Novel Curcumae Kwangsiensis mediated synthesis of silver nanoparticles for anti-lung cancer activity: a preclinical trial study

Type
Research paper

Keywords
Antioxidant, Silver nanoparticles, Curcumae Kwangsiensis Folium, Cytotoxicity, Anti-human lung cancer

Abstract
Introduction
The present work indicated the green synthesis and characterization and cytotoxicity, antioxidant, and anti-human lung cancer activities of silver nanoparticles containing Curcumae Kwangsiensis Folium leaf aqueous extract.

Material and methods
Ag nanoparticles have been produced by mixing the AgNO3 solution with aqueous Curcumae Kwangsiensis Folium leaf extract. Characterization of Ag nanoparticles was done by FE-SEM, FT-IR, TEM, and UV-Vis. FE-SEM and TEM images revealed an average diameter of 15-21 nm for the nanoparticles. MTT assay was used on common human lung cancer cell lines i.e., lung well-differentiated bronchogenic adenocarcinoma (HLC-1), lung moderately differentiated adenocarcinoma (LC-2/ad), and lung poorly differentiated adenocarcinoma (PC-14) cell lines to survey the cytotoxicity and anti-human lung cancer effects of Ag nanoparticles.

Results
They had very low cell viability and high anti-human lung cancer activities dose-dependently against HLC-1, LC-2/ad, and PC-14 cell lines without any cytotoxicity on the normal cell line (HUVEC). The IC50 of Ag nanoparticles were 249, 187, and 152 µg/mL against HLC-1, LC-2/ad, and PC-14 cell lines, respectively. The best results of cytotoxicity and anti-human lung cancer properties were seen in the concentration of 1000 µg/mL. Ag nanoparticles inhibited half of the DPPH molecules in the concentration of 135 µg/mL.

Conclusions
Maybe significant anti-human lung cancer potentials of Ag nanoparticles synthesized by Curcumae Kwangsiensis Folium leaf aqueous extract against common human lung cancer cell lines are linked to their antioxidant activities. After confirming the above results in the clinical trial researches, this formulation can be administrated to treat human lung cancers in humans.
Novel green synthesis of silver nanoparticles mediated by *Curcumae Kwangsiensis* for anti-lung cancer activities: a preclinical trial study

**Abstract:** The present work indicated the green synthesis and characterization and cytotoxicity, antioxidant, and anti-human lung cancer activities of silver nanoparticles containing *Curcumae Kwangsiensis* Folium leaf aqueous extract. Ag nanoparticles have been produced by mixing the AgNO$_3$ solution with aqueous *C. Kwangsiensis* Folium leaf extract. Characterization of Ag nanoparticles was done by FE-SEM, FT-IR, TEM, and UV-Vis. FE-SEM and TEM images revealed an average diameter of 15-21 nm for the nanoparticles. MTT assay was used on common human lung cancer cell lines i.e., lung well-differentiated bronchogenic adenocarcinoma (HLC-1), lung moderately differentiated adenocarcinoma (LC-2/ad), and lung poorly differentiated adenocarcinoma (PC-14) cell lines to survey the cytotoxicity and anti-human lung cancer effects of Ag nanoparticles. They had very low cell viability and high anti-human lung cancer activities dose-dependently against HLC-1, LC-2/ad, and PC-14 cell lines without any cytotoxicity on the normal cell line (HUVEC). The IC50 of Ag nanoparticles were 249, 187, and 152 µg/mL against HLC-1, LC-2/ad, and PC-14 cell lines, respectively. The best results of cytotoxicity and anti-human lung cancer properties were seen in the concentration of 1000 µg/mL. Ag nanoparticles inhibited half of the DPPH molecules in the concentration of 135 µg/mL. Maybe significant anti-human lung cancer potentials of Ag nanoparticles synthesized by *C. Kwangsiensis* Folium leaf aqueous extract against common human lung cancer cell lines are linked to their antioxidant activities. After confirming the above results in the clinical trial researches, this formulation can be administrated to treat human lung cancers in humans.

