

RESEARCH PAPER

## Eco-Friendly Synthesis of Ginkgo Biloba- Silver Nanoparticles and Bilobetein: Anti-Cancer Effects on serum for iraqi patients of Lung Cancer

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### ABSTRACT

The study focused on testing the anti-cancer effects of Ginkgo biloba extract-derived silver nanoparticles (Ag NPs), specifically examining cytokeratin21-1, alpha-1-antitrypsin, and retinol binding protein-1. Synthesis and characterization of Ag NPs from ginkgo biloba extract were conducted, followed by testing different concentrations of bilobetein to evaluate their anti-cancer effects and identify the optimal dose for lowering the concentrations of cytokeratin21-1, human alpha-1-antitrypsin, and retinol binding protein-1. XRD analysis revealed that the crystallite size and crystal structure of Ag-NPs were 29 nm and FCC-like, respectively. FE-SEM images showed spherical and cubic shapes with a particle size of 89 nm. UV-visible spectrum analysis indicated an optical energy gap of 3.5 eV for Ag-NPs. In the experiment, Bilobetein, an active compound in Ginkgo Biloba, and solutions containing varying concentrations of Ag-NPs from Ginkgo Biloba were synthesized. The impact of these solutions on the concentrations of CYFRA21-1,  $\alpha$ 1AT, and CRBP-1 was investigated to determine the optimal concentration for reducing the protein levels. After adding Bilobetein at a concentration of 8 ppm, the mean  $\alpha$ 1AT concentration values for control, adenocarcinoma, and small cell lung cancer were  $45.16 \pm 4.51$ ,  $47.72 \pm 1.01$ , and  $50.13 \pm 1.29$ , respectively. Similarly, after adding Ag NPs/ginkgo biloba at a concentration of 4 ppm, the  $\alpha$ 1AT concentration values for control, small cell lung cancer, and adenocarcinoma lung cancer were  $44.48 \pm 4.88$ ,  $45.89 \pm 2.18$ , and  $45.16 \pm 4.51$ , respectively. Regarding RBP-1 concentration values, after adding Bilobetein at a concentration of 8 ppm, the mean values for control, adenocarcinoma, and small cell lung cancer were  $2.22 \pm 0.19$ ,  $2.28 \pm 0.25$ , and  $2.91 \pm 0.46$ , respectively. Conversely, after adding Ag NPs/ginkgo biloba at a concentration of 4 ppm, the RBP-1 concentration values for control, small cell lung cancer, and adenocarcinoma lung cancer were  $2.07 \pm 0.13$ ,  $2.09 \pm 0.049$ , and  $2.62 \pm 0.149$ , respectively.

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## INTRODUCTION

Cytokeratin fragment 21-1, also known as CYFRA 21-1, serves as a tumor marker widely employed in the diagnosis and monitoring of lung cancer, particularly non-small cell lung cancer (NSCLC) [1]. Originating from epithelial cells, CYFRA 21-1 is a component of the soluble protein cytokeratin 19. Elevated blood levels of CYFRA 21-1 are indicative of the presence and progression of lung cancer, particularly squamous cell carcinoma, often suggesting an advanced disease stage. It is noteworthy that elevated CYFRA 21-1 levels are not exclusive to lung cancer but can also occur in various malignancies and non-cancerous disorders affecting epithelial tissues [3]. Alpha-1-antitrypsin (AAT or  $\alpha$ 1AT), a 54 kDa glycoprotein primarily produced in hepatocytes, belongs to the serpin superfamily. Recognized for its serine protease inhibition capability, AAT also exhibits anti-apoptotic, immunomodulatory, and caspase 3 regulatory properties in vitro and in vivo. Numerous studies have associated higher AAT levels with cancer and poorer prognosis, particularly in lung, breast, cervical, ovarian, and colorectal adenocarcinomas. AAT's involvement in the distal spread of various cancer types makes it a potential indicator of cancer progression and a patient's response to therapy [4-5]. Retinol Binding Protein-1 (RBP1) plays a crucial role in transporting retinol from the liver to retinal epithelial cells. As a member of the lipocalin protein family, RBP1 facilitates the delivery of retinol, also known as vitamin A, affecting the development and growth of epithelial cells. Retinoids, including retinol, exert their pleiotropic and transcriptional effects by binding to nuclear receptors such as retinoic acid receptors (RAR) and retinoid X receptors (RXR). The interaction with other receptors like peroxisome proliferator-activated receptors (PPARs), vitamin D3 receptors, and various orphan receptors in the normal respiratory epithelium regulates the cell cycle, promoting apoptosis or differentiation [6]. RBP1, involved in the conversion of retinol to retinyl esters, influences the production of retinoic acid. The balance of retinoic acid is crucial for controlling the proliferation and apoptosis of normal and abnormally differentiated cells, suggesting a role in the initiation and spread of cancer [8]. Silver nanoparticles (Ag NPs) may exert a transcriptional-level impact on the expression of cancer protein biomarkers, potentially influencing cancer-related processes [9-11].

Through a variety of methods, including electrostatic contacts, hydrophobic interactions, or particular binding sites on the nanoparticle surface, nanoparticles can interact with proteins. These interactions may have an impact on the stability, function, and structure of proteins [12]. It is feasible to improve nanoparticles' ability to target cancer cells specifically by adsorbing or conjugating RBP and CYFRA21-1 and AAT-1 onto their surface. Overexpressed receptors found on the surface of cancer cells can be selectively identified by RBP and CYFRA21-1 and AAT-1 bound to. By improving the accumulation of nanoparticles in malignant cells, this targeted delivery can deliver therapeutic drugs or imaging agents to the tumor location in a focused and effective manner [13]. Once proteins bound to siRNA or shRNA molecules enter the target cells, they form a double-stranded RNA complex by base-pairing with the mRNA protein. This complex is recognized by the cellular RNA-induced silencing complex (RISC), which breaks down the messenger RNA (mRNA) and stops it from translating into protein. Because fewer proteins are expressed, the amounts of proteins in the cells decrease, which prevents cancer [14].

