Anticancer, antimicrobial, and dye degradation activity of biosynthesised silver nanoparticle using *Artemisia kopetdaghensis*

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Biosynthesis of nanoparticles (NPs) is gaining attention due to the presence of functional plant macromolecules that benefit from NPs' synthesis and also due to their exceptional bioactive compounds. *Artemisia kopetdaghensis* extract acted as a reducing/capping agent. Characterisation of greener-synthesised AgNPs (Ak-AgNPs) was performed by various techniques, such as Ultraviolet (UV)−visible spectrophotometry, TEM, Fourier transform infrared, and XRD. Ak-AgNPs indicated great bactericidal properties in terms of zone of inhibition (ZI) against all of the pathogenic bacteria (i.e. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *and Staphylococcus aureus*). The maximum ZI of Ak-AgNPs was 22.3 and 17.8 mm against *K. pneumoniae* and *S. aureus*, respectively. Anti-proliferative activity of Ak-AgNPs on human hepatocellular cancer cell line (HepG2) using the MTT test showed dosage-dependent inhibition activity of Ak-AgNPs against HepG2 cancer cells. Also, green-synthesised Ak-AgNPs showed catalytic properties under UV-light in organic dye (methylene blue) degradation. This study revealed that the green-synthesised AgNPs using *A. kopetdaghensis* shoots extract had antibacterial and catalytic activities. Also, Ak-AgNPs had anti-proliferative potential against human liver cancer cell lines. The green-synthesised Ak-AgNPs have the potential to be exploited in anti-bacterial, anticancer, and biocatalyst technologies.

1. Introduction: Presently, the use of nanomaterials is very widespread and is used in many industries such as wastewater treatments, catalysts, sensors, and drug deliveries [1–5]. For many years, noble metal nanoparticles (NPs), especially silver (Ag), have been significantly applied in the biomedical field due to their physiochemical things [6–8]. In comparison with physical and chemical ways for the fabrication of NPs, the green approaches have various benefits such as economic, eco-friendly, and easily employed for large-scale production [5, 9, 10]. Additionally, green-based methods need not apply toxic and harmful substances [11, 12]. Herbal sources are utilised for the fabrication of NPs [13]. Also, due to the widespread spreading of plants, the synthesis of NPs using plants can be suggested as an economic way [14–16]. In comparison with the expensive ways, plant-mediated synthesis of NPs due to their pharmacological properties could be applied in drug delivery and health products [17, 18].

Artemisia, the genus belongs to the family Asteraceae, containing 200–400 species dispersed mainly in the mild zones of Asia, Europe, and North America [19, 20]. *Artemisia kopetdaghensis* (Fig. 1) is an aromatic shrub, which is traditionally used in Iran as anti-inflammatory, antimicrobial, antifungal, and sedative [21]. The purpose of this work is to explore the antimicrobial, anti-proliferative potential, and photo-catalytic activities of the AgNPs synthesised using *A. kopetdaghensis* shoot extract. This work highlights the potential use of the *Artemisia* genus in combination with nanosciences for various biomedicine usages.

2. Materials and methods

2.1. Plant source and chemicals: *A. kopetdaghensis* was collected from Zarrin-Kuh, a protected area in Dargaz, Iran. Ag nitrate ($AgNO_3$) was obtained from Sigma–Aldrich, USA. All tests were performed with distilled water. For bactericidal properties, the Tryptic Soy and the Mueller Hinton media were purchased from Quelab (UK). A voucher specimen was identified and deposited (No. 21533) in Dargaz Payame-Noor-University, Dargaz, Khorassan Razavi Province, Iran. The shoots were washed several times by tap water, then with distilled water and allowed to dry at room temperature. Five grams of fresh shoots powder was boiled in 300 ml distilled water. The prepared solution was initially filtered by normal filter paper. The obtained extract was stored at 3°C.

2.2. Green synthesis of AgNPs: First, 1 mM solution of $AgNO_3$ was prepared. Then, 90 ml of $AgNO_3$ solution was combined with 10 ml of *A. kopedaqensis* shoot extract. The colour of the resulting solution changed from yellow to dark brown after 8 h, indicating the fabrication of AgNPs.

