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## Green synthesis of silver nanoparticles using plant leaf extraction and phytochemical profile and biological potential of *Artemisia schrenkiana*

**Abstract.** This study explores the green synthesis of silver nanoparticles utilizing plant leaf extraction from *Artemisia schrenkiana* and evaluates their potential anticancer activity against liver cancer cells *in vitro*. One such method is the use of plant leaf extraction, which has shown promising results in the synthesis of silver nanoparticles. The use of *A. schrenkiana*, a plant native to Kazakhstan, for the green synthesis of silver nanoparticles is particularly intriguing. *A. schrenkiana* has been found to contain bioactive compounds that have potential anticancer properties. The green synthesis of silver nanoparticles using plant leaf extraction of *A. schrenkiana* presents an opportunity for sustainable and eco-friendly production methods. The process involves the utilization of environmentally friendly methods for nanoparticle synthesis, utilizing the natural properties of plant extracts. The synthesized nanoparticles are then tested for their efficacy in inhibiting the growth of pancreatic and liver cancer cells in laboratory settings. The findings shed light on the potential of these silver nanoparticles as a promising candidate for cancer therapy, emphasizing their biocompatibility and eco-friendly synthesis route.

**Key words:** *Artemisia schrenkiana*, medicinal plants, AgNO<sub>3</sub> solution, absorption spectra, green synthesis, leaf extraction, silver nanoparticles.

### Introduction

Medicinal plants possess potent healing properties, offering safety, efficacy, environmental friendliness, and minimal side effects, thus driving a growing demand worldwide [1]. According to the World Health Organization (WHO), traditional medicine encompasses practices, methods, and accumulated knowledge derived from life experiences, including plant, animal, and mineral-based remedies, spiritual therapies, and disease treatment techniques. Utilizing natural products for therapeutic purposes dates back to early medical practices [2].

Presently, medicines derived from plant sources are extensively utilized for treating and preventing various diseases, with their variety continually expanding [3, 4]. Herbal remedies offer distinct advantages over synthetic drugs, including pleasant effects and low toxicity. However, in modern Kazakhstan, the potential of medicinal plants from the domestic flora remains underexplored, despite their significance in producing essential medications, cosmetics, and dietary supplements [5, 6].

Humanity has long recognized the significance of plants, not only for their nutritional value but also for their medicinal properties, which have been utilized in the treatment of diseases for thousands of years. Many wild plants harbor rich reservoirs of raw materials in nature. However, the rapid depletion of forest land due to anthropogenic pressures, unplanned development, and excessive exploitation of cultivated plants has led to a decline in their numbers and the extinction of numerous species in nature. This loss of biodiversity and the extinction of endemic species have serious implications [7-9]. To address this challenge, growing medicinal plants in botanical gardens or agricultural fields has emerged as an alternative strategy to obtain more raw materials [10, 11]. Currently, many scientists are dedicated to cultivating medicinal plants using various methods, including organic cultivation without the use of fertilizers or agricultural chemicals, as well as utilizing agricultural byproducts as biogums [12]. The study of *Artemisia* plants is particularly significant due to the presence of a myriad of biologically active substances, which hold immense potential for

agricultural and pharmaceutical industries. However, excessive exploitation of these resources not only depletes plant reserves but also poses a threat to ecosystem destruction [13].

Natural plant extracts play a crucial role in green synthesis processes due to their sustainability and environmental friendliness [14]. Green synthesis minimizes adverse environmental impacts by avoiding the use of toxic chemicals and the generation of hazardous byproducts, unlike conventional chemical synthesis methods [15]. Silver nanoparticles synthesized from plant extracts are particularly advantageous for various biological applications, including wound healing, drug delivery, and imaging, owing to their non-toxic and biocompatible nature [16, 17]. Moreover, these nanoparticles exhibit potent antibacterial properties against a wide range of pathogens, including viruses, bacteria, and fungi [18]. The antibacterial activity of green-synthesized silver nanoparticles may be further enhanced by the presence of phytochemicals and biomolecules in plant extracts [19].

*A. schrenkiana*, commonly known as Schrenk's wormwood, is a member of the *Asteraceae* family and the *Artemisia* genus [20]. This versatile plant thrives in diverse ecosystems across Eastern Kazakhstan, including the Altai Mountains, salty steppes of the Central Tien-Shan Mountains, and the shores of forests and salt lakes. Its adaptability allows it to flourish in various climatic zones, such as deserts, semi-deserts, forests, wetlands, and rocky mountain surfaces [21]. *A. schrenkiana* is a cross-pollinated plant with very small seeds, weighing between 0.2–0.4 grams per thousand grains [22].

In the Center for Scientific Research of Medicinal Plants at Al-Farabi Kazakh National University, laboratory studies were conducted for the first time on the dried whole parts of the plant species *A. schrenkiana* in the East Kazakhstan region. These studies encompassed investigations into the humidity, ash content, extractive substances, organic acids, coumarins, polysaccharides, quantitative content of flavonoids, and macro-microelements such as quercetin (lead, cadmium, zinc, copper, nickel, iron, manganese, sodium, potassium) [23]. These trace elements play a crucial role in *A. schrenkiana*, comprising components of the plant's enzymes and vitamins. Additionally, the effects of *A. schrenkiana* plant extract on insulin, glucose, and HOMA-IR serum levels in diabetic rats were studied [24]. Despite its significance, this plant remains one of the few plants that have not been extensively studied in Kazakhstan.