**KEYWORDS:** Silver nanoparticles; *Curcumae Kwangsiensis* Folium; Antioxidant; Cytotoxicity; Anti-human lung cancer.

**Key point:** AgNPs@ *C. Kwangsiensis* against lung cancer

**Introduction**

Lung cancer is the most common cancers in men, also one of the most common cancers in the woman. The lung cancer signs are dysphagia, shortness of breath, cough, coughing up blood, weightloss, chestpain, shoulderpain, wheezing, fatigue, hoarseness, and weakness. The lung
cancer metastasis symptoms are blurred vision, seizures, headaches, and weakness (Hecht, 2012; Taylor et al., 2007; Thun et al., 2008). To diagnosis of lung cancer, positron emission tomography, magnetic resonance imaging, low-dose helical computerized tomography scan, computerized tomography, chest X-ray, molecular testing, blood tests, thoracentesis, needle biopsy, bronchoscopy, sputum cytology, and physical and history examination are used (Collins et al., 2007). For the treatment of lung cancer, chemotherapy, radiation therapy, and immunotherapy are used (Alsharairi, 2019; Collins et al., 2007). The main anti-human lung cancer chemotherapeutic supplements/drugs are alectinib, ceritinib, crizotinib, and brigatinib (Alsharairi, 2019). Due to severe side effects of the chemotherapeutic drugs and supplements, the formulation of the chemotherapeutic medications from Ag nanoparticles are the research priority of pharmacology, oncology, and organic chemistry researchers (Kayar and Agin, 2019; Jabłońska et al., 2019; Beyer et al., 2013; Raut et al., 2010; Varma, 2012).

Nanomaterials, which are the subject of our study, have unique competencies different from their macro-scale counterparts due to their low volume/surface ratio and many advanced and new physiochemical properties such as color, solubility, strength, prevalence, toxicity, magnetic, optical, thermodynamics (Arunachalam et al., 2013; Kaczmarczyk-Sedlak et al., 2019; Ball, 2018; Sintubin et al., 2009). In recent years, biological methods that non-toxic, cost-effective, and environmentally friendly have become the focus of interest compared to physicochemical nanoparticle synthesis methods (Hagh-Nazari et al., 2017; Farzaei et al., 2018; Sayyedrostami et al., 2018; Jalalvand et al., 2019; Ball, 2018; Mahdavi et al., 2020; Zangeneh, 2020; Zangeneh et al., 2019). Various pathways have been developed for the biogenic or biological formulation of nanomaterials from the salts of different metal ions (Goorani et al., 2019; Zhaleh et al., 2018; Mahdavi et al., 2020; Sintubin et al., 2009; Zangeneh, 2020). The basic principle in the biogenesis of nanoparticles is the reduction of metal ions of several biomolecules found in organisms. In addition to reducing the environmental impact of biological synthesis, it enables the production of large quantities of nanoparticles, which are well defined in size and morphology, independent of contamination. Microorganisms, marine algae, plant extracts, plant tissue, fruits, and all plants are administrated to formulate nanomaterials (Moradi et al., 2019; Ball, 2018; Beheshtkhoo et al., 2018; Hamelian et al., 2018; Hamelian et al., 2020; Mahdavi et al., 2020; Mohammadi et al., 2020; Sintubin et al., 2009; Zangeneh, 2020; Zangeneh et al., 2019; Zangeneh and Zangeneh, 2020). The reduction of metal ions using plant extracts has been a
known method since the 1900s. Due to the poor understanding of the mechanisms of the reducing agents, it has increased interest in the past 30 years (Sintubin et al., 2009). Recently, scientists have revealed that medicinal plants' green synthesized-metallic nanoparticles have excellent anti-cancer properties. Metallic nanoparticles have achieved notable consideration in the field of medicine. Some studies conducted today have shown that some nanoparticles have therapeutic properties and it is an excellent alternative to physicochemically different metal-supported nanoparticles, antibacterial, and especially anticancer drugs. Medicinal plants green synthesized-silver nanoparticles as a special type of them well-known metallic nanoparticles have recently been used for the cure of several types of tumors and cancers (Ghashghaii et al., 2017; Szymusik et al., 2019; Michalak et al., 2020; Sherkatolabbasieh et al., 2017; Goorani et al., 2020; Rashidi et al., 2018; Ball, 2018; Hamelian et al., 2018; Hamelian et al., 2020; Mahdavi et al., 2020; Mohammadi et al., 2020; Zangeneh, 2020; Zangeneh et al., 2019; Zangeneh and Zangeneh, 2020; Tahvilian et al., 2019). In this regard, in the study of Ahmeda et al. *Melissa officinalis* green-formulated Ag nanoparticles was used as a chemotherapeutic drug to treat leukemia (Ahmeda et al., 2020). A study was reported the anti-acute myeloid leukemia abilities of Ag nanomaterials in the cellular and molecular conditions. Ag nanoparticles significantly killed all malignant leukemia cells (32D-FLT3-ITD, Murine C1498, and Human HL-60/vcr) in the nano concentrations (Hamelian et al., 2018).