2.3. Characterisation of Ak-AgNPs: The fabrication of AgNPs was verified by Ultraviolet–visible (UV–Vis) spectrophotometry by using a spectrophotometer instrument (CE 9500). To specify the macromolecules, Fourier transform infrared (FTIR) examination was performed in the range of 400–4000 cm^{-1}. The crystalline construction of Ak-AgNPs was performed by XRD analysis

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Fig. 1 *Image of A. kopetdaghensis*

using an X' Pert PW 3040/60, Philips Holland at room temperature. The configuration and size of the Ak-AgNPs were determined using TEM (ZEISS LEO 912 AB).

2.4. Bactericidal activity: The bactericidal activity was carried out by the agar diffusion method. The antibacterial study was performed by diverse pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* as Gram-negative bacteria and *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* as Gram-positive bacteria. Nutrient broth agar is used for cultivating bacteria. The inoculum of each bacterium was established by growing the bacterium overnight in Mueller Hinton medium and then sub-cultured in Mueller Hinton agar at 37°C overnight. The clarity of the bacterium suspensions was regulated to the 0.5 McFarland standards. Antiseptic swabs were dipped into the inoculum cells. Mueller Hinton agar plates were inoculated with bacteria by spreading the swabs. The clean standard discs (6 mm) were soaked in Ak-AgNPs and extract. Streptomycin and gentamycin were used as positive control and *A. kopetdaghensis* extract and deionised water were used as negative control. Total discs were employed to evaluate the bactericidal activity against six infective bacterial on Mueller Hinton agar plates. The soaked discs were placed on the Mueller Hinton agar plates inoculated with bacteria. Plates were incubated for 24 h. The bactericidal properties of the greener synthesised AgNPs were measured by the zone of inhibition (ZI) nearby discs as bacterial growth reticence. The agar diffusion test was carried out in triplicate.

2.5. Anti-proliferative activity on cancer cell line (HepG2): Human liver carcinoma cell lines (HepG2) were purchased from the Institute Pasture of Iran. Cells were treated with different concentrations (0.016, 0.031, 0.063, 0.125, 0.250, and 0.500 mM) of Ak-AgNPs at different times (24, 48, and 72 h). After 24 h of incubation, the status of the cells was detected under a microscope, and then, 20 µl of the MTT substance was added and incubated at 37°C. A 96-cell plate reader was used to measure the absorbance at 570, and the cell-viability percentage was estimated.

2.6. Photo-catalytic property of Ak-AgNPs: The catalytic behaviour of AgNPs for photo-degradation of methylene blue (MB) was examined under UV radiance. Then, 1 ml of dispersed Ak-AgNPs (10^{-3} M) was increased with 99 ml of MB (10^{-5} M). Also, a control setup that did not comprise any Ak-AgNPs was examined. The solution was shacked in the dark to allow the physical adsorption to reach equilibrium. The different concentration of MB as a function of radiance time was estimated by a spectrophotometer. All the tests were carried out at room temperature.

3. Results and discussion

3.1. UV–Vis spectrophotometric analysis: UV–Vis spectroscopy test has carried out for NP preparation. As revealed in Fig. 2, blending the extract of *A. kopetdaghensis* with $AgNO_3$ solution leads to a change of solution colour from light yellow to dark brown due to Ak-AgNPs forming that have been biologically synthesised by *A. kopedaqensis* shoots extract showing a surface plasmon resonance (SPR) peak at 426 nm (Fig. 3), which proves their synthesis. Previous studies have described that AgNPs exhibit an absorption peak in the range of 410–450 nm because of the SPR [22, 23].

3.2. TEM study: The structure and size of green-synthesised Ak-AgNPs were studied by the TEM image (Fig. 4). Biosynthesised Ak-AgNPs are spherical in shape with diameter in the range of 3–35 nm. It is noticed from Fig. 4 that the NPs are fine.

3.3. XRD analysis: The XRD configuration of the Ak-AgNPs displays different diffraction peaks at $2\theta = 32.1$, 38.04, 46.1, 54.6, and 64.5° (Fig. 5). These main peaks in the spectrum, matching to the 111, 200, 120, 202, and 311 planes, respectively, reflect the configurations of the face-centred cubic and crystalline organisation of the Ak-AgNPs. Previous studies using herbal

Fig. 2 *Aqueous extract of A. kopetdaghensis and greener synthesised AgNPs (Ak-AgNPs)*