This research aims to delineate the characteristics of the plant community in the territory where *A. schrenkiana* grows, particularly in the Urzhar District of the Abai region of Kazakhstan. Furthermore, the study aims to investigate the synthesis of silver nanoparticles (AgNPs) using plant leaf extracts, specifically from *A. schrenkiana*. This process entails harnessing the biochemical compounds present in the plant extract as both reducing and stabilizing agents in AgNPs synthesis. Concurrently, this research focuses on analyzing the phytochemical composition of *A. schrenkiana*, which involves identifying and characterizing the bioactive compounds present in the plant extract, such as flavonoids, phenolic compounds, terpenoids, and alkaloids. Additionally, the study evaluates the biological potential of *A. schrenkiana* by conducting various biological assays to assess its antioxidant and membrane-stabilizing properties.

## Materials and methods

*Preparation of the plant extract for chemical composition.* *A. schrenkiana* specimens were collected in the southeastern region of Kazakhstan (Urzhar districts) in 2022 and dried following standard procedures for medicinal plants. Plant materials were acquired from a local pharmacy and identified by Dr. Alibek Ydyrys and Raushan Kaparbay. Specimens (*A. schrenkiana*—No. 2-36857) were deposited at the Biomedical Research Centre, Al-Farabi Kazakh National University, Almaty, Kazakhstan. The plants were crushed and powdered using a laboratory mill. The determination of biologically active substances in the plant extract was conducted at the laboratories of Akdeniz University Faculty of Science (Antalya, Turkey). As previously described [28], 20 mL of 80% methanol was added to a 2.00 g plant sample, followed by extraction in an orbital shaker for 1 hour. The tube was centrifuged at 5000 rpm for 5 minutes, and the liquid phase was collected by filtering the solution. This process was repeated three times by adding 5 mL of 80% methanol to the residual part in the tube. The resulting extracts were transferred to a 50 mL volumetric flask and diluted to volume.

*Determination of total phenolics.* The total phenolic contents of the plant samples were determined spectrophotometrically according to the method of Spanos and Wrolstad [29, 30]. For this purpose, 100  $\mu$ L of the extracted plant samples were mixed with 900  $\mu$ L of deionized water, 4 mL of  $\text{Na}_2\text{CO}_4$  solution (75 g/L), and 5 mL of 0.2 N Folin-Ciocalteu reagent. The mixture was incubated in darkness for 2 hours,

and the absorbance was measured at 765 nm using a spectrophotometer (Shimadzu UV-Vis 160A, Japan). The results were calculated as gallic acid equivalent [31].

**Determination of total flavonoids.** The total flavonoid contents of the plant samples were determined spectrophotometrically according to the method of Uysal et al. [32]. To achieve this, 1 mL of the extracted sample was mixed with 4 mL of deionized water and 0.3 mL of NaNO<sub>2</sub> solution (5%). After 5 minutes, 0.6 mL of AlCl<sub>3</sub> solution (10%) was added, followed by 2 mL of NaOH (4%) after another 5 minutes. The total volume was adjusted to 10 mL with deionized water, and the absorbance was measured at 510 nm using a spectrophotometer (Shimadzu UV-Vis 160A, Japan). The results were calculated as catechin equivalent.

**Antioxidant activity.** The antioxidant activity was determined by the DPPH (2,2-biphenyl-1-picrylhydrazyl) radical scavenging method. The DPPH radical solution (1 mm) was prepared by diluting the analysis with methanol after extraction. Different volumes of plant sample extracts (20-40-60-80-100 µL) were added to test tubes containing 600 µL of DPPH solution, and the total volume was adjusted to 6 mL with methanol. The tubes were vortexed and incubated in darkness at room temperature for 15 minutes. The absorbance values were measured at 517 nm using a spectrophotometer, and the percentage inhibition values were calculated accordingly.

$$\% \text{ Inhibition} = \frac{(\text{ADPPH} - \text{Aextract})}{\text{ADPPH}} \times 100$$

Certain inhibition values were plotted on a graph depicting the sample volume values, and a linear regression analysis was conducted to establish a curved equation dependent on the sample. The EC<sub>50</sub> value was then calculated based on this equation. The determination of the DPPH (1/IC<sub>50</sub>) value involved converting the reverse value to milligrams for the new plant, which inhibits 50% of the 1 g DPPH radical [33].

**Preparation of the plant extract for estimation of lipid peroxidation in liver microsomes. The estimation of total phenolic and flavonoid content.** The total phenolic content was determined using the Folin-Ciocalteu reagent technique [36]. Similarly, the total flavonoid concentrations were evaluated colorimetrically using rutin as the reference [37].