There isn’t study about the remedial capacities of Ag nanoparticles containing natural compounds in the treatment of human lung cancer. However, there are many studies about the anti-human lung cancer properties of medicinal plants including *Hibiscus syriacus, Memecylon umbellatum, Mirabilis multiflora, Oscillatoria acutissima, Chelidonium majus, Nigella sativa, Morinda citrifolia, Bruguiera sexangula, Camellia sinensis, Hypsizygus marmoreus, Lentinus edodes*, and *Viola odorata* (Monteiro et al., 2014).

*C. Kwangsiensis* Foliums one of the well-known plants in all of the world is from *Plantae* kingdom, *Zingiberaceae* family, *Zingiberoideae* subfamily, *Zingibereae* tribus, and *Curcuma* genus (Sacchetti et al., 2005). There are five species in the *Curcuma* genus including *Curcuma kwangsinensis, Curcuma phaeocaulis* Val., *Curcuma sichuanensis, C. Kwangsiensis* Folium, *Curcuma wenyujin*, and *Curcuma longa* L. All of these species are important due to significant therapeutic properties, of course remedial roles of *C. Kwangsiensis* Folium are unique. *C. Kwangsiensis* Folium is cultivated in Iran, Bangladesh, Taiwan, Nepal, India, and especially
China (Sacchetti et al., 2005; Tian and Liang). Recent researches have indicated *C. Kwangsiensis* Folium has notable anti-parasitic, antifungal, antiviral, antibacterial, larvicidal, antioxidant, hypoglycemic, anti-diabetic, nephroprotective, hepatoprotective, immunomodulatory, analgesic, anti-inflammatory, and anticancer activities (Tian and Liang). This species is shown to have steroids, saponins, tannin, terpenoids, glycosides, proteins, carbohydrates, alkaloids, phenolic compounds, flavonoids, and essential oils as the major phytochemical groups (Sacchetti et al., 2005). The antioxidant compounds of *Curcumae* genus are Oxygenated sesquiterpenes, Oxygenated monoterpenes, Limonene, Sesquiterpene hydrocarbons, *Trans*-caryophyllene, α-Zingiberene, *p*-Cymene, α-Pinene, Monoterpene hydrocarbon, β-Pinene, α-Terpinolene, *Trans*-β-elemenone, α-Terpineol, β-Sesquiphellandrene, and Camphor. Certainly the above phytochemicals have a unique role in the therapeutic effects of this plant. In the Chinese traditional medicine, *C. Kwangsiensis* Folium is administrated to treat cancers various types such as ovarian and uterine cancers (Sacchetti et al., 2005; Tian and Liang).

Accordingly, the present experiment was conducted to evaluate the possible anti-human lung cancer activity of synthesized Ag nanoparticles using *C. Kwangsiensis* Folium leaf aqueous extract against common cell lines of human lung cancer.

**Materials and Methods**

**Material**

Bovine serum, antimycotic antibiotic solution, 2,2-diphenyl-1-pikrilhydrazil (DPPH), dimethyl sulfoxide (DMSO), decamplmaneh fetal, 4-(Dimethylamino) benzaldehyde, hydrolyzate, Ehrlich solution, and borax-sulfuric acid mixture, DMED, all were afforded from the US Sigma-Aldrich company.

