Characterization and therapeutic activities of Syzygium aromaticum extract green-synthesized copper nanoparticles

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Abstract

In this study, Syzygium aromaticum extract as a stabilizing and reducing agent was utilized to synthesize copper nanoparticles in the aqueous medium. Various techniques containing UV-Vis. spectroscopy, FT-IR spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and Energy Dispersive X-ray Spectrometry (EDS) were used to characterize the synthesized nanoparticles (CuNPs). On the other hand, the MTT assay was run to evaluate anti lung cancer activity of CuNPs. The crystal size of CuNPs, according to the XRD analysis, was 30.73 nm. Moreover, the uniform spherical morphology ranging from 19.55 to 69.70 nm was detected in the SEM images for the biosynthesized nanoparticles. In the cellular and molecular part of the recent study, the treated cells with CuNPs@Syzygium aromaticum were assessed by MTT assay for 48 h about the cytotoxicity and anti-human lung adenocarcinoma properties on normal (HUVEC) and lung adenocarcinoma cell lines i.e. HLC-1, LC-2/ad, and PC-14. In the antioxidant test, the IC50 of CuNPs@Syzygium aromaticum and BHT against DPPH free radicals were 119 and 69 µg/mL, respectively. The viability of malignant lung cell line reduced dose-dependently in the presence of CuNPs@Syzygium aromaticum. The IC50 of CuNPs@Syzygium aromaticum were 443, 500, and 377 µg/mL against LC-2/ad, HLC-1, and PC-14 cell lines, respectively.

1. Introduction

Nanotechnology has grown rapidly in the manufacturing and production of nanoparticles with varied sizes, shapes and distribution. Although physical and chemical methods may have known and successful pure production, they are generally hazardous to the environment, time-consuming, and expensive (Ghashghaii et al., 2017; Kooti et al., 2017). Therefore, considering the nanoparticle production environmental aspects, the use of plant biomass, plant extracts, plant oils and microorganisms can be a main alternative to the chemical and physical ways. The biological production of nanoparticles greatly lowers the risk of danger to the environment and humans (Ishaq et al., 2020; Mahdavi et al., 2020). The nanoparticle synthesis by biological materials has become the interest of researchers because of their new physical and chemical characteristics and their uses in several medical sciences, optics, electronics and mechanics. Using physical ways needs high pressure and temperature as well as high cost (Ishaq et al., 2020; Mahdavi et al., 2020). Also, in many chemical ways, chemicals are dangerous and toxic not only for the environment but also for biological systems. The products of the chemical methods are so toxic. So, the need for a suitable way with low price, high efficiency, without environmental damage and toxic substances production is increasing (Hummers Jr & Offeman, 1958; Mahdavi et al., 2020). Biological production is one of the ways of solving the above cases and attention to this way of producing nanoparticles is increasing. There is a big list of resources that are used in the metal nanoparticle biological production. Things like microorganisms such as bacteria, actinomycetes, fungi and algae as well as plants and plant extracts are applied in the nanoparticle biological production(Hummers Jr & Offeman, 1958; Ishaq et al., 2020). The use of plants due to their compatibility with the environment and abundance are usually prioritized. Also, due to their lack of need for special nutrients and conditions for growth, plants are considered the best option for the nanoparticle

production by the biological method(Ghashghaii et al., 2017; Hummers Jr & Offeman, 1958; Ishaq et al., 2020; Mahdavi et al., 2020).

Nanoparticles are designed in such a way that they can carry a higher dose of medicine with them and deliver it to the target area that is affected by cancer. In fact, these particles are not a threat to healthy body cells and only affect cancer cells and destroy them (Hummers Jr & Offeman, 1958; Mahdavi et al., 2020). This method is considered a targeted treatment that attacks only the target cells and acts selectively. By this method on several patients, positive and satisfactory results have been obtained, which can be considered as part of the great developments of medical science in the field of cancer treatment (Ghashghaii et al., 2017; Kooti et al., 2017).

Clove is one of the medicinal plants that has received a lot of attention in traditional medicine. Cloves belong to the myrtaceae family and have the scientific name of *Syzygium aromaticum* (Cortés-Rojas et al., 2014; Karimi & Dabili, 2015; Mishra & Singh, 2008). Chemically, this plant contains a significant amount of volatile essential oil, tannin, caryophyllin substance, and amounts of triterpenes and esters (Kaur et al., 2010; Mishra & Singh, 2008; Rastiannasab et al., 2014; Slameňová et al., 2009). Also, clove glycosides include lighic alcohols, monoterpenoids, eugenol, isoeugenol, farnesol, sitosterol, nerolidol, and campestrol. Clove oil, which is obtained from its petals, has many medical uses and is used in the manufacture of medicines and cosmetics (Rastiannasab et al., 2014; Slameňová et al., 2009). Clove oil is known to be useful for treating wounds and injuries, the effects of insect bites, especially in sensitive skin (Karimi & Dabili, 2015; Kaur et al., 2010; Mishra & Singh, 2008). This oil is used in making anti-pimple compounds and is effective in treating purulent pimples. Cloves are widely used as a sedative and anesthetic in fish (Karimi & Dabili, 2015; Mishra & Singh, 2008; Slameňová et al., 2009). Also, clove extract is traditionally used as an analgesic in dentistry. Recently, researchers have realized that the clove has very complex antioxidant compounds and its anti-cancer effects are probably due to these compounds (Kaur et al., 2010; Rastiannasab et al., 2014).