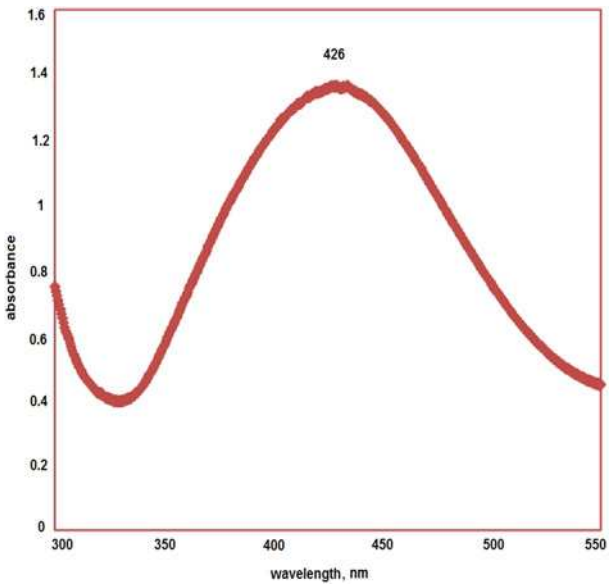

Fig. 3 *UV-Vis spectrometry of greener synthesised AgNPs (Ak-AgNPs)*

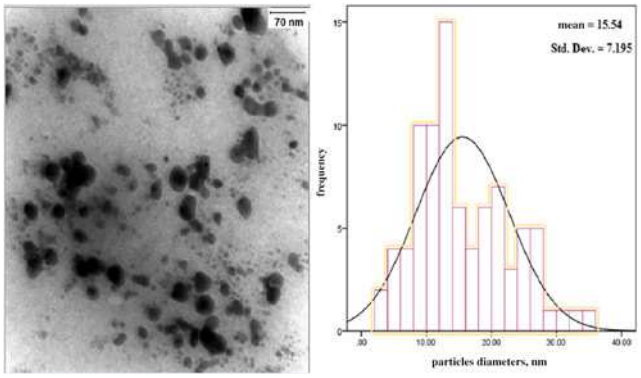

Fig. 4 *TEM image of greener synthesised AgNPs and the histogram of particles size distribution*

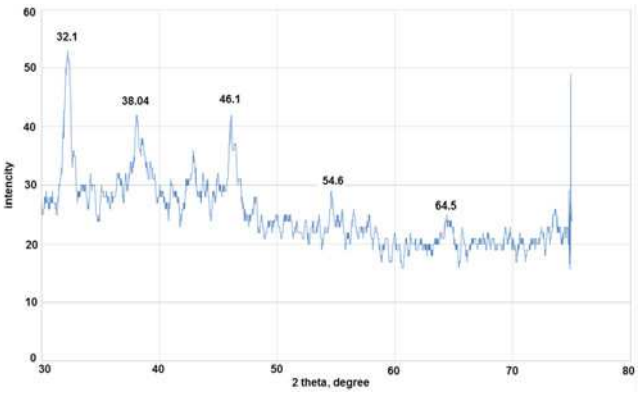

Fig. 5 *Powder X-ray diffraction (PXRD) pattern of the greener synthesised nanoparticles (Ak-AgNPs)*

extract to form AgNPs have reported similar peaks, and this result is consistent with previously reported results [24, 25]. The existence of different reducing factors in the extract is responsible for the steadiness of NPs and, therefore, for providing the crystalline configuration of Ak-AgNPs.

3.4. FTIR analysis: FTIR technique was applied to recognise the functional groups involved in the fabrication of Ak-AgNPs. As can be seen in Fig. 6, the peak at 3408 cm^{-1} revealed the O–H stretching vibration, which is induced as a result of the existence of alcohol and phenol [26]. The band at 2928 cm^{-1} is accredited to the C–H vibration (stretching) of aromatic compounds [26]. The band at 1629 cm^{-1} correlates with C–N and C–C stretching and shows the existence of proteins [26, 27]. The peak at 1384 cm^{-1} assigned to nitro N–O bending [28]. The band at 1035 cm^{-1} could be due to the C–O and C–N stretching vibrations

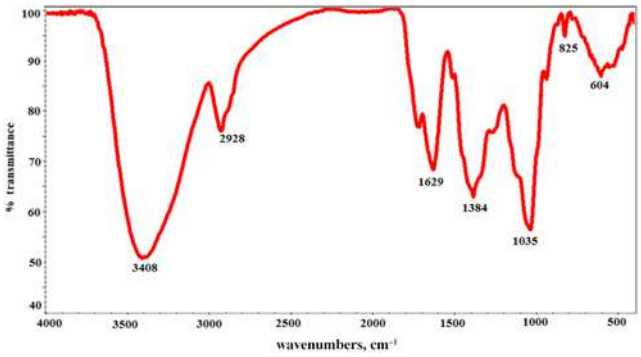

Fig. 6 *FTIR spectra of the Ak-AgNPs synthesised from the shoot extract*

of the SO_3H group [29]. Also, the 825 cm^{-1} band could be associated with the C–O stretching of phenols and alcohols [25]. The peak at 603 cm^{-1} may be attributed to the C–Br stretching which is a characteristic of alkyl-halides [26, 30].

