg/mL	2.58 µg/mL

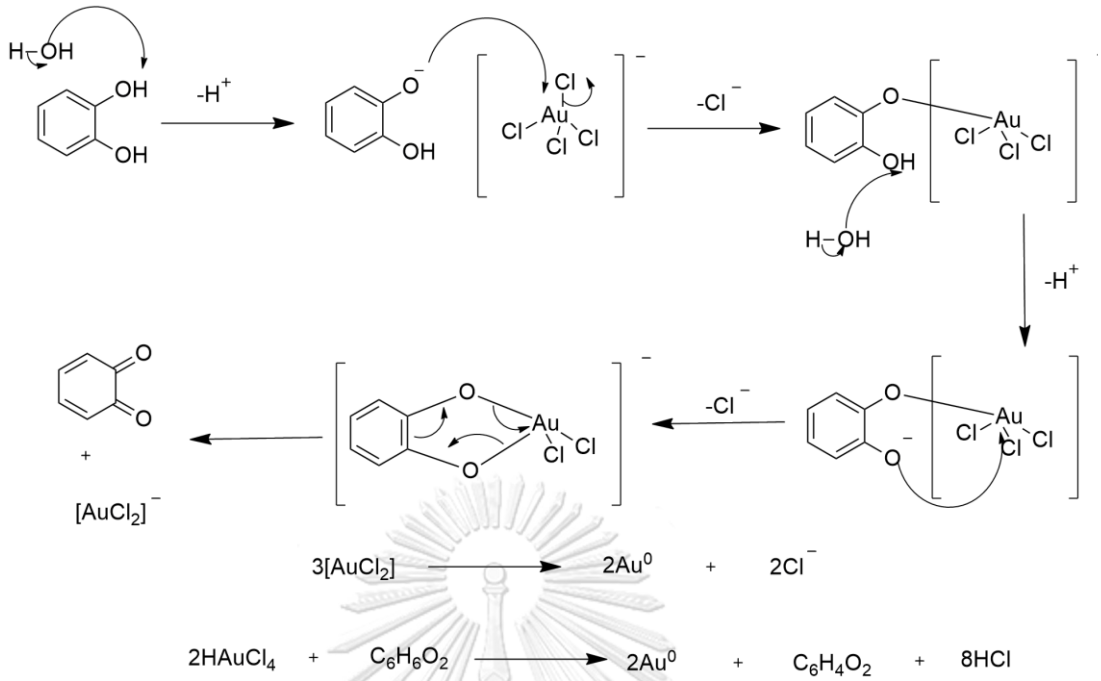


Figure 25 The mechanism of AuNPs formation which reduced by phenolic compound

Ratul Kumar Das et al. have reported the role of the plant latex biomolecule in the synthesis of AuNPs [62]. They suggested that shifting in the peak of amine and amide of latex-AuNPs from the originated position could confirm that proteins in latex could act as a stabilizer for AuNPs. For the FTIR analysis in this work, the similar trend was also observed. The total protein in latex-AuNPs was in the same range in the original latex which could confirm the role of the protein in latex.

From the FTIR analysis could suggest the role of phytochemical constituents and SDS in the latex stock solution toward the synthesis of AuNPs. Firstly, the polyol, glycoside and phenolic compounds in the latex could facilitate the reduction of Au^{3+}. At the growth stage, the proteins and SDS could act as stabilizers to prevent the self-agglomeration of AuNPs.

In this work, *C. buchan*ani latex was used to synthesize AuNPs, the green synthesis of AuNPs was performed without using of any toxic reagent. The obtained particles showed ultra-small around 5 nm at dried condition whereas the hydrodynamic size was observed at 123.4 nm. The particles showed high colloidal

stability with the negative charge on their surface due to the adsorption of biomolecule and SDS molecule on their surface. These results could suggest the possibility of the using of latex-AuNPs as a drug carrier.

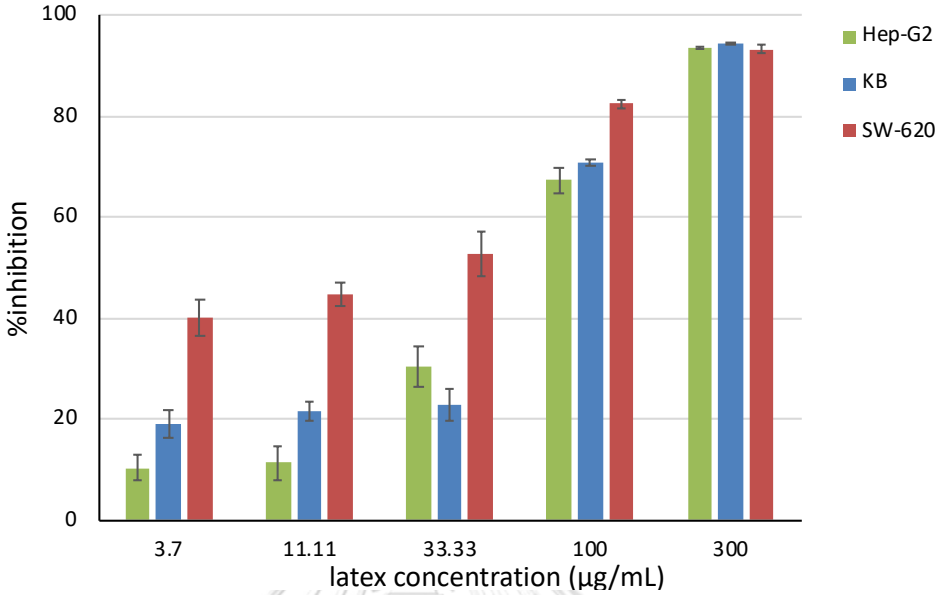
4.4 Evaluation of latex-AuNPs biological activity

According to the previous studies, the extract of *C. buchanani*, a Thai folk medicinal plant, showed the interesting biological activities such as anti-inflammatory and anti-dermatophyte activity. Thus, *C. buchanani* was used not only for the synthesis of AuNPs but also the anti-cancer and anti-inflammatory active agents. The cytotoxicity of latex and latex-AuNPs against cancer cell and normal cell was evaluated in order to investigate the anti-cancer activity. In addition, the anti-inflammatory activity was also investigated by NO assay.

