ORIGINAL ARTICLE

Characterisation of Silver Nanoparticles using a Standardised *Catharanthus roseus* Aqueous Extract

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ABSTRACT

Introduction: The biosynthesis of nanoparticles has been proposed as a cost-effective and environmental friendly alternative to chemical and physical methods. The present study was aimed to characterise *Catharanthus roseus* (*C. roseus*)-silver nanoparticles (AgNPs) using a standardised *C. roseus* aqueous extract. **Methods:** The standardisation was performed by using Liquid Chromatography/Time-of-Flight ion trap Mass Spectrometry. An optimised *C. roseus*-AgNPs have been previously synthesised. Further characterisation of *C. roseus*-AgNPs was evaluated by zeta potential analysis and fourier transform infrared spectroscopy (FTIR). **Results:** The chromatography analysis has revealed presence of thirteen possible indole alkaloids in *C. roseus* extract which were lochrovicine, lochnerine, vinleurosine, vindolinine, tabersonine, catharanthine, serpentine, catharosine, vincristine, catharine, ajmalicine, vinleurosine, and vindolicine. Zeta potential analysis exhibited the value at -16.6 mV. FTIR spectrum of *C. roseus* aqueous extract showed the absorption band at 3210.83 cm-1 (C-H stretch), 2934.11 (C-H bond), 1578.15 (N=O stretch), 1388.76 and 1314.89 (N=O bend), 1119.29 (C-O bond) and 729.94 (C-Cl bond). In comparison, FTIR spectrum of *C. roseus*-AgNP s showed the absorption band at 2925.01 and 2924.97 (C-H bond), 1622.93 (C-C=C symmetric stretch), 1383.19 and 1384.13 (N-O bend), 1037.92/1038.76/1238.3/1117.2 (C-O bond), 3169.4 (O-H bond), 774.59 and 691.53 (C-Cl bond). **Conclusion:** The present findings have shown that the *C. roseus* aqueous extract contains alkaloids that may responsible as reducing and stabilising agents in the synthesis of AgNPs.

Keywords: *Catharanthus roseus*, aqueous extract, silver nanoparticles, characterization

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INTRODUCTION

Nanotechnology is a rapid growing field in science, engineering and technology that involves a manipulation of materials at nanoscale level. Nanomaterials are widely applied in commercial products and biomedical field, for instance material packaging, cosmetics, coatings, targeted delivery and drug carriers (1, 2). Amongst various types of nanomaterials, nanoparticles have been reported to exhibit significant biological effects, suggesting its potential as an alternative treatment for diseases. Their nanosized particles with high surface area to volume ratio enable them to penetrate the cells more effectively, as compared to micro-sized particles (3, 4). These advantages have improved the limitations arise from the current conventional treatment.

Silver nanoparticles (AgNPs) can be defined as an inert inorganic metal element, ranging from 1 to 100 nm (5). Ultrafine particles of metallic silver have unique properties in terms of conductivity, stability, catalytic and antibacterial activity (6). Thus, they are commonly used as anti-inflammatory, antioxidant, antimicrobial and antibacterial agents. In addition, they are widely used in medical devices, catheters, dental fillers and wound dressing (7). AgNPs can be synthesised by chemical reduction, thermal decomposition, photo reduction and radiation (6, 8). However, these methods are time-consuming, expensive and highly hazardous. Chemicals used like sodium borohydride, potassium bitartrate, methoxy-polyethylene glycol and hydrazine have been reported can cause severe adverse effects. According to Hazardous Substance Fact Sheet issued by New Jersey Department of Health and Senior Services, sodium borohydride can cause irritation and burn to skin, eyes, nose, throat and lungs. Moreover, prolong exposure would lead to shortness of breath, damage of kidney and liver, and disruption of central nervous system (9). Due to these limitations, new development with cost-effective, non-toxic and natural approaches is in a great demand.