3.5. Antibacterial activity of biosynthesised AgNPs: The bactericidal properties of the aqueous shoots extract of *A. kopetdaghensis* and Ak-AgNPs were tested using the agar diffusion method against *S. aureus*, *S. epidermis*, *and S. pyogenes* (Gram-positive) bacteria, as shown in Fig. 7*a* and *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (Gram-negative) bacteria, as shown in Fig. 7*b*. Ak-AgNPs revealed high-bactericidal properties against all of the pathogenic bacteria when compared to the aqueous shoot extract of *A. kopetdaghensis*. The maximum ZI of Ak-AgNPs was 22.3 and 17.8 mm against *K. pneumoniae* and *S. aureus*, respectively. The aqueous shoot extract displayed very low antibacterial activity against pathogenic bacteria when compared with Ak-AgNPs (Table 1). The difference between Gram-positive bacteria and Gram-negative bacteria is due to the lack of an outer membrane in Gram-positive bacteria and, on the other hand, the cell wall thickness in Gram-negative bacteria, in which each of these properties can give different results depending on the type of antibacterial agent. The negative charge of the bacterial cell wall causes the positive charge of the Ag ions to be absorbed, which eventually causes ion leakage into the bacterial cell wall, destroying the bacterial cell due to electrostatic interactions [24, 31–33]. The bactericidal effect of Ak-AgNPs could be ascribed to their great surface to volume part and fine size, allowing them to interact closely with bacterial

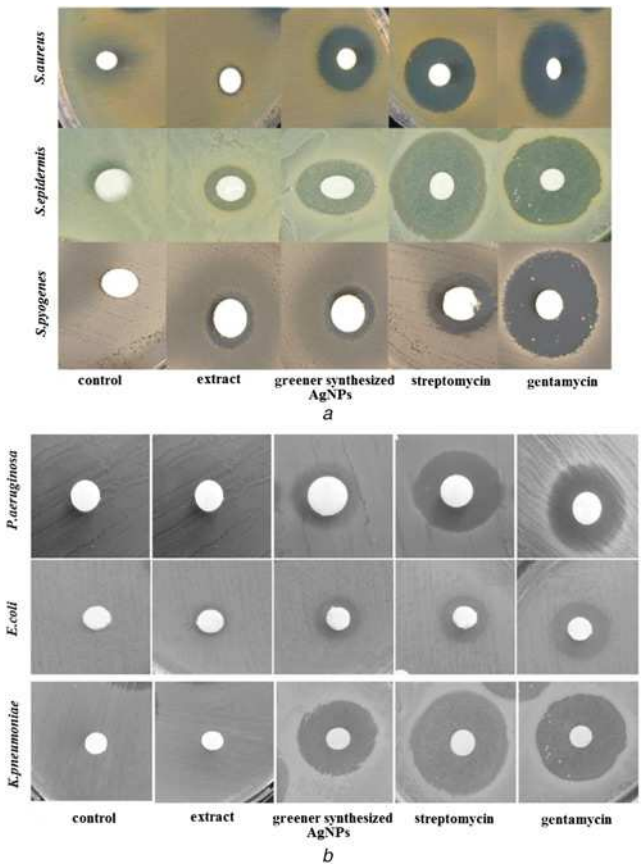

Fig. 7 *Antibacterial properties of A. kopetdaghensis extract and greener synthesized AgNPs (Ak-AgNPs) against gram-positive bacteria (a), and gram-negative bacteria (b)*
a Antibacterial properties of *A. kopetdaghensis* extract and greener synthesised AgNPs (Ak-AgNPs) against Gram-positive bacteria
b Bactericidal properties of *A. kopetdaghensis* extract and greener synthesised AgNPs (Ak-AgNPs) against Gram-negative bacteria

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Table 1 Bactericidal activity of *A. kopetdaghensis* and Ak-AgNPs against pathogenic bacteria