**Synthesis of Curcumae Kwangsiensis Folium green-synthesized Ag nanoparticles**
To obtain the aqueous extract of the plant, 250 gr of the dried branches of the *C. Kwangsiensis* Folium leaves were poured in a container containing 2000 mL boiled water, and the container lid was tightly closed for 4 h. Then, the content of the container was filtered, and the remaining liquid was placed on a bain-marie to evaporate. Finally, a tar-like material was obtained, which was powdered by a freeze dryer.

The extract of the *C. Kwangsiensis* Folium leaves was prepared for the green synthesis method was extracted with distilled water in the microwave. Green synthesis of silver nanoparticles was started with such a process combination of 200 mL AgNO$_3$×H$_2$O at concentrations of 10$^{-3}$ M and 400 mL of *C. Kwangsiensis* Folium leaf aqueous isolate (20 μg / mL) in a cylindrical flask.

The reaction mixture was kept under magnetic stirring for 12 h at room temperature. At the end of the reaction time, the black colored colloidal solution of Ag was formed. The solution was centrifuged at 10000 rpm for 15 minutes. The precipitate was sprayed with water and then resuspended (Hamelian et al., 2020).

**Chemical characterization of Curcumae Kwangsiensis Folium green-synthesized Ag nanoparticles**

Various analytical techniques were used to characterize the Ag nanoparticles:

The biomolecules related to the Ag nanoparticles reduction including secondary metabolites were detected by the FT-IR (Shimadzu IR affinity.1). In the UV-Vis spectroscopy analysis,
characteristic absorption bands of Ag metal were examined before and after the nanoparticles synthesis process. TEM and FE-SEM analysis of the Ag nanoparticles was performed with JEOL 200 kV by placing them on a carbon-coated copper grid.

**Determination of the antioxidant activities of CurcumaeKwangsiensis Folium green-mediated Ag nanoparticles**

The DPPH method is a common method for the assessment of the antioxidant activity of plant species and metallic nanoparticles. It is based on trapping the free radicals of the material, called DPPH, using antioxidant agents which reduce the absorption rate at 520 nm wavelength. When the DPPH solution is mixed with a material that can donate hydrogen atom, radical resuscitation is formed, which is followed by color reduction. This reaction eliminates the purple color, whose index is the formation of an absorption band at 520 nm (Hosseinimehr et al., 2011).

To determine the radical scavenging activity of the silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles, 1 mL of 50 µm DPPH was combined with 1 mL of variable concentrations (0-1000 µg/mL) of silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles. Then, they were transferred to the 37 °C for 1 h. The samples absorption rate was determined at 520 nm by a spectrophotometer, and the antioxidant activity was calculated by the below formula:
The blank sample contained 1 mL methanol and 1 mL silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles, and a sample of 1 mL DPPH and 2 mL silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles with the applied concentrations was regarded as the negative control (Hosseinimehr et al., 2011).

**Determination of the IC50 of Curcumae Kwangsiensis Folium green-mediated Ag nanoparticles in the antioxidant test**

Calculation of half-maximal inhibitory concentration (IC50) is a suitable method for comparison of the activity of pharmaceutical materials. In this method, the measurement and comparison criterion is the concentration in which 50% of the final activity of the drug occurs. In this experiment, the IC50 of various repeats is estimated and compared with the IC50 of BHT, which is introduced as the antioxidant activity index. The closer is the obtained value to the IC50 of BHT, the stronger is the antioxidant activity of the material. The graph of the IC50 of the extract was produced by drawing the percent inhibition curve versus the extract concentration. First, three stock samples with variable concentrations (0-1000 µg/mL) of silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles were prepared. Then, a serial dilution was prepared from each sample, and IC50 of the above samples was measured separately, following which
their mean was calculated. BHT, with different concentrations, was considered positive control.

All experiments were performed in triplicate (Hosseinimehr et al., 2011).

