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Discovery of high antibacterial and antitumor effects against multi-drug resistant clinically isolated bacteria and MCF-7 and AGS cell lines by biosynthesized silver nanoparticles using *Oxalis corniculata* extract

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RESEARCH ARTICLE



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ABSTRACT

The green technique is a unique way to produce functional nanoparticles. We examined the green synthesis of Ag nanoparticles (O-AgNPs) by the extract of *Oxalis corniculata*. Green-synthesized O-AgNPs were accomplished by monitoring critical factors such as concentration, pH, reaction time, and temperature. Several analytical techniques, including scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-ray diffraction analysis, and UV-Vis spectroscopy, were applied to characterize O-AgNPs. The SEM analysis showed O-AgNPs with a spherical shape and an average size of 33.57 nm. The XRD pattern indicated the face-centered cubic (fcc) structure of the prepared O-AgNPs. The anticancer activity of the synthesized O-AgNPs was investigated in MCF-7 (breast) and AGS (gastric) cell lines, indicating high anticancer effects against selected cell lines. The growth of all selected bacteria containing Gram+ and Gram- was inhibited by O-AgNPs. O-AgNPs showed greater inhibition in comparison to conventional antibiotics. As a result, our green synthesized AgNPs using plant extracts exhibited anticancer and antibacterial activities.

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1. Introduction

Nanotechnology is a scientific breakthrough that uses molecules at the level of atoms or the production of materials and devices with inimitable properties [1]. Nanomaterials were considered due to their high specific surface area and high reactivity [2,3]. In terms of shape, size, and crystal structure, nanoparticles significantly affect their catalytic, optical, and electronic properties [1]. The site-specific activity of the nanoparticles makes a safe dose in administration that induces the efficacy of the drug and reduces unwanted effects [4]. Nanoparticles have shown useful applications and may also have dangerous side effects on humans, aquatic organisms, and plants [5].

Cancer is a rapid and abnormal cell division [6-8] that is the second most current cause of death worldwide, killing more than eight million people per year. Cancer is predicted to improve by more than 50% in the future decades [9]. Current treatments are limited by side effects, drug resistance, and solubility [1]. The use of nanomaterials is a new strategy for cancer therapy that can be used as pharmaceutical carriers to increase drug efficacy [10,11]. Nanotechnology can lead to significant advances in the detection, diagnosis, and treatment

of cancer [7]. Fabrication of nanomaterials can be achieved using various physical, chemical, and biogenic synthesis techniques. Synthesis using physical and chemical methods may be created using toxic high radiation and reductants that affect both humans and other living organisms. However, the green synthesis method is a single step for low-cost and environmentally friendly synthesis of nanoparticles. The synthesized nanoparticles exhibited antifungal, anti-insecticidal, and antimicrobial activity. Due to their characteristics, all metallic nanoparticles have paved the way for nanoscale researchhers to advance medical research [1]. Green synthesis of Ag nanoparticles (AgNPs) was performed using various plant extracts such as Feijoa sellowiana [12], Crataegus pentagyna [13], Crataegus microphylla [14], Convolvulus fruticosus [15], Ferula persica [16], Stachys inflate [17], Scrophularia striata [18], Allium paradoxum [19], Allium sativum [20], Cleistanthus collinus [21], Piper longum fruit [22], Aloe vera [23], Melia azedarach [24], Pleurotus florida [25] and Dregea volubilis [26]. Effective anticancer, antibacterial, and antioxidant activities have been reported from biosynthesized Ag nanoparticles [14,22-24,27]. Various mechanisms have been proposed for the anticancer effects of AgNPs, including induction of apoptosis, cell cycle arrest, alteration in P53 utilization, and regulation of

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cytokine genes [4]. In this study, *Oxalis corniculata extract (O. corniculata)* extracts with phenol and flavonoids as useful bioactive compounds were used as stabilizing and capping agents to synthesize Ag nanoparticles from Ag ions.