Inhibition zone (mm ± SD)					
Bacteria	Control	Extract	Ak-AgNP	Streptomycin (SM)	Gentamycin (GM)
S. aureus	0	0	17.8 ± 1.1	21.9 ± 2.1	26.8 ± 1.2
S. epidermidis	0	4.3 ± 0.9	12.8 ± 0.9	28.9 ± 2.2	28.7 ± 3.2
S. pyogenes	0	2.7 ± 0.3	3.8 ± 0.8	11.7 ± 1.1	29.2 ± 3.1
P. aeruginosa	0	0	9.7 ± 1.2	18.7 ± 2.3	17.5 ± 1.2
E. coli	0	2 ± 0.5	8.5 ± 0.6	9.5 ± 1.0	12.5 ± 1.2
K. pneumoniae	0	0	22.3 ± 0.5	29.5 ± 1.2	26.8 ± 2.1

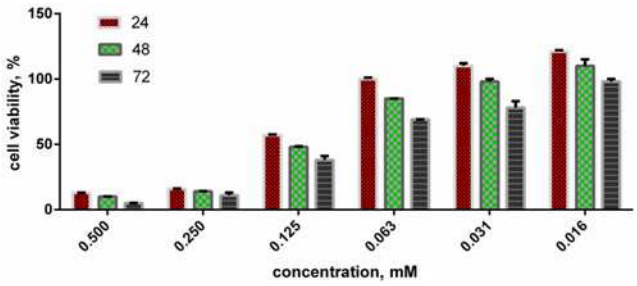

Fig. 8 *Anticancer activities of Ak-AgNPs against human hepatocellular cancer cell line (HepG2)*

membranes. Besides metabolites produced by the plant itself, there are other valuable materials that are formed by their symbiotic microbes, which by using modern genetic manipulation procedures, their production can be improved for bio-medicinal industries [34–36].

3.6. Assessment of anti-proliferative activity on cancer cell line (HepG2): Nowadays, cancers are the cause of many deaths. So far, various research studies have been carried out on different cancer cells such as U87, MCF7, and other different cancer cell lines [37–39]. According to the World Health Organisation report, liver cancer has become one of the lethal agents in the world [40]. To test the anti-proliferative action of the greener synthesised Ak-AgNPs, diverse concentrations of Ak-AgNPs were added to liver cancer cell lines (HepG2), and the cytotoxicity results checked at 24, 48, and 72 h. The MTT results shows that there is a gradual reduction in cell viability with increasing concentration of Ak-AgNPs (Fig. 8). Depending on the results of the anti-proliferative action examination, it may conclude that the Ak-AgNPs are lethal against HepG2, as shown in Fig. 8. The current project is the first study that investigates the cell viability of Ak-AgNPs against human hepatocellular cancer cell line. Owing to the greater cellular uptake and retention of Ak-AgNPs, the NPs exhibited high cytotoxicity towards HepG2 cell lines, and this was clearly influenced by the time and concentration of Ak-AgNPs.

3.7. Photo-catalytic activity and water purification of biosynthesised Ak-AgNPs: Photo-catalytic behaviour of Ak-AgNPs, for degradation of MB, an organic and stable compound was examined under UV irradiation. The irradiation process is performed every 30 min for 150 min (Fig. 9). The results indicated that Ak-AgNPs could be an efficient photocatalyst for the degradation of industrial dyes in environments. Under the optimum condition, the high activity of dye degradation (83.11%) has been observed. Ak-AgNPs provide the electron donor-to-receiver transmission. During electron transmission, the reactants are absorbed on the surfaces of the metal, and thus, the reactants' achieve an electron and are reduced. The present results are consistent with previous

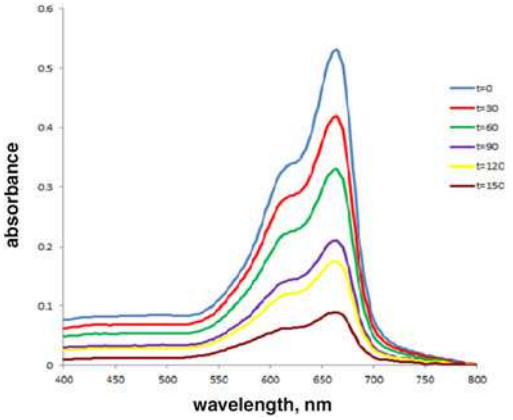

Fig. 9 *Photo-degradation of MB (10^{-5} M) in the presence of Ak-AgNPs*

reports on the removal of MB by biosynthesised AgNPs with herbal extracts [14, 41]. Numerous industries release unfavourable compounds such as organic materials and dyes into the environment, which has serious harmful effects. The consequences have demonstrated sorption peak declines rapidly in the presence of Ak-AgNPs and UV light that demonstrates the photo-degradation of MB.