The objective of this study is to develop a therapeutic agent for potential lung cancer treatment. The research involves a comparison of ginkgo biloba silver nanoparticles and bilobetin solution concentrations as anti-cancer substances. The aim is to identify the substances that most effectively reduce the concentrations of alpha-1-antitrypsin, retinol binding protein-1, and cytokeratin21-1 enzymes, as their decreased activity inhibits cancer cell growth, leading to cell death. The study conducted in vitro involved three groups of samples: the first group included 30 control blood serum samples from both male and female patients aged 23 to 45 years. The second group comprised 30 cases of small cell lung cancer blood serum from both male and female patients aged 45 to 80 years. The third group included 30 cases of adenocarcinoma lung cancer blood serum from both male and female patients aged 45 to 80 years. The study's findings demonstrate the anti-cancer effects of bilobetin compounds and Ag-NPs from ginkgo biloba extract by reducing the levels of cytokeratin21-1 (CYFRA21-1), alpha-1-antitrypsin (AAT), and retinol binding protein-1 (CRBP-1) in the blood serum of patients with adenocarcinoma and small cell lung cancer in Iraq.

## MATERIALS AND METHODS

### Material and methods

Ginkgo biloba and  $\text{Ag}_2\text{NO}_3$  salt were procured locally in Baghdad, Iraq. Patients at the Oncology Teaching Hospital of Baghdad Teaching Hospital, presenting with lung cancer symptoms, underwent blood sampling from January 2022 to June 2022. They were categorized into three groups as follows: Group 1 (G1) comprised 30 healthy individuals of both genders, with an average age range of 23 to 45 years. Group 2 (G2) consisted of 30 individuals of both genders diagnosed with small cell lung carcinoma, with an average age range of 45 to 80 years. Group 3 (G3) included thirty blood and plasma samples from individuals of both genders, aged 45 to 80 years, diagnosed with lung cancer (adenocarcinoma), and with no history of illnesses that could have influenced the characteristics investigated in this study [15].

### Preparation of Ginkgo biloba extract

To produce Ginkgo biloba extract, the powder was mixed with 10 grams of distilled water and 200 milliliters of water. The solution was then heated to  $70^\circ\text{C}$  and boiled for two hours using a magnetic stirrer. Subsequently, the mixture was allowed to cool to room temperature before being filtered through a Whatman filter sheet [12]. The process of transforming the two fresh Ginkgo biloba extracts is illustrated in Fig. 1.

### Preparation of Ag-NPs prepared from Ginkgo biloba extract

100 ml of 1 M silver nitrate ( $\text{Ag}_2\text{NO}_3$ ) were added to 10 ml of Ginkgo biloba extract to create the Ag NPs. After that, this solution was heated to  $60^\circ\text{C}$  for 45 minutes on a hotplate stirrer. By using this technique, Ag NPs were formed as the reaction solution's white hue became black. The

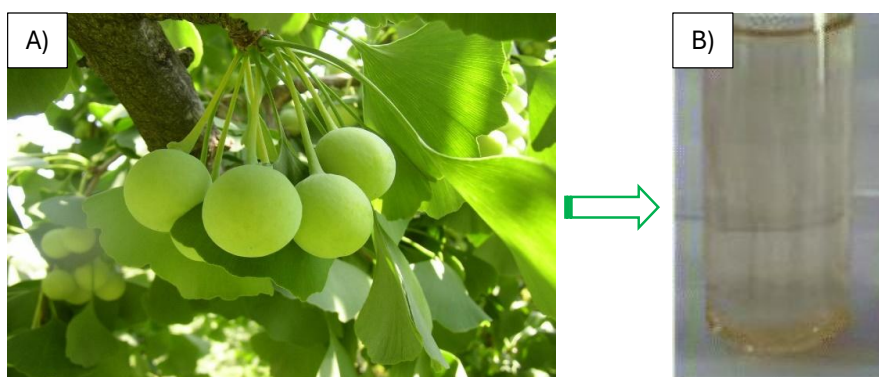


Fig. 1. (A-B). show the step for preparation of Ginkgo biloba extract.

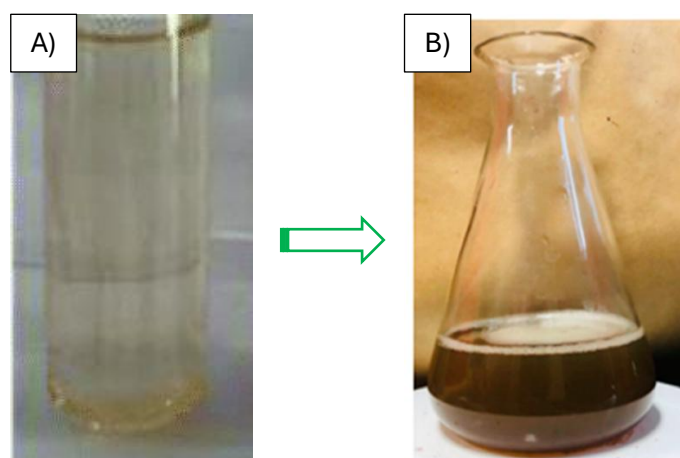


Fig. 2. (A-B). show the step for preparation of Ag NPs.

mixture was allowed to reach room temperature. 15 mL of Ag solution was put into a ceramic eyelid and baked for three hours at 250 °C to generate nanopowder. The powdered Ag NPs were then stored inside sealed tubes containing serum for subsequent analysis. Fig. 2 depicts the steps required in producing Ag NPs from *Ginkgo biloba* extract.

#### Specimen collection and preparation

Eight milliliters (ml) of venous blood were drawn using ten-milliliter single-use plastic needles. The blood samples from both participants and controls were collected in regular plastic containers without any anticoagulant. Subsequently, the blood was left to coagulate for 20 to 30 minutes at 37°C. After coagulation, the blood was divided into small Eppendorf tubes, centrifuged for 10 minutes at 3000 rpm, and then stored at -20°C until further analysis [16].

#### Materials

Chaina's Bilobetein provided the product,

Yirui Biotechnology, and prepared the standard in accordance with [17]. Silver nano particals of ginkgo biloba Prepared at different concentrations according to [17]. cytokeratin21-1(CYFRA21-1) kit, finetest.Human alpha-1-antitrypsin (AAT) kit, finetest.retinol binding protein-1 (RBP-1) kit, finetest.

