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Original article

Synthesis of silver nanoparticle (Ag NPs) using phytochemical rich medicinal plant *Lonicera japonica* for improve the cytotoxicity effect in cancer cells

Govindan Nadar Rajivgandhi <sup>a,b</sup>, Gnanasekaran Chackaravarthi <sup>b</sup>, Govindan Ramachandran <sup>b</sup>, Natesan Manoharan <sup>b,\*\*</sup>, R. Rangunathan <sup>c</sup>, Muhammad Zubair Siddiqi <sup>d</sup>, Naiyf S. Alharbi <sup>e</sup>, Jamal M. Khaled <sup>e</sup>, Wen-Jun Li <sup>a,f,\*</sup>

<sup>a</sup> State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources and Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China

<sup>b</sup> Department of Marine Science, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, 620024, India

<sup>c</sup> Centre for Bioscience and Nanoscience Research, Bharathiyar University, Echanari, Coimbatore 21, Tamil Nadu, India

<sup>d</sup> Department of Biotechnology, Hankyong National University, 327 Jungang Road, Gyeonggi-do 17579, South Korea

<sup>e</sup> Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

<sup>f</sup> State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, PR China

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## ABSTRACT

Initially, the excellent anti-oxidant property of the Ag NPs was synthesized from Chinese medicinal plant of *Lonicera japonica*, and their spectroscopic evidences were confirmed by UV-spectrometer. The morphology, size and shape of the synthesized Ag NPs were effectively identified by scanning electron microscope and transmission electron microscope. Both the spectroscopic results were more evident, which UV-spectrometer peak was exhibited at 456 nm, scanning electron microscope and transmission electron microscope results were also clearly shown ball like spherical shape. The potential anti-cancer effect of the Ag NPs was revealed, the selected Chinese plant *Lonicera japonica* was influenced the A549 lung cancer cells through excessive ROS production. The IC<sub>50</sub> concentration of 75 µg/mL was shown decreased viability in Ag NPs treated A549 lung cancer cells of MTT assay. Consecutively, the MTT assay result was proved by irregular shape and damaged morphology in A549 lung cancer cells by images of phase contrast microscope. Finally, the result was also clearly stated that the synthesized Ag NPs was very effective against A549 human lung cancer cells and it use for future drug discovery for inhibiting the lung cancer.

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\* Corresponding author at: State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources and Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China. (W.-J. Li)

\*\* Corresponding author at: Department of Marine Science, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, 620024, India (N. Manoharan)

E-mail addresses: [biomano21@yahoo.com](mailto:biomano21@yahoo.com) (N. Manoharan), [liwenjun3@mail.sysu.edu.cn](mailto:liwenjun3@mail.sysu.edu.cn) (W.-J. Li).

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## 1. Introduction

In modern years, metallic nanoparticles have enormous properties like chemical, physical, biological and etc. (Ghosal et al., 2020). Because, it has extended surface area with variety of application process including catalysis, anti-oxidant, electronic, pharmaceutical, environmental and other (He et al., 2016; Patil et al., 2020). It is used frequently in the entire field for improve the better yield than before standardized materials. Usually, metallic nanoparticles have increase efficiency, greatest potentiality, excellent bioactivities, extended biodegradable efficiency with lowest level of toxicity (Lyu et al., 2020; Mani et al., 2021a,b). Based on the greater ability in entire field, now day nanoparticles research is heightened word wide. Apart from this, the toxicity level is played a major concern in biomedical field, because of the side effect or chance to increase the infections and their virulence (Bolbanabad et al., 2020; Anna

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et al., 2017). It also leads to advantages some of some related infections. So, syntheses of lower toxicity level of the nanoparticles are very essential to eradicate any kind of infections (Hamed et al., 2017). Recent years, researchers are concentrated only in the safe method of nanoparticle synthesis that also increased high reproducibility and standard purity. So, all the researchers are focused biogenic nanoparticles with reduced toxicity index for all the applications including soil, agriculture, environment, food, pharmaceutical, biomedical and various other fields (Velázquez-Velázquez et al., 2015; Al-Brahim and Mohammed, 2020; Lakhan et al., 2020; Mani et al., 2021a,b). Also, utilized in large scale production with convenient size and shape of the nanomaterials. So far, most of the researchers are stated that the biogenic mediated nanomaterials are very effective than chemical, physical methods including microbes, plant, algae, and etc (Qais et al., 2020).