4.4.1 Anti-cancer

The selectivity of latex-AuNPs toward cancer cell lines was evaluated by MTT assay. The cytotoxicity of latex and latex-AuNPs with different concentration of latex were treated in various types of cancer cell lines including human mouth epidermal carcinoma (KB), human hepatocarcinoma cell lines (Hep G2) and colorectal cancer cells (SW 620). The cytotoxicity of latex and latex-AuNPs toward cancer cell lines was evaluated in the latex concentration ranging from 3-300 µg/ml. The inhibition percentage of latex concentration toward KB, Hep G2 and SW 620 cell lines shown in Figure 26. At the same concentration of latex, both latex and latex-AuNPs specifically inhibited to SW 620 cell lines compare to KB and Hep G2. These results suggest that the phytochemical constituents in latex and latex-AuNPs have a specificity to provide the highest inhibition percentage towards SW 620. Thus, this cell line was chosen to evaluate the cytotoxicity of latex and latex-AuNPs toward cancer cell line and normal cell line.

a)



b)

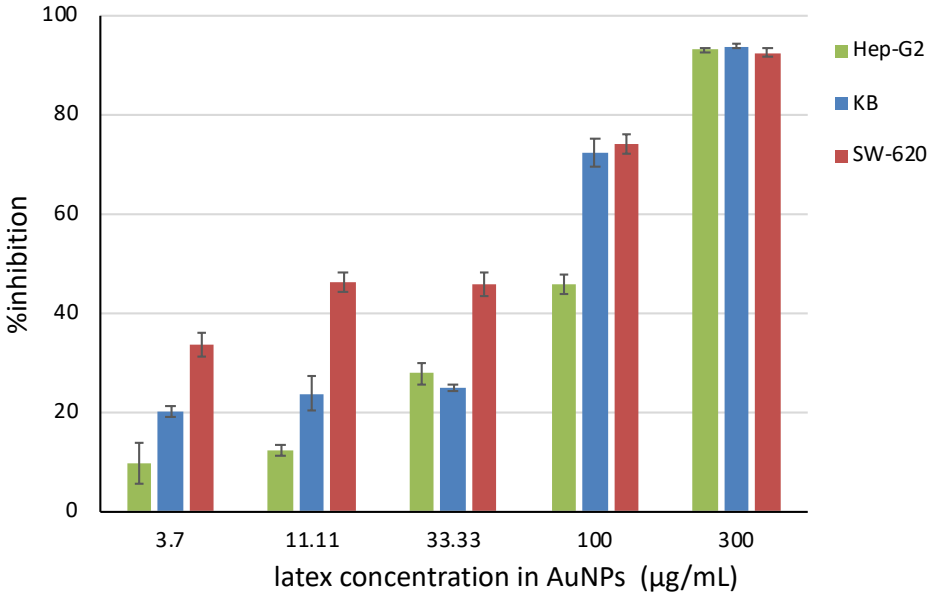


Figure 26 The percent inhibition of Hep G2, KB and SW 620 cell lines treated with a) latex and b) latex-AuNPs in the concentration range 3 to 300 µg/ml

Figure 27 showed the inhibition response of latex concentration toward SW 620 cancer cell line, the inhibition percentage was increased significantly as the function of latex concentration. This observation may cause by the phytochemical constituents in latex such as phenolic, alkaloid, glycoside and proteins. According to the previous work, the phenolic compound in the plant extract were responsible for both synthesis of AuNPs and bioactive compound [56]. In that case, the biological activity of synthesized AuNPs was significantly decreased compare to those originated plant extract as it was changed their important functional groups. In this work, the decreasing in the total phenolic content after the synthesis of AuNPs was also observed. However, the anticancer activity of originated latex and latex-AuNPs did not show any significantly different in the inhibition percentage which was not related to previous reports. This observation could be stated that the anticancer activity mainly affected by the major component in latex, proteins.