**The estimation of lipid peroxidation in liver microsomes.** Lipid peroxidation (LPO) was

determined by measuring malondialdehyde content as thiobarbituric acid-reacting substances (TBARS) using the Ohkawa et al. method [38].

**The isolation of rat erythrocytes.** Rat erythrocytes were isolated following euthanasia and centrifugation, and the erythrocyte pellets were used immediately for osmotic resistance testing after rinsing [39].

**The estimation of the osmotic resistance of erythrocytes.** The osmotic resistance of erythrocytes (ORE) was assessed by treating isolated erythrocytes with a hypotonic solution of NaCl and measuring hemoglobin absorbance in the supernatant to determine the extent of hemolysis [40-43].

**Green synthesis of silver nanoparticles.** AgNPs are now created using a variety of physical, chemical, and biological techniques. However, the most significant techniques are biological ones, including green synthesis, sometimes referred to as “eco-friendly” techniques. *A. schrenkiana* specimens were collected from the open spaces of Urzhar, Kazakhstan. Figure 1 shows crisp digital photos of the *A. schrenkiana* leaves. After giving the collected leaves a good rinse with running tap water and then with distilled water, they were allowed to air dry for two weeks at room temperature in the shade. The air-dried leaves were then put to storage in plastic bags for later usage after being ground into a powder using an electronic blender. Twenty grams of the final powder sample and one hundred milliliters of distilled water were combined to create the leaf broth solution. For thirty minutes, the mixture was agitated at 80-90 °C with a magnetic stirrer. Following cooling, the mixture was filtered using Whatman Filter Paper No. 1 and regular filter paper. The leftover extract was kept at 4 °C for further use, and the filtrates were used in the tests.



Figure 1 – *Artemisia schrenkiana* Ledeb

**Biosynthesis of silver nanoparticles.** The filtered plant extract was stored at 4 °C for use in another research. 6 mL of 0.01 mM prepared AgNO<sub>3</sub> solution (Merck, Germany) was incubated with 6 mL of *A. schrenkiana* extract for 5 minutes at 30 °C while being constantly stirred in order to produce silver nanoparticles (AgNPs). To make a stock solution of 1 mM AgNO<sub>3</sub>, 0.17 g of AgNO<sub>3</sub> was dissolved in 100 mL of distilled water in a volumetric flask. Every solution within the volumetric flasks was covered with a black covering and kept in a dark place for storage. Next, using a magnetic stirrer, 90 mL of the aqueous AgNO<sub>3</sub> solution was combined dropwise with 10 mL of plant extract, and the combination was stirred for 25 minutes at room temperature.

**Study of optical properties of AgNPs by UV-Vis spectrometry.** To verify the AgNP production, the UV-vis spectra were taken at various times. After 24 hours, the totally reduced solution was centrifuged using a Sorvall ST 8R centrifuge, with the settings set at 9000 rpm for 20 minutes at 25 °C. After discarding the supernatant liquid, the particle was again dispersed in distilled water [44-47]. To get rid of anything that clung to the silver nanoparticles' surface, the centrifugation procedure was performed three times.

**Statistical data analysis.** Statistical analysis was performed using the GraphPad Prism 6.0 Program (GraphPad Software, San Diego, CA, USA). Data were statistically analyzed using comparative and descriptive statistical techniques. The results of three separate experiments were provided as the mean standard deviation (SD). The correlation between extract concentration and lipid peroxidation was assessed, along with the degree of hemolysis. The Pearson correlation coefficient was generated using a nonlinear regression equation, with significance set at  $p \leq 0.05$ , and the T-criterion was used to determine the reliability of the registered changes in the indices.

## Results and discussion

**Phytochemical profile and biological potential of *A. schrenkiana*.** Biologically active substances found in plants serve as crucial indicators of plant quality, shaping their properties and overall value. The quantity and quality of these compounds are influenced by a multitude of internal and external factors. Variations in geographical growth areas and climatic conditions can lead to a diverse array of chemical compounds within the same plant species, thereby shaping the properties and diversity of biologically active compounds.

The collected plant materials of *A. schrenkiana* were carefully dried in shaded outdoor areas, ensuring

purity and the absence of fungi, while quantitatively and qualitatively assessing factors such as humidity and the quantity of extractive substances.

Minerals play a vital role in both plant and human life, with their composition in medicinal plants estimated through ash content analysis. Research indicates that mineral content can range from 3-25% depending on the type of raw material. In the case of *A. schrenkiana*, analysis revealed an ash content of 5.7% and humidity of 5.36% in the above-ground parts of the plant. Furthermore, it was determined that the surface part of the plant contains essential compounds such as amino acids, carbohydrates, polyphenols, flavonoids, and carotenoids (Table 1).

