

RESEARCH ARTICLE

Antimicrobial Potential of *Andrographis paniculata* Conjugated Gold Nanoparticle

Nurulshima Razalli¹, Subash C. B. Gopinath^{1,2*}, Farizul Hafiz Kasim^{1,3}, Ahmad Radi Wan Yaakub¹, Periasamy Anbu⁴

¹School of Bioprocess Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

²Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia

³Centre of Excellence for Biomass Utilization, School of Bioprocess Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

⁴Department of Biological Engineering, College of Engineering, Inha University, Incheon 402-751, Republic of Korea

*Correspondence to: Subash C. B. Gopinath, email: subash@unimap.edu.my

Received: May 23, 2019 **Accepted:** July 4, 2019 **Published:** July 17, 2019 **DOI:** doi.org/10.26689/itps.v2i1.744

Copyright: Razalli et al. This is an open-access article distributed under the terms of the Attribution-Non-Commercial 4.0 International 4.0 (CC BY-NC 4.0), which permits all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract: Background: Herbal extracts have been traditionally used as antibacterial agents, and different strategies have been proposed to enhance their antimicrobial activities. This research aimed to assess the antimicrobial potential of *Andrographis paniculata* conjugated gold nanoparticle (GNP) compounds against *Escherichia coli* and *Bacillus subtilis*.

Methods: Herbal extract was conjugated with GNP through electrostatic interaction, and characterization was carried out by Fourier-transmission infrared spectroscopy, screening electron microscopy, and transmission electron microscopy and supported by energy-dispersive X-ray spectroscopy. Antibacterial activity of the herbal extract and GNP conjugation was evaluated by disc diffusion assay.

Results: Chemical and morphological characterizations of the GNP and herbal extract conjugate revealed intactness of the GNP. In disc diffusion assay, the inhibition zone formed by these compounds was measured for both microorganisms, and they were in the range of 0.6-0.9 cm for the herbal extract. When the herbal extract conjugated with GNP, the zone was between 0.8 and 1.1 cm.

Conclusion: This study demonstrated the potentiality of GNP to carry the antibacterial compounds from the herbal extract.

Keywords: *Andrographis paniculata*; Gold nanoparticle; Antimicrobial potential; Herbal extract

1. INTRODUCTION

Before the existence of microbes was discovered, humans believed that plants have healing properties, reflecting the current antimicrobial characterization principles [1,2]. Formerly, humans utilized plants to treat infectious diseases, and the so-called “traditional medicines” are still being used for the treatment of various illnesses [3-5]. The original plants are the sources of natural compounds which have the antimicrobial potential and are likely to become the new anti-infection agents. In the previous studies, it was said that the search on novel anti-infection or antimicrobial agents has been underway in various fields including ethnopharmacology. Different studies

have focused on investigating the antimicrobial activity of plant extract. With these developments, many assays are generated involving the extracts from different parts of plant such as stem, leave, or root of the plant [6-8].

Hempedu bumi (*Andrographis paniculata*) is a Ayurveda Herb shrub that belongs to the Acanthaceae family [9,10]. *A. paniculata* grows predominantly in Taiwan, Sri Lanka, China, and India. In China, this plant is used as the herbal medicine for various illnesses. Recently, *A. paniculata* herb is widely used to treat different diseases [11-14].

Nanotechnology is an emerging area of study that gains increasing interest, and it focuses on the development of biological and synthetic methods in nanoparticle

engineering [15-18]. Due to the enhanced properties of nanoparticles, their application grows rapidly on various scopes such as biomedical, pharmaceutical, antimicrobial, and also, drug delivery [19-22]. Metallic nanoparticles have been used to capture the natural compounds to reveal the antimicrobial activity attributed to the high surface area to the volume ratio of the nanoparticles that allow accommodation of large amount of compounds. Various metallic nanoparticles such as, platinum, silver, and gold were focused recently because of their requisite importance [23,24]. Among the previously mentioned metal nanoparticles, gold nanoparticle (GNP) is more vital because of its history in medicinal application, e.g., treatment of cancer and arthritis with conjugated GNP and also due to their biocompatibility [25-29]. In this research, GNP was purchased, and compounds of *A. paniculata* herbal plant leaf were captured on the GNP.