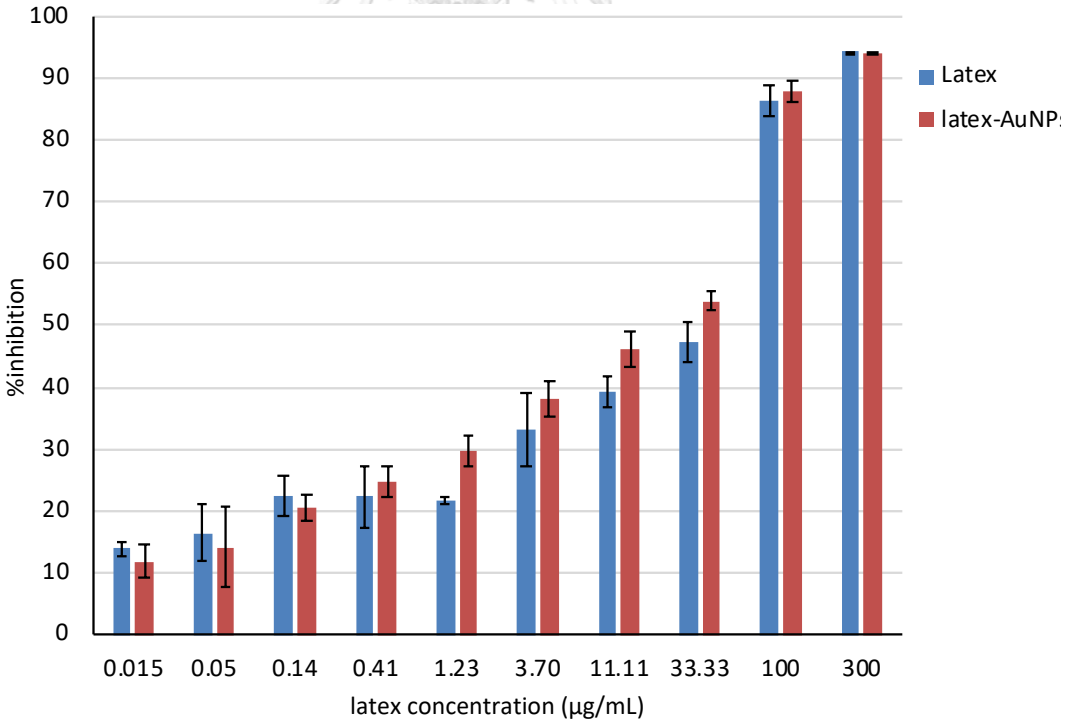


Figure 27 The percent inhibition toward SW 620 cell lines treated with latex and latex-AuNPs in the concentration range 0.015 to 300 µg/ml

The activity of the proteins in latex was reported. In 2018, Li Song *et al* reported that the cucumisin-like protease called serine protease TKP showed a potent activity to inhibit cell proliferation of human colorectal adenocarcinoma [81]. The same type of serine protease called cryptolepain was also discovered in *C. buchanani* latex [18]. Thus, we could hypothesize that the protease in the latex played a major role to inhibit the cancer cells growth.

The cytotoxicity of latex-AuNPs toward Wi 38, a normal cell line was also compared to the cancer cells. As shown in Figure 28, both latex and latex-AuNPs did not inhibit the normal cell at the latex concentration below 100 whereas the cancer cells showed 90% inhibition at the same concentration. These results implied that both latex and latex-AuNPs were specifically toxic toward cancer cell than normal cells which suggested the possibility to used latex-AuNPs as a drug carrier.

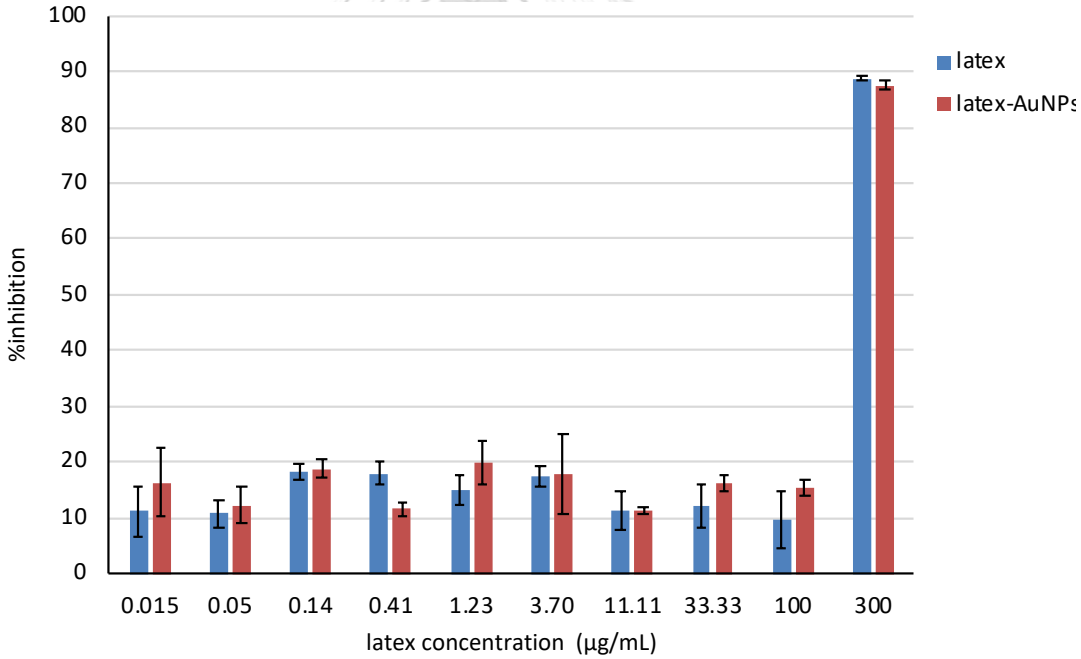


Figure 28 The percent inhibition toward Wi 38 cell lines treated with latex and latex-AuNPs in the concentration range 0.015 to 300 µg/ml

Moreover, doxorubicin was loaded on the latex-AuNPs to evaluate the capability of latex-AuNPs as a drug carrier. The latex concentration of latex-AuNPs at 3.7 µg/ml (40% inhibition) was mixed with various concentrations of doxorubicin ranging from 0.0001-10 µM. The inhibition percentage of doxorubicin loaded on latex-AuNPs and doxorubicin was investigated toward SW 620, cancer cell. The inhibition percentage of doxorubicin after loading on latex-AuNPs was improved significantly compared to the pure drug (Figure 29). This observation may cause by the binding between the negatively charged latex-AuNPs and positively charged doxorubicin. These results could confirm that latex-AuNPs could be used as a drug carrier for delivering the drug to the target site.