In the current research, the properties of copper nanoparticles formulated by *Syzygium aromaticum* leaf aqueous extract against common lung adenocarcinoma cell lines i.e. HLC-1, LC-2/ad, and PC-14 were evaluated.

2. Material and Methods

2.1. Preparation and extraction of aqueous extract

After preparing the *Syzygium aromaticum* leaves, they were dried away from sunlight. After grinding the plant, extraction was done from the plants. In this way, 15 g of the plant powder obtained was poured into an Erlenmeyer flask and soaked in 200 ml of distilled water for 3 days. After straining the obtained solution with a strainer, the evaporation of the solvent was done in wide plates on a bain-marie at a temperature of 40-50 °C. Then, a solution with a concentration of 10 mg/ml of DMEM medium was prepared for the remaining solid material. This extract was used for experiments.

2.2. Green synthesis and chemical characterization of CuNPs@Syzygium aromaticum

The CuNPs were biosynthesized based on the earlier study (Kumar et al., 2015). A 15 mL of the aqueous solution of *Syzygium aromaticum*extract (30 mg/mL) was added into 10 mL of 0.4M $Cu(NO_3)_2$ $.3H_2O$ (in all stages deionized water was used). After that, the mixture was refluxed at 75 °C for 12 hours. The obtained precipitate was rinsed three times and centrifuged at 12000 rpm for 15 min subsequently. Then, to dry the residuum, an oven was used at a temperature of 60 °C. Finally, the formed dark brown powder was kept in a vial to evaluate chemical characterization and its biological activity.

2.3 Chemical Characterization techniques

Different factors of the nanoparticles like shape, particle size, fractal dimensions, crystallinity, and surface area are characterized by FT-IR spectroscopy, XRD, SEM, and EDS. In the present study, the FT-IR spectra of the synthetic nanoparticles were recorded by a Shimadzu FT-IR 8400 ranging from 400 to 4000 cm^{-1}(KBr disc); The FE-SEM Images and EDS result were reported using MIRA3TESCAN-XMU. The CuNPs XRD

pattern was recorded in the 2ϑ which ranged from 20 to 80° by a GNR EXPLORER instrument at a 40 KV voltage, a current of 30 mA, and Cu-Kαradiation (1.5406 Å).

2.4. Antioxidant activities of CuNPs@Syzygium aromaticum

In this study, 100 µl of various dilutions of nanoparticles in methanol was added to 10 ml of 0.005% DPPH solution in methanol. After 1 hour of incubation at the absorption room temperature, the samples were read against Blank at 518 nm. The DPPH inhibition percentage was computed by the following formula (Mahdavi et al., 2020):

$$\text{Inhibition } (\%) = \frac{\text{Sample A.}}{\text{Control A.}} x100$$

2.5. Anti-human lung cancer properties of CuNPs@Syzygium aromaticum

In this research, the following cell lines were used to assess the anti-human lung adenocarcinoma properties of copper nitrate,*Syzygium aromaticum* leaf aqueous extract and CuNPs@*Syzygium aromaticum* using an MTT method.

a) Human lung cancer cell lines

- HLC-1: Lung well-differentiated bronchogenic adenocarcinoma.

- LC-2/ad: Lung moderately differentiated adenocarcinoma.

- PC-14: Lung poorly differentiated adenocarcinoma.

b) Normal cell line

- HUVEC

Cancer cell lines were placed in 1640-RPMI medium from GiBco manufacturer and were cultured after adding 10% bovine serum, 1% streptomycin and penicillin antibiotics and 2% glutamine. At this stage, the cell culture flasks were kept in an incubator with 5% CO_2 and 95% humidity at a temperature of 37°C, and the culture medium was replaced every three days. In this step, flasks with 80% cell density were used (flasks filled with cells up to 80% of the bottom). First, the culture medium was removed from the surface of the cells and by adding 1 ml of trypsin for 3 minutes and then adding the same volume of medium to neutralize the effect of trypsin, all the cells were separated from the flask bottom. This cell suspension was centrifuged at 1200 rpm for 4 minutes. The liquid above the sediment was discarded and 1 ml of culture medium was added to the sediment. By taking 10 µl of the cell suspension and adding the same amount of trypan blue on the surface of the neobar slide, the number of living cells was counted. The number of 10,000 cells from this cell suspension was added to each well of 96-well plates and 180 µl of culture medium was added to it. In the next step, 20 µl different concentrations of nanoparticles were added to the wells. In this research, based on the conventional concentrations of nanoparticles at the 0-1000 µg/ml, they were added to cancer cells. Another group of cells were tested as a control, without adding nanoparticles and only by adding water instead of nanoparticles, and each experiment was done in four replicates. After 24, 48 and 72 hours, the medium on the cells was replaced with a new medium. Then 20 µl MTT solution was added to each well and placed in a greenhouse for 4 hours in the dark in a CO2 incubator. During this time, the mitochondrial succinate dehydrogenase enzyme of living cells changes the yellow MTT solution into purple formazan crystals, which are insoluble in water. In the next step, 200 µl of DMSO (Dimethylsulfoxide) was added to the empty medium and shaken for 20 minutes to dissolve the light-producing crystals. In the last step, the absorbance was read with a wavelength of 492 and then 630 nm in an ELISA reader. Finally, the percentage of cell viability was calculated after dividing the optical absorbance (OD) of treated cells compared to control cells and multiplying by 100 (Arunachalam et al. 2013).