Gold is one of the metals that were widely used to produce nanoparticles for medicinal purposes, food packaging, and manufacturing. This forms the basis to investigate the antimicrobial potential of herbal extract captured by the GNPs in this study [30,31]. There are various methods for synthesizing nanoparticles, and they have been applied in different *in vitro* diagnostic utilities. The chemical compositions of plant extract might be involved and responsible for the bioreduction of metal ions [31-34]. Both gold and silver nanoparticles show the broad spectrum of antimicrobial potential against human and animal pathogens. Nanoparticles are also found to inactivate proteins and interfere with the DNA replication. Numerous literature have discussed the mechanisms of antimicrobial activity exerted by the gold and silver nanoparticles [35,36].

GNPs have diameter ranging from a few to several hundreds of nanometer, and they consist of gold core and surface coating. Gold core defines the fundamental properties of a GNP, and the layer surrounding the core is formed by the electrostatic interaction of the molecules in the biological milieu. GNPs were used for various applications such as gene delivery, drug delivery, antitumor, antimicrobial, cancer therapy, antioxidant, and catalytic use [23,37-39]. The present study aimed to demonstrate that the active components in *A. paniculata* are responsible for antimicrobial potential.

2. MATERIALS AND METHODS

2.1. Reagents and Biomolecules

GNP was selected to study the effectiveness of bactericidal agent against Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria. These bacterial species were provided by the School of Bioprocess Engineering, Universiti Malaysia Perlis. The main material used in the experiment to evaluate the inhibitory effects of compounds in *A. paniculata* leaves which were purchased from Ethno Resources Sdn. Bhd. in Selangor, Malaysia. GNP which was used to capture the herbal compounds was purchased from Sigma Aldrich, USA. Two samples namely, *A. paniculata* herbal extract and *A. paniculata* conjugated GNP were used in this experiment. Other materials used were nutrient agar, nutrient broth, and ampicillin which were purchased from Sigma-Aldrich, USA.

2.2. Extraction of *A. paniculata* and Conjugation of Herbal Extract with GNP

Five grams of *A. paniculata* powder was weighed, added to 50 mL of sterile distilled water, and allowed to suspend overnight. The collected pure herbal extract was then heated on a hot plate at 55°C for 10 min. The extracted solution was cooled and filtered using the filter paper. The extracted solution was also subject to fine contamination removal by filtration using syringe filter. Finally, sterile Falcon tube was used to store the herbal extract solution and it was kept at 4°C for further use [10].

To conjugate, the obtained herbal extract was mixed with the purchased GNP. After incubation for 30 min, the herbal extract conjugated GNP was centrifuged. Collected pellet was washed thrice with plenty of water and used for further characterization [10].

2.3. Characterization of *A. paniculata* Conjugated GNP

2.3.1. Morphological Observation

Scanning electron microscopy (SEM) (Hitachi, S-4300 SE, Japan) and transmission electron microscopy (TEM) were used for characterizing the morphology of the nanoparticles. Experiments were conducted with different magnifications to determine the shape, size, and distribution of the particles. In this experiment, herbal extract conjugated GNP was characterized by TEM (JEM-2100F; JEOL Ltd., Japan). The presence of GNP was confirmed using energy-dispersive X-ray (EDX) spectroscopy. Sample preparation for TEM was done by depositing on a copper grid, and the samples were analyzed after evaporating the liquid portion [2,10].

2.3.2. Fourier-Transform Infrared (Ftir) Spectroscopy

The FTIR spectroscopy was performed to analyze both dissolved leaf sample and the conjugated GNP. The analyzed samples include *A. paniculata* herbal extract, GNP, and *A. paniculata* herbal extract conjugated GNP. The samples were analyzed using Shimadzu 8400 s within a spectral range of 400-4000 cm⁻¹. The purpose of this method was to predict the chemical functional groups of the samples [2,10].

2.4. Evaluation of Antimicrobial Activity

2.4.1. Preparation of *Bacillus* Spp. And *E. Coli*

First, a pinpoint of inoculum of each microorganism was taken from the basic culture, and they were grown independently in the sterile culture tube containing 3 mL of sterilized broth. The bacterium inoculated broth was grown overnight, and then, the number of colonies with 10⁷ cells was used for the disc diffusion assay in nutrient agar plate. All the materials used were sterile and have been autoclaved at 121°C for 15 min [10].

2.4.2. Preparation of Nutrient Agar Plate

A 14 g of nutrient agar powder was weighed and suspended in 1 L of distilled water. The solution was sterilized in autoclave, and the nutrient agar solution was left to cool until reaching 50°C. It was then transferred into the sterile Petri dishes

under the sterile condition to prevent contamination. The Petri dishes were sealed and covered using Parafilm™ and stored in refrigerator until the next usage to avoid contamination.

