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ORIGINAL ARTICLE

Potency of phytosynthesized silver nanoparticles from *Lathraea squamaria* as anticandidal agent and wheat seeds germination enhancer

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In the field of nanobiotechnology, nanomedicine and nanobiomaterials, silver nanoparticles (AgNPs) are used as an antimicrobial and antifungal agent. One of the most efficient approaches to AgNPs production is eco-friendly or "green" synthesis. The present research is devoted to the phytosynthesis of AgNPs using, as bioreductant, the aqueous extract of different organs of common toothwort (*Lathraea squamaria* L.) – perennial root holoparasitic plant from the Broomrape family (Orobanchaceae). The extract from rhizomes tissue showed the highest level of DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition – 79%. Quantitative analysis revealed that the aqueous extracts from leaves and inflorescences tissues reduced DPPH with considerably less intense (by 21% and 63%, respectively) than the rhizomes extracts. The phytosynthesized AgNPs were characterized by UV-vis spectroscopy and scanning electron microscopy (SEM). The phytosynthesized AgNPs by *L. squamaria* were crystalline with a spherical and polygonal shape and diameters from 10 to 40 nm. The results of the agar diffusion method showed the potent anticandidal activity of phytosynthesized AgNPs (15–20 mm inhibition zone). Furthermore, the phytosynthesized AgNPs have shown significant potential in enhancing seed germination and early seedling development of emmer wheat (*Triticum dicoccum* Schrank.).

Abbreviations AgNPs – silver nanoparticles, ANOVA – analysis of variance, DPPH – 2,2-diphenyl-1-picrylhydrazyl, IE-AgNPs – silver nanoparticles prepared by inflorescences extract, LE-AgNPs – silver nanoparticles prepared by leaves extract, RE-AgNPs – silver nanoparticles prepared by rhizomes extract, SD – standard deviation, SEM – scanning electron microscopy, SPR - surface plasmon resonance, UV-Vis – ultraviolet-visible spectroscopy.

Introduction

Silver nanoparticles (AgNPs) show unique physical, chemical and biological properties, which determine their extensive use in various fields of materials science, optics, surgical and food handling tools, electronics, and cosmetology. At the same time, AgNPs succeed widely known bactericidal properties of silver, which leads to the use of silver-based nanomaterials in various medical applications, including wound repair, bone healing, dental materials, vaccine adjuvants, antidiabetic agents, and bioimaging (Xu et al. 2020).

The significance of AgNPs becomes much more apparent given their multipurpose ability to effectively reduce vitality and suppress the growth of numerous pathogens as broad-spectrum effectors. The potential of AgNPs as antibiotics is related to their various mechanisms of action, which attack

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microorganisms in multiple structures at a time and give them the ability to kill various types of pathogens (Bruna et al. 2021).

Most opportunistic pathogenic fungi cause disease in weakened immunity conditions, including long-term antibiotic use, steroid treatment, leukemia, neutropenia, organ transplants, or immunocompromised infections (Burduniuc et al. 2021). In particular, the genus *Candida* comprises over 200 species and is the most common microorganism among pathogenic fungi that causes the majority of fungal infections. (Gnat et al. 2021).

In the context of the COVID-19 pandemic, many patients have acute respiratory distress syndrome, which creates the preconditions for the development of superinfections. As a consequence, *Candida* strains can cause oral candidiasis and invasive fungal infections (Katz 2021). In particular, hospitalization for COVID-19 increases the risk of candidemia, the treatment of which requires using of broad-spectrum antibiotics specific to the pathogen.

Synthesis of AgNPs using aqueous plant extracts includes the reduction of Ag ions by widespread or unique plant metabolites from different parts of various species. These chemicals are involved in maintaining the pro-oxidative/antioxidative balance in plant cells and can be used in green chemistry comprises the use of chemicals as metal ions reducing agents (Odeniyi et al. 2020). The accumulation of these molecules in a vegetative mass of plants has been traditionally used since ancient times as alternative medications to treat various human diseases because of dedication to their remarkable antioxidative properties (Aras et al., 2021). These properties are also the basis for growing concern about the development of environmentally friendly, efficient, harmless to the environment, non-destructive, ecological and biological approaches to the synthesis of NPs. Afterward, increasingly, researchers presented high-quality review papers regarding the synthesis of metallic NPs using plant extracts as a green chemistry approach (Castillo-Henríquez et al. 2020).