Alternatively, the AgNPs can be synthesised biologically using microorganisms and plant extracts (10). This

approach is considered as a green synthesis, indicating that the microorganisms and plant extracts contain reducing agents for the silver ions. Plant extracts have been shown can produce higher rate of synthesis as compared to chemical methods and green synthesis by microorganisms (11, 12). Moreover, they are easily assessible and the synthesis procedures are simpler since it does not require a complex cell culture laboratory works and tedious purification techniques (13). Thus, the plant-mediated synthesis of AgNPs can be considered as a single-step process with rapid and low cost of production (14, 15). Plant extract is known to have diversity in structure and bioactivity, that include various phytochemicals such as carboxylic acid, ketone, aldehyde, alkaloid and flavonoid that may responsible as the reducing, stabilising and capping agents of the AgNPs. There are many plant extracts, particularly from the leaves part have been reported can synthesise AgNPs including Ganoderma neo-japonicum (7), Eucalyptus globulus, Ziziphus spina-christi, Camellia sinensi (16), Duranta repens (12), Annona squamosa (17), Rhinacanthus nasutus (18) and Catharanthus roseus (C. roseus) (19, 20).

C. roseus is a medicinal plant from Apocynaceae family that wildly grows in tropical and subtropical climate (21). This Madagascar periwinkle is a flowering herbaceous plant that usually grows upright or decumbent, up to one-meter height. *C. roseus* contains more than 120 terpenoid indole alkaloids including vinblastine, vincristine, ajmalicine, catharanthine, vindoline and serpentine. These alkaloids accumulated in different parts of plant and have pharmacological properties such as vinblastine and vincristine as anticancer, serpentine as sedative and ajmalicine as antihypertensive (22). Vinblastine and vincristine are the first natural drugs used in the cancer treatment including lymphoma, leukemia, neuroblastoma, rhabdomyosarcoma and breast cancer (23). Since the reports on the active compounds present in this plant are well documented, it is hypothesised that this plant contains various potential reducing agents responsible for the synthesis of AgNPs. The well characterised *C. roseus*-AgNPs would provide useful information on its potential applications. The screening of the possible active compounds, such as alkaloids present is crucial to correlate with its biological activity. Although the method of synthesis of *C. roseus*-AgNPs has been reported, there is a lack of information of the standardisation part. Therefore, this work was aimed to perform chromatographic analysis on the *C. roseus* aqueous extract for a standardisation purpose to be used in subsequent synthesis of AgNPs. This is followed by further characterisation of *C. roseus*-AgNPs to evaluate its characteristics and properties, particularly for anticancer.

MATERIALS AND METHODS

Liquid Chromatography/ Time-of-Flight Ion Trap Mass Spectrometry (LC/TOF-MS)

Analysis of the *C. roseus* aqueous extract synthesised in our previous study (20) was performed on Finnigan LTQ ion trap mass spectrometer (Thermo Fischer Scientific Inc., Waltham, MA, USA) equipped with binary pump, a UV detector, an autosampler, and a column thermostat. The method was adopted from a previous study (21) with slight modification. Chromatographic separations were performed on a ZORBAX Eclipse XDB-C18 Analytical (5.0 µm, 150 x 4.6 mm, Agilent USA) with a solvent flow rate of 400 uL/min. The sample injection volume was 10 µL with a detection wavelength of 280 nm. Ten mmoL of ammonium acetate in water (A) and acetonitrile (B) were used as a mobile phase. The gradient elution was adopted from the previous study (21) which was as follows: 5% B (15 min), 5–55% B (15 min), 55–95% B (5 min), 95–5% B (5 min), 5% B (5 min), and then re-equilibration of 5% B for 5 min before the next sample injection. The ESI source was operated in positive ion mode and the spectra was recorded by scanning from 100 to 1300 m/z. The ESI source conditions used were as follows: drying gas (N2) temperature of 350 °C, 5 L/min drying gas flow, 10 psi nebulizer gas (N2) pressure, and 4500 V of capillary voltage.