O. corniculata Linn. belongs to the Oxalidaceae family and is found in Asia, Europe, America, and Africa. These plants have essential phytochemical compounds for normal human health [28], including β-carotene, Vitamin C, and niacin. *The plant also* contains different phytomolecules, such as flavonoids, alkaloids, tannins, steroids, polyphenols, glycosidic compounds, lipids, and volatile oil. Leave infusion is used to remove warts and corneal opacities. It works for fever, cold, cough, diarrhea, dysentery, skin problems, urinary tract infections, and sprains [29]. Antiscorbutic, refrigerant, anti-inflammatory, diaphoretic, hepatoprotective [30], antiseptic, antidiabetic, diuretic activities [28], anticancer [31], antimicrobial and antifungal activities [32,33] have been reported in different studies for O. *corniculata* [34]. This plant is also used to improve cuts, anemia, dyspepsia, piles, dementia, and convulsions [29,35]. The extract exhibited antioxidant and radical scavenging activity [34].

The present study disclosed the biosynthesis route for silver nanoparticles using *O. corniculata* extract and investigated their anticancer and antimicrobial activities. The green synthesized nanoparticles were characterized using different analytical methods such as UV/vis spectroscopy, energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay exhibited that the prepared AgNPs possess anticancer activities against two cancer cell lines, MCF-7 (breast cancer) and AGS (human gastric carcinoma). In addition, the characterized 0-AgNPs were effective antibacterial agents against ATCC strains and resistant clinically isolated bacteria.

2. Experimental

2.1. Preparation of O. corniculata extract

The fresh plant was collected in September 2021 from the city of Lahijan in Gilan Province, in the north of Iran. The methanolic extract was obtained by percolating 20 g of powdered *O. corniculata* aerial parts for 24 h at room temperature (3×1). The extract was filtered using Whatman filter paper (No. 1) and then concentrated at 35 ° C using a rotary evaporator and freeze-dried.

2.2. Biosynthesis of O-AgNPs

Silver nitrate (AgNO₃) was provided by Fluka Company. Other reagents were used in analytical grade. In the procedure, 0.1 g of crude extract was diluted in 100 mL of deionised water to make it 1 mg/mL, and 15 mL of extract solution at pH = 10 was mixed with 15 mL of 1 mM of aqueous AgNO₃ solution, heated at 65 °C with constant stirring for 30 min, and AgNPs were gradually prepared. The same reactions were carried out with various concentrations of AgNO₃ at various temperatures and pH levels at various times during the reaction to determine the best conditions.

2.3. Characterization of O-AgNPs and instrumentation

The UV-Vis spectrum was recorded at 420 nm for Ag nanoparticles using a UV-Vis spectrophotometer (T80+, China.). The size and morphology of biosynthesized AgNPs were studied by SEM analysis (SCAN BRNO-Mira3 LMU, Germany), and XRD patterns were documented on (Philips PW 1800) using Cu K α (λ = 1.5406 Å, 40 kV, 40 mA) radiation.

2.4. Antibacterial activity

2.4.1. Minimal inhibitory concentration (MIC) of synthesized O-AgNPs

The antibacterial potential of silver nanoparticles was experienced at concentrations of 10 to 200 μ g/mL against *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), *Klebsiella pneumonia* (*K. pneumonia*, ATCC 700603), *Acinetobacter baumannii* (*A. baumannii*, ATCC 29606), *Proteus mirabilis* (*P. mirabilis*, ATCC 25933), *Staphylococcus aureus* (*S. aureus*, ATCC 27853), *Enterococcus faecalis* (*E. faecalis*, ATCC 29213) and *Escherichia coli bacteria* (*E. coli*, ATCC 25922) bacteria.

In the process, 100 μL of Mueller Hinton broth culture medium was added to all wells in the microplate, and the appropriate concentrations of the antibiotics examined were obtained. Subsequently, 100 µL of dilute bacterial suspension was moved to all wells; MIC results were studied after 24 hours of incubation. The MIC was stated to be the first well to show no growth. The positive control (bacterial culture medium without extracts) and the negative control (non-bacterial culture medium) were applied for this study. For ATCC strains, ciprofloxacin was utilised as a reference chemical for antibacterial activity. Several clinical centers provided seven bacterial pathogens and were known for their biochemical ways. The influence of synthesized O-AgNPs was studied on these resistant bacteria S. aureus, A. baumannii, and P. aeruginosa (the presence of the genes encoding the enzymes of carbapenemase OXA-24 and OXA-23 is confirmed), P. mirabilis (the presence of the genes encoding OXA-23 is positive and NMD, VIM, PhosA3 is negative), peripheral E. faecalis (positive for ermB, TetM, TetL, VanA, VanB), E. coli (positive for OXA-23, PhosA3, and VIM and negative for NMD), K. pneumonia (positive for OXA-23 and NMD also negative for VIM). According to the approach previously stated, the MIC was estimated as the maximum dilution of O-AgNPs, demonstrating complete suppression of the pathogens [16,36].