The potentially effective concept of AgNPs green synthesis should be based on the aqueous plant extract that contains a substantial amount of bioreducers. *Lathraea squamaria* L. (*L. squamaria* or common toothwort) is a perennial root holoparasitic plant from the Broomrape family (Orobanchaceae), formerly classified in the Figwort family (Scrophulariaceae) (Ahmeti et al. 2021). *L. squamaria* has a wide system of underground rhizomes and stems, covered with small tightly overlapping scale-like non-chlorophyllous leaves and aboveground inflorescences (Fig. 1).

Phytochemical screening of aqueous extract of the common toothwort raw material indicates potential antitumoral, biligenic and diuretic activities, it also stimulates cardiac, hepatic and renal system functions (Bokov et al., 2020). The presence of polyfunctional secondary metabolites makes it possible to consider a promising source for the phytosynthesis of silver nanoparticles with new properties.

The purpose of the work was to study the potency of phytosynthesized silver nanoparticles from aqueous extracts of *Lathraea squamaria* L. rhizomes, leaves and inflorescences as anticandidal agents and germination enhancer of emmer wheat seeds.

Materials and methods

Collection of plant samples

The sample material – rhizomes, leaves and inflorescences of *Lathraea squamaria* L. were collected from Holosiivskyi National Natural Park (50°22’33.4"N 30°30’39.2"E) in Holosiivskyi district, Kyiv, Ukraine and were brought to the laboratory for further studies.

Preparation of plant extract and antioxidant activity evaluation

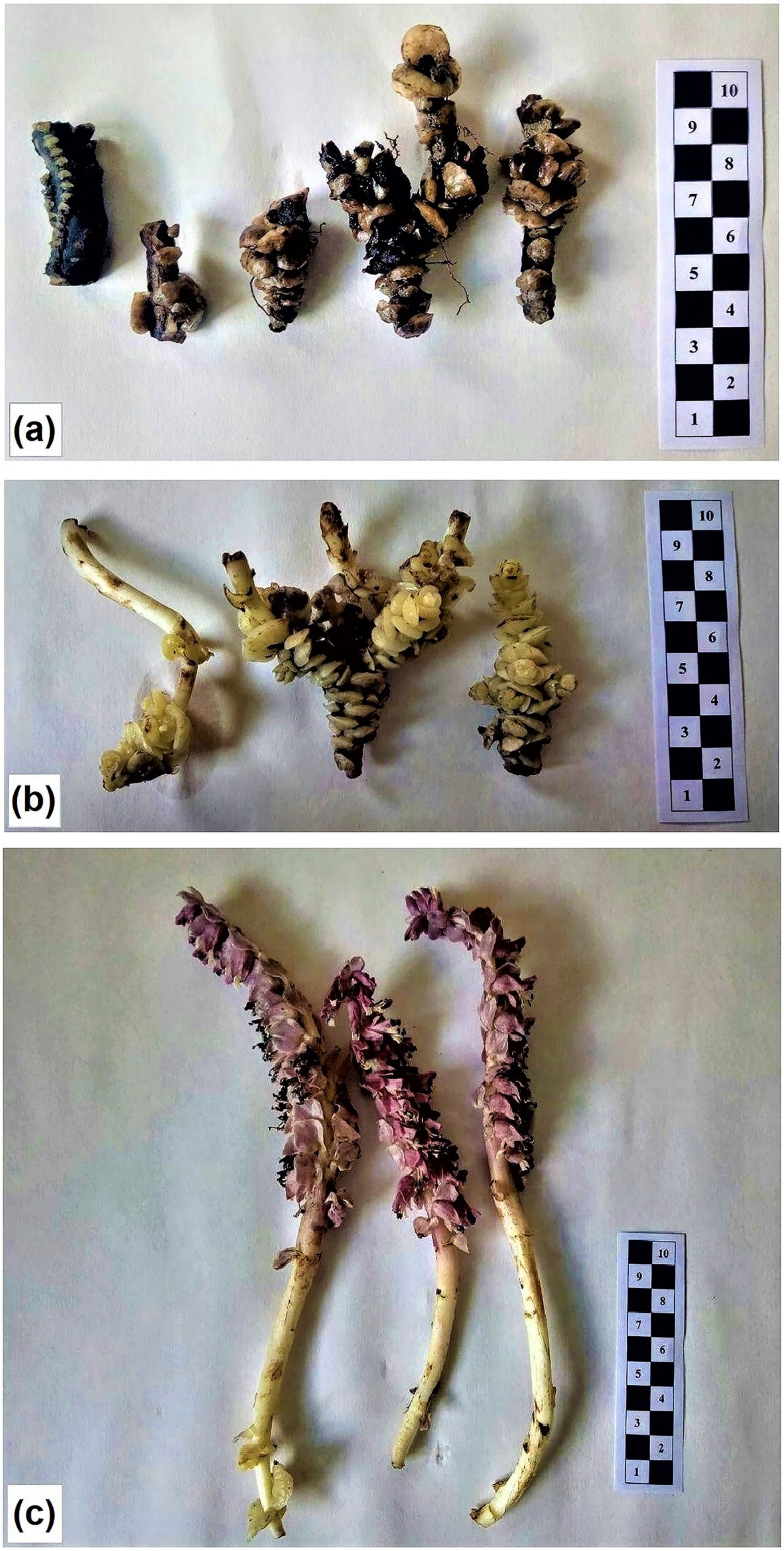
Rhizomes, leaves and inflorescences of *L. squamaria* were washed several times in deionized water to remove the soil particles and any organic impurities and then air dried at 60 ºC to eliminate the residual moisture. The cleaned and dried organs of *L. squamaria* were cut into small pieces and powdered into finely dispersed flour. Two grams of flour were put in a flask with a flat bottom with 100 ml deionized water and boiled for 20 min at 80 ºC. The obtained extracts of rhizomes, leaves and inflorescences were cooled at room temperature and filtered with Whatman No.1 filter paper.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay was used to quantify the ability to reduce components of plant extracts to quench the DPPH radical. The color of the reaction mixture changes from purple to yellow with a decreasing absorbance at 517 nm wavelength because of the reduction of DPPH to a non-radical form. Changes of absorbance were recorded with UV-1800 «Shimadzu» (Japan) UV-Vis spectrophotometer. The percentage of inhibition was calculated against blank (Nakagawa et al. 2021).