#### Measurement the concentration of cytokeratin21-1(CYFRA21-1) and Human alpha-1-antitrypsin (AAT) and retinol binding protein-1 (RBP-1) in serum

The basis of this reagent lies in the sandwich enzyme-linked immunosorbent assay (ELISA) technique. Initially, 96-well plates were coated with capture antibodies, and detecting antibodies linked with biotin were employed. Following the addition of standards, test samples, and biotin-conjugated detection antibodies, the wells were washed with a washing solution. Subsequently, HRP-Streptavidin was added, and unbound conjugates were removed using the wash solution. The enzymatic activity of HRP was visualized

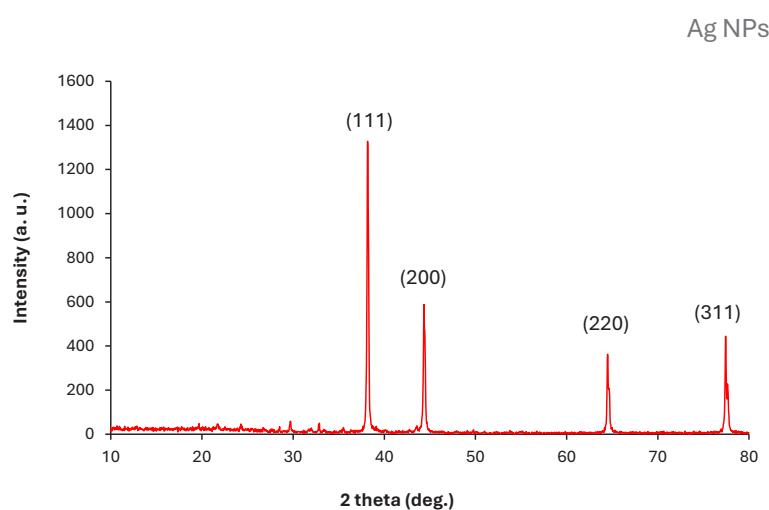


Fig. 3. XRD device of Ag-NPs from mixing *Ginkgo biloba* extract with  $\text{Ag}_2\text{NO}_3$  salt by chemical method.

Table 1. XRD measurement of Ag-NPs from mixing *Ginkgo biloba* extract with  $\text{Ag}_2\text{NO}_3$  salt by chemical method.

| Material                     | Phase  | 2 $\theta$ (deg.) | $\beta$ (deg.) | $\theta$ (rad) | $\beta$ (rad) | D(nm)    | $\delta$ | $\xi$    |
|------------------------------|--------|-------------------|----------------|----------------|---------------|----------|----------|----------|
| <i>Ginkgo biloba</i> extract | Ag-NPs | 38.34             | 0.55           | 0.336316       | 0.009649      | 15.21647 | 43.18888 | 22.77137 |
|                              |        | 44.47             | 0.5            | 0.390088       | 0.008772      | 17.08381 | 34.26341 | 20.28236 |
|                              |        | 64.65             | 0.69           | 0.567105       | 0.012105      | 13.57453 | 54.26884 | 25.52575 |
|                              |        | 77.21             | 0.34           | 0.677281       | 0.005965      | 29.81713 | 11.24782 | 11.62084 |

using TMB substrates. As a catalyst, TMB, in conjunction with HRP, produced a blue product, turning yellow upon the addition of an acidic stop solution. The yellow density in the plate indicated the appropriate amount of material captured. To determine the target concentration, the absorbance at 450 nm was read using a microplate scanner [18–20].

#### Statistical investigation

The data were presented with means and standard deviations, and statistical analysis was conducted using the Student's t-test. Any observed differences in mean values between two groups with a p-value of 0.05 or less were deemed statistically significant. Office Excel 2010 was employed to compute the overall predictive values for the outcomes in each testing group [21].

### RESULT AND DISCUSSION

#### XRD device of Ag NPs from Ginkgo biloba extract

The XRD measurement confirmed the crystalline nature of Ag nanoparticles. The presence of four prominent Bragg reflections at approximately 38.34, 44.47, 64.65, and 77.69° corresponds to the four diffraction peaks assigned to fcc silver planes (111), (200), (220), and (311). Notably, the Bragg reflections are fainter and broader than the robust (111) reflection (Fig. 3), underscoring the highly anisotropic nature of the nanocrystals. The mean size of nanoparticles was determined using

the Debye-equation Scherrer's method based on the width of the (111) peak. Additional peaks were observed, indicating that the nanoparticle surfaces were coated [22].

#### The FE-SEM images of Ag NPs from Ginkgo biloba extract

The FE-SEM images of the generated Ag samples are depicted in Fig. 4. The images illustrate the presence of nanoparticles that are unevenly dispersed and exhibit spherical and cubic shapes, with varying sizes. Agglomeration of nanoparticles was observed due to the disparate sizes and solvent evaporation during sample preparation. These results align with previous studies, confirming the effectiveness of the Ag NPs manufacturing process from the extract. The particle sizes ranged from 50 to 62 nm as determined by the FE-SEM device [23].

#### UV-visible spectrum of Ag NPs from Ginkgo biloba extract

The production of Ginkgo Biloba-Ag-NPs induced a significant change in the color of the working solution, progressing from an initial color to a gradual transformation into brown-black as the surface was developed. Plasmon resonance was utilized to activate the synthesized Ag-NPs. The UV-Vis spectra obtained (a-b) are presented in Fig. 5. The spectra were examined between 350 and 550 nm for both Ginkgo Biloba extracts (GB)