Zeta Potential Analysis

The *C. roseus*-AgNPs was previously synthesised and optimised using uv-vis spectroscopy, X-ray diffraction analysis and transmission electron microscopy (20). The sample was sent to School of Chemical Engineering, USM to observe the colloidal stability in dispersion using Zetasizer Nano ZS (Malvern, UK). Stock solution at a concentration of 30 mg/ml in 0.001 M KNO3 was prepared. Three different pH solutions of 0.001 M KNO_3 were prepared by adjusting the pH to 3, 6 and 9 by using HNO_3 and 0.01 M KOH. Each suspension at a concentration of 1.5 mg/ml was prepared by mixing 1.0 ml of the stock suspension to 20 ml of either pH 3, 6 or 9 KNO_3 solutions. After soaking the suspensions, in an ultrasonic bath for 5 min, they were then transferred into the measurement cell. The measurements were performed twice per sample at each pH.

Fourier Transform Infrared (FTIR) Spectroscopy

The *C. roseus*-AgNPs samples were submitted to School of Chemical Sciences, Universiti Sains Malaysia for FTIR analysis. The FTIR analysis of the dried *C. roseus* aqueous extract and extract mediated synthesis AgNPs powders were carried out using FTIR Spotlight 200 (Perkin Elmer, USA).

RESULTS

Liquid Chromatography/ Time-of-Flight Ion Trap Mass Spectrometry (LC/TOF-MS)

The analysis was carried out for both *C. roseus* aqueous extract samples, either prepared in 40°C water bath or boiled at 100°C (20) to study the presence of active compound in the extract. LC method was optimised

and developed to the best chromatographic peak shapes. Various gradients of mobile phases at a flow rate of 400 µL/min were used for method optimisation purposes. From Fig. 1, both samples exhibit same active compounds despite the difference in preparation methods. The analysis has revealed the presence of thirteen possible indole alkaloids which were lochrovicine (m/z 339) lochnerine (m/z 325), vindoline (m/z 457), serpentine (m/z 349), catharosine (m/z 385), vincristine (m/z 825), catharine m/z (823), ajmalicine (m/z 353), vinleurosine (m/z 809), and vindolicine (m/z 925). Vindolinine, tabersonine and catharanthine are isomers that exhibited identical protonated molecule ions of m/z 337. Fig 2 shows the chemical structure of the identified compounds. The mass spectra, retention times and molecular weight (MW) from the analysis were compared with the previous studies to identify the alkaloids in *C. roseus* aqueous extract (21-26).

Zeta Potential Analysis

The stability of *C. roseus*-AgNPs was evaluated by using zeta potential analysis. From Fig 3, the zeta potential measurement of AgNPs exhibited at -16.6 mV, indicates that these AgNPs are incipiently stable (27). The capping of biomolecules was further confirmed using FTIR analysis.