2.4.3. Disc Diffusion Assay

Disc diffusion assay is one of the methods that can be used to evaluate antimicrobial activity. Filter paper was used to absorb the solution on the surface of nutrient agar plate [10]. The filter paper was punched into small circles and sterilized by autoclaving. The sterilized filter paper discs were kept in a sealed container for further use. Experiments were performed in triplicates.

2.4.4. Preparation of Dilutions

Dilutions were made and tested to get the minimum inhibition concentration by the microbial activity using disc diffusion method. The ratio of the sterile distilled water:*A. paniculata* herbal extract solution was 1:1. Initially, 500 µL of *A. paniculata* herbal extract solution was transferred into the first test tube (1) using micropipette. Then, 250 µL of *A. paniculata* herbal extract solution from test tube (1) was transferred into test tube (2), and 250 µL of the sterile distilled water was added. These steps were repeated for following test tubes (3-5). The dilutions were made using micropipette with sterile tip to prevent contamination.

2.4.5. Disc Diffusion Method and Determination of Minimum Inhibitory Concentration (Mic)

Initially, 100 µL of *B. subtilis*. (10^7 cells) was pipetted onto the agar plate then spread well. Using forceps, sterile filter paper discs were transferred onto the surface of the agar plate carefully. The filter paper discs were labeled properly, and each filter paper disc was loaded with different amounts of solutions (20, 25, 30, 35, 40, and 45 µL) from the dilutions made. Then, the agar plates were placed in the incubator set at 37°C overnight and monitored on the next day. Different dilutions containing 20, 25, 30, 35, 40, and 45 µg of compounds from the plant extract were tested to determine the MIC. These steps for the disc diffusion method were repeated for *E. coli* as well to find the MIC. A clear zone of inhibition formed at the end of the incubation and diameter was measured using a ruler [10]. The experiment was repeated using conjugated GNP sample. Experiments were performed in triplicates.

3. RESULTS AND DISCUSSION

In this study, compounds of *A. paniculata* leaf extract were collected and conjugated with GNP. The complex compounds were characterized morphologically and chemically by SEM, TEM, and FTIR. Furthermore, the antimicrobial potential of the conjugated GNP and herbal extract was tested separately (Fig. 1).

3.1. SEM

SEM analysis was performed to determine the size of the particles consisted in the complex of herbal extract and GNPs

(Fig. 2A and B). This study confirmed the intactness and sizes of GNPs, indicating that the GNP nanoparticles can be used for this study. The mechanism of the properly sized GNP entry into the cell wall is unclear. However, a few hypotheses have been proposed to give some ideas on the antimicrobial effect of GNPs at nanolevel. The bactericidal activity of GNP greatly relies on the particle size. Thus, these SEM results clearly indicated that GNPs were intact with the compounds from the herbal extract.

3.2. TEM

TEM was utilized to determine the size and shape of the purchased GNPs. It has been found that used particles were polydispersed and spherical in shape. The size of molecules of the purchased GNPs with herbal extract conjugate was approximately 20 nm as shown in Fig. 3. In addition, EDX spectrum clearly confirmed the presence of gold elements.

3.3. FTIR Spectroscopy

Measurement using FTIR spectroscopy was carried out to identify the possible molecules from *A. paniculata* herbal

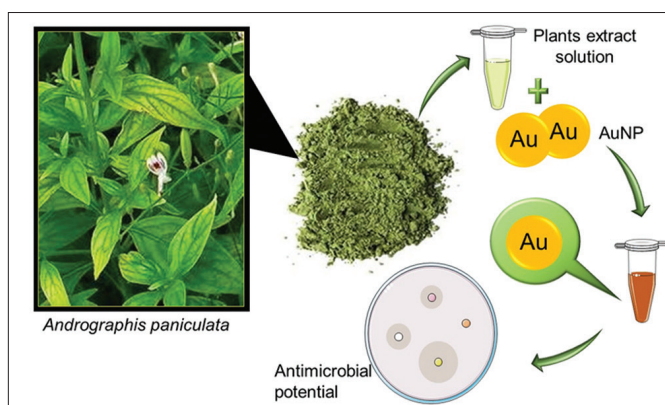


Fig. (1). Diagrammatic representation of extraction and conjugation. Extract of *Andrographis paniculata* is conjugated with gold nanoparticle for the assessment of antibacterial activity.