3. Results and Discussion

3.1. Chemical characterization of CuNPs@Syzygium aromaticum

FT-IR Analysis

In laboratories, a large part of the measurements is based on absorbance reactions. The activity of most cholesterol, triglycerides, enzymes, lipoproteins, creatinine, urea, sugar and a wide range of analytes with research and clinical applications, drugs and metabolites can be measured by spectrophotometry. Investigating the molecular structure, identifying compounds, comparing structures and finding the maximum absorption wavelength are other spectrophotometry applications in research problems (Cheirmadurai et al., 2014).

The formation of copper oxide nanoparticles is certified by the presence of the peaks at 484, 592, and 667 cm^{-1}, which belongs to the bending vibration of Cu-O (Cheirmadurai et al., 2014). Take peaks in 3404 and 2927 cm^{-1} as examples, which are related to O-H and aliphatic C-H, in order (Figure 1). Likewise, the peaks extending from 1431 to 1675 cm^{-1} correspond to C=C and C=O stretching, and the peaks related to -C-O and C-N stretching are detectable at 1261 and 1085 cm^{-1}, respectively. The presence of various compounds like alkaloids, carbohydrates, phenolic compounds, flavonoids, glycosides, terpenoids, which have been reported previously, are certified by these peaks (Cortés-Rojas et al., 2014; Karimi & Dabili, 2015; Mishra & Singh, 2008).

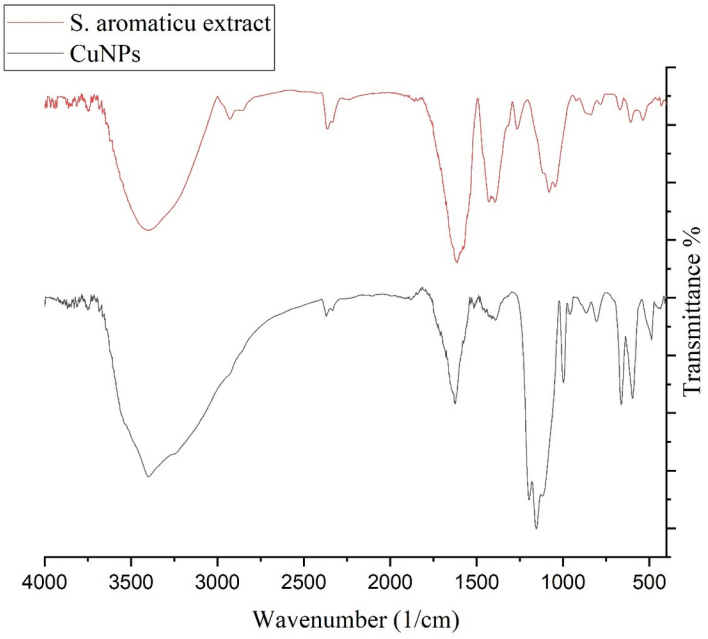

Figure 1: FT-IR spectra of biosynthesized CuNPs@Syzygium aromaticum

XRD Analysis

The peaks at 2ϑ values of 32.75, 35.59, 38.88, 48.82, 58.53, 61.63, and 68.32 are indexed as (110), (11-1), (111), (20-2), (202), (-113), and (220) planes (Figure 2). These peaks are in good agreement with standard database ICDD PDF card no. 96-901-6327. The peaks at various degrees were also described previously (Kumar et al., 2015). The average crystal size of CuNPs@Syzygium aromaticum was 30.73 nm that was calculated using X-Ray diffraction based on Scherrer equation

$D=k\lambda/\beta\cos\vartheta$

In our literature review, the smallest crystal size of the green-synthesized copper nanoparticles using plant extracts ranged from 8 to 15 nm, belonging to CuNPs@Syzygium aromaticum produced using the fruit extracts of Ziziphus spina-christ [20]. However, in the other studies, the CuNPs crystal size has been

reported in the range of 8.71 to 19.21 nm (Devi & Ahmaruzzaman, 2018; Ijaz et al., 2017; Rajesh et al., 2018; Sorbiun et al., 2018; Vaidehi et al., 2018).