Mechanistically, the water soluble bioactive compounds are heightened in the nanoparticle synthesis and act as an effective reducing agent for metal ions especially for metal oxide nanoparticles (Elshaarawy et al., 2020). The Chinese medicinal plant of *Lonicera japonica* has rich flavonoid derivatives and excellent bioactive compounds have excellent anti-cancer properties against various cancer cells and it has excellent reducing agent for various metal oxide nanoparticles (Shang et al., 2011). Plant extract, bioactive derivatives, chemical molecules and respective synthesized nanoparticles are having tremendous anti-oxidant, anti-malarial, anti-biofilm, anti-microbial, anti-cancer and anti-diabetics (Wang et al., 2016; Rantang et al., 2018). As same as the plant of *Lonicera japonica* synthesized nanoparticles are also having same kind of activities with higher efficiency (Annappurna, 2015).

Taken to consideration, the current study is focused on synthesis of silver nanoparticles using *Lonicera japonica* against human lung cancer cells of A549 and its anti-cancer effects of plant extract and silver nanoparticles are performed using various in vitro assays. In addition, this is the first time approach to realize the use of Chinese plant extract and respective silver nanoparticles to perform against A549 lung cancer cells.

## 2. Materials and methods

### 2.1. Needed chemicals and materials

#### 2.1.1. Extraction of plant extract

The Chinese medicinal plant of *Lonicera japonica* leaves were grinded well and taken 1 Kg in sterile distilled water and followed by heat vigorously until the extract was separated from mixture of the samples. Taken Whatman No. 1 filter paper and filtered the liquid portion of the filtrate from extracted samples. Then needed amount of methanol was added into the samples and taken together in soxhlet apparatus. The process was conducted for three hours at 37 °C. After, the sample was put into the hot air oven and maintained at 45 °C until the color changes from green to dark green color in the evaporated samples. In this process, rotary evaporator was used for evaporation process and dark green filtrate for nanoparticles synthesis (Dirar et al., 2019).

### 2.2. Available chemical derivatives by LC–MS

The available phytochemical compounds, flavonoid, phenols, secondary metabolites and other chemical compounds of the selected plant was analyzed by LC–MS spectroscopy with the help of previously report of Rahul et al. (2014). In LC–MS analysis, the solvent of dichloromethane was used as an injection solution, the split ration, and 1:20 and 70 Ev respectively. All the chemical compound detection, dichloromethane (95%) and phenyl (5%) combination was taken together and performed with the procedure of

40 m × 0.45 mm × 0.25 μm. The temperatures and helium gas of 1 mL/m were used as a format of 40 °C initial and 250 °C extension. Then, maintained in oven at 4 °C, and followed by sample with chloroform at the ratio of 1:10 taken together. After completion of all the procedure, the available chemical derivatives present in the selected plant were used by NIST and report effectively.

### 2.3. Amount of phenol and flavonoid content

The detection of phenol and flavonoid derivatives detection, the successful method of Folin–Ciocalteu's was performed to our sample with the followed procedure of Fui et al. (2021). For measurement, gallic acid is act as a standard and compared with standard, the result was interpreted by UV-spectrometer. One in double concentration of our sample and Folin–Ciocalteu's solution was taken together. Continuously, the sample was taken with 2 mL of Na<sub>2</sub>CO<sub>3</sub> also. All the solutions were mixed tightly for 10 min and maintained at sterile dark place. After incubation, the sample was read at 410 nm using spectrophotometer and compared with standard before conclude the result. As same as 10–100 μg/mL of the gallic acid was acted as a positive control. The result was positive when 1 μg of powder sample to result.