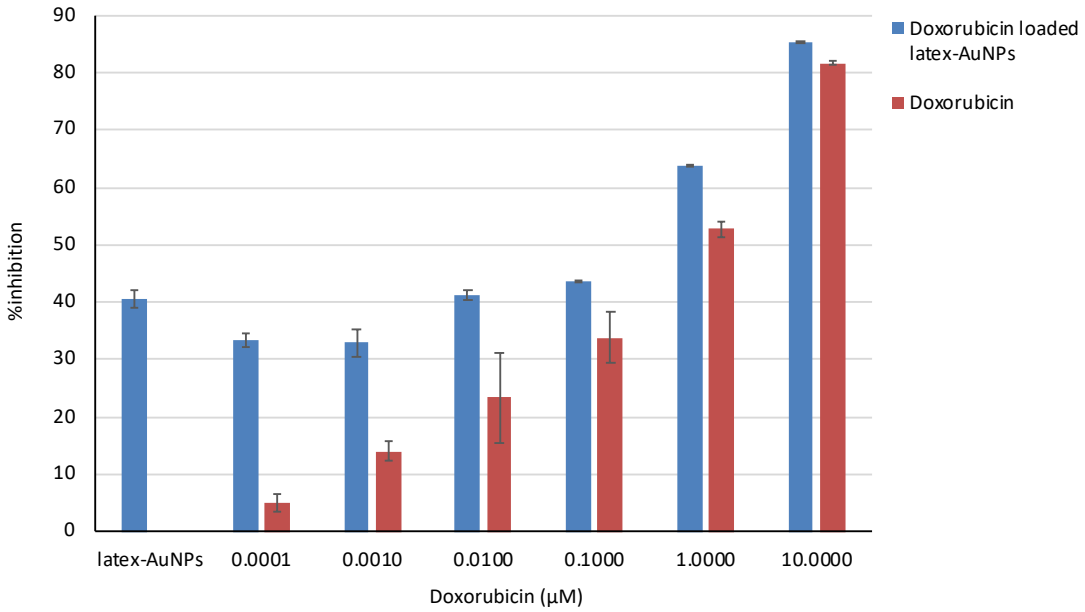


Figure 29 The percent inhibition toward SW 620 cell lines treated with latex-AuNPs and doxorubicin loaded latex-AuNPs in the doxorubicin concentration range 0.0001 to 10 µM

4.4.2 Anti-inflammatory

The latex-AuNPs were also used in the evaluation of anti-inflammatory activity. The results showed that by treating latex and latex-AuNPs in the latex concentration range 1.9-150 µg/ml showed cell viability above 80% toward RAW 264.7 cells (Figure 30). The NO content was also investigated at various concentrations. The results

showed that the increasing in latex concentration could not inhibit the NO production (Figure 31). In addition, the slightly decreasing in NO production to 28% inhibition at latex-AuNPs concentration 150 µg/ml. According to previous reports, the bark extract of *Acacia mearnsii* could be exhibited the same percent inhibition of NO production by using the extract concentration at only 50 µg/ml [82]. These results suggested that *C. buchanani* latex exhibited the lower activity toward anti-inflammatory in the comparison of other plant extract.