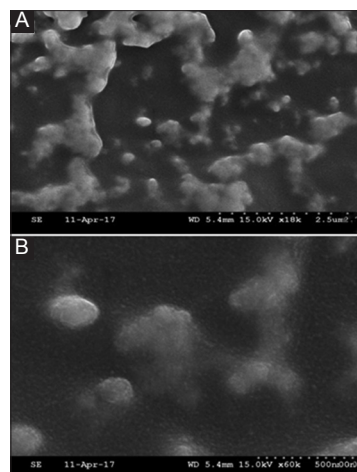


Fig. (2). Scanning electron microscopy observation on herbal extract conjugated gold nanoparticle. (A) Image with 2.5 µm scale; (B) image with 500 nm scale.

extract and *A. paniculata* herbal extract conjugated GNP that exhibit efficient antimicrobial properties. FTIR has been performed with three samples such as the herbal extract, GNP, and herbal extract conjugated GNP. The spectrum of *A. paniculata* herbal extract displayed a broadband at 3364.99 cm^{-1} , which represented the stretching of O-H functional group while the weak band at 1640.12 cm^{-1} was corresponding to the asymmetric stretching of C=O group (Fig. 4A). The spectrum with a broadband of GNP molecule was at 3372.44 cm^{-1} , and the weak band was at 1638.60 cm^{-1}

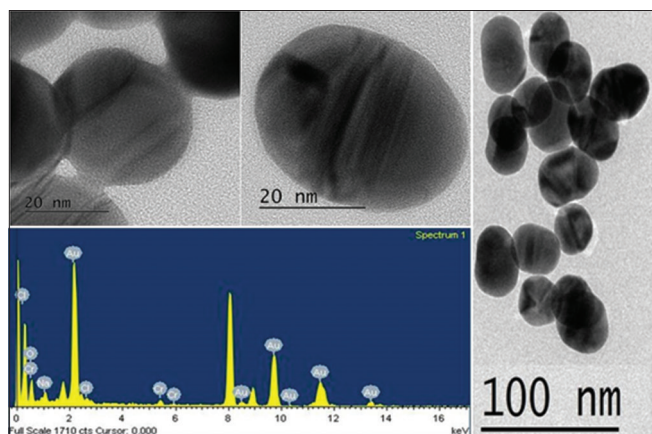


Fig. (3). Transmission electron microscopy observation on herbal extract conjugated gold nanoparticle. Images with 20 and 100 nm scales are shown. Energy-dispersive X-rayspectrum clearly indicates the presence of gold.

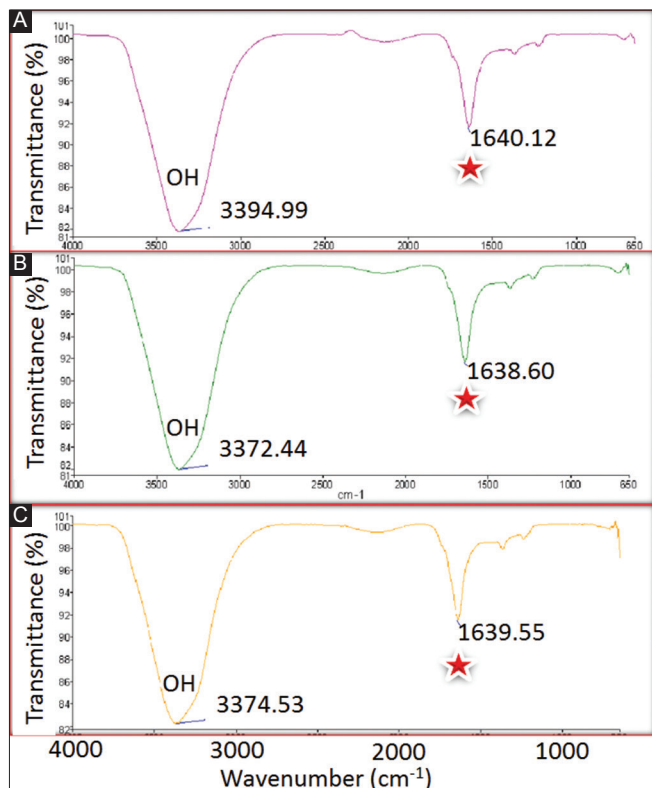


Fig. (4). (A-C) Fourier-transform infrared spectroscopy has been done with three samples, namely the herbal extract, gold nanoparticle (GNP), and herbal extract conjugated GNP. The scanning is from 500 to 4000 nm. The significant peaks other than hydroxyl phase are indicated by the red stars.