2.4.2. Minimum bactericidal concentration (MBC) of synthesized AgNPs

The minimum bactericidal concentration (MBC) was determined according to the MIC values; the MIC dilutions were taken and grown on Muller Hinton agar medium separately. After 24 hours of incubation, the plates were investigated for bacterial growth. The lowest concentration of NPs that did not show bacteria growth was assigned as the minimum bactericidal concentration (MBC) [16,37].

2.5. Anticancer activity of synthesized O-AgNPs

The MTT assay was performed; Two cancer cell lines comprising AGS and MCF-7 cells were prepared from the pasture Institute, Iran. Briefly, cells in a suspension containing 6-10×10³ were seeded to a 96-well plate and incubated for 24 hours at 37 °C in a humidified atmosphere of 95% air and 5% CO2. All tests were performed three times. Cell cultured supernatant was eliminated after 24 hours, and different concentrations of silver nanoparticles were diluted in culture medium supplemented with 10% fetal bovine serum (FBS) and antibiotics. Treatment was carried out with varying concentrations of synthesised O-AgNPs (0.1-50 µg/mL) for 24 hours of incubation. One mL of MTT (5 mg/mL, yellow tetrazole) was taken into each well and incubated for 4 hours. The control wells are made of cell culture medium only. In this test, the conversion of MTT to formazan (insoluble) by 'dehydrogenase enzymes in intact mitochondria' of live cells is evaluated.



Figure 1. Optimization of concentration of silver nitrate in green synthesis of silver nanoparticles.



Figure 2. Optimization of temperature in green synthesis of silver nanoparticles.

Pipetting two to three times gradually dissolved the created crystals. A Synergy H1 hybrid multimodel microplate reader (Biotek Instruments, Winooski, Vermont, NE, USA), was used to measure the absorbance at 570-620 nm. Equation (1) was used to convert the optical density (OD) value to the percentage of viability [16]:

Percentage of cell viability =
$$\frac{OD \text{ value of samples}}{OD \text{ value of control}} \times 100$$
 (1)

3. Results and discussions

3.1. Green synthesis and characterization

The green synthesis of metal NPs such as silver, selenium, gold, titanium, etc., makes available advancements over chemical and physical methods because of their cost-effective, eco-friendly responsive, natural origin, and ease of scaled-up synthesis. There is no need for high energy and temperature [38]. The better mechanism for the green synthesis of AgNPs is the plant reduction process related to their phytochemicals [39]. In plant extracts, biomolecules such as alkaloids, flavonoids, terpenoids, amino acids, tannins, saponins, phenols, carbohydrates, etc., play reducing, capping, and stabilising roles. The different parts of a medical plant, such as stem, roots, leaves, flower, fruit, seed, and bark, can be used for the green synthesis of AgNPs [40]. Studies indicated that AgNPs could be prepared using a green reducing agent. The silver salt will be converted to silver nanoparticles. AgNPs have received great attention for their 'unusual chemical, physical, catalytic, electronic, magnetic, and biological activities'. The properties of silver nanoparticles are significantly different from those of bulk silver metal. These are large surface volume ratios that lead to a large fraction of surface atoms, high surface energy, spatial confinement, and fewer imperfections [41]. Silver nanoparticles have strong bactericidal activity against a wide

variety of multidrug resistant strains [42,43]. In addition, they exhibited antifungal [40,44], anti-neoplastic, and antiviral activities [45-47]. Various factors affect the synthesis of metal nanoparticles, including temperature, pH, reaction time, extract, and metal salt concentration.

UV-vis spectroscopy is a valuable technique for the initial characterisation of NPs. The synthesis of AgNPs by reducing silver ions was evaluated by ultraviolet-visible (UV-vis) spectroscopy. As a result of surface plasmon vibrations, AgNPs appear brown in an aqueous medium [48]. The shape, temperature, and dielectric constant of the medium could affect the peak locations of the UV-vis spectra. In contrast, the shape of the UV-Vis peak exhibited a nanoparticle morphology [49]. Surface plasmon resonance (SPR) occurs from the collective oscillations of valence electrons in incident radiation's electromagnetic field. This phenomenon plays a key role in determining the absorption spectra of Ag NPs [50]. The λ_{max} for O-AgNPs was observed in the range of 400-500 nm [50].