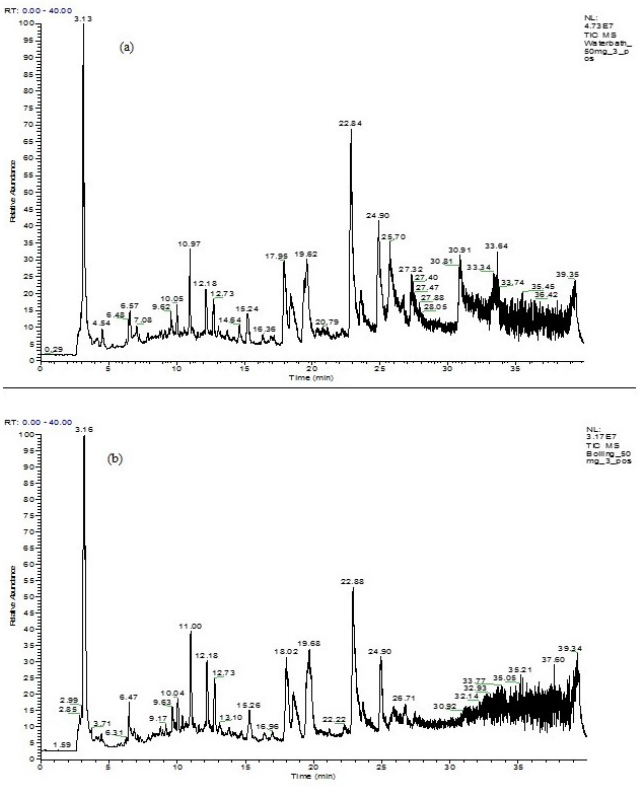

Figure 1: Full-scan ESI-MS spectra of *C. roseus* aqueous extract, prepared in 40°C waterbath (a) and boiling (b), in positive ion mode by direct loop injecting in ion trap mass.

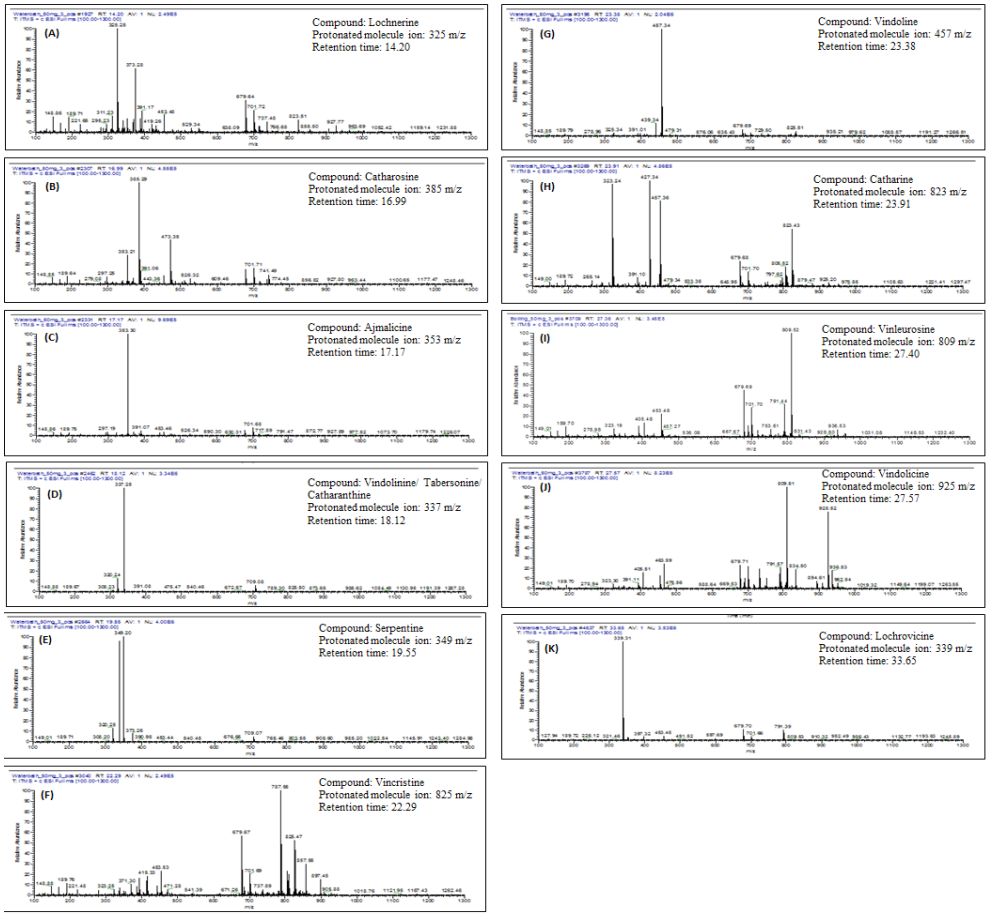

Figure 2: Chemical structure and ESI-MS spectra of each compound in *C. roseus* aqueous extract analysed by ion trap liquid chromatography time off flight/mass spectroscopy (A) lochnerine, (B) catharosine, (C) ajmalicine, (D) vindolinine/tabersonine/catharanthine, (E) serpentine, (F) vincristine, (G) vindoline and (H) catharine, (I) vinleurosine, (J) vindolicine and (K) lochrovicine

