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Research Article

In-vitro Anticancer Potential of Phytogenic Ag-Au Bimetallic Nanoparticles using *Clitoria ternatea* Flower Extract

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ABSTRACT

Bimetallic nanoparticles (BNPs) have gained significant attention in the field of biomedical and pharmaceutical because of its tunable size, shape, high surface to volume and enhanced biological properties. In this study, we report the ecofriendly route to produce the noble (Ag-Au) bimetallic nanoparticles using aqueous flower extract of *Clitoria ternatea* as a reducing and capping agent. The synthesized C.T-Ag-Au BNPs were characterized by using physicochemical techniques such as UV-visible, FTIR, XRD and SEM-EDX analysis. The UV-visible spectra reveal the formation of homogeneous bimetallic nanoparticles by the single blue-shifted peak at 541 nm of C.T-Ag-Au BNPs. The phyto fabrication of synthesized C.T-Ag-Au BNPs was analyzed using FTIR spectroscopy. XRD confirms the formation of phase pure cubic Ag-Au alloy bimetallic nanoparticles with crystallite size is 14.5 nm. The surface morphology and elemental analysis of C.T-Ag-Au BNPs were examined by using SEM-EDX analysis. The synthesized C.T-Ag-Au BNPs were evaluated for their cytotoxicity against A549 human lung cancer cells through standard MTT assay. From this assay, green synthesized bimetallic nanoparticles induced cell apoptosis, suggesting that the synthesized C.T-Ag-Au BNPs gave best anticancer properties against lung cancer A549 cell lines.

Introduction

Cancer is one of the multifactorial diseases which were caused by a complex mixture of environmental and genetic factors and it leading to cause of mortality in worldwide.[1] Due to the development of more potent and effective antineoplastic drugs for the treat the cancer is one of the persuaded goals.[2] The production of phytogenic mediated noble metal nanoparticles achieved this. Nobel metal nanoparticles have gained considerable attention due to their facile properties and fascinating applications.[3] The combinations of two distinctive metal atoms are nanoparticles bound together into single nanoparticles. Bimetallic (BNPs) are favored over monometallic nanoparticles counterparts because of their enhanced optical, electrical, thermal, magnetic and catalytic properties and biological attributes.[4] BNPs are eminent in various fields such as biological, pharmacological and catalysis due to their promising properties. Two metals induced the synergistic actions and multifunctional properties present in the BNPs. Based on orientation BNPs were further categorized into four types such as core-shell, subclusters, intermetallic and alloy.[5] There are numerous methods are available for the production of BNPs such as chemical, physical and biological methods.[6] Among them biological method is a prior need because the naturally occurring bio-molecules such as the phytochemical constituent of plant extract and the biomolecules of microorganism and alga play a significant role in the production of bimetal nanoparticles. Due to the production of stable and sustainable BNPs, plant-mediated method is preferable to the microorganism-mediated method.[7] *Clitoria ternatea* is a perennial herbaceous plant with conspicuous blue or white color which belongs to the Fabaceae family and it is commonly known as a butterfly pea.[8] *C. ternatea* contains a variety of ethnic medical

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uses and has been shown to a number of pharmacological applications such as antimicrobial, anticancer, antioxidant, anticonvulsant, anti-inflammatory, anti-stress, anti-depressant and analgesics.[9] The *C. ternatea* plant extract contains many bioactive compounds.[10] Mainly the flower extract contains anthocyanin delphinidin which gives the color of C.T also they have phytochemical constituents such as flavonoids, alkaloids, terpenoids, tannins, saponins, resins, glycosides and bioactive compounds are Kaemferol, qucertin, myricetic, tannic acid, phytosterols.[11] These phytochemical constituents may act as reducing and capping agent in the production of nanoparticles. This present research highlights the facile, rapid, green, sustainable, cost-effective and environmentally friendly synthesis of Ag-Au BNPs using *C. ternatea* aqueous flower extract. The synthesized nanoparticles were characterized by using spectroscopic techniques such as UV-visible, FTIR, XRD and SEM-EDX analysis. Furthermore, Ag-Au BNPs were studied for their anticancer activity toward human lung cancer cells (A549).