Further, the rich flavonoid content of the selected plant was measured by colorimetric method and universal standard of rutin was also used in this study. In this experiment, 500 μL of extract and 200 μL of sodium nitrate were taken together in test tube. Apart from this, 150 μL of sodium nitrate was taken separately before process start. Then, potassium iodide and aluminium chloride solutions were added gradually into side wall in tubes. Then, the mixture solutions were maintained at room temperature 1 h, whereas without addition of extract containing control also maintained with same procedure. Finally, the O.D value of the control and treated were taken separately and noted based on the 1 mg of flavonoid equal to 1 μg gallic acid (Nastaran and Haldi, 2021) (Fig. 1).

### 2.4. Synthesis and characterization of Ag NPs

The 1 mL of selected plant extract filtrate and 10 mL of silver nitrate were taken for synthesis of silver nanoparticles based on the recent reports of Masum et al., (2019). Briefly, the mentioned amount of the required materials were taken together in a single test tube and heated in heavy sun light 1 h, also 1 N H<sub>3</sub>PO<sub>4</sub> was used as a pH maintenance. After, the color was changed to green to yellow or brown to yellow in the test tube was suggested that the plant extract influenced the Ag NO<sub>3</sub> to form silver ions and then converted to silver nanoparticles. Basically, the formed silver nanoparticles were initially proved by UV-spectroscopy and structurally confirmed with size and shape by SEM and TEM (Sahoo et al., 2020) (Fig. 2).

#### 2.4.1. Anti-cancer activity of synthesized Ag NPs by cell viability assay

The viability of A549 lung cancer cells in the presence of Ag NPs treatment plate was monitored by UV-spectrometer with the help of dimethylthiazol-diphenyltetrazolium bromide (MTT) solution interruption (Krishnamoorthy et al., 2020). The 96-well plate was evenly filled with 24 h cancer cells before filled with complete medium of RPMI and followed necessary procedure. Initially, ~2 × 10<sup>4</sup> of cancer cells were diluted into the 96-wells eventually in the 5% CO<sub>2</sub> availability incubator for 4 h. In addition, the humidity of 95% was very effective to pure process. Then, 25–250 μg/mL concentration of synthesized Ag NPs was added into all the 96-well containing A549 lung cancer cells without control and blank. The plate was maintained based on the above said procedure for 1 day. Then, MTT solution was added after complete treatment and also control wells of the plate eventually and sealed by using