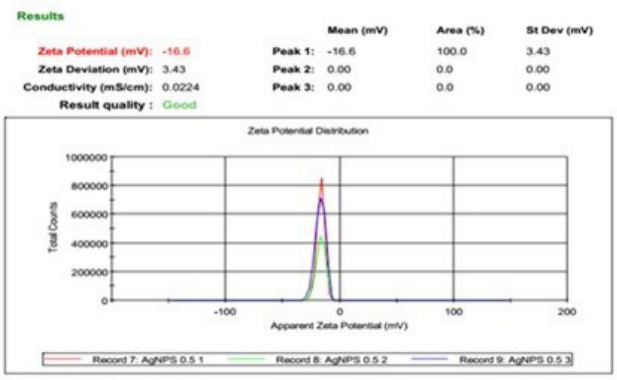

Figure 3: Zeta potential analysis of *C. roseus*-Agnps.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed to find the possible functional groups and biomolecules for capping and efficient stabilisation of the synthesised AgNPs. In Fig. 4, the FTIR spectrum of *C. roseus* aqueous extract showed the absorption band at 3210.83 cm-1 (C-H stretch), 2934.11 (C-H bond), 1578.15 (N=O stretch), 1388.76 & 1314.89 (N=O bend), 1119.29 (C-O bond) and 729.94 (C-Cl bond). While, the FTIR spectrum of *C. roseus*-AgNPs showed the absorption band at 2925.01 & 2924.97 (C-H bond), 1622.93 (C-C=C symmetric stretch), 1383.19 & 1384.13 (N-O bend), 1037.92/1038.76/1238.3/1117.2 (C-O bond), 3169.4 (O-H bond), 774.59 & 691.53 (C-Cl bond).

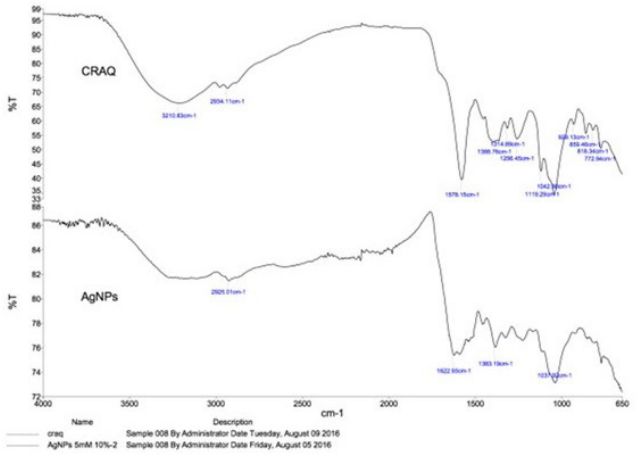

Figure 4: Fourier transform infrared spectroscopy (FTIR) of *C. roseus* aqueous extract and *C. roseus*-Agnps.

DISCUSSION

The synthesis of AgNPs requires three components, which are the silver salt, reducing agent and stabilising or capping agent. The mixture between the silver salt and crude extract is performed extracellularly, and therefore the synthesis is more convenient and cost-effective. In this study, the screening of compounds present in *C. roseus* aqueous extract was performed using high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS). This is assumed to be one of the methods for extract standardisation, so that a uniform extract that contains similar alkaloids will be used in subsequent experiments. The standardisation is very crucial to ensure the reproducibility of the plant extract is taken into account throughout the study. The analysis using this instrument serves more advantages in terms of its high precision and high sample loading. Generally, the analysis of the alkaloids can be performed through electrospray ionisation, electron impact and atmospheric pressure chemical ionisation. However, electrospray ionisation has been reported to have the highest sensitivity (21). Thus, this analysis was chosen in this study.