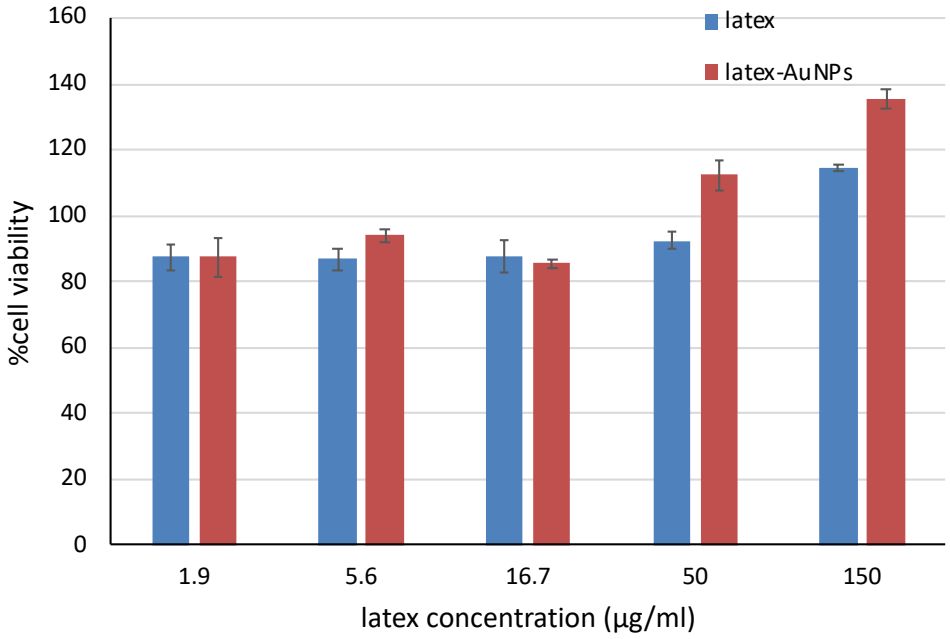


Figure 30 Cytotoxicity of latex and latex-AuNPs toward RAW 264.7 cell line

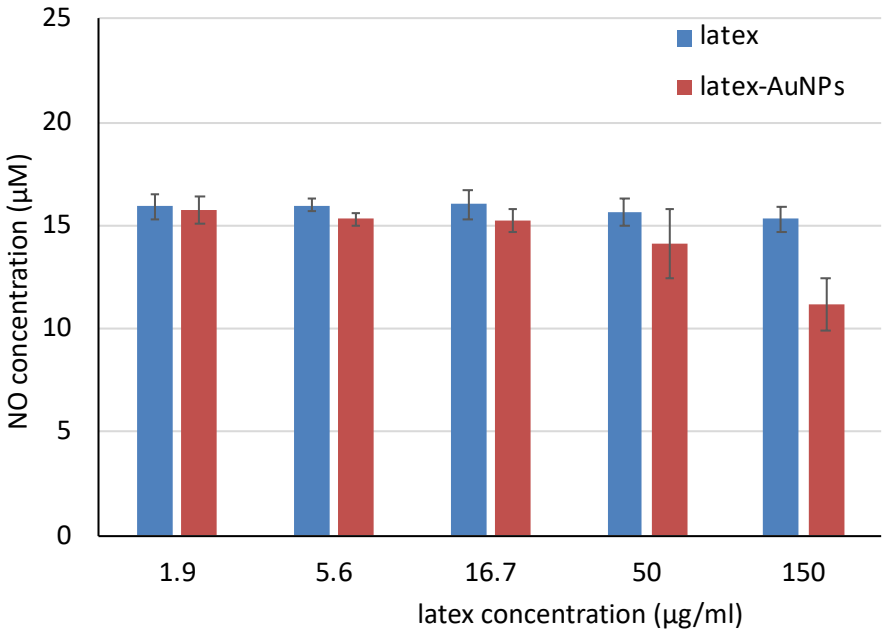


Figure 31 NO production of RAW 264.7 treated with various concentration of latex and latex-AuNPs

Chapter V
CONCLUSION

In this work, the green synthesis of AuNPs was done by using *C. buchanai* latex as reducing and stabilizing agents. The *C. buchanani* latex was collected and dissolved in SDS solution in order to obtain the homogeneous latex stock solution. To synthesize AuNPs, the latex stock solution was used as reducing and stabilizing agents. The effects latex concentration, volume of 1%NaOH, reaction temperature and volume of 1%$HAuCl_4$ were studied toward the LSPR of synthesized AuNPs. The results showed that the smallest size of AuNPs could be formed by mixing of 20:1 of the ratio latex to $HAuCl_4$ with 30 µl of 1%NaOH and 20 µl of 1%$HAuCl_4$ which they were mixed and stirred continuously at 60°C of reaction temperature. This method was performed without the using of toxic reagent so it could be considered as a simple, low-cost and non-toxic method to obtained AuNPs.

The latex-AuNPs showed the small size around 5 nm and the zeta potential exhibited the highly negative charge on their surface which imply the long-term stability of the particles. The FTIR analysis was used to confirm the adsorption of biomolecule and SDS on AuNPs surface which could support the zeta potential result. The decreasing in total phenolic contents after the synthesis of AuNPs suggested the role of phenolic compound in the reduction of Au^{3+} which could be used to confirm the FTIR results. In addition, the role of the proteins in latex as a stabilizing agent was confirmed by the determination of total protein contents which it was not shown any significant change after the synthesis of AuNPs.

The evaluation of biological activity of latex and latex-AuNPs showed potential inhibition toward cancer cells whereas they showed lower cytotoxicity toward cancer cells. Both of latex and latex-AuNPs did not show significantly different for the cytotoxicity toward cancer and normal cell lines. These results implied that the

cytotoxicity of latex and latex-AuNPs mainly affected by those phytoconstituents which was not changed after the synthesis of AuNPs. Moreover, the possibility to use latex-AuNPs as a drug carrier was confirmed by loading of doxorubicin, a commercial drug, into the latex-AuNPs solution. The results showed that doxorubicin loaded latex-AuNPs could improve the inhibition percentage toward cancer cell of doxorubicin at the same concentration.

REFERENCES

[1] Misra, R., Acharya, S., and Sahoo, S.K. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discovery Today 15(19) (2010): 842-850.

[2] Khlebtsov, N. and Dykman, L. Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. Chemical Society Reviews 40(3) (2011): 1647-1671.

[3] Alkilany, A.M. and Murphy, C.J. Toxicity and cellular uptake of gold nanoparticles: what we have learned so far? Journal of Nanoparticle Research 12(7) (2010): 2313-2333.

[4] Raveendran, P., Fu, J., and Wallen, S.L. Completely "Green" Synthesis and Stabilization of Metal Nanoparticles. Journal of the American Chemical Society 125(46) (2003): 13940-13941.

[5] Kimling, J., Maier, M., Okenve, B., Kotaidis, V., Ballot, H., and Plech, A. Turkevich Method for Gold Nanoparticle Synthesis Revisited. The Journal of Physical Chemistry B 110(32) (2006): 15700-15707.

[6] Chahardoli, A., Karimi, N., and Fattahi, A. Nigella arvensis leaf extract mediated green synthesis of silver nanoparticles: Their characteristic properties and biological efficacy. Advanced Powder Technology 29(1) (2018): 202-210.

[7] Smitha, S.L., Philip, D., and Gopchandran, K.G. Green synthesis of gold nanoparticles using Cinnamomum zeylanicum leaf broth. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 74(3) (2009): 735-739.

[8] Gopinath, K., Gowri, S., Karthika, V., and Arumugam, A. Green synthesis of gold nanoparticles from fruit extract of Terminalia arjuna, for the enhanced seed germination activity of Gloriosa superba. Journal of Nanostructure in Chemistry 4(3) (2014): 115.

[9] Suman, T.Y., Radhika Rajasree, S.R., Ramkumar, R., Rajthilak, C., and Perumal, P. The Green synthesis of gold nanoparticles using an aqueous root extract of Morinda citrifolia L. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 118 (2014): 11-16.

[10] Shameli, K., et al. Green Biosynthesis of Silver Nanoparticles Using Callicarpa maingayi Stem Bark Extraction. Molecules 17(7) (2012).