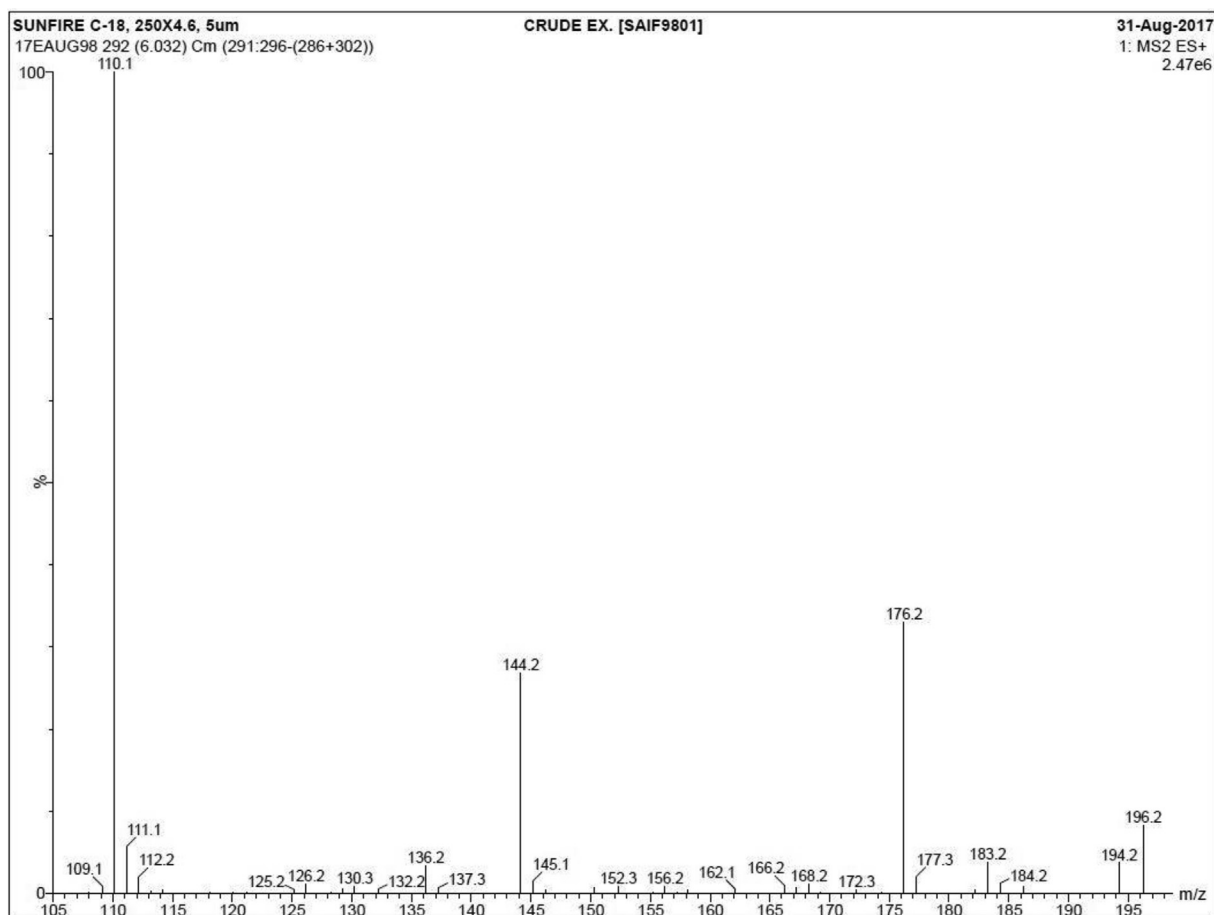


Fig. 1. LC-MS analysis of selected plant extract for detection of available chemical derivatives.

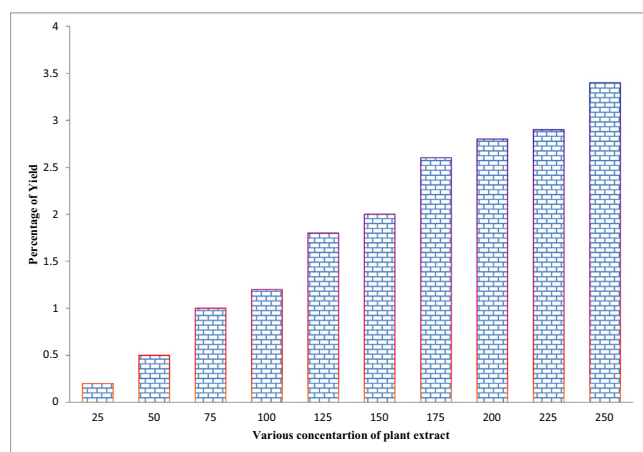


Fig. 2. Measurement of available phenolic content percentage in the selected medicinal plant extract of *Lonicera japonica*.

aluminum foil. The plate was clearly monitored until the formation of formazan crystal production, once it formed; the plate was taken to consideration for measurement by UV-spectroscopy at 500 nm of O.D. Experiment was conducted three times without any changes and calculated each and every plate. Then, the result of  $IC_{50}$  value of the tested Ag NPs against A549 lung cancer cells was calculated based on the universal available formula,

$$\text{Percentage} = IC_{50} \left[ \frac{\text{Control O.D} - \text{Test O.D}}{\text{Control O.D}} \right] \times 100.$$