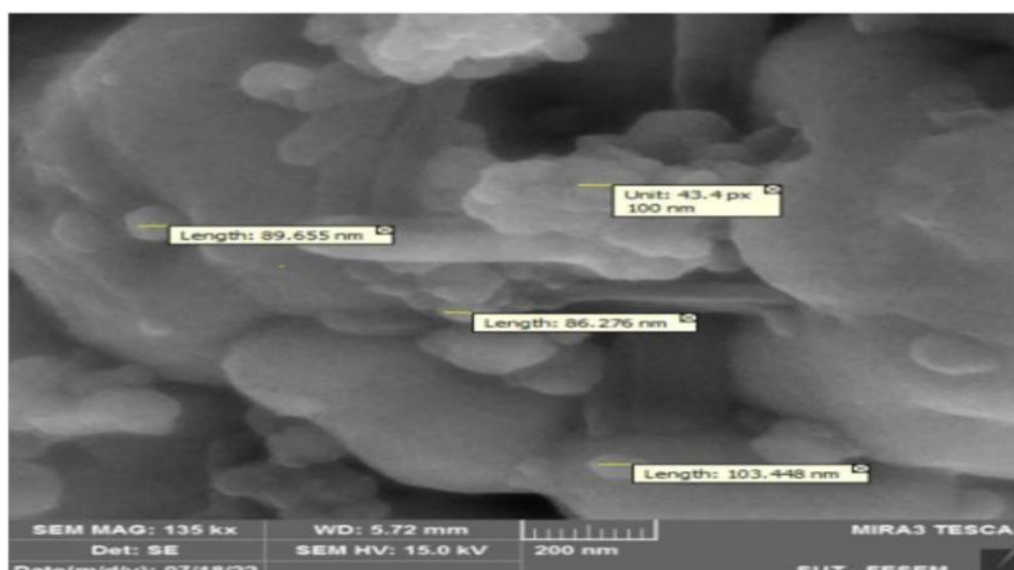


Fig. 4. FE-SEM images of Ag-NPs from mixing *Ginkgo biloba* extract with  $\text{Ag}_2\text{NO}_3$  salt by chemical method.

and synthetic GB-Ag-NPs. While the Ginkgo Biloba extract failed to identify a peak between 200 and 400 nm, the Ginkgo Biloba absorption spectrum-Ag-NPs clearly exhibited a peak at 448 nm [24].

The optical energy gap of the samples (plant extract and Ag NPs) is illustrated in Fig. 6 (a-b). In Fig. 6a, the optical energy gap value for the plant (Ginkgo biloba extract) is 5 eV. However, in Fig. 6b, the optical energy gap value decreases to 3.5 eV for Ag-NPs derived from Ginkgo biloba extract.

#### FT-IR spectra

Fourier-transform infrared spectroscopy (FTIR) analyses were employed to ascertain whether the potential Ginkgo biloba leaf extract contained biomolecules that could effectively stabilize silver nanoparticles. The FTIR spectrum (Fig. 7) displayed a comparison between the spectra of the plant

extract before and after treatment with silver nanoparticles. The peaks at 3506.59  $\text{cm}^{-1}$  and 3402  $\text{cm}^{-1}$  shifted (attributed to N-H bending and amides). The bands at 1450.47  $\text{cm}^{-1}$  and 1381.03  $\text{cm}^{-1}$  were observed due to the C-N and C-O stretching modes, associated with the stretching mode of the aromatic amine group. The peaks at 1654.92  $\text{cm}^{-1}$  and 1246.02  $\text{cm}^{-1}$  were attributed to the vibrational stretching of the C-OH bond in proteins, polyphenols, and alkene groups present in the plant extract.

#### Anti-cancer lung using A-NPs from Ginkgo biloba extract

Table 2 presented the mean  $\pm$  standard deviation of the cytokeratin21-1 (CYFRA21-1) concentration in the serum of patients with small cell and adenocarcinoma lung cancer in

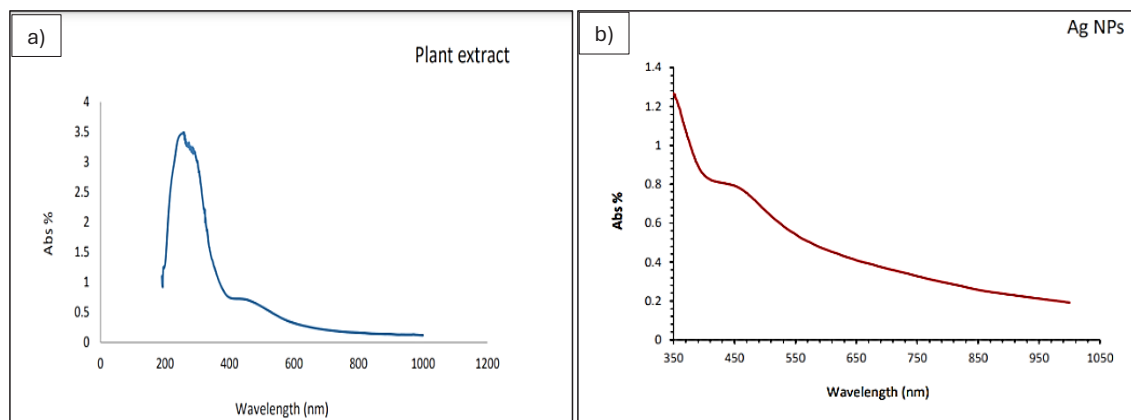


Fig. 5. UV-visible spectrum of sample, a) Plant extract (*Ginkgo biloba*) extract, b) Ag NPs.

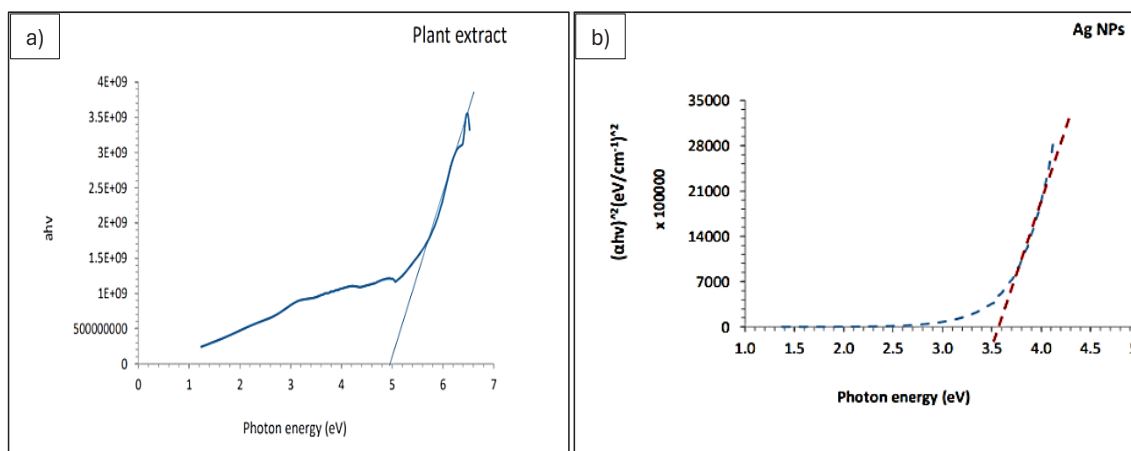


Fig. 6. Optical energy gap of sample, a) Plant extract (*Ginkgo biloba*) extract, b) Ag NPs.