**Evaluation of anti-lung cancer properties of Curcumaes Kwangsiensis Folium green-mediated Ag nanoparticles**

The human lung cancer cell lines (Lung well-differentiated bronchogenic adenocarcinoma: HLC-1, Lung moderately differentiated adenocarcinoma: LC-2/ad, and Lung poorly differentiated adenocarcinoma: PC-14) and the normal cell line (HUVEC) in the MTT assay were used as follows. They were then cultured as a monolayer culture in 90% RPMI-1640 medium and 10% fetal serum and supplemented with 200 mg/mL streptomycin, 125 mg/mL penicillin, and 8 mg/mL amphotericin B. The culture was then exposed to 0.5 atmospheric carbon dioxide at 37 °C, on which the tests were performed after at least ten successful passages. MTT assay a method used to investigate the toxic effects of various materials on various cell lines, including non-cancer and cancer cells. To evaluate the cell toxicity effects of the compounds used in this research, the cells were transferred from the T25 flask to the 96-well flasks. In each cell of the 96-cell flasks, 7000 cells of cancer and fibroblast cell lines were cultured, and the volume of each cell was eventually increased to 100 µL. Before the treatment of the cells in the 96-well flak, the density of cells was increased to 70%, so the 96-well flasks were incubated for 24 h to
obtain the cell density of $7 \times 10^3$. Next, the initial culture medium was discarded, and variable concentrations (0-1000 µg/mL) of silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles were incubated at 37 °C and 0.5 CO$_2$ for 24, 48, and 72 h. Then, 20 µL MTT was added to each well after a certain amount of time. Next, 100 µL DMSO solvent was added to each well. They were then kept at room temperature for 25 min and read at 490 and 630 nm by a microtitre plate reader.

The cell lines were treated with the hydroalcoholic extract (1.25 mg/mL), which inhibited about 20% of the cell growth. Annexin/PI method was used to determine the apoptosis level in the treated and control cell lines using a flow cytometry machine. To perform experiment, the cell lines were treated with a variable concentrations (0-1000 µg/mL) of silver salt, *C. Kwangsiensis* Folium leaf extract, and Ag nanoparticles for 24 h. Cells were irrigated with phosphate-buffered saline (PBS). After centrifugation, buffer binding was added to the obtained precipitate. Then, 5 µl Annexin V dye was added and incubated for 15 min at 25 °C. Cells were washed with the binding solution, following which 10 µL PI dye was added. Finally, cell analysis was done by a flow cytometry machine according to the below formula:

$$\text{Cell viability (\%)} = \frac{\text{Sample A.}}{\text{Control A.}} \times 100$$

**Qualitative Measurement**

The obtained results were analyzed by SPSS (version 20) software using one-way ANOVA, followed by Duncan post-hoc test ($P \leq 0.01$).
Results

In the recent study, Ag nanoparticles were formulated using *C. Kwangsiensis* Folium leaf extract. Also, we assessed the anti-human lung cancer activities of Ag nanoparticles against common human lung cancer cell lines in the *in vitro* condition.

*Chemical characterization of Ag nanoparticles*

UV-Vis spectroscopic analysis showed the presence of an absorption peak at 422 nm which confirmed the formation of the Ag nanoparticles (Figure 1). Mohammadi *et al.* (2019) observed the peak of Ag nanoparticles containing *Phoenix dactylifera* seed ethanolic extract at the wavelength of 438 nm (Mohammadi et al., 2020). Hamelian *et al.* reported *Thymus kotschyanus* aqueous extract synthesized AgNPs with a peak at 440 nm in the UV-Vis spectrum (Hamelian et al., 2018). Zangeneh *et al.* revealed the absorbance at 462 nm for Ag nanoparticles synthesized by *Spinacia oleracea* L. (Zangeneh, 2020). These reports support the results of the current work. The *C. Kwangsiensis* Folium mediated synthesis of Ag nanoparticles showed excellent stability even after 20 days and no considerable changes occurred in UV-absorbance.

Figure 1

In the FT-IR test, the antioxidant and secondary compounds are determined based on several peaks in special wavelengths. The analysis of the IR spectra of the Ag nanoparticles revealed the
peaks at 590, 1089, 1401, 1613, and 3429 cm\(^{-1}\) related to the Ag-O, C-OH, C=O, C-O, and OH, respectively (Figure 2).