Materials and Methods

Materials

C. ternatea flowers were collected from the rural area of Usilampatti, Madurai district. Silver nitrate ($AgNO_3$), Tetrachloroauric acid ($HAuCl_4$) was purchased from sigma Aldrich without any purification. All the solutions and experiments were done by using double distilled water.

Preparation of *C. ternatea* Flower Extract

Fresh flowers of *C. ternatea* are washed several times with double distilled water, shade dried and powdered. About 2 grams of the powdered flower was located into 100 mL of beaker containing 50 mL of double distilled water and mixed well. The extract was filtered through whatman no 1 Filter paper and the collected filtrate was stored at 4°C for future use.[12]

Green Synthesis of Ag-Au Bimetallic Nanoparticles

The Ag-Au bimetallic nanoparticles were synthesized by using *C. ternatea* flower extract. 10 mL of plant extract was added to 60 mL mixture of 1 mm silver nitrate and tetrachloroauric acid in the ratio of 1:3 at room temperature. After adding C.T flower extract change in the color of the reaction mixture was an initial indication of the reduction of metal salts to nanoparticles. This reaction was monitored by using UV-visible spectroscopy analysis. Thus the synthesized nanoparticles were centrifuged at 12000 rpm for 15 minutes and followed by dispersion. The process of centrifugation and redispersion was repeated for thrice and dried overnight in oven at 80°C.[13]

Characterization of the Ag-Au Bimetallic Nanoparticles

The aqueous *C. ternatea* flower extract-mediated bimetallic nanoparticles were subjected to characterize by using physicochemical techniques such as UV-vis, FTIR, XRD and SEM-EDX analysis. The UV-vis absorption spectroscopy were obtained by using Shimadzu UV-2600 with respect to the time at regular intervals from 200 to 800 nm range. This absorption spectrum was used to confirm the formation of Ag-Au BNPs. The photo-fabrication of synthesized bimetallic nanoparticles was identified by using FTIR- 8400s Shimadzu. The synthesized nanoparticles were exposed to FTIR spectroscopy with scanning range was 4000–400 cm^{-1}. The nanoparticles crystalline nature, phase and size were identified by X-ray diffraction (XRD) technique. The data was recorded using PANalytical EMPYREAN at 45Kv, 40 MA with a scanning rate of 2θ/min and over a range of 2θ, which is 20–80°. The surface morphology and elemental analysis of synthesized nanoparticles were examined by using TESCAN VEGA3 SBH model scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis.[14]

Anticancer (MTT) assay

The synthesized CT-Ag-Au BNPs has the potential to inhibit the growth of A549 human lung cancer cells were investigated through MTT assay. In this assay, the yellow-colored water-soluble MTT tetrazolium is reduced to form an insoluble formazon crystal in the presence of mitochondrial dehydrogenase enzyme which occurs only in viable cells. The insoluble formazon crystals were dissolved in DMSO solvent. The obtained violet-colored solution was quantified by using a spectrophotometer. For this assay, the cells were cultured in 96-well micro titre plate containing 10^3 to 10^4 cells per well for 24 hours with 100 µL of complete media. The cell lines were maintained in CO_2 incubator at 37°C and 5% CO_2. The cells were treated with 10, 25, 50, 75 and 100 μg^{-1} concentrations of the BNPs and CT extract prepared in incomplete media. The cells were kept under incubation at same conditions of 24 hours. Later 20 µL of MTT solution (5 mg/mL stock solution prepared in phosphate buffer saline) was added and kept for 4 hours at 37°C. The cell contents were discarded without disturbing the formazon crystals formed by adding 200 µL of DMSO. The color intensity of the solution is irreversibly proportional to dead cells. The absorbance was measured at 570 nm and the dead cells' percentage were calculated using the equation.[4]

$$(\%)\ \text{Inhibition} = 100 - (\text{OD of sample}/\text{OD of Control}) \times 100 \quad (1)$$