4. Conclusion: The fabrication of AgNPs by *A. kopetdaghensis* via a green method is an economic and eco-friendly method, which could be helpful to human health and environmental subjects. The potentially active phytoconstituents involved in the green fabrication of NPs such as phenols, carbohydrates, proteins, and aromatic compounds are bio-compatible for an extensive range of biomedical uses. Also, the Ak-AgNPs were studied to display their bactericidal property against infective bacteria and anti-proliferative effects on human liver cancer cell lines (HepG2), in a dose-dependent manner. Furthermore, green-synthesised Ak-AgNPs showed noble photocatalytic activity in the decomposition of MB dye. Therefore, this study adds another feature to the medicinal hub plant *A. kopetdaghensis*, i.e. its capability to effectively formulate AgNPs, which could be used successfully for their antimicrobial and anticancer properties in biocatalyst technologies.

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6 References

[1] Rahdar A., Aliahmad M., Samani M., *ET AL.*: 'Synthesis and characterization of highly efficacious Fe-doped ceria nanoparticles for cytotoxic and antifungal activity', *Ceram. Int.*, 2019, **45**, pp. 7950–7955

[2] Nikazar S., Sivasankarapillai V.S., Rahdar A., *ET AL.*: 'Revisiting the cytotoxicity of quantum dots: an in-depth overview', *Biophys. Rev.*, 2020, **12**, (3), pp. 703–718

[3] Sivasankarapillai V.S., Pillai A.M., Rahdar A., *ET AL.*: 'On facing the SARS-Cov-2 (COVID-19) with combination of nanomaterials and medicine: possible strategies and first challenges', *Nanomaterials*, 2020, **10**, (5), p. 852

[4] Darroudi M., Yazdi M.E.T., Amiri M.S.: 'Plant-mediated biosynthesis of nanoparticles' in '21st century nanoscience – a handbook' (CRC Press, USA, 2020), pp. 1-1–1-18

[5] Es-haghi A., Javadi F., Taghavizadeh Yazdi M.E., *ET AL.*: 'The expression of antioxidant genes and cytotoxicity of biosynthesized cerium oxide nanoparticles against hepatic carcinoma cell line', *Avicenna J. Med. Biochem.*, 2019, **7**, (1), pp. 16–20

[6] Hileuskaya K., Ladutska A., Kulikouskaya V., *ET AL.*: 'Green' approach for obtaining stable pectin-capped silver nanoparticles: physico-chemical characterization and antibacterial activity', *Colloids Surf. A, Physicochem. Eng. Aspects*, 2020, **585**, p. 124141

[7] Moshfegh A., Jalali A., Salehzadeh A., *ET AL.*: 'Biological synthesis of silver nanoparticles by cell-free extract of polysiphonia algae and their anticancer activity against breast cancer MCF-7 cell lines', *Micro Nano Lett.*, 2019, **14**, (5), pp. 581–584

[8] Ga'al H., Fouad H., Tian J., *ET AL.*: 'Synthesis, characterization and efficacy of silver nanoparticles against *Aedes albopictus* larvae and pupae', *Pestic. Biochem. Physiol.*, 2018, **144**, pp. 49–56

[9] Yazdi M.E.T., Khara J., Housaindokht M.R., *ET AL.*: 'Role of *Ribes khorasanicum* in the biosynthesis of silver nanoparticles and their antibacterial properties', *IET Nanobiotechnol.*, 2019, **13**, (2), pp. 189–192

[10] Fouad H., Hongjie L., Hosni D., *ET AL.*: 'Controlling *Aedes albopictus* and *Culex pipiens* pallens using silver nanoparticles synthesized from aqueous extract of *Cassia fistula* fruit pulp and its mode of action', *Artif. Cells Nanomed. Biotechnol.*, 2018, **46**, (3), pp. 558–567

[11] Rahdar A., Aliahmad M., Hajinezhad M.R., *ET AL.*: 'Xanthan gum-stabilized nano-ceria: green chemistry based synthesis, characterization, study of biochemical alterations induced by intraperitoneal doses of nanoparticles in rat', *J. Mol. Struct.*, 2018, **1173**, pp. 166–172