**Table 1** – Quantitative composition of biologically active compounds (quantitative composition, mg/g of dry matter)

Metabolites	Amount of biologically active substances, %
Extractive substances	29.57±0.10
Amino acids	1.45±0.16
Coumarins	0.135±0.31
Carbohydrates	2.32±0.4
Flavonoids	3.8±0.11
Tannin	10.06±0.02
Alkaloids	1.0±0.31
Phenols	2.83±0.22

Extractives refer to the weight of the dry residue obtained by evaporating the dried powder of *A. schrenkiana* individually with 80% ethanol alcohol. According to the research findings, this plant contained extractive substances at a concentration of 29.57%, along with amino acids at 1.45%. A total of 12 amino acids were identified in the extract of *A. schrenkiana*, of which 8 are considered essential amino acids. The quantities of amino acids determined in the plant extract using the capillary electrophoresis system "Capel 105M" are presented in Table 1 (chromatogram).

As depicted in Table 2, it can be observed that the content of non-essential amino acids, specifically leucine + isoleucine, is relatively high, at 190.0 mg/l. Additionally, arginine, histidine, methionine, and threonine were determined to be present at levels of 25.0 mg/l, 23.0 mg/l, 12.0 mg/l, and 15.0 mg/l, respectively. Among the 13 different amino acids identified, 8 types (leucine, isoleucine, lysine, methionine, threonine, phenylalanine, arginine, histidine) are considered non-essential amino acids.

**Table 2** – Number of amino acids determined in *A. schrenkiana* plant extract

No.	Time	Component	Height	Start	End	Area	Conc., mg/l	% of amino acids
1	6.198	arginine	0.505	6.167	6.233	22.02	25.0	0.103±0.041
2	8.522	lysine	0.157	8.483	8.563	3.495	1.70	0.007±0.002
3	8.652	tyrosine	0.102	8.570	8.697	3.948	4.10	0.017±0.005
4	9.025	phenylalanine	0.140	8.945	9.087	6.859	6.50	0.027±0.008
5	9.243	histidine	0.396	9.123	9.335	24.95	23.0	0.095±0.047
6	9.493	leucine + isoleucine	9.190	9.335	9.590	524.5	190.0	0.781±0.203
7	9.632	methionine	0.468	9.590	9.708	14.29	12.0	0.049±0.017
8	9.802	proline	0.198	9.708	9.838	8.101	5.00	0.021±0.005
9	9.893	threonine	0.711	9.843	9.967	22.44	15.0	0.062±0.025
10	10.237	serin	0.051	10.198	10.302	2.143	1.10	0.005±0.001
11	10.367	alanine	0.316	10.302	10.443	10.7	4.50	0.018±0.005
12	10.867	glycine	0.204	10.832	10.918	3.677	1.30	0.005±0.002

According to our research findings, *A. schrenkiana* contains various compounds, including coumarins (0.135%), carbohydrates (2.32%), sucrose (0.30g/100g), fructose (11.02g/100g), and tannins (10.06%). Additionally, the plant exhibited a relatively low level of polyphenols (265.01±1.2mg GAE/g), while the total quantitative number of flavonoids, determined by standard colorimetric analysis, was notably high (142.1±2.1mg CAE/g).

The DPPH radical scavenging activity of these plants ranged from 0.45 µg/ml to 5.01 µg/ml (EC50) respectively. The crude methanolic extracts of *A. schrenkiana* showed antioxidant activity, with (EC50) values of (3.7 µg/ml) respectively.

Yields of essential oils are 0.37 % for *A. schrenkiana*. The main components of *A. schrenkiana* essential oil were Camphene – 3.65%, 1,8-Cineole – 29.05%,  $\gamma$ -Terpinene – 0.30%, p-Cymene – 1.08%,  $\alpha$ -Thujone – 0.35%, Camphor – 44.79%, Bornyl acetate – 0.93%, Terpinene-4-ol – 1.16%, Borneol – 4.26%, Carvone – 0.70%,  $\beta$ -Oplophenone – 1.57%, Spathulenol – 3.76%, and Unidentified compounds – 8.40%.

*The properties of plant extracts.* The results of the analysis of the IC50 for plant extracts, total

flavonoids, and concentration of polyphenolic components are shown in Table 3. These findings provide an evaluation of certain biochemical characteristics of *A. schrenkiana*, a plant or material under investigation for possible health advantages.

Based on the estimation of membrane-stabilizing attributes linked to the IC50 values, the most significant membrane-stabilizing effects of *A. schrenkiana* may be identified. The plant extracts from *A. schrenkiana* were arranged according to the amount of flavonoids and phenolic compounds they contained. The knowledge acquired was in line with studies on plant extracts' antioxidant and membrane-stabilizing properties.

*The Impact of Herbal Extracts of A. schrenkiana.* Table 4 illustrates that the *A. schrenkiana* extract exhibited the best membrane-protective qualities. Plant ethanolic extracts efficiently decreased erythrocyte hemolysis at concentrations ranging from 0 to 100 g dry substance/mL. At a concentration of 5 g/mL, erythrocyte fragility therefore dropped by as much as 50.9 ± 0.9% and 93.1 ± 1.2%. The antihemolytic action of plantains varies with dosage.