The UV-Vis spectra of O-AgNPs using a constant concentration of the extract (2 mg/mL) with various concentrations of AgNO₃ at room temperature are shown in Figure 1. While the concentration of the silver nitrate enhances, the absorption peak gets more sharpness. Furthermore, Figure 2 displays UVvis spectra of O-AgNPs synthesised at various temperatures. It is clear that as the temperature increases, the absorbance increases with it. So, the best green synthesis was observed at 85 °C. Figure 3 exhibited the effect of pH on the fabrication of AgNPs, raising absorbance with increasing pH at 12. The reaction was monitored for four hours; the UV-vis spectra of O-AgNPs as a function of time are depicted in Figure 4. Furthermore, the brown colour of AgNPs appeared shortly after adding the plant extract to the AgNO₃ solution.

The UV-Vis spectrophotometer records the surface plasmon resonance (SPR) as an absorbance peak around 420 nm. Growth in AgNPs yield led to a higher SPR band by increasing the collision frequency [38].



Figure 3. Optimization of pH in green synthesis of silver nanoparticles.



Figure 4. Optimization of time of incubation in green synthesis of silver nanoparticles.



Figure 5. SEM image of AgNPs synthesized by O. corniculata in 500 nm.

Furthermore, the creation of bigger nanoparticles generates a redshift in the UV-vis spectrum or a broadening of the peak. On the basis of the SPR bands in our study, the produced O-AgNPs showed small spherical particles.

The SEM technique was used to characterise the morphological and structural properties of green synthesised O-AgNPs [50-52]. In Figures 5 and 6, the size and shape of O-AgNPs were seen using the SEM technique. The biosynthesised O-AgNPs had a spherical shape with an average size of 33.57 nm. The EDS results showed the percentage of composition of the mixture. This technique defined Ag0 as the significant element [53]. The elemental silver signal of AgNPs on the vertical axis displays the number of X-ray counts, whereas the horizontal axis exhibits energy in keV [54]. Figure 7 presents the EDS spectrum; A strong peak at 3 keV confirms the formation of AgNPs. X-ray diffraction (XRD) analysis approved the crystallographic structure of green-prepared AgNPs using the characteristic peaks of the X-ray diffraction pattern [42]. An

increase or decrease in peak intensity is associated with the amount of constituents [55]. Figure 8 shows the XRD pattern of the prepared O-AgNPs; The diffraction peaks at $2\theta = 38.46, 44.5, 64.7$, and 77.9° corresponded to Bragg's from (111), (200), (220) and (311) planes, respectively. The obtained data demonstrated the face-centered cubic (fcc) structure of the synthesized O-AgNPs. Three peaks at 27.94, 32.5, and 46.36° have corresponded to the impurity of AgCl.

3.2. Antibacterial activity of AgNPs

AgNPs with multivalent mechanisms display antibacterial activities. Studies have shown that AgNPs possess broad antibacterial activity against infectious microorganisms such as *E. coli* and *S. aureus* [56]. The antibacterial effect of AgNPs is related to the oxidation and release of Ag⁺ ions, so it is an ideal bactericidal agent.



Figure 6. SEM image of AgNPs synthesized by O. corniculata in 200 nm.



Figure 7. EDS spectrum of AgNPs synthesized by O. corniculate.



Figure 8. X-ray diffraction analysis of AgNPs synthesised by O. corniculata.

The large surface-to-volume ratio and a large fraction of silver nanoparticles' surface atoms create a tremendous antimicrobial effect compared to silver metal [57]. Furthermore, because of their small size, AgNPs quickly penetrate cell membranes and affect intracellular processes. The excellent antibacterial activity of AgNPs is attributed to their well-

developed surface, which makes it available to high contacts with the environment. Another considered mechanism is the production of free radicals from the body of the synthesised silver nanoparticles. Free radicals can damage the cell membrane and create a porous layer that eventually kills the cell [58].

Table 1. ATCC study of green synthesized silver nanoparticles *.