[12] Manshad A.K., Ali J.A., Imani I., *ET AL.*: 'Green synthesize of CuO@Fe_3O_4@Xantan nanocomposites and its application in enhanced oil recovery by considering IFT and wettability behaviors', *Micro Nano Lett.*, 2020, **15**, pp. 550–555

[13] Rostamizadeh E., Iranbakhsh A., Majd A., *ET AL.*: 'Green synthesis of Fe_2O_3 nanoparticles using fruit extract of *Cornus mas* L. and its growth-promoting roles in barley', *J. Nanostruct. Chem.*, 2020, **10**, pp. 125–130

[14] Yazdi M.E.T., Hamidi A., Amiri M.S., *ET AL.*: 'Eco-friendly and plant-based synthesis of silver nanoparticles using *Allium giganteum* and investigation of its bactericidal, cytotoxicity, and photocatalytic effects', *Mater. Technol.*, 2019, **34**, (8), pp. 490–497, doi: 10.1080/10667857.2019.1583408

[15] Taghizadeh S.-M., Lal N., Ebrahiminezhad A., *ET AL.*: 'Green and economic fabrication of zinc oxide (ZnO) nanorods as a broadband UV blocker and antimicrobial agent', *Nanomaterials*, 2020, **10**, (3), p. 530

[16] Yazdi M.E.T., Khara J., Housaindokht M.R., *ET AL.*: 'Assessment of phytochemical components and antioxidant activity of *Rheum turkestanicum* janisch', *Studies Med. Sci.*, 2020, **31**, (2), pp. 75–81

[17] Amiri M.S., Joharchi M.R., TaghavizadehYazdi M.E.: 'Ethno-medicinal plants used to cure jaundice by traditional healers of Mashhad, Iran', *Iran. J. Pharm. Res., IJPR*, 2014, **13**, (1), p. 157

[18] Chung I.-M., Park I., Seung-Hyun K., *ET AL.*: 'Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications', *Nanoscale Res. Lett.*, 2016, **11**, (1), p. 40

[19] Taleghani A., Emami S.A., Tayarani-Najaran Z.: '*Artemisia* a promising plant for the treatment of cancer', *Bioorg. Med. Chem.*, 2020, **28**, p. 115180

[20] Emami S.A., Nadjafi F., Amine G.H., *ET AL.*: 'Les espèces de plantes médicinales utilisées par les guérisseurs traditionnels dans la province de Khorasan, nord-est de l'Iran', *J. Ethnopharmacol.*, 2012, **48**, pp. 48–59

[21] Hosseini A., Bakhtiari E., Khajavi Rad A., *ET AL.*: 'The evaluation and comparing of cytotoxic effects of *Ferula gummosa* gum, *Scutellaria lindbergii*, *Kelussia odoratissima* and *Artemisia kopetdaghensis* extracts on ACHN cell line', *Iran. J. Pharm. Res.*, 2017, **16**, (3), p. 1104

[22] Yazdi M.E.T., Amiri M.S., Hosseini H.A., *ET AL.*: 'Plant-based synthesis of silver nanoparticles in *Handelia trichophylla* and their biological activities', *Bull. Mater. Sci.*, 2019, **42**, (4), p. 155

[23] Heikal Y.M., Şuţan N.A., Rizwan M., *ET AL.*: 'Green synthesized silver nanoparticles induced cytogenotoxic and genotoxic changes in *Allium cepa* L. varies with nanoparticles doses and duration of exposure', *Chemosphere*, 2020, **243**, p. 125430

[24] Hamidi A., Taghavizadeh Yazdi M.E., Amiri M.S., *ET AL.*: 'Biological synthesis of silver nanoparticles in *Tribulus terrestris* L. extract and evaluation of their photocatalyst, antibacterial, and cytotoxicity effects', *Res. Chem. Intermed.*, 2019, **45**, (5), pp. 2915–2925

[25] Taghavizadeh Yazdi M.E., Khara J., Sadeghnia H.R., *ET AL.*: 'Biosynthesis, characterization, and antibacterial activity of silver nanoparticles using *Rheum turkestanicum* shoots extract', *Res. Chem. Intermed.*, 2018, **44**, (2), pp. 1325–1334

[26] Jyoti K., Baunthiyal M., Singh A.: 'Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics', *J. Radiat. Res. Appl. Sci.*, 2016, **9**, (3), pp. 217–227