It was observed that the *C. roseus* aqueous extract contains thirteen possible alkaloids. It is interesting to note that the presence of these alkaloids indicate that the compounds are highly soluble in aqueous solution and contain significant medicinal properties. The commercially available compounds such as serpentine and ajmalicine have been prescribed for antihypertensive while vincristine has been clinically used to treat various types of cancers, particularly leukemia (28). The screening of the extract suggested that some of these compounds may act as reducing and stabilising agents of the AgNPs. Moreover, previous studies have shown that plant-mediated AgNPs possessed significant anticancer (29, 30) and antibacterial (31, 32) activity, indicating that the AgNPs are coupled with particular active compounds and produced the synergistic effects. The identification of the alkaloids with their respective properties may rule out the potential application of *C. roseus*-AgNPs.

The data from zeta potential analysis revealed that the colloid behaviour of biosynthesised *C. roseus*-AgNPs were incipiently stabilised, with -16.6 mV. Literature suggested that the zeta potential values of colloidal particles within of less than -30 and more than 30 are considered stable (33, 34). In comparison, previous study obtained -63.1 mV of *C. roseus*-AgNPs (2). Although similar plant was used, different method and plant sources might be the probable factors contributing to various potential stability. The biosynthesised *C. roseus*-AgNPs exhibit an electric potential from its surface to other points which is referred to as shear plane. The values of zeta potential determine the suspension stability and particle surface morphology based on the shear plane. Therefore, this analysis is not only influenced by the characteristics of the nanoparticles, but also the environment such as pH and ionic strength (35). It can be concluded that these parameters might vary among the synthesised nanoparticles.

The characteristics of C-H bond, C-O bond, C-Cl bond, N=O bend and C-N bond stretching vibrations are common in both *C. roseus* aqueous extract and *C. roseus*-AgNPs indicating that these biomolecules were involved in the reduction and capping of silver nanoparticles (32).

C-H bond stretching mode found in alkane, C-O bond stretching mode are the carbonyl functional groups of ester and alcohols, C-Cl bond are alkyl halides, N=O bend represents nitro groups and C-N stretching bands are available in amine stretch of proteins and amino acids, present in the leaves extract of *C. roseus* (27). This further confirmed the AgNPs obtained in this study might be surrounded by biomolecules that contain similar functional groups as the extract. Based on this finding, the biologically active compounds in *C. roseus*-AgNPs may include fatty acid and esters such as palmitic acids, lauric acid, myristic acid and methyl palmitate. Previous works have also suggested the involvement of other compounds that may act as reducing and stabilising agents such as aldehyde, ketone and thirteen carbon compound that include theaspirance a, theaspirance b, (E)-α-ionone and (E)-β-ionone-5 and 6-epoxide (36, 37). However, further analysis is required to determine the specific compounds which are responsible in reducing Ag+ ions in the production of *C. roseus*-AgNPs.

CONCLUSION

As a conclusion, the findings indicate that the AgNPs have been successfully synthesised by a standardised *C. roseus* aqueous extract. The standardisation of *C. roseus* extract is crucial to ensure uniformity of the active compounds present. The presence of indole alkaloids suggest that they may responsible to act as reducing, stabilising and synergistic agents of the biosynthesised AgNPs. Moreover, its characteristics play an important role to correlate with future biological effects. This work has strengthened the evidence of using the alternative plant-mediated synthesis to produce AgNPs.