(Fig. 4B). FTIR spectrum confirmed the presence of hydroxyl and carbonyl group in *A. paniculata* herbal extract. In addition, the broadband and weak band stretching for GNP indicated the presence of C=O stretching. Different reports have revealed the role of hydroxyl and carbonyl groups in the preparation of herbal extract including their phytochemical and phenolic compounds that can act as a reducing and stabilizing agent. The sample of herbal extract conjugated with GNP molecule showed the highest broadband as compared to the spectrum of herbal extract and GNP. The herbal extract conjugated GNP showed the strong broadband at 3374.53 cm^{-1} and the weak broadband at 1639.55 cm^{-1} (Fig. 4C). This result proved that *A. paniculata* herbal extract can be combined with GNP to make bioreductant and capping agent.

3.4. Antimicrobial Activity of Herbal Extract and GNP Conjugate

Antimicrobial activity of herbal extract and *A. paniculata* herbal extract conjugated GNP against two different microorganisms, namely *E. coli* and *B. subtilis* was studied (Figs. 5 and 6). The conjugated GNPs and herbal extract exhibited an inhibitory activity against the bacterial growth. Zone of inhibition was recorded, and the inhibition zone for herbal extract was in the range from 0.6 ± 0.05 to $0.9 \pm 0.08\text{ cm}$. For the herbal extract conjugated GNP, the zone was between 0.8 ± 0.06 and $1.1 \pm 0.1\text{ cm}$. The antibacterial effect was noticed in both Gram-negative and Gram-positive bacteria. However, this finding was not consistent with the antimicrobial activity

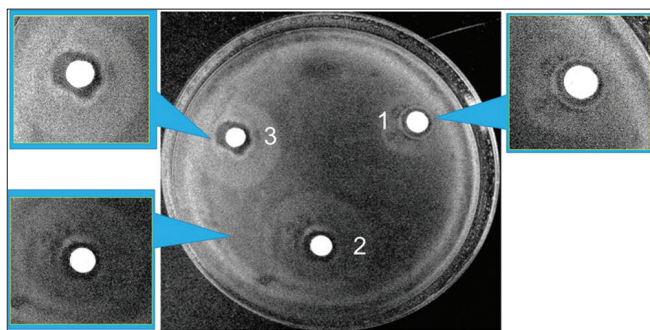


Fig. (5). Antibacterial potential against *Escherichia coli*. (1) Ampicillin control (5 µg), (2) herbal extract, and (3) herbal extract conjugated gold nanoparticle.

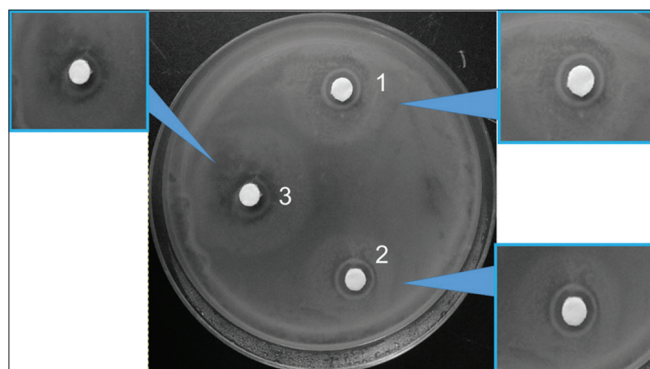


Fig. (6). Antibacterial potential against *Bacillus subtilis*. (1) Ampicillin control (5 µg), (2) herbal extract, and (3) herbal extract conjugated gold nanoparticle.

of GNP and herbal extract conjugates as stated in other studies although applications of gold (Au) are claimed to be better. It is widely accepted that metal nanoparticles are harmful to fungi and bacteria [40], and this characteristic allows them to adhere with cell wall of microorganisms, resulting in the destruction and death of the cell. In this study, different concentrations (20, 25, 30, 35, 40, and 45 µg) of herbal extracts were tested. The MIC was determined at 45 µg and it was compared with the conjugation with GNPs (Figs. 5 and 6). Further, there is no significant difference in the antimicrobial activity against *B. subtilis* and *E. coli*. This study is well supported by and can be correlated with the previous findings which were demonstrated using graphene and silver nanoparticle conjugating the herbal extract for microbial inhibitions [2,10].