Results and Discussion

Phytochemical Analysis of *C. ternatea* Flower Extract

The phytochemical analysis of *C. ternatea* aqueous flower extract was done by using standard procedure.[8] The *C. ternatea* flower extract shows the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, cardiac glycosides, phenols, glycosides, resins, saponins and tannins.[11] Table 1 shows the phytochemical screening

of C.T flower extract. The green synthesis of Ag-Au BNPs was achieved using *C. ternatea* aqueous flower extract as a reducing and capping agent.

Synthesis of Ag-Au bimetallic Nanoparticles

The formation of Ag-Au BNPs was observed from the initial reaction mixture color change to the solution final color change, indicating the reduction of metal salts to nanoparticles. This change was observed after adding of *C. ternatea* flower extract. The initial conformation of the synthesis of Ag-Au BNPs was visually confirmed by the transformation of pale yellow to reddish-brown color. This color change indicates Ag-Au bimetallic nanoparticles formation.[13]

UV-visible spectroscopy analysis

The UV-vis spectroscopy technique is widely used for confirmed the formation of nanoparticles. After the visual conformation of color change of the solution, the formation of nanoparticles was further confirmed by UV-vis spectroscopy analysis. Fig. 1 shows the UV-vis spectra of CT flower extract and Ag-Au BNPs. The characteristic surface plasmon resonance (SPR) band for Ag-Au BNPs was seen at 541 nm.[15] The presence of single blue-shifted peak at 541 nm indicates the formation of homogenous bimetallic nanoparticles. The intensity of blue shift peaks were indicates the reduction of $Au^{3+} \rightarrow Au^{0}$ and $Ag^{1+} \rightarrow Ag^{0}$ thus proves the C.T flower extract can be used as a natural reducing agent.[16] The surface plasmon resonance band (SPR) mainly depends on the structure, size, shape, and composition of the nanoparticles formed. The pure *C. ternatea* flower extract was measured and two peaks were observed at around 575 and 620 nm. Due to the presence of delphinidin anthocyanin with the color of purplish blue which is responsible for peaks at 575 and 620 nm and color of C.T.[17]

FTIR spectroscopy analysis

FTIR spectroscopy analysis was done to identify the functional groups of biomolecules in the C.T flower extract. C.T aqueous flower extract contains anthocyanin as a major reducing and capping agent component. Fig. 2 represents the FTIR spectra of C.T extract and CT-Ag-Au BNPs. The distinct clear peaks at 3391, 2924, 1642, 1468 1386, 1058, 1026 and 988 cm^{-1} was observed in CT flower extract which were shifted in the case synthesized Ag-Au BNPs.[17] The absorption peak was observed at 3391 cm^{-1}, corresponding to free O-H stretching band of hydroxyl groups. The active compounds of *C. ternatea* flower extract such as anthocyanin and flavonol, are identified by the peaks at 2924, 1468 1026 and 988 cm^{-1} were corresponding to the symmetric and asymmetric stretching vibration of CH_3, aliphatic C-H stretches, aromatic and vibration of –C-CH. The intensive band at 1235 and 1386 cm^{-1} indicates the plane bending of the hydroxyl groups of two phenolic compounds. The absorption peak at 1408 cm^{-1} represents the carbonyl groups. The peak at 1624 and 1358 cm^{-1} confirm the existence of alcoholic and phenolic functional groups.[18] After the formation of BNPs some peaks have reduced the intensity, but these peaks confirmed the presence of bioactive compounds at 1023, 1234 and 1457 cm^{-1} was still existed. This is the indication of presence of bioactive compounds on the surface of synthesized bimetallic nanoparticles.