relation to the control groups with and without the addition of a concentrated bilobetin (8 ppm) solution and a concentrated Ag NPs of Ginkgo biloba (4 ppm) solution. Table 2 and Fig. 8 demonstrate a substantial ( $P \leq 0.05$ ) rise in CYFRA21-1 concentration levels between the patient groups G2 and G3 and the control group

G1, which did not receive any further treatment. These findings concur with those of earlier studies that discovered a noteworthy rise in lung cancer patients' blood CYFRA21-1 levels [25]. Also a significant decrease with added bilobetin solution (8 ppm) and Ag NPs of *ginkgo biloba* (4 ppm) was observed in the patients group G2 and

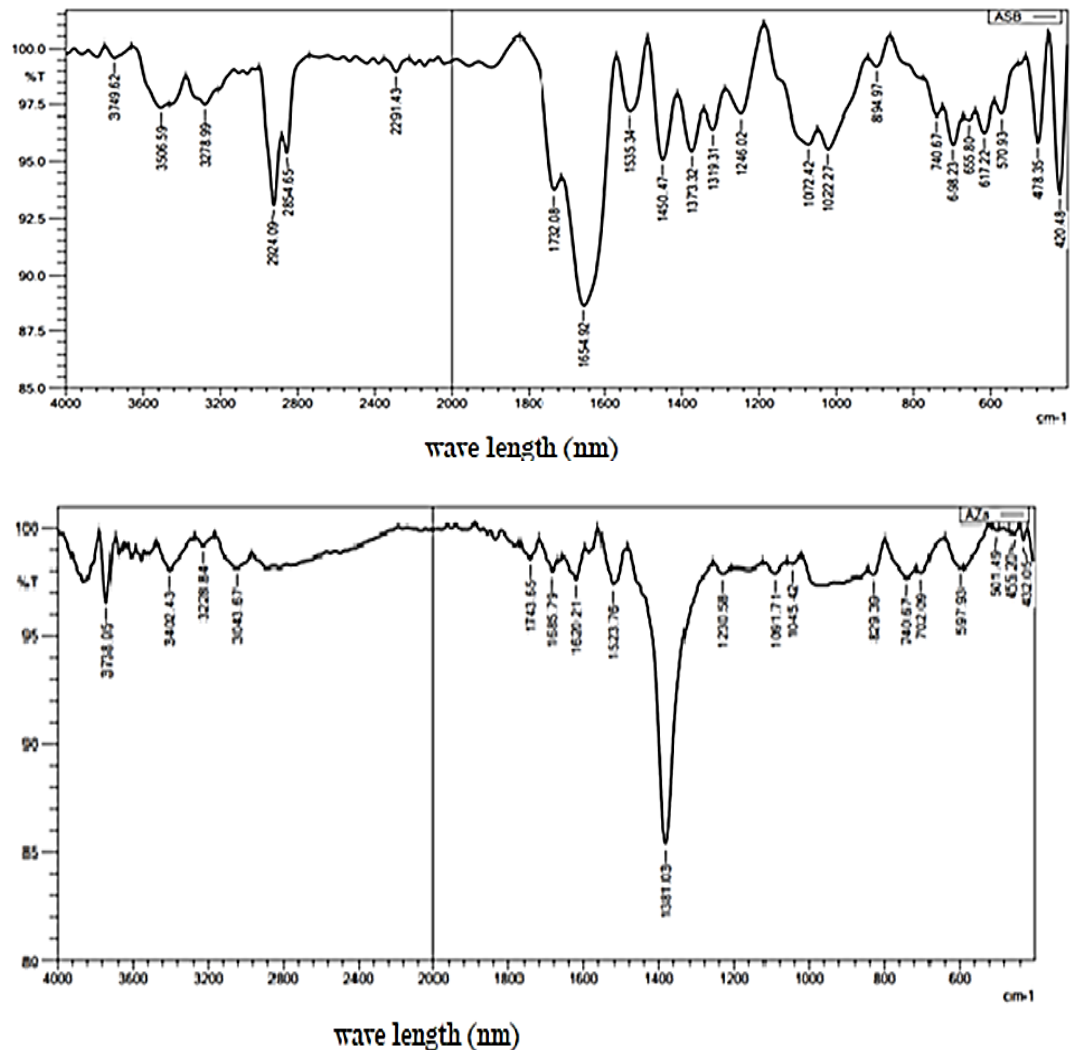


Fig. 7. FT-IR spectra of (a) GB extract only (b) Ag NPs from GB extract.

Table 2. Level of cytokeatin21-1(CYFRA21-1) concentrations in sera of three studies groups.

| Groups                      | No | AAT (ng/ml) without add means $\pm$ SD | P        | AAT (ng/ml) with bilobeleiton add means $\pm$ SD | P       | AAT (ng/ml) with Ag NPs using ginkgbiloba add means $\pm$ SD | P        |
|-----------------------------|----|----------------------------------------|----------|--------------------------------------------------|---------|--------------------------------------------------------------|----------|
| Control G1                  | 30 | 151.31 $\pm$ 60.92                     | 0.558613 | 145.22 $\pm$ 43.05                               | 0.14273 | 85.27 $\pm$ 78.42                                            | 0.005232 |
| Small cell lung cancer (G2) | 30 | 164.94 $\pm$ 103.72                    | 0.10736  | 159.29 $\pm$ 28.94                               | 0.02748 | 95.57 $\pm$ 90.47                                            | 0.007731 |
| Adenocarcinoma              | 30 | 191.64 $\pm$ 57.52                     | 0.002197 | 172.06 $\pm$ 48.72                               | 0.22197 | 102.53 $\pm$ 63.20                                           | 0.025671 |