[27] Ahmed S., Saifullah Ahmad M., *ET AL.*: 'Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract', *J. Radiat. Res. Appl. Sci.*, 2016, **9**, (1), pp. 1–7

[28] Sankar R., Rizwana K., Shivashangari K.S., *ET AL.*: 'Ultra-rapid photocatalytic activity of *Azadirachta indica* engineered colloidal titanium dioxide nanoparticles', *Appl. Nanosci.*, 2015, **5**, (6), pp. 731–736

[29] Neelgund G.M., Hrehorova E., Joyce M., *ET AL.*: 'Synthesis and characterization of polyaniline derivative and silver nanoparticle composites', *Polymer Int.*, 2008, **57**, (10), pp. 1083–1089

[30] Sadeghi B., Mohammadzadeh M., Babakhani B.: 'Green synthesis of gold nanoparticles using *Stevia rebaudiana* leaf extracts: characterization and their stability', *J. Photochem. Photobiol. B, Biol.*, 2015, **148**, pp. 101–106

[31] Jalilian F., Chahardoli A., Sadrjavadi K., *ET AL.*: 'Green synthesized silver nanoparticle from *Allium ampeloprasum* aqueous extract: characterization, antioxidant activities, antibacterial and cytotoxicity effects', *Adv. Powder Technol.*, 2020, **31**, pp. 1323–1332

[32] Vanti G.L., Kurjogi M., Basavesha K.N., *ET AL.*: 'Synthesis and antibacterial activity of solanum torvum mediated silver nanoparticle against *Xxanthomonas axonopodis pv. punicae* and *Ralstonia solanacearum*', *J. Biotechnol.*, 2020, **309**, pp. 20–28

[33] Fouad H., Hongjie L., Yanmei D., *ET AL.*: 'Synthesis and characterization of silver nanoparticles using *Bacillus amyloliquefaciens* and *Bacillus subtilis* to control filarial vector *Culex pipiens* pallens and its antimicrobial activity', *Artif. Cells Nanomed. Biotechnol.*, 2017, **45**, (7), pp. 1369–1378

[34] Rahnama M., Forester N., Ariyawansa K.G. S.U., *ET AL.*: 'Efficient targeted mutagenesis in *Epichloë festucae* using a split marker system', *J. Microbiol. Methods*, 2017, **134**, pp. 62–65

[35] Rahnama M., Johnson R.D., Voisey C.R., *ET AL.*: 'The global regulatory protein VelA Is required for symbiosis between the endophytic fungus *Epichloë festucae* and *Lolium perenne*', *Mol. Plant–Microbe Interact.*, 2018, **31**, (6), pp. 591–604

[36] Rahnama M., Maclean P., Fleetwood D.J., *ET AL.*: 'The LaeA orthologue in *Epichloë festucae* is required for symbiotic interaction with *Lolium perenne*', *Fungal Genet. Biol.*, 2019, **129**, pp. 74–85

[37] Mousavi S.H., Tavakkol-Afshari J., Brook A., *ET AL.*: 'Role of caspases and bax protein in saffron-induced apoptosis in MCF-7 cells', *Food Chem. Toxicol.*, 2009, **47**, (8), pp. 1909–1913

[38] Rahimi V.B., Mousavi S.H., Haghighi S., *ET AL.*: 'Cytotoxicity and apoptogenic properties of the standardized extract of *Portulaca oleracea* on glioblastoma multiforme cancer cell line (U-87): a mechanistic study', *EXCLI J.*, 2019, **18**, p. 165

[39] Zarei M., Karimi E., Oskoueian E., *ET AL.*: 'Comparative study on the biological effects of sodium citrate-based and apigenin-based synthesized silver nanoparticles', *Nutr. Cancer*, 2020, pp. 1–9, doi: 10.1080/01635581.2020.1801780

[40] Ahmadian E., Dizaj S.M., Rahimpour E., *ET AL.*: 'Effect of silver nanoparticles in the induction of apoptosis on human hepatocellular carcinoma (HepG2) cell line', *Mater. Sci. Eng., C*, 2018, **93**, pp. 465–471

[41] Taghavizadeh Yazdi M.E., Modarres M., Amiri M.S., *ET AL.*: 'Phyto-synthesis of silver nanoparticles using aerial extract of *Salvia leriifolia* benth and evaluation of their antibacterial and photo-catalytic properties', *Res. Chem. Intermed.*, 2019, **45**, (3), pp. 1105–1116

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