4. CONCLUSION

The herbal extract from *A. paniculata* conjugated GNPs was analyzed using TEM, SEM, and FTIR spectroscopy. The characterization experiment determined the morphology, size, shape, and chemical nature of the herbal extract conjugated GNPs. Furthermore, the antimicrobial potential of *A. paniculata* conjugated GNPs was assessed. Disc diffusion method was used to evaluate the antimicrobial activity. From this study, the zones of inhibition found for both *B. subtilis* and *E. coli* by herbal extract conjugated GNPs were in the range between 0.8±0.06 and 1.1±0.1 cm. The inhibition zone for herbal extract was slightly smaller than the conjugated GNPs, ranging from 0.6±0.05 to 0.9±0.08 cm. The obtained results clearly indicate better antimicrobial potential of herbal extract conjugated GNPs than the herbal extract alone, and a larger zone of inhibition is probably due to the better dispersion of the compound in aqueous solution. Validation with additional inhibition studies should be performed for more insights.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- [1] Ramanathan, S.; Gopinath, S.C.B. Potentials in Synthesizing Nanostructured Silver Particles. *Microsyst. Technol.*, **2017**, 10, 1-13.
- [2] Gopinath, S.C.B.; Ramanathan, S.; Suk, K.H.; Ee Foo, M.; Anbu, P.; Uda, M.N.A. Engineered Nanostructures to Carry the Biological Ligands. *MATEC Web Conf.*, **2018**, 150, 6002.
- [3] Cowan, M.M. Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev.*, **1999**, 12(4), 564-82.
- [4] Krishnaiah, D.; Sarbatly, R.; Nithyanandam, R. A Review of the Antioxidant Potential of Medicinal Plant Species. *Food Bioprod. Process.*, **2011**, 89(3), 217-33.
- [5] Bono, D. R. S.; Krishnaiah, D.; Sarbatly, R.; Bono, A. Phytochemical Antioxidants for Health and Medicine a Move Towards Nature. *Biotechnol. Mol. Biol. Rev.*, **2007**, 1, 97-104.
- [6] Williams, G.C.; Nesse, R.M. The Dawn of Darwinian Medicine. *Q. Rev. Biol.*, **2004**, 66(1), 1-22.
- [7] Pandey, K.B.; Rizvi, S.I. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxid. Med. Cell. Longev.*, **2009**, 2(5), 270-8.
- [8] Daszak, P.; Tabor, G.M.; Kilpatrick, A.M.; Epstein, J.; Plowright, R. Conservation Medicine and a New Agenda for Emerging Diseases. *Ann. N. Y. Acad. Sci.*, **2004**, 1026, 1-11.
- [9] Wasman, S.Q.; Mahmood, A.A.; Chua, L.S.; Alshawsh, M.A.; Hamdan, S. Antioxidant and Gastroprotective Activities of *Andrographis paniculata* (*Hempedu bumi*) in Sprague Dawley rats. *Indian J. Exp. Biol.*, **2011**, 49(10), 767-72.
- [10] Foo, M.E.; Anbu, P.; Gopinath, S.C.B.; Lakshmi Priya, T.; Lee, C.G.; Yun, H. S.; Uda, M.N.A.; Yaakub, A.R.W. Antimicrobial Activity of Functionalized Single-walled Carbon Nanotube with Herbal Extract of *Hempedu bumi*. *Surf. Interface Anal.*, **2018**, 50(3), 354-61.
- [11] Kumar, R.A.; Sridevi, K.; Kumar, N.V.; Nanduri, S.; Rajagopal, S. Anticancer and Immunostimulatory Compounds from *Andrographis Paniculata*. *J. Ethnopharmacol.*, **2004**, 92(2-3), 291-5.
- [12] Zaidan, M.R.; Rain, A.N.; Badrul, A.R.; Adlin, A.; Norazah, A.; Zakiah, I. *In Vitro* Screening of Five Local Medicinal Plants for Antibacterial Activity Using Disc Diffusion Method. *Trop. Biomed.*, **2005**, 22(2), 165-70.