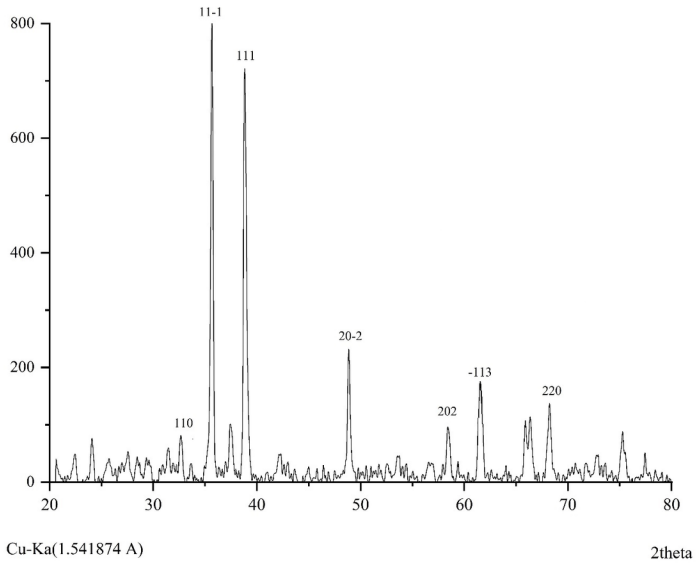

Figure 2. XRD Pattern of CuNPs@Syzygium aromaticum

SEM Analysis

SEM analysis is a member of the scanning electron microscope family and is used to examine the surface characteristics and morphology of different samples. In this method, electron beams with specific energy and wavelength sweep the sample surface (Ghidan et al., 2016; Harshiny et al., 2015; Rao & Pennathur, 2017). By the detector data that have collected the return sample surface electrons, benefits data is obtained from the sample surface. It should be noted that image quality and high resolution in the images have a direct relationship with the structure of the sample and the quality of synthesis and the absence of contamination and unwanted particles, and samples with a specific structure provide acceptable images (Kumar et al., 2015; Sulaiman et al., 2018; Taghavi Fardood & Ramazani, 2016).

The FE-SEM images of CuNPs@*Syzygium aromaticum* depict a spherical morphology which has been reported by another research group (Fig 3)(Ghidan et al., 2016). The uniformity, well dispersed, and homogeneity of the CuNPs@*Syzygium aromaticum* is confirmed by the figure as well as a propensity to aggregate, like the other metallic nanoparticles that are synthesized using green chemistry approaches. This property of metallic nanoparticles can be found in other similar studies for FeNPs, CdNPs, CuNPs, AgNPs, and TiNPs (Baghayeri et al., 2018; Ghidan et al., 2016; Harshiny et al., 2015; Rao & Pennathur, 2017). The diameter range size for the synthesized nanoparticle was obtained from 19.55 to 69.70 nm. In our review of literature on the green-synthesized CuNPs@*Syzygium aromaticum* revealed that the paarrticle size is in the range of 5 to 100 nm (Kaur et al., 2016; Sulaiman et al., 2018; Taghavi Fardood & Ramazani, 2016).

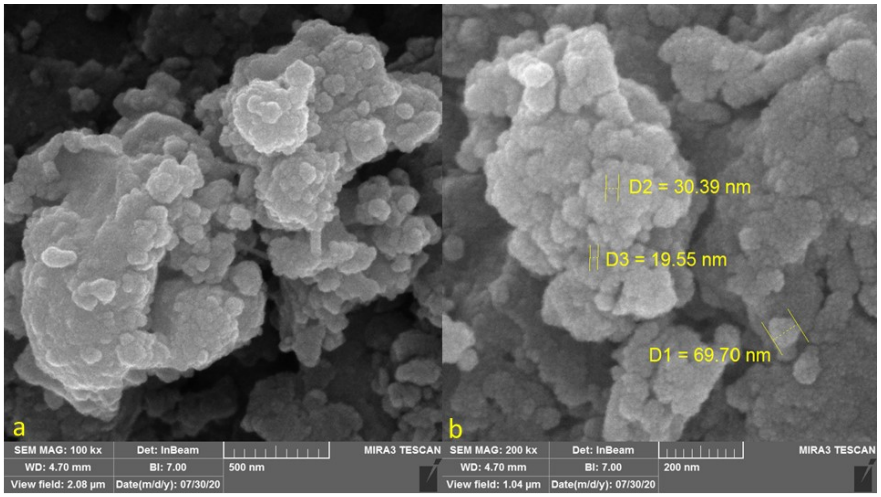

Figure 3. SEM Images of CuNPs@Syzygium aromaticum

EDS Analysis

In the EDS analysis, the signals which are at 0.93Kev (for CuLα), and at 8.02 Kev (for Cu Kα) approve the presences of copper, matchable to previous biosynthesized CuNPs which were reported (Devi & Ahmaruzzaman, 2018). The oxygen in copper oxide nanoparticles and some organic molecules existing in *Syzygium aromaticum* extract that linked to the CuNPs surface result in the formation of a signal around 0.5 Kev. A signal around 0.3 Kev certifies the presence of carbon on the CuNPs surface (Figure 4).