Table 1: Phytochemical analysis of *Clitoria ternatea* flower extract

Phytochemicals	*Aqueous C.T Flower extract*
Alkaloids	+
Carbohydrates	+
Terpenoids	+
Cardiac glycosides	-
Tannins	+
Glycoside	+
Flavonoids	+
Saponins	-
Resins	+
Phenols	+

XRD spectroscopy analysis

The phase purity, crystalline nature and size of C.T mediated Ag-Au BNPs were analyzed through the XRD spectroscopy analysis. Fig. 3 shows the diffraction patterns of CT- Ag-Au BNPs. The spectrum provides the distinguishable peaks at 38.06, 44.81, 64.71 and 77.87, which correspond to the planes of (111), (200), (220)

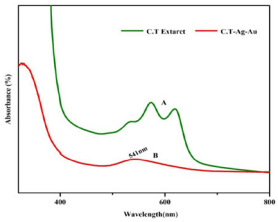

Fig. 1: UV-visible spectra of C.T flower extract and CT-Ag- Au BNPs

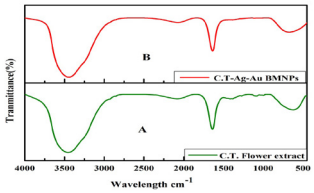

Fig. 2: FTIR spectra of C.T extract and CT Ag-Au BNPs

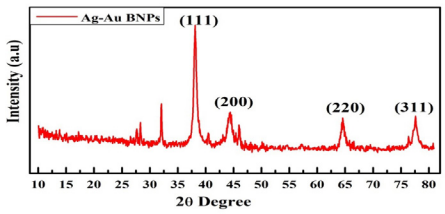

Fig. 3: XRD spectra of C.T- Ag-Au BNPs

and (311). The diffraction peaks are well matched with JCPDS file no-04-0783 for (FCC) cubic Ag and JCPDS file no-04-0784 for (FCC) cubic Au.[13, 16] The Ag-Au bimetallic nanoparticles are not different than Ag and Au mono metal nanoparticles, which can be explained by their similar phase and crystal lattice structure. The CT mediated Ag-Au BNPs crystallite size can be estimated using Debye-Scherrer equation.

$$D=0.9\lambda / \beta \cos\theta \qquad (2)$$

Where

D - Particle size,

λ - Wavelength of the incident x-ray beam,

θ - Bragg's diffraction angle,

β - Full width at half maxima (FWHM) of the C.T-Ag-Au BNPs peak.[19]

This equation shows that the estimated average crystal size of CT mediated Ag-Au BNPs was 14.5 nm. Based on the XRD patterns, the synthesized C.T-Ag-Au BNPs were at high purity of crystalline nature as no other impurity peak was observed.

SEM-EDX analysis

The surface morphology of the CT- Ag-Au BNPs were investigated using SEM analysis, represented by Fig. 4. The CT-mediated Ag-Au BNPs were shown in cubic shape and seem to be agglomerated.[20] The energy-dispersive X-ray spectroscopy analysis showed the elemental analysis of synthesized Ag-Au BNPs. Fig. 5 shows the EDX spectra of Ag-Au BNPs. The peak at 2.5 and 3eV suggest the peak of Ag and Au elements. Additional peaks of C and O were seen which are present in aqueous solution of C.T. flower extract.[13]