**Table 3** – Lipid peroxidation and membrane-stabilizing properties of several *A. schrenkiana* plant extracts (mean  $\pm$  standard deviation (SD),  $n = 3$ )

Species	Total Polyphenols ( $\mu\text{g GAE/mg}$ )	Total Flavonoids ( $\mu\text{g RE/mg}$ )	Lipid Peroxidation IC50 ( $\mu\text{g/mg protein}$ )	Membrane Stabilizing Properties IC50 ( $\mu\text{g/mL of RBC}$ )
<i>A. schrenkiana</i>	342.1 $\pm$ 2.1	265.1 $\pm$ 1.2	3.4 $\pm$ 0.07*	195.1 $\pm$ 7.0*

Note: A log dosage concentration–inhibition curve was used to calculate the 50% inhibitory concentration (IC50) values in  $\mu\text{g/mL}$ . The concentration of total flavonoids and polyphenols was given as the mean  $\pm$  SD of studies conducted in triplicate. Values represented as mean  $\pm$  SE ( $n = 3$ ).  $p$  value (\*\*): very highly significant; (\*): highly significant; (\*): significant.

**Table 4** – Influence of herbal extracts of the *A. schrenkiana* on the osmotic resistance of the erythrocyte membrane. Note: mean  $\pm$  SD,  $n = 3$ . The extent of hemolysis was calculated as the percentage of total hemolysis caused by 0.1%  $\text{Na}_2\text{CO}_3$ 

Species	Extract Concentration ( $\mu\text{g Dry Substance/mL ES}$ )					
	0	5	10	50	100	IC50
<i>A. schrenkiana</i>	100	93. $\pm$ 1.2*	86.3 $\pm$ 3.4**	53.9 $\pm$ 5.7***	50.9 $\pm$ 0.9*	50 $\mu\text{g} <$

Note: The data are expressed as the mean  $\pm$  SD ( $n = 3$ ). Values represented as mean  $\pm$  SE ( $n = 3$ ).  $p$  value (\*\*\*): very highly significant; (\*\*): highly significant; (\*): significant.

Nevertheless, it was discovered that at concentrations higher than 10 g/mL, the protective benefits of *A. schrenkiana* extracts on erythrocyte membranes were strengthened. After the research results were analyzed, it was shown that all plant extracts could maintain membranes and lessen hemolysis of red blood cells. Most of the extracts had a considerable impact on the stability of erythrocyte membranes; however, the concentration needed to achieve a 50% effect was outside the range that was investigated. On erythrocyte membranes, *A. schrenkiana* had a significant effect of stabilizing the membrane.

Herbal extracts were used to preincubate the erythrocyte suspension prior to testing it in a hypoosmotic NaCl solution. Osmotic resistance was measured using the degree of hemolysis. Nearly all

of the extracts reduced cell hemolysis in a dose-dependent manner throughout the concentration range of 0–100 g/mL, as Table 4 illustrates. With a 42.2% reduction in hemolysis, respectively, these results unambiguously validated the antihemolytic effect of *A. schrenkiana* extracts. The extracts of *A. schrenkiana* also showed anti-hemolytic activity at concentrations of 100 g/mL, lowering erythrocyte hemolysis by 50.9  $\pm$  0.9%.

Table 5 describes the results of studies on the effect of plant extracts on lipid peroxidation processes in the microsomal fraction of liver membranes. It is widely known that raw materials obtained from plant raw materials have beneficial properties for humans and contain many compounds. Medicinal plants contain various categories of phytochemicals that are organic sources of antioxidants.

**Table 5** – Effect of *A. schrenkiana* herb extracts on the liver microsome's degree of lipid peroxidation

Species by common name	Extract Concentration ( $\mu\text{g Dry Substance/mg Protein}$ )					
	0	250	500	1250	2500	IC50
<i>A. schrenkiana</i>	100	81.5 $\pm$ 3.2*	26.6 $\pm$ 3.7**	19.4 $\pm$ 2.5**	14.5 $\pm$ 2.9*	392 $\mu\text{g}$