G3 and control group G1 compared without any added. A previous study by [26-27] demonstrated that curcumin, a polyphenol, has the ability to inhibit cancer by blocking the NF- $\kappa$ B pathway [28], one of which is to enable the expression of the protein cytokeratin21-1. because of antioxidant properties of curcumin can also influence the expression of cytokeratins, Oxidative stress can activate various signaling pathways, including transcription factors such as nuclear factor-kappa B (NF- $\kappa$ B) involved in regulating various genes, including those related to inflammation, cell survival, and immune response. that curcumin particularly targets the IKK (IB kinase) complex is a group of enzymes that plays a role in spreading the cellular response to inflammation., which is in charge of phosphorylating NF- $\kappa$ B [29]. Since our compound under study bilobetein is also a polyphenol compound, we believe that it may act similar to that of curcumin, as it has an anti-cancer effect by inhibiting the NF- $\kappa$ B pathway. Which includes reducing the expression of cytochrome protein and thus preventing the growth and progression of lung cancer. The hydroxyl groups on the surface of the ginkgo biloba silver nanoparticles [30] may potentially interact with the molecules of cytokeratin 19-9 once they have entered the cancer cells through

hydrogen bonding. Because hydrogen and oxygen have different electronegativity, the hydrogen atom in the hydroxyl group has a partial positive charge. This partial positive charge may draw the partial negative charge of an oxygen or nitrogen atom or other electronegative atom present in the amino acid residue of cytokeratin 19-9, resulting in the creation of a hydrogen bond. These hydrogen bonds may help to maintain the contact between cytokeratin (19-9) and ginkgo biloba silver nanoparticles, which may have an impact on the conformation, stability, or activity of cytokeratin 19-9 in cancer cells, Electrostatic forces attract Ag NPs and CK(19-9), allowing positively charged amino acid residues to interact with negatively charged Ag-NPs, potentially facilitating their attachment. and inhibit cancer cells [31-33].

Table 3 presents the mean  $\pm$  standard deviation of serum levels of Human alpha-1-antitrypsin ( $\alpha$ 1AT) in patients with small cell and adenocarcinoma lung cancer, compared to control groups with and without the addition of a concentrated (8 ppm) bilobetein solution and a concentrated (4 ppm) Ag NPs of Ginkgo biloba solution. The table and Fig. 9 depict that concentrations of human alpha-1-antitrypsin ( $\alpha$ 1AT) in patient groups G2 and G3 were significantly higher ( $P \leq 0.05$ ) compared to the control group G1 without any additional

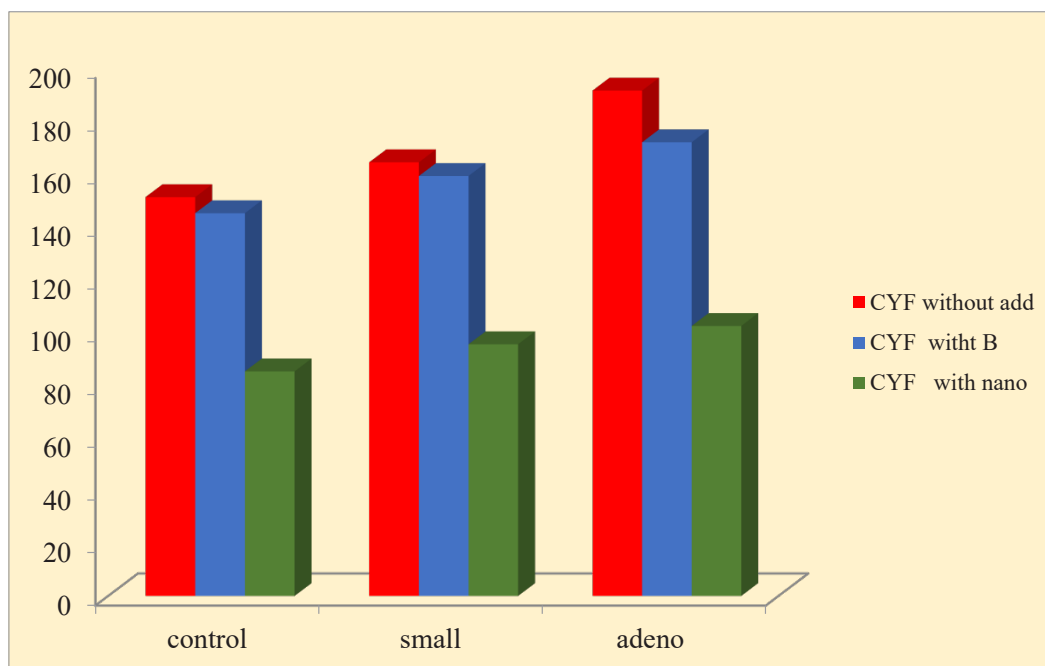


Fig. 8. Level of CYFRA21-1 concentrations in sera of three studies groups.

treatment. This observation aligns with previous studies indicating that lung cancer patients exhibit significantly elevated levels of the A1AT protein compared to control groups [34].

A previous study by [35-36] demonstrated that, Epigallocatechin-3-gallate (EGCG) a polyphenol [37], has the ability to inhibit cancer by blocking PI3K (phosphoinositide 3-kinase). It plays a critical role in regulating cell growth and survival by transmitting signals from cell surface receptors to downstream effectors. The PI3K pathway promotes cell survival by inhibiting apoptosis (programmed cell death). EGCG's inhibition of the PI3K pathway can counteract the anti-apoptotic signals, leading to increased apoptosis in cancer cells. This disruption of cell survival signals may indirectly affect AAT expression and function, as AAT levels can be influenced by PI3K pathway

and inhibit lung cancer. EGCG-coated Ag-NPs can generate reactive oxygen species (ROS) through their surface chemistry or by interactions with cellular components. Elevated levels of ROS can induce oxidative stress, which can affect various cellular processes, including protein function. ROS can directly oxidize critical amino acid residues in AAT, leading to conformational changes and loss of function. Additionally, ROS can promote the formation of disulfide bonds or other oxidative modifications in AAT, the oxidative stress induced by EGCG-coated Ag-NPs may interfere with the normal function of AAT, leading to its inhibition in cancer cells [38]. Bilobetin, a polyphenol compound, may act similarly to EGCG, inhibiting the PI3K pathway and reducing AAT protein expression, potentially preventing lung cancer growth and progression.