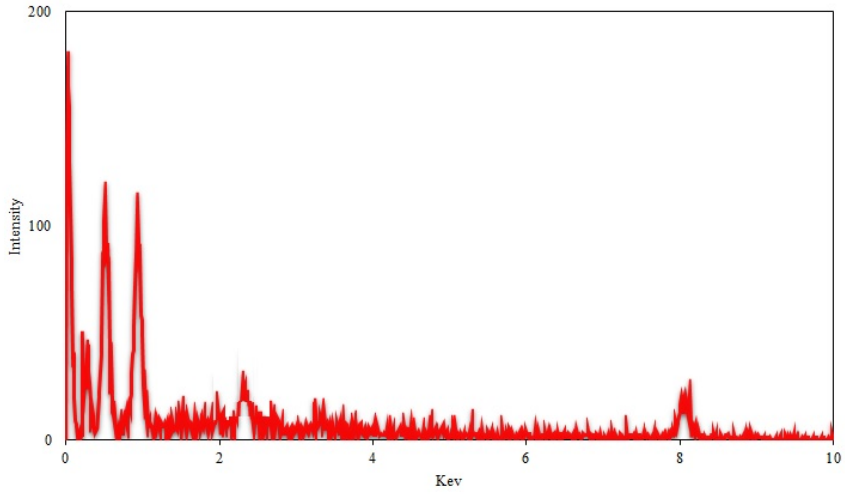

Figure 4. EDS analysis of CuNPs@Syzygium aromaticum

3.2. Cytotoxicity, anti-human lung cancer, and antioxidant activities of CuNPs@Syzygium aromaticum

A living cell is a collection of microscopic components with specific functions and functions. The plasma membrane surrounds the cell contents and is in contact with the external environment (Ghidan et al., 2016). The selective permeability of the membrane maintains the stability of the cell with the help of various transport mechanisms. Small-sized nanoparticles easily pass through the membrane and due to their special surface properties, they interact with important cellular components such as mitochondria, lysosomes, and

nuclei (Seydi et al., 2019; Sulaiman et al., 2018). The continuity of the structure and maintenance of the function of cells is often determined by biological macromolecules such as carbohydrates, lipids, and proteins. The structure of biomolecules changes under the influence of interaction with various types of nanoparticles (Seydi et al., 2019; Sulaiman et al., 2018; Taghavi Fardood & Ramazani, 2016). Molecular oxygen dissolved in biological fluids is transformed into singlet oxygen under the influence of light energy required for biological transfer reactions. Superoxide radical is formed by a reduction reaction of oxygen molecule [29,30]. This reaction can be done as a result of the redox cycle, or enzymatically with the catalysis of NADPH oxidase, and also as a byproduct of enzymatic reactions with Xanthine oxidase and a byproduct of the electron transport chain in mitochondria (Seydi et al., 2019). By upregulating the xanthine oxidase and NADPH oxidase enzymes, nanomaterials increase the production of superoxide radicals in some cells such as macrophages and neutrophils and trigger inflammatory reactions. The superoxide anion is suddenly converted to hydrogen peroxide (H_2O_2) by the catalysis of the superoxide dismutase enzyme and with the help of copper, manganese or zinc as a cofactor (Seydi et al., 2019; Sulaiman et al., 2018). The dissolution of nanomaterials based on iron or copper catalyzes the formation of active oxygen species and through the Fenton reaction leads to the production of hydroxyl (OH) and peroxyl (OOH) free radicals (Hamelian et al., 2018; Taghavi Fardood & Ramazani, 2016). For example, TiO_2 nanoparticles used in sunscreens produce singlet oxygen and superoxide under the influence of light rays. An increase in the level of ROS leads to inflammatory responses such as an increase in cells with polymorphous nuclei and disturbances in the phagocytosis process of macrophages in some model animals such as rodents (Hamelian et al., 2018; Taghavi Fardood & Ramazani, 2016). Oxidative stress is caused by the predominance of free radicals on antioxidants and is one of the main mechanisms of toxicity of most metal nanoparticles such as gold, zinc oxide, and silver (Hamelian et al., 2018; You et al., 2012). Oxidative stress by regulating redox-sensitive transcription factors, activates some kinase enzymes and intermediate proteins of inflammatory reactions and causes tissue damage such as damage to the cell membrane, genetic material and biological macromolecules. At very high levels, disruption of the signaling pathways inside the cell leads to apoptosis and cell necrosis (Katata-Seru et al., 2018; Mao et al., 2016; Namvar et al., 2014; Sankar et al., 2014; You et al., 2012). Reactive oxygen species disrupt the function of the central nervous system by peroxidizing the unsaturated fatty acids of neuronal cells. Clogging of blood vessels, blood pressure and re-narrowing of arteries after angioplasty due to ROS cause disorders of the cardiovascular system (Mao et al., 2016; You et al., 2012). Mitochondria are one of the ROS production main sources through the electron transport respiratory chain. Several clinical syndromes such as stroke, Duchenne muscular dystrophy, cardiac conduction defects due to ROS oxidative attack and mitochondrial DNA double-strand break occur (Seydi et al., 2019). By producing reactive oxygen species in cancer cells, nanoparticles create changes in the chemical structure of histones or other proteins that are effective in shaping the structure of DNA (Seydi et al., 2019; Sulaiman et al., 2018). Unfolding of the helical structure of DNA disrupts gene expression and malfunction of regulation of cellular function of cancer cells. In addition to ROS toxicity, some nanoparticles use other effective mechanisms for cell damage by releasing metal ions, accumulating in some cellular components, and interacting with nuclear components (Hamelian et al., 2018; Sulaiman et al., 2018). In general, the cell damage mechanisms by nanoparticles include (1) membranes physical damage (2) Structural changes in the cell skeleton components (3) Disturbance in oxidative and transcription damage (4) Mitochondria damage (5) Dysfunction of lysosome (6) ROS production (7) Membrane proteins dysfunction (8) Mediators and inflammatory factors synthesis (Mao et al., 2016; Namvar et al., 2014; Sankar et al., 2014).