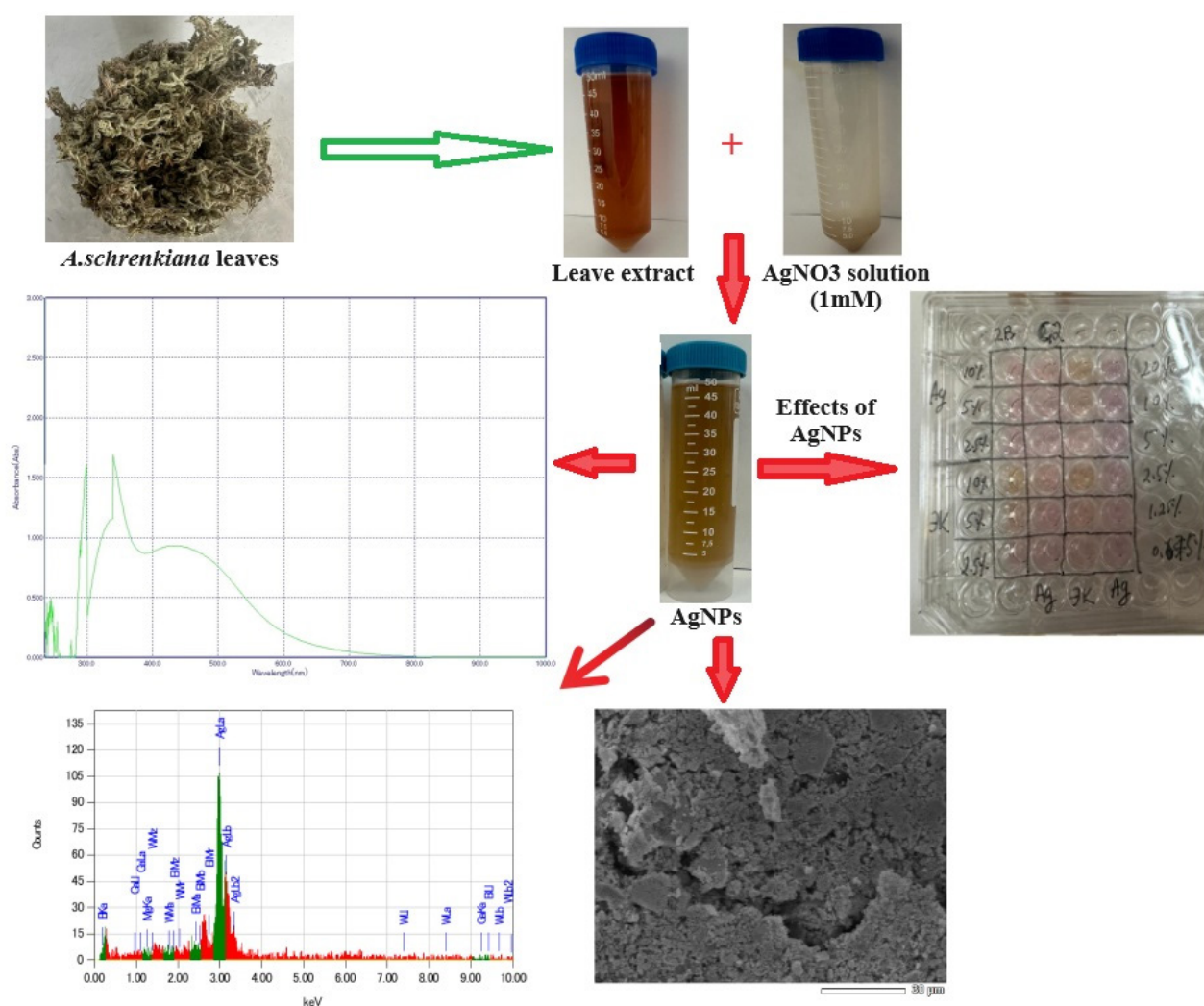
Note:  $n = 3$ , mean  $\pm$  SD. In comparison to the control group, the data are presented as the mean  $\pm$  SD ( $n = 3$ ); \* indicates  $p < 0.05$ ; \*\* indicates  $p < 0.01$ ; and \*\*\* indicates  $p < 0.001$ . *A. schrenkiana* rxy =  $-0.9736$ .

Analyzing the data, it can be concluded that the membrane protective effect and antioxidant properties of extracts are not manifested to the same extent in the same plant species. This is primarily due to the different mechanisms of action of bioactive substances in plants on membranes.

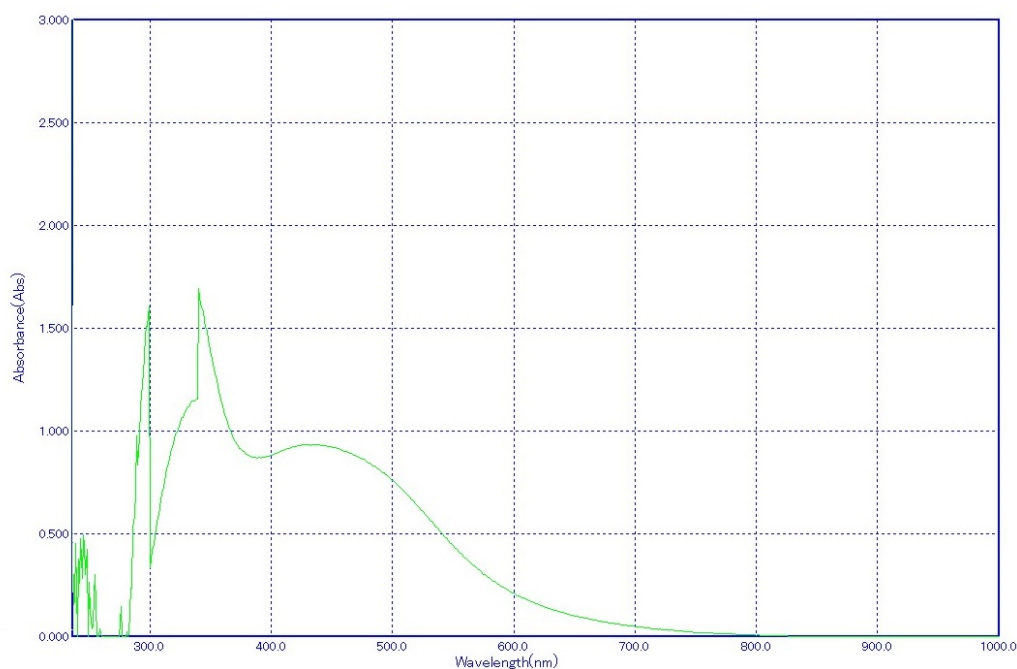
*Green synthesis of silver nanoparticles.* This study examined various amounts of *A. schrenkiana* leaves extract from 10 ml with 100 ml aqueous solution of  $\text{AgNO}_3$  (1 mM) in order to standardize the nanoparticles manufacturing technique. The color of the  $\text{AgNO}_3$  solution rapidly changed from light yellowish to dark brown after 30 minutes due to the dissolution of the 15 ml leaf extract (Figure 2).