### 3. Result

#### 3.1. Detection of available phytochemical derivatives using LC-MS spectroscopy

In chemical composition of medicinal plant Chinese medicinal plant of *Lonicera japonica* was shown more than 40 different peaks in LC-MS analysis. Among these peaks, 13 different peaks were observed with phytochemical derivatives of *Lonicera japonica* and relatively confirmed to previous reported data of Shang et al. (2011). In addition, all these peaks were confirmed by Wiley chemical compounds Library that attached with International Repository of phytochemical derivatives, Sun Yat-Sen University, China. Previously, these 13 peaks were screened based on the retention time, occupied percentage and occupied area and have excellent biomedical inhibition effect. All those peaks were possible to relate with phenolic, flavonoids, bioactive and alkaloids derivatives. Further, the phytochemical rich derivatives of *Lonicera japonica* were reported with high rate of anti-oxidant property and various biomedical activities (Rantang et al., 2018). Yuke et al., 2020 also documented for anti-microbial and anti-cancer properties of *Lonicera japonica*. The present result was more similar to previously reported author of Tang et al. (2021) and the Chinese medicinal plant of *Lonicera japonica* suggested with anti-oxidant and anti-cancer properties. Based on the Wiley Repository Library reports, the list of phytochemicals, bioactive compounds, phenols and flavonoid derivatives were listed below Phenol, 2,4-bis(1,1-dimethylethyl) (206.32), 1-acetyl-3-amino-4-cyano-3-pyrroline, methyl 2-amino-3-methylbutanoate hydrochloride, Pyrrolo[1,2-a]

pyrazine-1,4-dione, pyrimido[1,2-a]azepine, 2,3,4,6,7,8,9,10-octahydro-, 1,2,5-oxasilaborolane, 4,4,5-triethyl-2,2,3-trimethyl, hexahydro-3-(2-methylpro (210.27), butanoic acid, 2-hydroxy-3-methyl- (146.18 g/Mol), 5 h,10 h-dipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione, octahydro-, (5*as-cis*, 4-cyanobenzoic acid, 1-(cyclopentyl)ethyl ester), phenylethyl Alcohol (122.16 g/Mol, formamide, n-(4-[2-(1,1-dimethylethyl)-5-oxo-1,3-dioxolan-4-yl]butyl), benzoic acid, 2-amino (137.14), n,n-diethyl-2-formyl-5-methoxybenzamide, dodecanoic acid. Their total retention time was also covered with 10, 50,000 and their percentages were matched to these percentages. Further, the potential compounds were also closely matched to respective retention time, occupied area and occupied percentages. Similar result evidences were also reported by previous researcher for medicinal plant of *Lonicera japonica* with increased anti-oxidant activities. Previously similar result was published by Annapurna (2015), and the plant bioactive compounds were identified based on the LC-MS analysis using NIST Wiley Repository Library (Fig. 3).

### 3.2. Calculation of available photochemical amounts

In result of phenol content amount detection, the result was more favor to *Lonicera japonica* extract and it confirmed that the Chinese medicinal plant *Lonicera japonica* has more phenol content and the control of gallic acid contain  $R_2 = 0.95052$  was closely related to the extract. After comparison with universal control was revealed that the Chinese medicinal plant *Lonicera japonica* was very higher and effective. Further, the concentration of 10–100  $\mu\text{g/mL}$  was also revealed that the extract has more phenol content and it was comparatively very high. As same as, the 10–100  $\mu\text{g/mL}$  concentration of extract was also very effective for flavonoid content production. The universal positive control result of  $R_2 = 0.9423$  was clearly lower than extract production. In addition, both the result was suggested that the Chinese medicinal plant was efficiently produced phenol and flavonoid contents very high in rate, and it confirmed by after comparison of positive control of rutin. Recently, Tang et al., 2021, Yuke et al., 2020 reported that the high phenol and flavonoid content was extracted from Chinese medicinal plant of *Lonicera japonica*. In addition, the biological