In this research, the viability of malignant lung cell line reduced dose-dependently in the presence of copper nitrate, *Syzygium aromaticum* leaf aqueous extract, and CuNPs@*Syzygium aromaticum* . The IC50 of CuNPs@*Syzygium aromaticum* were 199, 226, and 319 µg/mL against PC-14, LC-2/ad, and HLC-1 cell lines, respectively (Tables 1,2). The IC50 of *Syzygium aromaticum* leaf aqueous extract were 377, 433, and 500 µg/mL against PC-14, LC-2/ad, and HLC-1 cell lines, respectively (Tables 1,2).

Table 1. The anti-lung adenocarcinoma properties of copper nitrate, *Syzygium aromaticum* leaf aqueous extract, and CuNPs@*Syzygium aromaticum* against human lung adenocarcinoma cell lines.

ὃνςεντρατιον (μγ/μλ)	Cell Viability (%)	Cell Viability (%)	Cell Viability (%)	Cell V
	HUVEC	**PC-14**	**LC-2/ad**	**HLC-1**
Copper nitrate (0)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Copper nitrate (1)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Copper nitrate (2)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Copper nitrate (3)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Copper nitrate (7)	100±0^{a}	100±0^{a}	100±0^{a}	99±0.7
Copper nitrate (15)	98.8±0.44^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Copper nitrate (31)	95.2±1.3^{a}	98.4±0.54^{a}	100±0^{a}	100±0^{a}
Copper nitrate (62)	91.2±0.83^{a}	97.4±0.89^{a}	99.2±1.3^{a}	100±0^{a}
Copper nitrate (125)	85±0.7^{a}	94±1^{a}	96.4±1.34^{a}	98.4±0
Copper nitrate (250)	77.2±0.44^{ab}	89.2±1.3^{a}	92.4±0.54^{a}	95.4±1
Copper nitrate (500)	70.4±0.54^{ab}	81.2±0.44^{ab}	84.6±0.89^{a}	89±1.2
Copper nitrate (1000)	60.6±1.14^{b}	72.4±1.34^{ab}	73.2±1.3^{ab}	80.2±1
Syzygium aromaticum **(0)**	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Syzygium aromaticum **(1)**	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Syzygium aromaticum **(2)**	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Syzygium aromaticum **(3)**	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Syzygium aromaticum **(7)**	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
Syzygium aromaticum **(15)**	100±0^{a}	98.2±1.3^{a}	99.4±0.54^{a}	100±0^{a}
Syzygium aromaticum **(31)**	99.2±0.44^{a}	95±0.7^{a}	95.4±0.54^{a}	98.8±0
Syzygium aromaticum **(62)**	98±1^{a}	89.4±1.34^{a}	90.4±0.89^{a}	93.4±1
Syzygium aromaticum **(125)**	96.4±1.34^{a}	83.2±1.3^{ab}	83.2 ± 0.83^{ab}	84.4±0
Syzygium aromaticum **(250)**	93±1.22^{a}	70±1.22^{ab}	72.4±1.34^{ab}	73.6±0
Syzygium aromaticum **(500)**	89±1^{a}	57.4±0.54^{b}	60.2±0.44^{b}	61.4±0
Syzygium aromaticum **(1000)**	83±0.7^{ab}	37.8±0.44^{bc}	41.4±1.34^{bc}	43.2±1
CuNPs@*Syzygium aromaticum* (0)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
CuNPs@*Syzygium aromaticum* (1)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
CuNPs@*Syzygium aromaticum* (2)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
CuNPs@*Syzygium aromaticum* (3)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
CuNPs@*Syzygium aromaticum* (7)	100±0^{a}	98.4±0.54^{a}	99.2±1.3^{a}	100±0^{a}
CuNPs@*Syzygium aromaticum* (15)	100±0^{a}	96.6±0.89^{a}	94.4±0.54^{a}	97.6±0
CuNPs@*Syzygium aromaticum* (31)	99±0.7^{a}	93.2±1.3^{a}	89.2±1.3^{a}	91.2±0
CuNPs@*Syzygium aromaticum* (62)	96.4±1.34^{a}	86.6±1.14^{a}	84.4±0.54^{a}	85.4±0
CuNPs@*Syzygium aromaticum* (125)	91±1.22^{a}	75.6±0.89^{ab}	76.2±0.83^{ab}	77.6±1
CuNPs@*Syzygium aromaticum* (250)	86.2±1.3^{a}	60.4±0.54^{b}	62.4±1.34^{b}	67.8±0
CuNPs@*Syzygium aromaticum* (500)	80±1^{ab}	40±1.22^{bc}	46.4±0.54^{b}	50±0.7
CuNPs@*Syzygium aromaticum* (1000)	73±1.22^{ab}	12.2±1.3^{c}	20.2±0.44^{c}	18.2±1