[11] Ramos, M.V., Demarco, D., da Costa Souza, I.C., and de Freitas, C.D.T. Laticifers, Latex, and Their Role in Plant Defense. Trends in Plant Science 24(6) (2019): 553-567.

[12] Das, R.K., Babu, P.J., Gogoi, N., Sharma, P., and Bora, U.J.I.N. Microwave-mediated rapid synthesis of gold nanoparticles Using Calotropis procera latex and study of optical properties. 2012 (2012).

[13] Panthong, A., Kanjanapothi, D., and Taylor, W.J.J.o.E. Ethnobotanical review of medicinal plants from Thai traditional books, Part I: Plants with anti-inflammatory, anti-asthmatic and antihypertensive properties. 18(3) (1986): 213-228.

[14] Laupattarakasem, P., Houghton, P., Hoult, J., and Itharat, A.J.J.o.E. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. 85(2-3) (2003): 207-215.

[15] Hanprasertpong, N., et al. Analgesic, Anti-Inflammatory, and Chondroprotective Activities of Cryptolepis buchanani Extract: In Vitro and In Vivo Studies %J BioMed Research International. 2014 (2014): 8.

[16] Vinayaka, K., Prashith, K.T., Mallikarjun, N., and Sateesh, V.J.P.J. Anti-dermatophyte activity of Cryptolepis buchanani Roem. & Schult. 2(7) (2010): 170-172.

[17] Padmalochana, K. and Rajan, M.D.J.J.o.A.P.S. Evaluation of the antioxidant and hepatoprotective activity of Cryptolepis buchanani. 3(2) (2013): 99.

[18] Pande, M., Dubey, V.K., Yadav, S.C., and Jagannadham, M.V. A Novel Serine Protease Cryptolepain from Cryptolepis buchanani: Purification and Biochemical Characterization. Journal of Agricultural and Food Chemistry 54(26) (2006): 10141-10150.

[19] Schloman, W.W. Reduced-lipid natural rubber latex. 1999, Google Patents.

[20] Cabrera, G.F.S., et al. Green synthesis of gold nanoparticles reduced and stabilized by sodium glutamate and sodium dodecyl sulfate. Biochemical and Biophysical Research Communications 484(4) (2017): 774-780.

[21] Norhanifah, M.Y., Nurulhuda, A., and Asrul, M. The Influence of Deproteinisation in the Morphology of Natural Rubber Latex Particles and Subsequent Film Formation. Procedia Chemistry 16 (2015): 31-38.

[22] De Jong, W.H. and Borm, P.J.A. Drug delivery and nanoparticles:applications and hazards. International journal of nanomedicine 3(2) (2008): 133-149.

[23] Ferrari, M. Cancer nanotechnology: opportunities and challenges. Nature Reviews Cancer 5(3) (2005): 161-171.

[24] Park, J.H., Lee, S., Kim, J.-H., Park, K., Kim, K., and Kwon, I.C. Polymeric nanomedicine for cancer therapy. Progress in Polymer Science 33(1) (2008): 113-137.

[25] Parveen, S. and Sahoo, S.K. Polymeric nanoparticles for cancer therapy. Journal of Drug Targeting 16(2) (2008): 108-123.

[26] Mitra, S., Gaur, U., Ghosh, P.C., and Maitra, A.N. Tumour targeted delivery of encapsulated dextran–doxorubicin conjugate using chitosan nanoparticles as carrier. Journal of Controlled Release 74(1) (2001): 317-323.

[27] Pandey, S.K., et al. Controlled release of drug and better bioavailability using poly(lactic acid-co-glycolic acid) nanoparticles. International Journal of Biological Macromolecules 89 (2016): 99-110.

[28] Bolotin, E.M., et al. Ammonium Sulfate Gradients for Efficient and Stable Remote Loading of Amphipathic Weak Bases into Liposomes and Ligandoliposomes. Journal of Liposome Research 4(1) (1994): 455-479.

[29] Sapra, P. and Allen, T.J.P.i.l.r. Ligand-targeted liposomal anticancer drugs. 42(5) (2003): 439-462.

[30] Jiang, T., et al. Dual-functional liposomes based on pH-responsive cell-penetrating peptide and hyaluronic acid for tumor-targeted anticancer drug delivery. Biomaterials 33(36) (2012): 9246-9258.

[31] Guo, X. and Szoka, F.C. Steric Stabilization of Fusogenic Liposomes by a Low-pH Sensitive PEG–Diortho Ester–Lipid Conjugate. Bioconjugate Chemistry 12(2) (2001): 291-300.

[32] Okazaki, M., Yoshida, Y., Yamaguchi, S., Kaneno, M., and Elliott, J.C. Affinity binding phenomena of DNA onto apatite crystals. Biomaterials 22(18) (2001): 2459-2464.

[33] Lee, M.S., et al. Target-specific delivery of siRNA by stabilized calcium phosphate nanoparticles using dopa–hyaluronic acid conjugate. Journal of Controlled Release 192 (2014): 122-130.

[34] Raynal, I., Prigent, P., Peyramaure, S., Najid, A., Rebuzzi, C., and Corot, C.J.I.r. Macrophage endocytosis of superparamagnetic iron oxide nanoparticles: mechanisms and comparison of ferumoxides and ferumoxtran-10. 39(1) (2004): 56-63.

[35] Lim, Z.-Z.J., Li, J.-E.J., Ng, C.-T., Yung, L.-Y.L., and Bay, B.-H. Gold nanoparticles in cancer therapy. Acta Pharmacologica Sinica 32 (2011): 983.

[36] Senapati, S., Mahanta, A.K., Kumar, S., and Maiti, P. Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduction and Targeted Therapy 3(1) (2018): 7.