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REFERENCES

1. Chung IM, Park I, Seung-Hyun K, Thiruvengadam M, Rajakumar G. Plant-mediated synthesis of silver nanoparticles: their characteristics properties and therapeutic applications. Nanoscale Research Letters. 2016; 11:40.
2 Kotakadi VS, Rao RY, Gaddam SA, Prasad TN, Reddy AV, Gopal DV. Simple and rapid biosynthesis of stable silver nanoparticles using dried leaves of *Catharanthus roseus* Linn. G. Donn and its anti microbial activity. Colloids and Surface Biointerfaces. 2013; 105:194-198.
3 Ovais M, Khalil AT, Raza A, Khan MA, Ahmad I, Islam NU et al. Green synthesis of silver nanoparticles via plant extracts: beginning a new era in cancer theranostics. Nanomedicine, 2016; 11(23):3157-3177.
4 Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicine: a review of FDA-approved materials and clinical trials to date. Pharmaceutical Research, 2016; 13(10):2373-2387.
5 Calderyn-Jimйnez B, Monique E. Johnson ME, Montoro Bustos AR, Murphy KE, Michael R. Winchester MR, Josй R. Vega Baudrit JR. Silver Nanoparticles: Technological Advances, Societal Impacts, and Metrological Challenges. Frontiers in Chemistry. 2017; 5(6).
6 Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. International Journal of Molecular Sciences. 2016;17(9):1534.
7 Sangiliyandi G, Jegadeesh R, Sri Nurestri AM, Priscilla AJ, Sabaratnam V. Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: a potential cytotoxic agent against breast cancer cells. International Journal of Nanomedicine. 2013b; 8:4399-4413.
8 Navaladian S, Viswanathan B, Viswanath RP, Varadarajan TK. Thermal decomposition as route for silver nanoparticles. Nanoscale Research Letters. 2007; 2:44-48.
9 Prabhu D, Arulvasu C, Babu G, Manikandan R, Srinivasan P, Govindaraju K, Ashokkumar T. Synthesis and characterization of silver nanoparticles using crystal compound of sodium para-hydroxybenzoate tetrahydrate isolated from Vitex negundo L. leaves and its apoptotic effect on human colon cancer cell lines. European Journal of Medicinal Chemistry, 2014; 84.
10 Jang SJ, Yang IJ, Tettey CO, Kim KM, Shin HM. In-vitro anticancer activity of green synthesized silver nanoparticles on MCF-7 human breast cancer cells. Materials Science and Engineering C. 2016; 68:430-435.
11 Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J et al. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. Asperellum. Nanotechnology. 2008;19(7):075103.
12 Basanagowda MP, Ashok AH. Green Synthesis of Silver Nanoparticles by Duranta repens leaves and their antimicrobial efficacy. Nano Trends: A Journal of Nanotechnology and Its Application. 2013; 14(1):13-18.
13 Bhatttacharya D, Gupta RK. Nanotechnology and potential of microorganism. Critical Review of Biotechnology. 2005; 25:199-204.
14 Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. Journal of Nanoparticles Research. 2007; 10:507-517.
15 Saifuddin N, Wong CW, Yasumira AAN. Rapid biosynthesis of silver nanoparticles using culture