Anticancer MTT Assay

To evaluate the anticancer potential of CT mediated Ag-Au BNPs on the cell viability of cancer cells, they were treated against human lung cancer (A549) cell lines. The concentrations of the CT flower and synthesized bimetallic nanoparticles were as 10, 25, 50, 75 and 100 µg/mL. The results exhibited a concentration dependent manner. When increasing the concentration of nanoparticles will decrease the cell viability of cancer cells. The IC_{50} values were calculated by using dose dependent curve. The results were represented in graphs as the percentage of cell viability post-treatment compared to negative control (untreated cells). Table 2 shows the half-minimum inhibitory concentration (IC_{50}) of CT extract and Ag-Au BNPs while Fig. 6 shows the percentage of cell viability to concentration of CT extract and Ag-Au BNPs. Fig. 7 shows the inverted microscopic cell morphology images of A549 cell lines treated with 100 µg/mL of CT extract and Ag-Au BNPs. The IC_{50} values of CT flower extract and CT-mediated Ag-Au BNPs were 85.45 ± 0.05 and 57.35 ± 0.05 µg/mL, respectively. At the concentration of 10 µg/mL the extract shows 75% cell viability and CT-mediated Ag-Au BNPs showed 70% of cell viability. On increasing the concentration of CT extract and Ag-Au BNPs the cell viability was decreased further and reached 25 and 18% at 100 µg/mL. However, the results indicate that the C.T-Ag-Au BNPs exhibit increased cytotoxic potential compared to CT flower extract. This was achieved due to the synergistic action of both Ag and Au metal nanoparticles.[21] When the concentration of nanoparticles, more CT-mediated Ag-Au BNPs enter the cells, which may arrest the working function of mitochondria and produce more reactive oxygen species (ROS). This increases in ROS can damage more cancer cells by damaging the cells DNA, leading to apoptosis/necrotic or cell death.[22] The phytogenic mediated Ag-Au BNPs by using CT flower extract that have shown cytotoxicity effect in the human lung cancer cells in our study have not been reported yet to best of our knowledge.

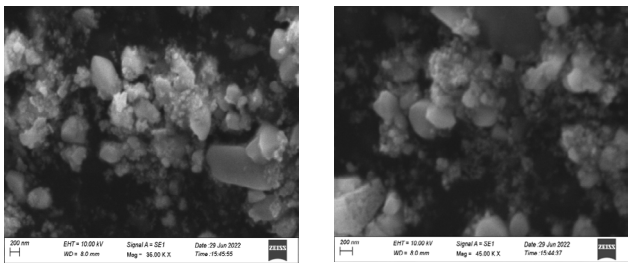

Fig. 4: SEM micrographs of CT-Ag-Au BNPs

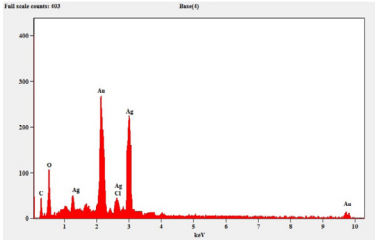

Fig. 5: Energy dispersive X-ray spectra of CT- Ag-Au BNPs

Table 2: IC_{50} Value of CT flower extract and C.T-Ag-Au BNPs

IC_{50} (µg/mL) ± SD Values	
Tested samples	A549 human lung cancer cells
C.T. flower extract	85.45 ± 0.05
C.T-Ag-Au BNPs	57.35 ± 0.05

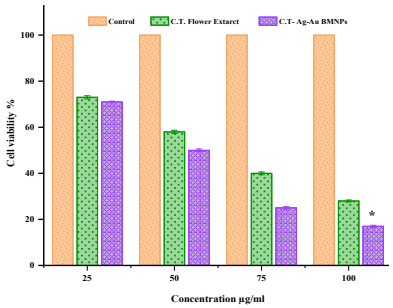

Fig. 6: MTT assay for the CT flower extract and C.T-Ag-Au BNPs treated with A549 cell lines. Control is without addition of flower extract and NPs. The asterisk (*) indicates significant difference between the control and Ag-Au BNPs (100 µg /ml) treated A549cell lines

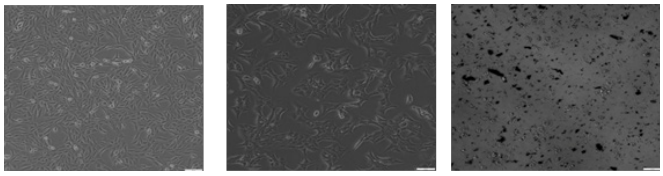

Fig. 7: Inverted microscopic cell morphology images of the A549 cancer treated with 100 µg/mL concentration for a) Control b) CT extract and c) CT- Ag-Au BNPs