The IR spectra investigated for the Ag nanoparticles revealed the absorption peaks at (I) 3287 cm\(^{-1}\) (OH group of alcohols and phenols); (II) 1623 cm\(^{-1}\) (C-O group of carboxylic acid group); (III) 1383 cm\(^{-1}\) (C=O stretching of carboxylic acid group); (IV) 1038 cm\(^{-1}\) (C-OH vibrations of the protein/polysaccharide)\(^{[13]}\).

Figure 2.

The size of the nanoparticles (15-21 nm) calculated through TEM images (Figure 3). Furthermore, the histogram plot from the TEM image showed the particle size distribution of biosynthesized Ag nanoparticles ranges of 11 to 24 nm. In the previous studies, the size of Ag nanoparticles formulated by aqueous extract of medicinal plants had been calculated in the ranges of 5-50 nm with the shape of spherical (Ahmeda et al., 2020; Hamelian et al., 2018). These reports support the results of the current work.

Figure 3.

In the present study, the FE-SEM image of Ag nanoparticles is shown in Figure 4. The Ag nanoparticles appeared as an agglomerated structure. The hydroxyl groups present in \textit{C. Kwangsiensis} Folium could be responsible for agglomeration(Ahmeda et al., 2020; Hamelian et
al., 2018). Also, FE-SEM images indicated a diameter of 15-21 nm and the shape of spherical for Ag nanoparticles.

Figure 4

Antioxidant properties of Ag nanoparticles synthesized using Curcumae Kwangsiensis Folium leaf aqueous extract

For determining of antioxidant properties of several materials such as medicinal plants and metallic nanoparticles green-synthesized by medicinal plants the free radicals are used that the most main of them is DPPH. In the high antioxidant capacities of several materials, the color of the DPPH molecules changes from violet to the pale yellow or colorless (Mahdavi et al., 2020; Mohammadi et al., 2020; Zangeneh, 2020; Zangeneh et al., 2019).

In the recent study, the concentration of 1000 µg/mL or high concentration showed the best result. Also, the antioxidant properties increased as dose dependent (Figure 5). Among all sampled examined (silver salt, C. Kwangsiensis Folium leaf aqueous extract, and Ag nanoparticles), the Ag nanoparticles revealed the most remarkable inhibition activities against free radicals. BHT revealed similar antioxidant activities compared to the AgNPs. The exact IC50 of C. Kwangsiensis Folium, butylated hydroxytoluene, and AgNPs were 399, 207, and 135 µg/mL, respectively (Table 1).

Figure 5.
Table 1.

Anti-human lung cancer potentials of Ag nanoparticles synthesized using Curcumae Kwangsiensis Folium leaf aqueous extract

In our research, the treated cell lines with silver salt, *C. Kwangsiensis* Folium, and Ag nanoparticles were tested by a well-known cytotoxicity test, i.e. MTT test for 72 h regarding the cytotoxicity activities on normal (HUVEC) and common human lung cancer (well-differentiated bronchogenic adenocarcinoma (HLC-1), moderately differentiated adenocarcinoma (LC-2/ad), and poorly differentiated adenocarcinoma (PC-14)) cell lines (Figures 6-9). Ag silver salt, *C. Kwangsiensis* Folium leaf aqueous extract, and Ag nanoparticles didn’t show any cytotoxicity against HUVEC cells in the MTT assay.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

About human lung cancer cell lines, the cell viability dose-dependently decreased in the presence of silver salt, *C. Kwangsiensis* Folium, and Ag nanoparticles. The IC50 of *C. Kwangsiensis* Folium and Ag nanoparticles against HLC-1 cell line were 461 and 358 µg/mL, respectively;
against LC-2/ad cell line were 431 and 300 µg/mL, respectively; and against PC-14 cell line were 480 and 252 µg/mL, respectively. The best results of cytotoxicity and anti-human lung cancer potentials of Ag nanoparticles against the above cell lines were seen in the case of the PC-14 cell line (Table 2).