Bacteria	ATCC	Ciprofloxacin (µg/mL) MIC	MIC of extract (µg/mL)	MBC (µg/mL)	MIC (µg/mL)		
S. aureus	ATCC 29213	0.21	>4000	12	0.7		
E. faecalis	ATCC 29212	0.21	>4000	12	10		
P. aeruginosa	ATCC27853	0.23	>4000	50	1.5		
A. baumannii	ATCC 19606	0.25	>4000	6	1.5		
E. coli	ATCC 25922	0.1	>4000	1.5	1.5		
K. pneumoniae	ATCC 700603	0.1	>4000	12	3		
P. mirabilis	ATCC 25933	0.1	>4000	1.5	3		

* MIC: Minimal inhibitory concentration; MBC: Minimal bactericidal concentration.

Table 2. Clinical isolated bacteria treatment using green synthesized O-AgNPs *.

Bacteria	Treatment with AgNPs		Susceptibility to antimicrobial agents										
	MBC (µg/mL)	MIC (µg/mL)	AK	СМ	ERM	CEZ	CIF	PC	GM	TC	00	VC	MC
S. aureus	180	6	R	R	R	R	R	R	R	R	R	S	R
E. faecalis	180	6	S	R	R	R	R	R	S	R	R	R	R
P. aeruginosa	45	3	R	R	R	R	R	R	R	R	R	R	R
A. baumannii	90	3	R	R	R	R	R	R	R	R	R	R	R
E. coli	180	3	R	R	R	R	R	R	R	R	R	R	R
K. pneumoniae	90	11.5	R	R	R	R	R	R	R	R	R	R	R
P. mirabilis	22.5	6	R	R	R	R	R	R	R	R	R	R	R

* MIC: Minimal inhibitory concentration; MBC: Minimal bactericidal concentration; MC: Meticillin; VC: Vancomycin; OC: Oxacillin; TC: Tetracycline; GM: Gentamicin; PC: Penicillin; CIF: Ciprofloxacin; CEZ: Ceftazidime; ERM: Erythromycin; CM: Clindamycin; AK: Amikacin.



Figure 9. Cell viability of (a) AGS and (b) MCF-7 cancer cell lines (μ g/mL).

AgNPs cannot simply affect Gram-positive bacteria that have a thick cell wall comprising peptidoglycan. Gram-negative bacteria have a thin lipid layer in their cell wall that causes AgNPs to penetrate them easily [59].

The silver nanoparticles synthesized from *O. corniculata* showed antibacterial activity against two series of bacteria, including ATCC and clinically isolated strains. The bactericidal mechanism of silver colloid particles against bacteria has not been identified very well until now. The antibacterial activity of AgNPs was organized on *P. aeruginosa, K. pneumonia, A. baumannii, P. mirabilis, S. aureus, E. faecalis,* and *E. coli.* In ATCC strains, the lowest MIC was observed for *A. baumannii, E. coli, P. mirabilis,* which was around 1.5 μ g/mL, and MBCs were 6.0, 1.5, and 3.0 μ g/mL in treated with O-AgNPs. The results in Table 1 displayed that the AgNPs prepared using *O. corniculata* affect the growth of the selected bacteria. Subsequently, for further antibacterial evaluation, synthesized nanoparticles were studied against clinically isolated resistant strains (Table 2).

Seven clinically isolated bacteria were chosen for this study to test their susceptibility to our manufactured AgNPs.

In this regard, *A. baumannii* and *P. aeruginosa* were isolated from wounds and phlegm, and the genes encoding carbapenemase enzymes were found to be resistant to cefepime, piperacillin/tazobactam, ciprofloxacin, aztreonam, ceftazidime, levofloxacin, gentamicin, tobramycin, amikacin, imipenem, and doripenem meropenem. While carbapenems are the last line of treatment, the MIC values for colistin sulphate were reported at 256 and 8 μ g/mL, respectively. Our fabricated 0-AgNPs considerably inhibited the growth of resistant *A. baumannii* and *P. aeruginosa* with a MIC of 3 μ g/mL and MBCs of 90 and 45 μ g/ml.