In contrast, other samples underwent color changes after 2–8 hours of incubation in a dark room, and the control sample remained colorless.

Additionally, the results of UV-visible spectrophotometers, which displayed a spectrum of surface plasmon resonance (SRP) ranging from 300 to 360 nm of absorption band, validated the production of AgNPs in the solution (Fig 3). It should be mentioned, nevertheless, that 1.5 ml of fruit extract in 100 ml of  $\text{AgNO}_3$  solution was also used to create AgNPs. In fact, a more pronounced and intense absorption band at 360 nm was visible in the SPR spectra of AgNPs produced from the increased concentration of leaf extract (Figure 3).



**Figure 2** – Schematic diagram for biosynthesis of AgNPs using leaves extract of *A. schrenkiana*



**Figure 3** – Absorption spectra of extract of *A. schrenkiana* leaves

Additionally, the results of the UV-vis spectra demonstrated that the reaction mixture's absorbance intensity increased with time, and the solution remained stable after 24 hours of incubation, indicating that the creation of the nanoparticles in solution had been completed. As a result, the AgNPs mediated by the blend of 100 ml AgNO<sub>3</sub> solution and 15 ml leaf extract were freeze-dried and utilized in subsequent research.

*Study of optical properties of AgNPs by UV-Vis spectrometry.* The final stage was using 100% ethanol. Morphological studies and size examination of the synthesized AgNPs were conducted by a field-emission scanning electron microscope and a transmission electron microscopy. The SEM image (Figure 4) and the TEM images both demonstrated that the majority of AgNPs were extremely mono-dispersed in spherical forms.

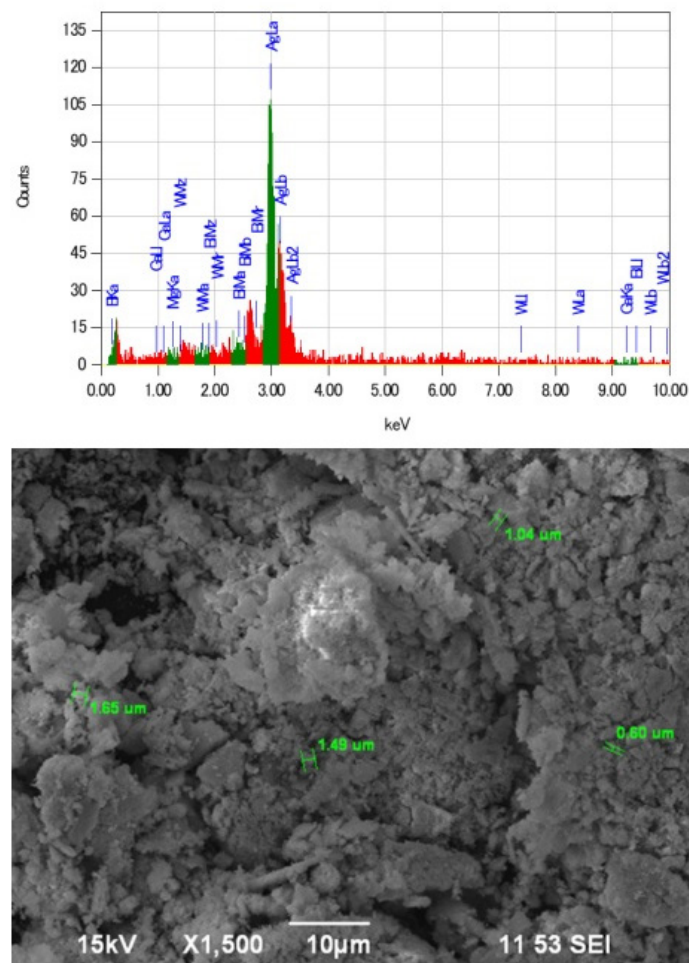
The SAED pattern's brilliant circular spots indicated the (1.04), (1.65), and (1.49) planes and confirmed the particles' crystalline structure. These statistics agree with the findings of the XRD. The AgNPs' size distributions ranged from 0.60 to 1.65

nm. Additionally, the presence of the silver element in the produced AgNPs was verified using the EDX equipment.