Table 2

Discussion

One of the reasons of synthesizing of the metallic nanoparticles by plants is the significant antioxidant properties of them. Several studies have reported that plants green-formulated Ag nanoparticles can trap the free radicals and save the body cells. (del Mar Delgado-Povedano et al., 2016; Rehana et al., 2017). The reason behind the antioxidant activity of green or biosynthesized nanoparticles could be due to the presence of metabolites compounds (del Mar Delgado-Povedano et al., 2016; Jeong et al., 2012; Rehana et al., 2017; Sankar et al., 2014). In the previous study, it was revealed that C. Kwangsiensis Folium leaf has many antioxidant compounds such as Oxygenated sesquiterpenes, Oxygenated monoterpenes, Germacrone, iso-Curcumeneol, cis-α-Elemenone, Caryophyllene oxide, Germacrene A, β-Selinene, α-Humulene, β-Bourbonene, Myrtenol, Endo-borneol, Limonene, Camphene, Sesquiterpene hydrocarbons, β-Turmenone, Curzerenone, Trans-calamenene, β-Bisabolene, AR-Curcumene, Trans-
caryophyllene, α-Zingiberene, Aromadendrene, p-Cymene, α-Pinene, Monoterpene hydrocarbon, epi-Curzerenone, 2-Undecanol, Isoborneol, Pinocarveol, β-Pinene, ar-Turmenone, Germacrene B, Curzerene, β-Elemene, 2-Undecanone, 4-Vinyl-2-methoxy-phenol, p-Cymen-8-ol, α-Terpinolene, Trans-β-elemenone, α-Selinene, Germacrene D, α-Terpineol, β-Sesquiphellandrene, Borneyl acetate, and Camphor (Sacchetti et al., 2005). Various researches were performed in the nanobiotechnology field using several herbs, but still, no report is available on C. Kwangsiensis Folium green-formulated Ag nanoparticles.

Among the different parameters of metallic nanoparticles such as, nature of surface functions, texture, size, and morphology, the size effect is most necessary in the anticancer test using standard cancer cells. Previous reports revealed that the anticancer activity increases with a decrease in particle size based on their better penetration ability over the cell lines. It has been surveyed that particle size lower than 50 nm displays better activity in the corresponding cancer cell lines (Namvar et al., 2014). As can be observed in Figures 4 and 5 of our study, the sizes of Ag nanoparticles synthesized by C. Kwangsiensis Folium leaf aqueous extract are at the ranges of 15-21 nm.

The anticancer effects of green-formulated Ag nanoparticles have been confirmed in the previous studies (Jacob et al., 2012; Suman et al., 2013; Vivek et al., 2012). In the study of Suman et al. (2013) was clarified the anti-cervix cancer effects of Ag nanoparticles containing natural compound (Morindacitrifolia) against HeLa cell line. In the previous study, the Ag nanoparticles
killed all HeLa cells in high doses (Suman et al., 2013). In another study, the anti-liver cancer properties of Ag nanoparticles containing Piper longum leaf against Hep-2 cell lines were proved (Jacob et al., 2012). In the previous experiment has been reported that Ag nanoparticles green-synthesized by Annona quamosal leaf have excellent anti-breast cancer potentials against MCF-7 cell line (Vivek et al., 2012).

Likely the significant anti-human lung cancer potentials of Ag nanoparticles synthesized by C. Kwangsiensis Folium against human lung cancer cell lines are linked to their antioxidant activities. The similar researches have revealed the antioxidant materials such as metallic nanoparticles especially Ag nanoparticles and ethno medicinal plants reduce the volume of tumors by removing free radicals (Katata-Seru et al., 2018). In detail, the high presence of free radicals in the normal cells make many mutation in their DNA and RNA, destroy their gene expression and then accelerate the proliferation and growth of abnormal cells or cancerous cells (Beheshtkhoo et al., 2018; Sangami and Manu, 2017). The free radicals high presences in all cancers such as skin cancers, throat, ovarian, testicular, bladder, colon, small intestine, gastrointestinal, stromal, stomach, breast, lung, vaginal, prostate, pancreatic, liver, gallbladder, hypopharyngeal, fallopian tube, thyroid, esophageal, parathyroid, bileduct, and rectal indicate significant role of these molecules in making tumorigenesis and angiogenesis (Beheshtkhoo et al., 2018; Radini et al., 2018). Many researchers reported that Ag nanoparticles synthesized by
ethno medicinal plants have remarkable role in the removing free radicals and growth inhibition of all cancerous cells (Oganesvan et al., 1991; Radini et al., 2018).