The isolated *K. pneumonia* and *E. coli* from urine were found to be resistant to carbapenems. Colistin sulfate had MICs of 128 and 32 μ g/ml, although O-AgNPs had MICs of 11.5 and 3 μ g/mL, respectively. Although *P. mirabilis* isolated urine was resistant to imipenem and meropenem with MICs of 2 and 32 μ g/mL, and

the synthesized AgNPs showed a MIC value of 6 μ g/ml with a MBC value of 22.5 μ g/mL. *E. faecalis* isolated from the intestine was resistant to ampicillin, vancomycin, teicoplanin, erythromycin, tetracycline, levofloxacin and kanamycin, but was a little sensitive to gentamicin; O-AgNPs could inhibit resistant *P. mirabilis* with a MIC of 6 μ g/mL and a MBC ~180 μ g/mL. *The S. aureus* from the chip was susceptible to vancomycin. Our synthesized O-AgNPs exhibited a MIC of 6 μ g/ml and a MBC of 180 μ g/mL, respectively.

3.3. Anticancer activity of O-AgNPs

AgNPs have exhibited a wide range of biological activities that make them promising agents for infection, cancer, and multidrug resistant cancer cells [51,60]. AgNPs demonstrated unique anticancer activity against various cancer cells [61]. In this work, the anticancer activity (in vitro) of green synthesized AgNPs by O. corniculata extract against AGS and MCF-7 cell lines was proven by MTT assay. The MTT assay determines cell growth inhibitory activity using mitochondrial dehydrogenase activity in living cells [54]. Active mitochondria have these dehydrogenase enzymes, so the reaction occurs only in living cells [53]. Cell viability (%) treated with various concentrations of silver nanoparticles is shown in Figure 9. This study powerfully revealed the antiproliferative activity of biosynthesized silver nanoparticles with a low effect on normal cells compared to the control. As a result, the inhibitory concentration at 50% (IC₅₀) for MCF-7 and AGS cell lines was found to be 3.3 and 3.2 μg/mL of O-AgNPs, respectively.

Cell inhibition of AgNPs was reported in ranging from 3.043 to 25 µg/mL for the MCF-7 cell line [62]. The reported results showed that Ag nanoparticles could have induced reactive oxygen species [63]. The NPs enter the cells, interact with the biological components, and damage DNA and/or the mitochondria-dependent apoptosis pathway leading to cell death. In some cases, the inhibition of the AGS cell line treated with silver nanoparticles was observed, ranging from 10 to 100 µg/mL. Silver nanoparticles in the size of 5-35 nm induce apoptosis through the mitochondrial pathway and target function [61]. Studies reported that silver nanoparticles with spherical shapes and a mean diameter of less than 100 nm indicated considerable toxicity against breast cancer cells. In contrast, it showed less toxicity in normal cells [64,65]. Furthermore, silver nanoparticles can induce the apoptosis pathway lacking p53 and cause reactive oxygen species (ROS) **[4,61]**.

4. Conclusions

In the current research, the one-pot biosynthesis of AgNPs in plant extracts (*O. corniculata* extract) was explained. The polyphenols contained are reducing and capping agents. Various methods confirmed the synthesis of O-AgNPs; the SEM images exhibited NPs with a spherical shape with an average size of 33.57 nm. In addition, the EDS and XRD results proved the formation of O-AgNPs. The Green prepared NPs demonstrated antibacterial activity against some pathogenic Grampositive and negative bacteria. Furthermore, AgNPs indicated anticancer efficacy against MCF-7 and AGS cells, making them a potent chemotherapeutic agent. Therefore, according to the current results, AgNPs synthesized using the plant can be considered effective compounds in cancerous cell treatment.

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Disclosure statement 📭

Conflict of interest: The authors declare that they have no conflict of interest. Ethical approval: All ethical guidelines have been adhered to. Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement GR

Conceptualization: Mohammad Ali Ebrahimzadeh; Methodology: Mohammad Ali Ebrahimzadeh, Seyedeh Roya Alizadeh; Formal analysis: Mohammad Ali Ebrahimzadeh, Seyedeh Roya Alizadeh; Investigation: Mohammad Ali Ebrahimzadeh, Seyedeh Roya Alizadeh; Resources: Zahra Hashemi; Funding: Zahra Hashemi; Supervision: Zahra Hashemi; Writing - Original Draft: Mohammad Ali Ebrahimzadeh, Seyedeh Roya Alizadeh, Zahra Hashemi; Review and Editing: Zahra Hashemi. All authors have read and agreed to the published version of the manuscript.

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210