The several words present significant differences between experimented groups (P [?]0.01).

Table 2. The IC50 of copper nitrate, *Syzygium aromaticum*leaf aqueous extract, and CuNPs@*Syzygium aromaticum* in cytotoxicity and anti-lung adenocarcinoma tests.

	ὃππερ νιτρατε (μγ/μΛ)	Syzygium aromaticum (μγ/μΛ)	ὐNΠσ˜*Σψζψγιυμ αρομαπιςυμ* (μγ/μΛ)
IC50 against HUVEC	-	-	-
IC50 against PC-14	-	688±0^{b}	377±0^{d}

	ὀππερ νιτρατε (μγ/μΛ)	Syzygium aromaticum (μγ/μΛ)	ὐΝΠσ˜Σψζψγιυμ αρομαπιςυμ (μγ/μΛ)
IC50 against LC-2/ad	-	774±0[a]	443±0[cd]
IC50 against HLC-1	-	813±0[a]	500±0[c]

The several words present significant differences between experimented groups (P [?]0.01).

In this study, we determined the CuNPs@*Syzygium aromaticum*antioxidant properties by the free radical (DPPH) test. Free radicals (FRs) are unstable molecules or atoms that have an unpaired electron.

Medicinal plants are of special interest to researchers due to having valuable secondary metabolites and many medicinal properties (Jalalvand et al., 2019). During the past years, much research has been conducted on various aspects of these plants. One of the things that has received a lot of attention is the therapeutic and significant effects of medicinal plants compared to chemical drugs (Jalalvand et al., 2019; Kooti et al., 2017). For example, hydroalcoholic extract of safflower flower is effective in preventing diabetes. The effect of this extract is due to the presence of secondary metabolites from the group of flavonoids and their antioxidant properties (Cortes-Rojas et al., 2014; Mahdavi et al., 2020). Another aspect of plant research is using them in producing nanoparticles instead of chemical methods, which is a faster and cheaper method than chemical methods and has less risk for humans and the environment (Cortes-Rojas et al., 2014; Ishaq et al., 2020; Mahdavi et al., 2020). Therefore, in recent years, more attention has been paid to this method of producing nanoparticles called green chemistry. In this research, the copper nanoparticle production by the plant aqueous extract and the anticancer effects of the produced CuNPs were studied (Rajesh et al., 2018). The basis of nanoparticle production is the regeneration of copper salt by plant extract and the neutralizing its electric charge (Zangeneh et al., 2019). The anticancer property of copper nanoparticles has been studied in many research projects, and various reasons have been mentioned for this phenomenon (Zangeneh et al., 2019). Attacking the surface of the cell membrane through interaction with sulfur-containing proteins, disrupting cell permeability and respiration, and as a result, cell death, inhibiting the respiratory enzymes of cancer cells by combining with the thiol group and also stopping the cell from replicating DNA and thus preventing reproduction are among the reasons mentioned for the anticancer properties of copper nanoparticles (Ijaz et al., 2017; Seydi et al., 2019). So far, various nanoparticles have been produced using the extracts of many plants. For example, in a study, they studied the plant production of copper nanoparticles by the medicinal plant yarrow and used the extract of the yarrow plant as a reducing agent for the biological production of CuNPs (Khani et al., 2018).

In the antioxidant test, the IC50 of *Syzygium aromaticum* leaf aqueous extract, CuNPs@*Syzygium aromaticum* , and BHT against DPPH free radicals were 385, 119, and 69 μg/mL, respectively (Tables 3,4).

Table 3. The antioxidant activities of copper nitrate,*Syzygium aromaticum* leaf aqueous extract, CuNPs@*Syzygium aromaticum* , and BHT against DPPH.