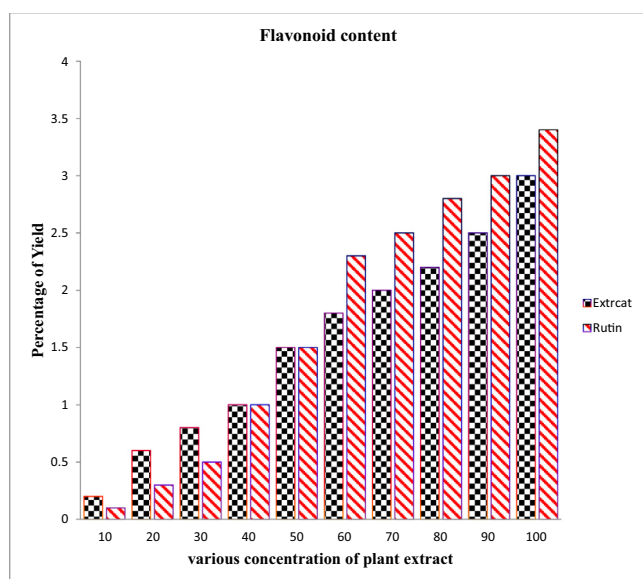


Fig. 3. Identification of available flavonoid content percentage in the selected medicinal plant extract of *Lonicera japonica*.

applications like anti-bacterial, anti-fungal, and anti-cancer were very high rate and evidently proved by Annapurna, 2015; Shang et al., 2011. Plant has more nutrients, hormones, nitrogen content, carbon contents and some other related contents for improve their productivity and activity (Wang et al., 2016). As same as, the medicinal plant and their secondary metabolites were eradicated most of the infectious pathogens with the help of excessive nutrients. In the stress environment, the plant produced excessive bioactive metabolites and phytochemical derivative; they are also acted as excellent biological properties and used in various biomedical and pharmaceutical properties Rantang et al., 2018.

### 3.3. Silver nanoparticle synthesis using Chinese medicinal plant of *Lonicera japonica*

Based on the MiO theory, the present Ag NPs peak was observed at the nm of 456 and it effectively checked in Fig. 4. In the silver nitrate plus extract containing test tube was slightly changed their original color after addition of Chinese medicinal plant *Lonicera japonica* extract at 30 min. Later, the color formation was changed to increasing time interval and changed the color from brown to pale yellow and clear nature of the sample was observed at 1 h. The surface plasma resonance between the silver nitrate and extract was clearly stated the synthesized material was Ag NPs and the plant extract was an effective precursor molecule. The color variations of the tube were suggested that the silver nitrate was converted to silver nanoparticles with the help of plant extract at 1 h time duration. Crystalline structure of the biosynthesized Ag NPs were carried out by XRD. The Ag nanostructure employing by *Lonicera japonica* plant extract was additional established by the distinguishing peaks detected in the XRD analysis in Fig. 4b. The four separate diffraction peaks of the  $2\theta$  values of  $36.3^\circ$ ,  $47.5^\circ$ ,  $66.4^\circ$  and  $76.9^\circ$  corresponded to the plane of (111), (200), (220) and (311) respectively. The designates Ag NPs are fcc and crystalline in nature and exhibited planes were originate to be a faultless match with the JCPDS card number 04-0783 and earlier conclusions also indicated that same JCPDS card number (Suman et al., 2013; Lee et al., 2013).

In addition, the SEM result of minute agglomerate structure was clearly formed in the viewed structure and it confirmed the Ag NPs presence in the viewed picture (Fig. 5a). The morphological appearance of present result was agreed by previously reported evidences of Ruiz-Baltazar et al. (2018); Velmurugan et al. (2016), and the morphology was spherical in shape. Supportively, the TEM micrograph picture was clearly delivered the original spherical morphology of Ag NPs. Also, the clear spherical shape of the Ag NPs and their size was clearly zoomed. Both the scanning electron microscopic method and transmission electron microscopic method was clearly mingled each other in the stage of shape and size (Kup et al., 2020). So, present result was accepted that the Chinese medicinal plant *Lonicera japonica* as an excellent precursor for synthesis of Ag NPs. Similarly Sathiyaseelan et al., 2020; reported that the plant extract is the best choice for synthesis of Ag NPs due to the low cost and environmental free nature. Recently, Wong et al., 2017; Hussain et al., 2019 reported that the biosynthesized Ag NPs was very efficient than chemical and physical method synthesis due to the less toxic nature. This statement was agreed with Que et al. (2019) and reported contrary chemical and physical methods.