- [13] Roy, S.; Rao, K.; Bhuvanawari, C.; Giri, A.; Mangamoori, L.N. Phytochemical Analysis of *Andrographis Paniculata* Extract and its Antimicrobial Activity. *World J. Microbiol. Biotechnol.*, **2010**, 26(1), 85-91.
- [14] Subramanian, R.; Asmawi, M.Z.; Sadikun, A. *In Vitro* α-Glucosidase and α-Amylase Enzyme Inhibitory Effects of *Andrographis Paniculata* Extract and Andrographolide. *Acta Biochim. Pol.*, **2008**, 55(2), 391-8.
- [15] Gopinath, S.C.B.; Kumar, P.K.R.; Tominaga, J. A BioDVD Media with Multilayered Structure is Suitable for Analyzing Biomolecular Interactions. *J. Nanosci. Nanotechnol.*, **2011**, 11(7), 1-7.
- [16] Gopinath, S.C.B.; Kumaresan, R.; Awazu, K.; Fujimaki, M.; Mizuhata, M.; Tominaga, J.; Kumar, P.K.R. Evaluation of Nucleic Acid Duplex Formation on Gold over Layers in Biosensor Fabricated Using Czochralski-Grown Single-Crystal Silicon Substrate. *Anal. Bioanal. Chem.*, **2010**, 398(2), 751-8.
- [17] Tanisellass, S.; Md Arshad, M.K.; Gopinath, S.C.B. Current State of Green Reduction Strategies: Solution-Processed Reduced Graphene Oxide for Healthcare Biodetection. *Mater. Sci. Eng. C*, **2018**, 96, 904-14.
- [18] Anasdas, J.R.; Kannaiyan, P.; Raghavachary, R.; Gopinath, S.C.B.; Chen, Y. Palladium Nanoparticle-Decorated Reduced Graphene Oxide Sheets Synthesized Using Ficus Carica Fruit Extract: A Catalyst for Suzuki Cross-Coupling Reactions. *PLoS One*, **2018**, 13(2), 1-13.
- [19] Lakshmi Priya, T.; Fujimaki, M.; Gopinath, S.C.B.; Awazu, K.; Horiguchi, Y.; Nagasaki, Y. A High-Performance Waveguide-Mode Biosensor for Detection of Factor IX Using PEG-Based Blocking Agents to Suppress Non-Specific Binding and Improve Sensitivity. *Analyst*, **2013**, 138(10), 2863.
- [20] Lakshmi Priya, T.; Horiguchi, Y.; Nagasaki, Y. Co-Immobilized Poly(Ethylene Glycol)-Block-Polyamines Promote Sensitivity and Restrict Biofouling on Gold Sensor Surface for Detecting Factor IX in Human Plasma. *Analyst*, **2014**, 139(16), 3977-85.
- [21] Lakshmi Priya, T.; Gopinath, S.C.B.; Citartan, M.; Hashim, U.; Tang, T.H. Gold Nanoparticle-Mediated High-Performance Enzyme-Linked Immunosorbent Assay for Detection of Tuberculosis ESAT-6 Protein. *Micro Nanosyst.*, **2016**, 8(2), 92-8.
- [22] Lakshmi Priya, T.; Gopinath, S.C.B.; Tang, T.H. Biotin-Streptavidin Competition Mediates Sensitive Detection of Biomolecules in Enzyme Linked Immunosorbent Assay. *PLoS One*, **2016**, 11(3), e151153.
- [23] Gopinath, S.C.B.; Awazu, K.; Fujimaki, M.; Shimizu, K.; Shima, T. Observations of Immuno-Gold Conjugates on Influenza Viruses Using Waveguide-Mode Sensors. *PLoS One*, **2013**, 8(7), 1-10.
- [24] Gopinath, S.C.B.; Awazu, K.; Fujimaki, M.; Shimizu, K.; Mizutani, W.; Tsukagoshi, K. Surface Functionalization Chemistries on Highly Sensitive Silica-Based Sensor Chips. *Analyst*, **2012**, 137(15), 3520.
- [25] Fujimaki, M.; Nomura, K.; Sato, K.; Kato, T.; Gopinath, S.C.B.; Wang, X.; Awazu, K.; Ohki, Y. Detection of Colored Nanomaterials Using Evanescent Field-Based Waveguide Sensors. *Opt. Express*, **2010**, 18(15), 15732-40.
- [26] Arshad, M.K.M.; Adzhari, R.; Fathil, M.F.M.; Gopinath, S.C.B.;