Concentration (μγ/μλ)	**DPPH inhibition (%)**	**Concentration (μγ/μλ)**	**DPPH inhibition (%)**
Copper nitrate (0)	0±0[a]	*Syzygium aromaticum* **(0)**	0±0[a]
Copper nitrate (1)	0±0[a]	*Syzygium aromaticum* **(1)**	0±0[a]
Copper nitrate (2)	0±0[a]	*Syzygium aromaticum* **(2)**	0±0[a]
Copper nitrate (3)	2.6±0.89[a]	*Syzygium aromaticum* **(3)**	2±1[a]

Concentration (μγ/μλ)	**DPPH inhibition (%)**	**Concentration (μγ/μλ)**	**DPPH inhibition (%)**
Copper nitrate (7)	4.2±1.3[a]	*Syzygium aromaticum* **(7)**	4.2±1.3[a]
Copper nitrate (15)	8.2±1.3[a]	*Syzygium aromaticum* **(15)**	10.4±0.89[a]
Copper nitrate (31)	13.6±1.14[a]	*Syzygium aromaticum* **(31)**	17.4±1.34[ab]
Copper nitrate (62)	19.2±1.3[ab]	*Syzygium aromaticum* **(62)**	24±1.22[ab]
Copper nitrate (125)	25.4±1.34[ab]	*Syzygium aromaticum* **(125)**	34.4±0.54[ab]
Copper nitrate (250)	33.4±0.54[ab]	*Syzygium aromaticum* **(250)**	45.2±0.44[b]
Copper nitrate (500)	40±0.7[b]	*Syzygium aromaticum* **(500)**	56.2±1.3[b]
Copper nitrate (1000)	48.4±0.89[b]	*Syzygium aromaticum* **(1000)**	69.8±0.44[bc]
Concentration (μγ/μλ)	**DPPH inhibition (%)**	**Concentration (μγ/μλ)**	**DPPH inhibition (%)**
CuNPs@*Syzygium aromaticum* (0)	0±0[a]	**BHT (0)**	0±0[a]
CuNPs@*Syzygium aromaticum* (1)	0±0[a]	**BHT (1)**	0±0[a]
CuNPs@*Syzygium aromaticum* (2)	1.2±0.44[a]	**BHT (2)**	2.4±1.34[a]
CuNPs@*Syzygium aromaticum* (3)	4±1.22[a]	**BHT (3)**	6±0.7[a]
CuNPs@*Syzygium aromaticum* (7)	10.8±0.44[a]	**BHT (7)**	12.6±0.89[a]
CuNPs@*Syzygium aromaticum* (15)	18.4±1.34[ab]	**BHT (15)**	20.4±0.54[ab]
CuNPs@*Syzygium aromaticum* (31)	26±1[ab]	**BHT (31)**	30.6±1.14[ab]
CuNPs@*Syzygium aromaticum* (62)	37.2±1.3[b]	**BHT (62)**	48.4±0.89[b]
CuNPs@*Syzygium aromaticum* (125)	51.2±1.3[b]	**BHT (125)**	65.4±1.34[bc]
CuNPs@*Syzygium aromaticum* (250)	67.6±0.89[bc]	**BHT (250)**	87.2±1.3[c]
CuNPs@*Syzygium aromaticum* (500)	85.2±1.3[c]	**BHT (500)**	100±0[c]
CuNPs@*Syzygium aromaticum* (1000)	100±0[c]	**BHT (1000)**	100±0[c]

The several words present significant differences between experimented groups (P [?]0.01).

Table 4. The IC50 of copper nitrate, *Syzygium aromaticum*leaf aqueous extract, CuNPs@*Syzygium aromaticum* , and BHT in the antioxidant test.

ὃνςεντρατιον (μγ/μλ)	**Copper nitrate**	Syzygium aromaticum	**CuNPs@*Syzygium aromaticum***	**BHT**
Γ50 (μγ/μλ)	-	385±0[a]	119±0[b]	69±0[b]

The several words present significant differences between experimented groups (P [?]0.01).

4. Conclusion

In conclusion, we like to introduce a green method to synthesis copper nanoparticles for the first time using the extract of *Syzygium aromaticum* . The structural features of nanoparticles were evaluated through various analytical techniques such as FT-IR, XRD, FE-SEM, and EDS analyses. The techniques approved that CuNPs@*Syzygium aromaticum* had been synthesized in the best possible condition. The viability of malignant lung cell line reduced dose-dependently in the presence of CuNPs@*Syzygium aromaticum* . The IC50 of CuNPs@*Syzygium aromaticum* were 377, 433, and 500 μg/mL against PC-14, LC-2/ad, and HLC-1 cell lines, respectively. The CuNPs@*Syzygium aromaticum* showed the best antioxidant activities against DPPH. The IC50 of CuNPs@*Syzygium aromaticum* and BHT against DPPH free radicals were 122 and 124 μg/mL, respectively. After the clinical study, CuNPs@*Syzygium aromaticum* containing*Syzygium aromaticum* leaf aqueous extract can be utilized as an efficient drug to treat the lung cancer in humans.

Conflict of interest statement

There is no conflict of interest

Data Availability Statement

The data that support the findings of this study are available from the corresponding author

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