### 3.4. Inhibition of A549 lung cancer cells by MTT assay

Among the concentrations of 5–75  $\mu\text{g/mL}$ , the result was proved at the concentration of 75  $\mu\text{g/mL}$  with 52% of cell viability. In this concentration, the viability was observed half rate due to the influence of Chinese medicinal plant of *Lonicera japonica* extract and it

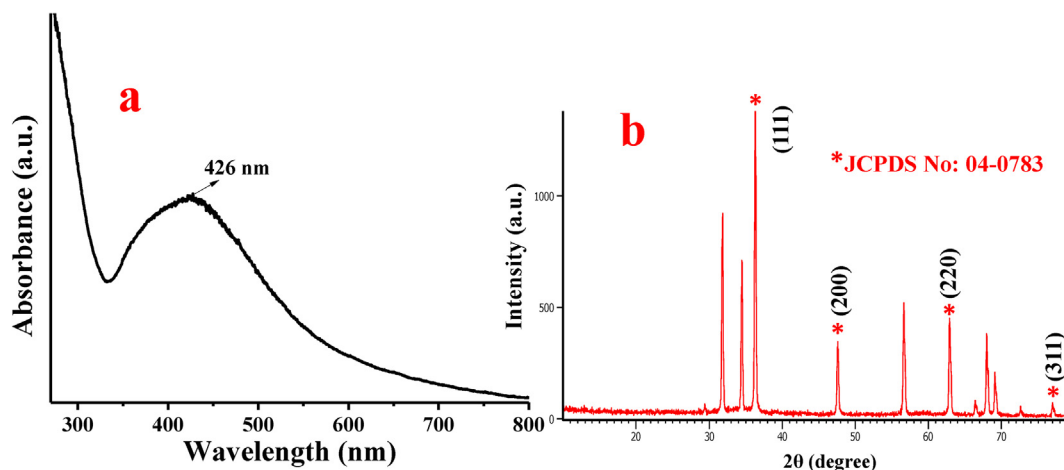


Fig. 4. Detection of synthesized Ag NPs by UV-spectroscopy (a) and XRD (b) using medicinal plant extract of *Lonicera japonica*.