- Md Nor, M.N.M. Field-Effect Transistor-Integration with TiO₂ Nanoparticles for Sensing of Cardiac Troponin I Biomarker. *J. Nanosci. Nanotechnol.*, **2018**, 18(8), 5283-91.
- [27] Perumal, V.; Hashim, U.; Gopinath, S.C.B.; Haarindraprasad, R.; Poopalan, P.; Liu, W.W.; Ravichandran, M.; Balakrishnan, S.R.; Ruslinda, A.R. A New Nano-Worm Structure from Gold-Nanoparticle Mediated Random Curving of Zinc Oxide Nanorods. *Biosens. Bioelectron.*, **2016**, 78, 14-22.
- [28] Letchumanan, I.; Md Arshad, M.K.; Balakrishnan, S.R.; Gopinath, S.C.B. Gold-Nanorod Enhances Dielectric Voltammetry Detection of c-Reactive Protein: A Predictive Strategy for Cardiac Failure. *Biosens. Bioelectron.*, **2019**, 130, 40-7.
- [29] Letchumanan, I.; Gopinath, S.C.B.; Md Arshad, M.K.; Anbu, P.; Lakshmipriya, T. Gold Nano-Urchin Integrated Label-Free Amperometric Aptasensing Human Blood Clotting Factor IX: A Prognosticative Approach for "Royal Disease." *Biosens. Bioelectron.*, **2019**, 131, 128-35.
- [30] Liu, J.; Qin, G.; Raveendran, P.; Ikushima, Y.; Ikushima, Y. Facile "Green" Synthesis, Characterization, and Catalytic Function of Beta-D-Glucose-Stabilized Au Nanocrystals. *Chemistry*, **2006**, 12(8), 2131-8.
- [31] Kuppusamy, P.; Yusoff, M.M.; Govindan, N. Biosynthesis of Metallic Nanoparticles Using Plant Derivatives and Their New Avenues in Pharmacological Applications an Updated Report. *Saudi Pharm. J.*, **2014**, 24(4), 473-84.
- [32] Khan, M.; Al-Marri, A.H.; Khan, M.; Shaik, M.R.; Mohri, N.; Adil, S.F.; Kuniyil, M.; Alkhatlan, H.Z.; Al-Warthan, A.; Tremel, W.; Tahir, M.N.; Siddiqui, M.R.H. Green Approach for the Effective Reduction of Graphene Oxide Using *Salvadora Persica* L. Root (Miswak) Extract. *Nanoscale Res. Lett.*, **2015**, 10(1), 281.
- [33] Iravani, S. Green Synthesis of Metal Nanoparticles Using Plants. *Green Chem.*, **2011**, 13, 2638-50.
- [34] Kasthuri, J.; Kathiravan, K.; Rajendiran, N. Phyllanthin-Assisted Biosynthesis of Silver and Gold Nanoparticles: A Novel Biological Approach. *J. Nanopart. Res.*, **2009**, 11(5), 1075-85.
- [35] Bankura, K.; Maity, D.; Mollick, M.M.R.; Mondal, D.; Bhowmick, B.; Roy, I.; Midya, T.; Sarkar, J.; Rana, D.; Acharya, K.; Chattopadhyay, D. Antibacterial Activity of Ag-Au Alloy NPs and Chemical Sensor Property of Au NPs Synthesized by Dextran. *Carbohydr. Polym.*, **2014**, 107(1), 151-7.
- [36] Rónavári, A.; Igaz, N.; Gopisetty, M.K.; Szerencsés, B.; Kovács, D.; Papp, C.; Vágvölgyi, C.; Boros, I.M.; Kónya, Z.; Kiricsi, M.; Pfeiffer, I. Biosynthesized Silver and Gold Nanoparticles Are Potent Antimicrobials against Opportunistic Pathogenic Yeasts and Dermatophytes. *Int. J. Nanomed.*, **2018**, 13, 695-703.
- [37] Gopinath, S.C.B.; Perumal, V.; Kumaresan, R.; Lakshmipriya, T.; Rajintraprasad, H.; Rao, B.S.; Arshad, M.K.M.; Chen, Y.; Kotani, N.; Hashim, U. Nanogapped Impedimetric Immunosensor for the Detection of 16 kDa Heat Shock Protein against *Mycobacterium tuberculosis*. *Microchim. Acta*, **2016**, 183(10), 2697-703.
- [38] Perumal, V.; Saheed, M.S.M.; Mohamed, N.M.; Saheed, M.S.M.; Murthe, S. S.; Gopinath, S.C.B.; Chiu, J.M. Gold Nanorod Embedded Novel 3D Graphene Nanocomposite for Selective Bio-Capture in Rapid Detection of *Mycobacterium tuberculosis*. *Biosens. Bioelectron.*, **2018**, 116, 116-22.
- [39] Anniebell, S.; Gopinath, S.C.B. Polymer Conjugated Gold Nanoparticles in Biomedical Applications. *Curr. Med. Chem.*, **2018**, 25(12), 1433-45.
- [40] Ramanathan, S.; Gopinath, S.C.B.; Anbu, P.; Lakshmipriya, T.; Kasim, F.H.; Lee, C.G. Eco-friendly Synthesis of *Solanum trilobatum* Extract-Capped Silver Nanoparticles is Compatible with Good Antimicrobial Activities. *J. Mole. Struct.*, **2018**, 1160, 80-91. 20 and 100 nm scales are shown. Energy-dispersive X-ray spectrum clearly indicates the presence of gold.