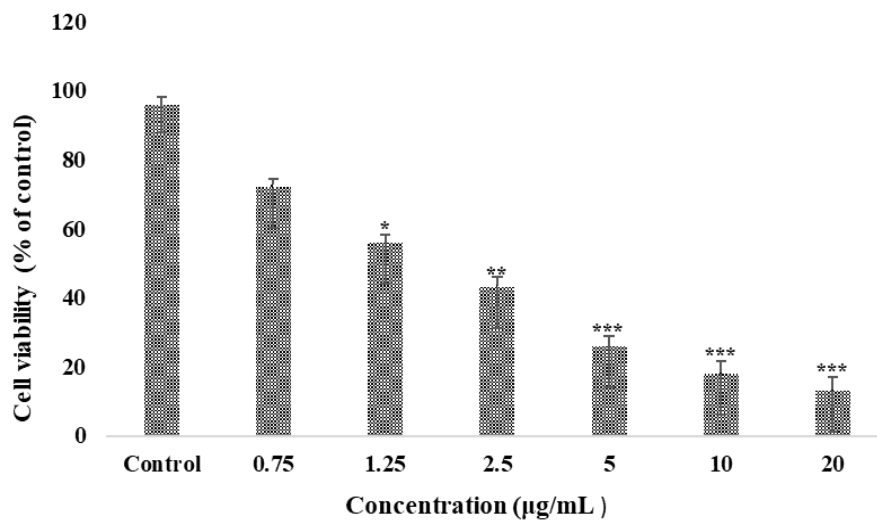
*Anticancer activity against pancreatic and liver cells lines in vitro.* The MTT assay (Sigma Aldrich, Germany) was used to examine the inhibitory effects of manufactured nanoparticles and *A. ciniformis* extract on the proliferation of liver cancer cells. For a whole day, the liver cancer cell was exposed to varying concentrations (0.75, 1.25, 2.5, 5, 10, 20 µg/mL) of both commercial and biological silver nanoparticles. At a dose of 20 µg/mL, the greatest inhibitory effect on the growth of liver cancer cells was found; this effect was statistically significant when compared to the control group ( $p < .001$ ).

When comparing the bioavailability percentage of commercial and phytosynthesized silver nanoparticles at 10 and 20 µg/mL to the untreated cells, there was no significant difference ( $p > .05$ ) (Figure 5). Biological and commercial nanoparticles with a 20 µg/mL bioavailability of 78% and 91%, respectively.





**Figure 4** – EDX spectrum of the synthesized AgNPs. A strong peak at 3 keV indicates the existence of Ag



**Figure 5** – Cell viability of liver cancer cells following treatment with biosynthesized AgNPs after 24 (results are reported as viability in comparison with control group;  $p \leq 0.001$ \*\*\*,  $p \leq 0.01$ \*\* ,  $p \leq 0.05$ \*).

Using varying AgNP concentrations, the colorimetric MTT test was used to assess the cytotoxicity of AgNPs. The findings showed that the cytotoxicity of AgNPs was dose dependant and that they had the greatest inhibitory effect on liver cancer cells at doses of 10 µg/mL and 20 µg/mL ( $p < .001$ ). Moreover, 2.5 µg/mL was determined to be the IC<sub>50</sub> of AgNPs. Furthermore, the identical quantities of AgNPs were applied to liver cancer cells for a duration of 24 hours. The cytotoxicity of AgNPs against normal liver cancer cells revealed that these cells were less susceptible to AgNPs' effects (IC<sub>50</sub> = 10 µg/mL). Figure 5 displays the outcomes of liver cancer cell survival after 24 hours. Following treatment with AgNPs, the morphological alterations in liver cancer cells were examined. AgNPs treatment of liver cancer cells resulted in wrinkling, rounding, and membrane bulging, all of which are signs indicating the AgNPs will probably cause the cancer cells to undergo apoptosis.

### Conclusion

In conclusion, *A. schrenkiana* emerges as a significant medicinal plant, particularly in the context of the domestic flora of Kazakhstan. Through comprehensive phytochemical analysis, it has been revealed to contain a rich array of biologically active compounds, including extractive substances, coumarins, carbohydrates, tannins, amino acids, flavonoids, and phenolic compounds [43]. These constituents contribute to its pharmacological properties, which encompass antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Overall, *A. schrenkiana* represents a promising avenue for the development of novel therapeutic and preventive drugs, offering potential solutions

for addressing various health challenges. Continued investigation into this plant species holds the promise of uncovering new avenues for drug discovery and development, ultimately benefiting both traditional and modern healthcare systems.

AgNPs made by green synthesis from plant extracts seem to be more suitable for clinical applications than those made by physical or chemical means. Furthermore, the green synthesis of AgNPs is an easy, secure, economical, and environmentally beneficial process. Green AgNPs appear to be some promising anti-cancer drugs as a result. To ascertain their biocompatibility and adverse effects in animal samples, more investigation is necessary. The current study's findings demonstrated that green AgNPs produced by *A. schrenkiana* have anti-metastatic effects on liver cancer cells, and that these effects are achieved by causing the cancer cells to undergo apoptosis. As a result, biosynthesized nanoparticles may be applied as cancer-fighting chemotherapeutic drugs.

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### Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

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