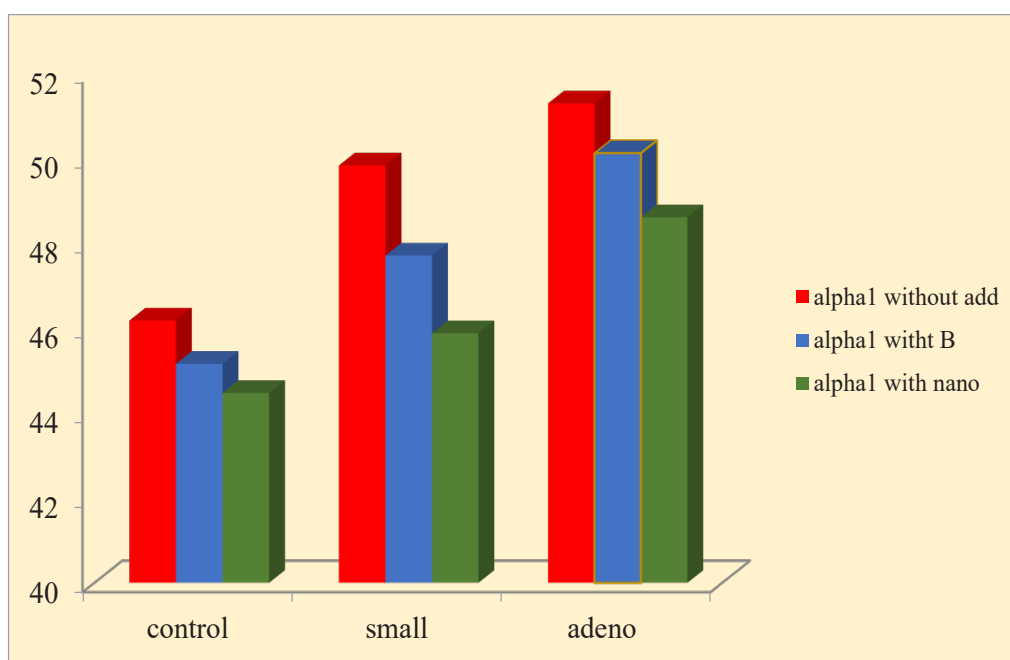


Fig. 9. Level of Human alpha-1-antitrypsin concentrations in sera of three studies groups.

Table 3. Level of Human alpha-1-antitrypsin concentrations in sera of three studies groups.

| Groups                      | N o | AAT (ng/ml) without add means ±SD | P                         | AAT (ng/ml) with bilobeleiton add means ±SD | P                         | AAT (ng/ml) with Ag NPs using ginkgbiloba add means ±SD | P                         |
|-----------------------------|-----|-----------------------------------|---------------------------|---------------------------------------------|---------------------------|---------------------------------------------------------|---------------------------|
| Control G1                  | 30  | 46.19±4.026                       | 2.87404x10 <sup>-05</sup> | 45.169±4.51                                 | 0.00368337                | 44.48±4.879                                             | 0.242571                  |
| Small cell lung cancer (G2) | 30  | 49.84±9.11                        | 2.22584x10 <sup>-08</sup> | 47.72±1.015                                 | 3.07758x10 <sup>-07</sup> | 45.88±2.947                                             | 1.52125X10 <sup>-03</sup> |
| Adenocarcinoma              | 30  | 51.302±1.55                       | 0.001071097               | 50.125±1.29                                 | 9.56405x10 <sup>-11</sup> | 48.62±2.18                                              | 1.34535X10 <sup>-21</sup> |

The concentrations of retinol binding protein-1 (RBP-1) in the serum of patients with small cell and adenocarcinoma lung cancer were assessed and compared to control groups, as presented in Table 4. The measurements were conducted both with and without the addition of concentrated Ag NPs of Ginkgo biloba (4 ppm) and bilobetin (8 ppm) solutions. The concentration values of retinol binding protein-1 (RBP-1) exhibited a statistically significant increase ( $P \leq 0.05$ ) between patient groups G2 and G3 and the control group G1, which did not undergo any additional therapy, as shown in Table 4 and Fig. 10. These findings align with prior research associating cellular retinol binding protein-1 with a notable elevation in hepatocellular cancer [39]. Apigenin, a naturally occurring flavonoid present in numerous plants, has demonstrated diverse biological roles, including anticancer properties. Apigenin can upregulate RBP1, induce apoptosis, and suppress proliferation by interacting with

RBP1 and modifying its expression and activity. This modulation can impact the initiation and progression of lung cancer, mediated through the upregulation of the p53 tumor suppressor pathway [40–41]. This is a critical regulator of cell survival and proliferation, and it has demonstrated interactions with other signaling pathways, including the NF- $\kappa$ B signaling pathway, as well as transcription factors [42]. Van der Waals forces refer to the attractive forces between nonpolar molecules. The carbonyl groups in apigenin and the hydrophobic regions of RBP can potentially interact via van der Waals forces. Through interaction with the hydrophobic regions of RBP or by modifying its overall structure, apigenin may inhibit its activity [43]. The non-covalent interactions between the functional groups on Ag-NPs and RBP-1 facilitate the attachment of Ag-NPs to RBP-1. Specifically, electrostatic interactions occur between the negatively charged COOH group on Ag-NPs and the positively charged