Conclusion

This study successfully produced the *C. ternatea* aqueous flower extract mediated Ag-Au bimetallic nanoparticles using a simple, facile, and sustainable eco-friendly green approach. *C. ternatea* plant is a medicinal herb with natural bioactive compounds. This phytochemical constituent of flower extract was act as a reducing and capping agent of synthesized bimetallic nanoparticles. The formation photo fabrication, size, shape, crystalline nature and purity of the synthesized Ag-Au BNPs were analyzed by using spectroscopic techniques. Additionally, the bimetallic nanoparticles show their anticancer activity against human lung cancer (A549) cells. The synthesized bimetallic nanoparticles effectively reduced cancer cells viability in their presence in different concentrations. Thus, the report adds another feature to the medicinal herb CT flower extract to successfully formulate bimetallic nanoparticles and other metal/metal oxide nanoparticles that could be used effectively for their cytotoxicity effect.

References

1. Doria G, Conde J, Veigas B, Giestas L, Almeida C, Assunção M, Rosa J, Baptista PV. Noble metal nanoparticles for biosensing applications. Sensors. 2012;12(2):1657-1687. Available from: https://doi.org/10.3390/s120201657
2. Babar VB, Nagarale SN, Khapale PR. Biosynthesis of silver nanoparticles of aqueous extract of *plectranthus amboinicus* (lour.) Spreng for anticancer activity. Journal of Pharmacognosy and Phytochemistry. 2019;8(6):61-68.
3. Geetha R, Ashokkumar T, Tamilselvan S, Govindaraju K, Sadiq M, Singaravelu G. Green synthesis of gold nanoparticles and their anticancer activity. Cancer Nanotechnology. 2013;4:91-98. Available from: https://doi.org/10.1007/s12645-013-0040-9
4. Velidandi A, Pabbathi NP, Dahariya S, Baadhe RR. Green synthesis of novel Ag–Cu and Ag–Zn bimetallic nanoparticles and their in vitro biological, eco-toxicity and catalytic studies. Nano-Structures & Nano-Objects. 2021;26:100687. Available from: https://doi.org/10.1016/j.nanoso.2021.100687
5. Loza K, Heggen M, Epple M. Synthesis, structure, properties, and applications of bimetallic nanoparticles of noble metals. Advanced functional materials. 2020;30(21):1909260. Available from: https://doi.org/10.1002/adfm.201909260
6. Mazhar T, Shrivastava V, Tomar RS. Green synthesis of bimetallic nanoparticles and its applications: a review. Journal of Pharmaceutical Sciences and Research. 2017;9(2):102.
7. Fanoro OT, Oluwafemi OS. Bactericidal antibacterial mechanism of plant synthesized silver, gold and bimetallic nanoparticles. Pharmaceutics. 2020;12(11):1044. Available from: https://doi.org/10.3390/pharmaceutics12111044
8. Manjula P, Mohan CH, Sreekanth D, Keerthi B, Devi BP. Phytochemical analysis of *Clitoria ternatea* Linn., a valuable medicinal plant. The Journal of Indian Botanical Society. 2013;92(3-4):173-178.
9. Gupta GK, Chahal J, Bhatia M. *Clitoria ternatea* (L.): Old and new aspects. J Pharm Res. 2010;3(11):2610-2614.
10. Kavitha R, Premalakshmi V. Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. International Journal of Pharma and Bio Sciences. 2013;4(4).
11. Lijon MB, Meghla NS, Jahedi E, Rahman MA, Hossain I. Phytochemistry and pharmacological activities of *Clitoria ternatea*. International Journal of Natural and Social Sciences. 2017;4(1):1-10.