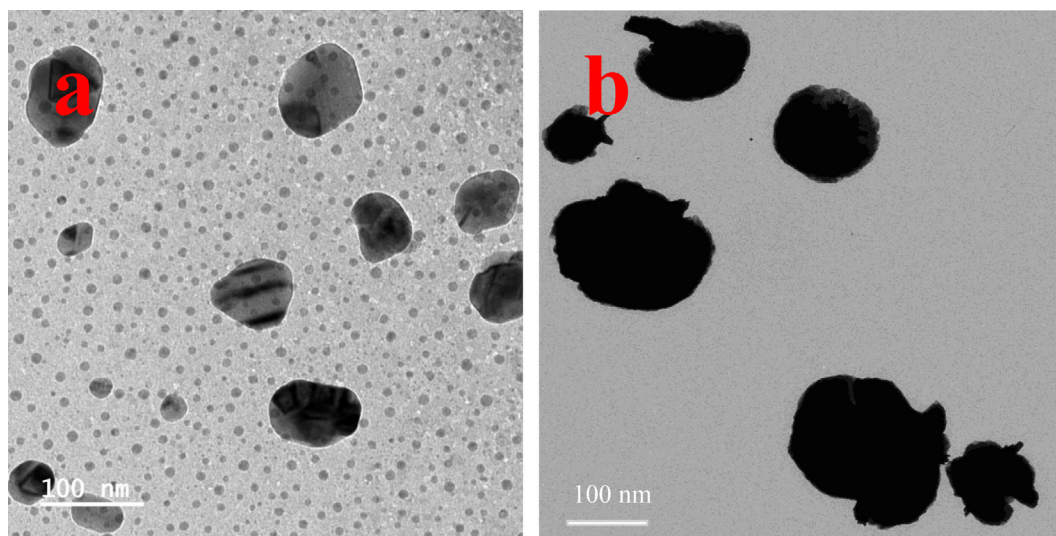


Fig. 5. Size and shape morphology of *Lonicera japonica* mediated Ag NPs confirmed by SEM (a) and TEM (b).

may helped to regenerate the ROS when ROS level is decreasing. The apoptosis level was very high at this concentration because of the Ag NPs. Particularly, 5–10  $\mu\text{g}/\text{mL}$  concentration of Ag NPs was tried to stabilize the cells in decreased phase and helped to produce the ROS initially. After 50  $\mu\text{g}/\text{mL}$  the apoptosis was increased due to the continuous production of ROS level. In addition, the nucleus was collapsed and unformed. Due to this deficient, the cancer cells failed their regeneration process and gene alteration was stimulated. Further, late apoptosis was more severe to the cancer cells and damaged the structural integrity. These evidences were clearly indicated by [Jeyaraj et al. \(2015\)](#); [Sangour et al. \(2021\)](#). In this stage, the cells were not able to produce gene expression and failed to produce the favorable enzymes ([Malvin et al., 2020](#)). Chromatin condensation was clearly happened into the cancer cells and it shrunked totally. Ag NPs may enter into the internal membranes and helped to continuous ROS production and leads to death ([Ramar et al., 2015](#)). Hence, the concentration of 75  $\mu\text{g}/\text{mL}$  Ag NPs was more efficient than other concentration for damaged the A549 lung cancer cells and this concentration was fixed as an  $\text{IC}_{50}$ . Recently, [Almalki and Khalifa \(2020\)](#), [Rajesh Kumar et al. \(2018\)](#) reported that the plant mediated Ag NPs was very efficient for ROS production and helped to more apoptosis process ([Fig. 6](#)).

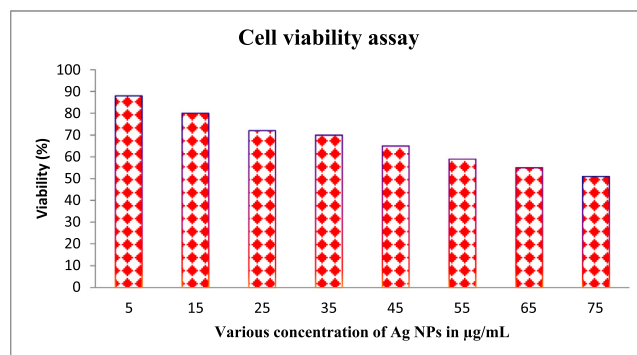


Fig. 6. Anti-cancer ability of Ag NPs proved against A549 lung cancer cells by cell viability assay.

#### 4. Conclusion

Based on the above study, the excellent anti-oxidant property of the Chinese medicinal plant of *Lonicera japonica* was chosen in this study for synthesis of Ag NPs. As per result, the SPR peak of the synthesized Ag NPs was confirmed by UV-spectroscopy and mor-

phological with size and shape of the synthesized Ag NPs were confirmed by microscopic observations. In biomedical process, the result was proved that the selected plant extract has anti-cancer properties against tested A549 lung cancer cells and confirmed by decreased viability formation. The phase contrast images were supported to MTT result, and clearly stated that the synthesized Ag NPs damaged the morphology of A549 human lung cancer cells at very lowest concentration. Finally, the present study was proved that the synthesized Ag NPs were very efficient against A549 lung cancer cells and it will be used in future for best drug choice with complete inhibition of cancer cells.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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