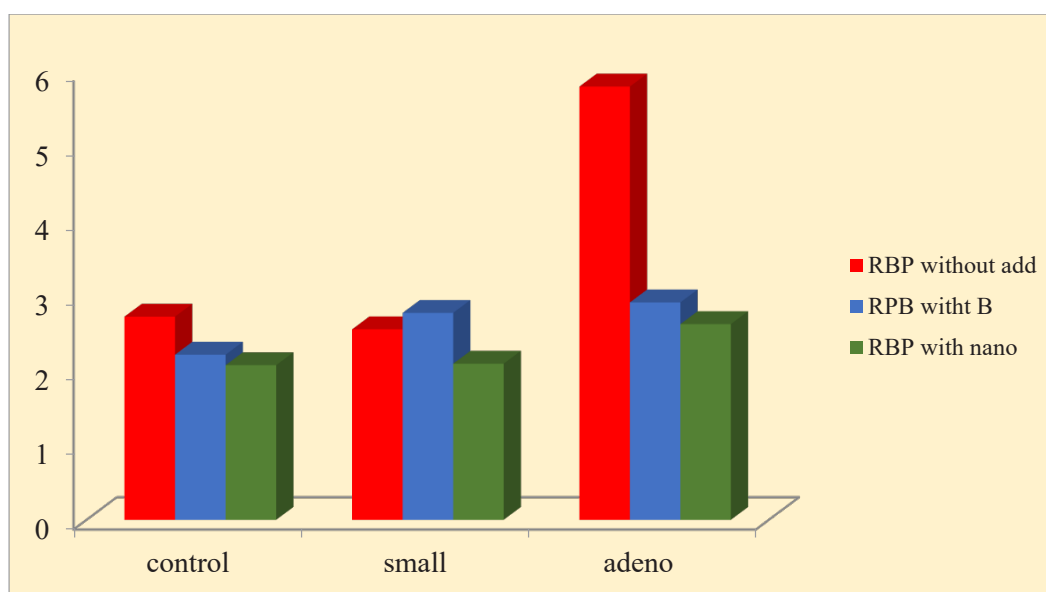


Fig. 10. Level of retinol binding protein-1(RBP-1) concentrations in sera of three studies groups.

Table 4. Level of retinol binding protein-1(RBP-1) concentrations in sera of three studies groups.

| Groups                      | No | RBP-1 (ng/ml) without add means $\pm$ SD | P                                  | RBP-1 (ng/ml) with bilobeleiton add means $\pm$ SD | P                                  | RBP-1 (ng/ml) with Ag NPs using ginkgbiloba add means $\pm$ SD | P                                  |
|-----------------------------|----|------------------------------------------|------------------------------------|----------------------------------------------------|------------------------------------|----------------------------------------------------------------|------------------------------------|
| Control G1                  | 30 | 2.72 $\pm$ 0.633                         | 0.167645                           | 2.218 $\pm$ 0.198472                               | 0.245836882                        | 2.074 $\pm$ 0.134102                                           | 0.342571                           |
| Small cell lung cancer (G2) | 30 | 2.547 $\pm$ 0.193                        | 1.76887 $\times$ 10 <sup>-27</sup> | 2.276 $\pm$ 0.253300058                            | 3.06825 $\times$ 10 <sup>-1</sup>  | 2.099 $\pm$ 0.049921                                           | 1.52125 $\times$ 10 <sup>-21</sup> |
| Adenocarcinoma (G3)         | 30 | 5.80 $\pm$ 0.573                         | 1.29358 $\times$ 10 <sup>-36</sup> | 2.91 $\pm$ 0.458730883                             | 1.23779 $\times$ 10 <sup>-08</sup> | 2.62 $\pm$ 0.14989498                                          | 1.34535 $\times$ 10 <sup>-23</sup> |

amino acid residues on RBP-1. Hydrogen bonding occurs when specific functional groups, such as COOH or NH<sub>2</sub> groups on Ag-NPs, interact with complementary groups on RBP-1. Examples of hydrophobic interactions involve the hydrophobic regions of apigenin and the hydrophobic surface of RBP-1; these interactions contribute to cancer prevention [44–47]. Bilobetin and apigenin are two polyphenol chemicals that may work similarly by preventing noncovalent interaction and reducing RBP-1 protein production, which may halt the growth and spread. The interaction of RBP-1 with Ag-NPs may enhance Ag-NPs' anticancer potential by inhibiting the PI3K/Akt/mTOR pathway and promoting apoptosis in cancer cells [48].

## CONCLUSION

In the blood serum of Iraqi patients with adenocarcinoma and small cell lung cancer, bilobetin compounds and Ag-NPs from *Ginkgo biloba* extract have anti-cancer substances effects by reducing the levels of the proteins cytokeratin21-1 (CYFRA21-1), alpha-1-antitrypsin (AAT), and retinol binding protein-1 (CRBP-1). XRD device show the crystallite size and crystal structure of Ag-NPs are 29 nm and FCC-like structure. FE-SEM images show the particle size and the morphology are 89 nm, spherical and cubic shapes. UV-visible spectrum of Ag-NPs show the optical energy gap is 3.5 eV. The Bilobetin compound, one of the active substances in the *Ginkgo Biloba* plant, as well as solutions of varying concentrations of Ag-NPs from the *Ginkgo Biloba* plant, were synthesized in this study. The Bilobetin compound, one of the active substances in the *Ginkgo Biloba* plant, as well as solutions of varying concentrations of Ag-NPs from the *Ginkgo Biloba* plant, were synthesized in this study. The effects of (Ag NPs) from solutions of the *Ginkgo Biloba* plant on the concentrations of (CYFRA21-1) and ( $\alpha$ 1AT) and (CRBP-1) were investigated in order to identify the ideal concentration at which the concentrations of the proteins diminish with lowering. The (mean  $\pm$  SD) of CYFRA21-1 concentration values after adding Bilobetin with concentration (8ppm) for control and adenocarcinoma and small cell lung cancer were (145.22  $\pm$  43.05 pg/ml) (159.29  $\pm$  28.49 pg/ml) (172.06  $\pm$  48.72 pg/ml) respectively. after adding (Ag NPs)/ginkgo biloba with concentration (4ppm) for control and lung cancer in small cell and adenocarcinoma lung cancer were (85.27  $\pm$  78.42),

(95.57  $\pm$  90.47), (102.53  $\pm$  63.20), respectively. The (mean  $\pm$  SD) of  $\alpha$ 1AT concentration values after adding Bilobetin with concentration (8ppm) for control and adenocarcinoma and small cell lung cancer were (45.16  $\pm$  4.51), (47.72  $\pm$  1.01), (50.13  $\pm$  1.29) respectively after adding (Ag-NPs)/ ginkgo biloba with concentration (4ppm) for control and lung cancer in small cell and adenocarcinoma lung cancer were (44.48  $\pm$  4.88), (45.89  $\pm$  2.18) respectively. The (mean  $\pm$  SD) of RBP-1 concentration values after adding Bilobetin with concentration (8ppm) for control and adenocarcinoma and small cell lung cancer were (2.22  $\pm$  0.19), (2.28  $\pm$  0.25), (2.91  $\pm$  0.46) respectively. after adding Ag-NPs/ginkgo biloba with concentration (4ppm) for control and lung cancer in small cell and adenocarcinoma lung cancer were (2.07  $\pm$  0.13), (2.09  $\pm$  0.049), (2.62  $\pm$  0.149), respectively.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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