[37] Wong, I.Y., Bhatia, S.N., Toner, M.J.G., and development. Nanotechnology: emerging tools for biology and medicine. 27(22) (2013): 2397-2408.

[38] Li, H., Jin, H., Wan, W., Wu, C., and Wei, L.J.N. Cancer nanomedicine: mechanisms, obstacles and strategies. 13(13) (2018): 1639-1656.

[39] Bertrand, N., Wu, J., Xu, X., Kamaly, N., and Farokhzad, O.C. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Advanced Drug Delivery Reviews 66 (2014): 2-25.

[40] Arvizo, R.R., Bhattacharyya, S., Kudgus, R.A., Giri, K., Bhattacharya, R., and Mukherjee, P. Intrinsic therapeutic applications of noble metal nanoparticles: past, present and future. Chemical Society Reviews 41(7) (2012): 2943-2970.

[41] Roma-Rodrigues, C., Raposo, R.L., Cabral, R., Paradinha, F., Baptista, V.P., and Fernandes, R.A. Tumor Microenvironment Modulation via Gold Nanoparticles

Targeting Malicious Exosomes: Implications for Cancer Diagnostics and Therapy. International Journal of Molecular Sciences 18(1) (2017).

[42] Kundu, S., Peng, L., and Liang, H. A New Route to Obtain High-Yield Multiple-Shaped Gold Nanoparticles in Aqueous Solution using Microwave Irradiation. Inorganic Chemistry 47(14) (2008): 6344-6352.

[43] Lee, J.-H., Choi, S.U.S., Jang, S.P., and Lee, S.Y. Production of aqueous spherical gold nanoparticles using conventional ultrasonic bath. Nanoscale Research Letters 7(1) (2012): 420.

[44] Mafuné, F., Kohno, J.-y., Takeda, Y., Kondow, T., and Sawabe, H. Formation of Gold Nanoparticles by Laser Ablation in Aqueous Solution of Surfactant. The Journal of Physical Chemistry B 105(22) (2001): 5114-5120.

[45] Lunardi, C.N., Barros, M.P.F., Rodrigues, M.L., and Gomes, A.J. Synthesis of gold nanoparticles using Euphorbia tirucalli latex and the microwave method. Gold Bulletin 51(4) (2018): 131-137.

[46] Turkevich, J., Stevenson, P., and Hiller, J.J.D.F.S. Synthesis of gold nanoparticles Turkevich method. 11 (1951): 55-75.

[47] Ngumbi, P.K., Mugo, S.W., Ngaruiya, J.M., and King'ondu, C.K. Multiple plasmon resonances in small-sized citrate reduced gold nanoparticles. Materials Chemistry and Physics 233 (2019): 263-266.

[48] Leff, D.V., Brandt, L., and Heath, J.R. Synthesis and Characterization of Hydrophobic, Organically-Soluble Gold Nanocrystals Functionalized with Primary Amines. Langmuir 12(20) (1996): 4723-4730.

[49] Martin, M.N., Basham, J.I., Chando, P., and Eah, S.-K. Charged Gold Nanoparticles in Non-Polar Solvents: 10-min Synthesis and 2D Self-Assembly. Langmuir 26(10) (2010): 7410-7417.

[50] Brust, M., Walker, M., Bethell, D., Schiffrin, D.J., and Whyman, R. Synthesis of thiol-derivatised gold nanoparticles in a two-phase Liquid–Liquid system. Journal of the Chemical Society, Chemical Communications (7) (1994): 801-802.

[51] Shao, Y., Jin, Y., and Dong, S. Synthesis of gold nanoplates by aspartate reduction of gold chloride. Chemical Communications (9) (2004): 1104-1105.

[52] Perrault, S.D. and Chan, W.C.W. Synthesis and Surface Modification of Highly Monodispersed, Spherical Gold Nanoparticles of 50–200 nm. Journal of the American Chemical Society 131(47) (2009): 17042-17043.

[53] Sujitha, M.V. and Kannan, S. Green synthesis of gold nanoparticles using Citrus fruits (Citrus limon, Citrus reticulata and Citrus sinensis) aqueous extract and its characterization. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 102 (2013): 15-23.

[54] Iravani, S. Green synthesis of metal nanoparticles using plants. Green Chemistry 13(10) (2011): 2638-2650.

[55] Sadeghi, B., Mohammadzadeh, M., and Babakhani, B. Green synthesis of gold nanoparticles using Stevia rebaudiana leaf extracts: Characterization and their stability. Journal of Photochemistry and Photobiology B: Biology 148 (2015): 101-106.

[56] Chahardoli, A., Karimi, N., Sadeghi, F., and Fattahi, A. Green approach for synthesis of gold nanoparticles from Nigella arvensis leaf extract and evaluation of their antibacterial, antioxidant, cytotoxicity and catalytic activities. Artificial Cells, Nanomedicine, and Biotechnology 46(3) (2018): 579-588.

[57] Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P., and Misra, A. Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and Surfaces A: Physicochemical and Engineering Aspects 339(1) (2009): 134-139.

[58] Webb, A.R., Mythen, M.G., Jacobson, D., and Mackie, I.J. Maintaining blood flow in the extracorporeal circuit: haemostasis and anticoagulation. Intensive Care Medicine 21(1) (1995): 84-93.

[59] Padmalochana, K. and Elangovan, L. Evaluation of the Antioxidant and Hepatoprotective Activity of Cryptolepis Buchanani. 2013.

[60] Dutta, S.K., Sharma, B.N., and Sharma, P.V. Buchananine, a novel pyridine alkaloid from Cryptolepis buchanani. Phytochemistry 17(11) (1978): 2047-2048.