In this research, the Ag nanoparticles were attained from the reaction between AgNO$_3$ and $C. Kwangsiensis$ leaf aqueous extract in vitro condition. TEM, FE-SEM, UV–Vis, and FT-IR methods were utilized to evaluate nanoparticle characteristics. The results of these techniques revealed that Ag nanoparticles had been synthesized in the best way. Base on the FT-IR spectrum the presence of a great number of antioxidant compounds produced appropriate conditions for the reduction of silver. In the TEM technique, the mean size of Ag nanoparticles was assessed to be 18 nm, which is favorable.

The Ag nanoparticles showed the best antioxidant activities against DPPH. Ag nanoparticles had appropriate anti-lung cancer activities dose-dependently against HLC-1, LC-2/ad, and PC-14 cell lines without any cytotoxicity on the normal cell line (HUVEC). After clinical study Ag nanoparticles containing $C. Kwangsiensis$ leaf aqueous extract can be utilized as an efficient drug in the treatment of lung cancer and diseases in humans.

Acknowledgment

The authors extend their appreciation to the Researchers supporting Project number (RSP-2020/98) King Saud University, Riyadh, Saudi Arabia for financial support.

Limitation
Due to the COVID19 pandemic conditions, we might not to investigate the anti-human lung adenocarcinoma effects of other metallic nanoparticles such as palatine, gold, iron and copper green-synthesized by C. Kwangsiensis leaf aqueous extract in the in vitro conditions.

Authors’ contributions

All authors contributed and discussed in the analysis and results and commented on the manuscript. All authors read and approved the final manuscript.

Funding No funding was received.

Compliance with ethical standards

Conflict of interest All authors declare that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

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**Table 1.** The IC50 of silver salt, *Curcuma* *Kwangsiensis* *Folium*, Ag nanoparticles, and BHT in the antioxidant test.

| IC50 against DPPH(µg/mL) | AgNO₃ (µg/mL) | *Curcuma* *Kwangsiensis* *Folium* | AgNPs | BHT |
|--------------------------|---------------|-----------------------------------|-------|-----|
|                          | -             | 399±0                             | 135±0 | 207±0 |
Table 2. The IC50 of silver salt, *Curcumae Kwangsiensis* Folium, and Ag nanoparticles in the cytotoxicity test.

|                  | AgNO₃     | *Curcumae Kwangsiensis* Folium | AgNPs |
|------------------|-----------|--------------------------------|-------|
| IC50 against HUVEC(µg/mL) | -         | -                              | -     |
| IC50 against HLC-1(µg/mL) | -         | 539±0                          | 247±0 |
| IC50 against LC-2/ad(µg/mL) | -         | 461±0                          | 189±0 |
| IC50 against PC-14(µg/mL)  | -         | 366±0                          | 152±0 |
Figure 1. The UV–Vis spectrum of biosynthesized Ag nanoparticles.
Figure 2. FT-IR spectra of biosynthesized Ag nanoparticles.
Figure 3. TEM image of Ag nanoparticles.
Figure 4. FE-SEM image of Ag nanoparticles.
Figure 5. The antioxidant effects of silver salt, *Curcumae Kwangsiensis* Folium, Ag nanoparticles, and BHT against DPPH.
Figure 6. The cytotoxicity effects of silver salt, *Curcumae Kwangsiensis* Folium, and Ag nanoparticles against HUVEC cell line.
Figure 7. The anti-human lung cancer effects of silver salt, *Curcumae Kwangsiensis* Folium, and Ag nanoparticles against lung well-differentiated bronchogenic adenocarcinoma (HLC-1) cell line.
Figure 8. The anti-human lung cancer effects of silver salt, Curcuma Kwangsiensis Folium, and Ag nanoparticles against lung moderately differentiated adenocarcinoma (LC-2/ad) cell line.
Figure 9. The anti-human lung cancer effects of silver salt, *Curcumae Kwangsiensis* Folium, and Ag nanoparticles against lung poorly differentiated adenocarcinoma (PC-14) cell line.