supernatant of bacteria with microwave irradiation. E-Journal of Chemistry. 2009; 6(1): 61-70.
16 Mohammed AE. Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticlesmediated by Eucalyptus camaldulensis leaf extract. Asian Pacific Journal of Tropical Biomedicine. 2015; 5:382-386.
17 Vivek R, Thangam R, Muthuchelian K, Gunasekaran P, Kaveri K, . Kannana S. Green biosynthesis of silver nanoparticles from Annona squamosa leaf extract and its in vitro cytotoxic effect on MCF 7 cells. Process Biochemistry. 2012;47: 2405-2410.
18 Pasupuleti VR, Prasad TN, Shiekh RA, Balam SK, Narasimhulu G, Reddy CS, Ab Rahman I, Gan SH. Biogenic silver nanoparticles using Rhinacanthus nasutus leaf extract: synthesis, spectral analysis, and antimicrobial studies. International Journal of Nanomedicine. 2013; 8:3355-3364.
19 Mukunthan KS, Elumalai EK, Patel TN, Murty VR. Catharanthus roseus: A Natural Source for the synthesis of silver nanoparticles. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(4):270-274.
20 Ghozali SZ, Vuanghao L, Ahmad NH. Biosynthesis and Characterization of Silver Nanoparticles using Catharanthus roseus Leaf Extract and its Proliferative Effects on Cancer Cell Lines. J Nanomed Nanotechnol. 2015; 6:4.
21 Chen Q, Zhang W, Zhang Y, Chen J, Chen Z, Identification and quantification of active alkaloids in Catharanthus roseus by liquid chromatography-ion trap mass spectrometry. Food Chemistry. 2013;139: 845-852.
22 Kumar S, Singh A, Bajpai V, Srivastava M, Sigh BP, Kumar B. Structural characterization of monopertene indole alkaloids in ethanolic extracts of Rauwolfia species by liquid chromatography with quadrupole time-of-flight mass spectrometry. Journal of Pharmaceutical Analysis. 2016;6(6):363-373
23 Liu Z, Wu HL, Li Y, Gu HW, Yin XL, Xie LX, Yu RQ. Rapid and simultaneous determination of five vinca alkaloids in Catharanthus roseus and human serum trilinear component modeling of liquid chromatography-diode array detection data. Journal of Chromatography B. 2016; 1026:114-123.
24 Musharraf SG, Goher M, Zareena B. Quantification of steroidal alkaloids in Buxus papillosa using electrospray ionization liquid chromatography-triple quadrupole mass spectrometry," Steroids. 2015;100: 5-10.
25 Pan Q, Saiman MZ, Mustafa NR, Verpoorte R, Tang K. A simple and rapid HPLC-DAD method for simultaneously monitoring the accumulation of alkaloids and precursors in different parts and different developmental stages of Catharanthus roseus plant. Journal of Chromatography B. 2016; 1014:10-16.
26 Martinello M, Cristofoli C, Gallina A, Mutinelli F. Easy and rapid method for the quantitative determination of pyrrolizidine alkaloids in honey by ultra performance liquid chromatography-mass spectrometry: An evaluation in commercial honey. Food Control. 2014; 37:146-152.
27 Chaudhuri SK, Chandela S, Malodia L. Plant mediated green synthesis of silver nanoparticles using Tecomella undulata leaf extract and their characterization. Nano Biomed Eng. 2016; 8(1): 1-8.
28 Van der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthus alkaloids: pharmacognosy and biotechnology. Current Medicinal Chemistry. 2014;11, 607-628.
29 Boca G, Potara M, Gabudean AM, Juhem A, Baldeck PL, Astilean S. Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible, highly effective photothermal transducers for in vitro cancer cell therapy. Cancer Letters. 2011; 311,131-140.
30 Ramy SY, Hashem AS. Biosynthesis and characterization of silver nanoparticles produced by Pleurotus ostreatus and their anticandidal and anticancer activities. World Journal of Microbiology and Biotechnology. 2014,30,2797-2803.
31 Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. Trend in Biotechnology. 2010,28:580-588.
32 Manisha DR, Alwala J, Kudle KR, Rudra MPP. Biosynthesis of silver nanoparticles using flower extracts of Catharanthus roseus and evaluation of its antibacterial efficacy. World Journal of Pharmacy and Pharmaceutical Science. 2014;3(5):877-885.
33 Wang P, Shi Q, Liang H, Steuerman DW, Stucky GD, Keller AA. Enhanced environmental mobility of carbon nanotubes in the presence of humic acid and their removal from aqueous solution. Small. 2008;4(12):2166–70.
34 Saeb ATM, Alshammari AS, Al-Brahim H, Al-Rubeaan KA. Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. The Scientific World Journal. 2014; 1-9.
35 Xu R. Progress in nanoparticles characterization:Sizing and zeta potential measurement. Particuology. 2007; 6:112-115.\
36 Sottomayor M, Cardoso IL, Pereira L, Barcelo AR. Peroxidase and the biosynthesis of terpenoid indole alkaloids in the medicinal plant Catharanthus roseus (L.) G. Don. Phytochemistry Reviews. 2004; 159-171.
37 Brun G, Bessiere JM, Dijoux-France MG, David B, Mariotte AM. Volatile components of Catharanthus roseus (L.) G. Don (Apocynaceae. Flavor and Fragrance Journal. 2001; 16; 116-119.