[61] Venkateswara, R., Sankara Rao, K., and Vaidyanathan, C.S. Cryptosin — a new cardenolide in tissue culture and intact plants of Cryptolepis buchanani Roem. & Schult. Plant Cell Reports 6(4) (1987): 291-293.

[62] Das, R.K., Sharma, P., Nahar, P., and Bora, U. Synthesis of gold nanoparticles using aqueous extract of Calotropis procera latex. Materials Letters 65(4) (2011): 610-613.

[63] Moreno-Luna, F.B., Tovar-Corona, A., Herrera-Perez, J.L., Santoyo-Salazar, J., Rubio-Rosas, E., and Vázquez-Cuchillo, O. Quick synthesis of gold nanoparticles at low temperature, by using Agave potatorum extracts. Materials Letters 235 (2019): 254-257.

[64] Annur, S., Santosa, S.J., and Aprilita, N.H.J.O.J.o.C. pH Dependence of Size Control in Gold Nanoparticles Synthesized at Room Temperature. 34(5) (2018): 2305-2312.

[65] Jana, N.R., Gearheart, L., and Murphy, C.J. Seeding Growth for Size Control of 5−40 nm Diameter Gold Nanoparticles. Langmuir 17(22) (2001): 6782-6786.

[66] Huang, X. and El-Sayed, M. Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. Vol. 1, 2010.

[67] Njoki, P.N., et al. Size Correlation of Optical and Spectroscopic Properties for Gold Nanoparticles. The Journal of Physical Chemistry C 111(40) (2007): 14664-14669.

[68] Khlebtsov, N.G. Determination of Size and Concentration of Gold Nanoparticles from Extinction Spectra. Analytical Chemistry 80(17) (2008): 6620-6625.

[69] Zayed, M.F., Eisa, W.H., El-kousy, S.M., Mleha, W.K., and Kamal, N. Ficus retusa-stabilized gold and silver nanoparticles: Controlled synthesis, spectroscopic characterization, and sensing properties. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 214 (2019): 496-512.

[70] Guo, M., Li, W., Yang, F., and Liu, H. Controllable biosynthesis of gold nanoparticles from a Eucommia ulmoides bark aqueous extract. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 142 (2015): 73-79.

[71] R, N., S, M., S, J.P., and P, P. Green synthesis and characterization of bioinspired silver, gold and platinum nanoparticles and evaluation of their synergistic antibacterial activity after combining with different classes of antibiotics. Materials Science and Engineering: C 96 (2019): 693-707.

[72] Piella, J., Bastús, N.G., and Puntes, V. Size-Controlled Synthesis of Sub-10-nanometer Citrate-Stabilized Gold Nanoparticles and Related Optical Properties. Chemistry of Materials 28(4) (2016): 1066-1075.

[73] Kataki, S., Hazarika, S., and Baruah, D.C. Investigation on by-products of bioenergy systems (anaerobic digestion and gasification) as potential crop nutrient using FTIR, XRD, SEM analysis and phyto-toxicity test. Journal of Environmental Management 196 (2017): 201-216.

[74] Chen, S., Wang, S., Li, L., Qu, J., Tao, X., and He, H. Exploration on the mechanism of enhancing low-rank coal flotation with cationic surfactant in the presence of oily collector. Fuel 227 (2018): 190-198.

[75] Hamed, S.A.A.K.M. and Hassan, M.L. A new mixture of hydroxypropyl cellulose and nanocellulose for wood consolidation. Journal of Cultural Heritage 35 (2019): 140-144.

[76] Vishnoi, N., Dixit, S., and Singh, D.P. Surface binding and intracellular uptake of arsenic in bacteria isolated from arsenic contaminated site. Ecological Engineering 73 (2014): 569-578.

[77] Laksee, S., Puthong, S., Kongkavitoon, P., Palaga, T., and Muangsin, N. Facile and green synthesis of pullulan derivative-stabilized Au nanoparticles as drug carriers for enhancing anticancer activity. Carbohydrate Polymers 198 (2018): 495-508.

[78] Osibe, D.A. and Aoyagi, H. A novel strategy for the synthesis of gold nanoparticles with Catharanthus roseus cell suspension culture. Materials Letters 238 (2019): 317-320.

[79] Zeng, X., Xu, L., Tian, J., Yin, W., Yang, Y., and Deng, W. Effect of a CA depressant on flotation separation of celestite from fluorite and calcite using SDS as a collector. Minerals Engineering 111 (2017): 201-208.

[80] Swargiary, M. and Kumar, S. One pot phytosynthesis of gold nanoparticles using aqueous extract of elephant apple- an eco-friendly approach. Oriental Pharmacy and Experimental Medicine 17(3) (2017): 285-289.

[81] Song, L., Xu, X., and Li, Z. A serine protease extracted from Trichosanthes kirilowii inhibits epithelial-mesenchymal transition via antagonizing PKM2-

mediated STAT3/Snail1 pathway in human colorectal adenocarcinoma cells. Journal of Functional Foods 40 (2018): 639-647.

[82] Xiong, J., Grace, M.H., Esposito, D., Komarnytsky, S., Wang, F., and Lila, M.A. Polyphenols isolated from Acacia mearnsii bark with anti-inflammatory and carbolytic enzyme inhibitory activities. Chinese Journal of Natural Medicines 15(11) (2017): 816-824.

VITA

NAME	Kittiphong Thongsuk
DATE OF BIRTH	28 September 1992
PLACE OF BIRTH	Chachoengsao
INSTITUTIONS ATTENDED	Burapha University
HOME ADDRESS	150/2 Nongnae, Phanomsarakham, Chachoengsao