12. Khwannimit D, Maungchang R, Rattanakit P. Green synthesis of silver nanoparticles using *Clitoria ternatea* flower: an efficient catalyst for removal of methyl orange. International Journal of Environmental Analytical Chemistry. 2022;102(17):5247-5263. Available from: https://doi.org/10.1080/03067319.2020.1793974
13. Gupta S, Hemlata H, Tejavath K. Synthesis, characterization and comparative anticancer potential of phytosynthesized mono and bimetallic nanoparticles using *Moringa oleifera* aqueous leaf extract. Beilstein Arch. 2020;1:95. Available from: https://doi.org/10.3762/bxiv.2020.95.v1
14. Renuga D, Jeyasundari J, Athithan AS, Jacob YB. Synthesis and characterization of copper oxide nanoparticles using *Brassica oleracea* var. italic extract for its antifungal application. Materials Research Express. 2020;7(4):045007. Available from: https://doi.org/10.1088/2053-1591/ab7b94
15. Kuppusamy P, Ilavenil S, Srigopalram S, Kim DH, Govindan N, Maniam GP, Yusoff MM, Choi KC. Synthesis of bimetallic nanoparticles (Au–Ag alloy) using *Commelina nudiflora L.* plant extract and study its on oral pathogenic bacteria. Journal of inorganic and Organometallic Polymers and Materials. 2017;27:562-568. Available from: https://doi.org/10.1007/s10904-017-0498-8
16. Malapermal V, Mbatha JN, Gengan RM, Anand K. Biosynthesis of bimetallic Au-Ag nanoparticles using *Ocimum basilicum* (L.) with antidiabetic and antimicrobial properties. Advanced materials letters (Online). 2015;6(12):1050-1057. Available from: https://doi.org/10.5185/amlett.2015.5997
17. Chan JZ, Ali RR, Shameli K, Tarmizi ZI, Salleh MS. Biosynthesis of Gold Nanoparticles: A Simple Method of Size Controlled using *Clitoria ternatea* Flower Extract. In IOP Conference Series: Materials Science and Engineering 2021;1051(1):012090. IOP Publishing. Available from: https://doi.org/10.1088/1757-899X/1051/1/012090
18. Fatimah I, Hidayat H, Nugroho BH, Husein S. Ultrasound-assisted biosynthesis of silver and gold nanoparticles using *Clitoria ternatea* flower. South African Journal of Chemical Engineering. 2020;34:97-106. Available from: https://doi.org/10.1016/j.sajce.2020.06.007
19. Athithan AS, Jeyasundari J, Renuga D, Naveena A. Nature inspired synthesis, physico-chemical characterization of Zn doped Fe_3O_4 nanoparticles using *Andrographis paniculata* (burm. F.) nees leaf extract and assessment of in vitro pancreatic alpha amylase inhibitory activity. International Journal of Applied Pharmaceutics. 2020; 12(6):229-235. Available from: http://dx.doi.org/10.22159/ijap.2020v12i6.39278
20. Gopinath K, Kumaraguru S, Bhakyaraj K, Mohan S, Venkatesh KS, Esakkirajan M, Kaleeswarran P, Alharbi NS, Kadaikunnan S, Govindarajan M, Benelli G. Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the *Gloriosa superba* leaf extract and their antibacterial and antibiofilm activities. Microbial pathogenesis. 2016;101:1-1. Available from: https://doi.org/10.1016/j.micpath.2016.10.011
21. Ali S, Sharma AS, Ahmad W, Zareef M, Hassan MM, Viswadevarayalu A, Jiao T, Li H, Chen Q. Noble metals based bimetallic and trimetallic nanoparticles: controlled synthesis, antimicrobial and anticancer applications. Critical reviews in analytical chemistry. 2021;51(5):454-81. Available from: https://doi.org/10.1080/10408347.2020.1743964
22. Patil MP, Kim GD. Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. Applied microbiology and biotechnology. 2017;101:79-92. Available from: https://doi.org/10.1007/s00253-016-8012-8

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