Phytosynthesis of AgNPs

Silver nanoparticles were synthesized using filtered extracts *L. squamaria* rhizomes, leaves and inflorescences by the addition of 0.001 M $AgNO_3$ silver nitrate (Vanlalveni et al. 2021). For the reduction of silver ions, 10 mL of extract was

Fig. 1 Different organs of *Lathraea squamaria* L. plants: underground rhizomes (a), underground stems with leaves (b) and aboveground – inflorescences (c)



mixed with 40 mL of silver nitrate. The resulting solution was incubated under a light-emitting diode lamp (Secret Jardin, 42 W, 6500 K) for 1.5 h at room temperature for the development of AgNPs according to Smirnov et al. 2021.

Characterization of phytosynthesized AgNPs and its stability

UV-vis spectra of obtained AgNPs solutions from extracts of rhizomes (RE-AgNPs), leaves (LE-AgNPs) and inflorescences (IE-AgNPs) were recorded with UV-1800 «Shimadzu» (Japan) UV-Vis spectrophotometer at a resolution of 1 nm. The dynamics of synthesis and stability of AgNPs solutions were also analyzed by measuring the absorbance in the range of 200–700 nm at regular time intervals during synthesis (15 min, 30 min, 45 min, 60 min, 75 min, and 90 min) and in 24 h (Kithiyon et al. 2019). The size distribution was estimated after desiccation of the purified silver nanoparticles at 60 ºC by scanning electron microscopy (SEM, Tescan Mira 3 MLU).

Anticandidal activity of phytosynthesized AgNPs

The antimicrobial activity of test samples against common pathogenic microbe *Candida albicans* was evaluated using the agar diffusion test method (Alves et al. 2021). The culture of the test bacteria was grown in nutrient broth (Himedia) and adjusted to 0.5 McFarland turbidity standards. 1 mL of 24-hour *Candida albicans* culture suspension was inoculated on the surface of solidified Mueller-Hilton agar in Petri plates. Then 5 wells per one agar plate were made by cylindrical steel drill and 100 µL aliquots of test samples were added to the wells, using 0.001 M $AgNO_3$ as positive control. The samples were incubated at 37 °C for 18–24 h, whereafter the diameters of inhibition zones were recorded for the six separate determinations.

Seed germination phenotyping

Emmer wheat *Triticum dicoccum* Schrank. (cv. 'Holikovska') seeds were sterilized with 10% (v/v) hydrogen peroxide, and further within 6 h were vernalized in distilled water at 4 °C to stimulate germination. Then seeds were transferred to a Petri dish on wet filter paper with 10 mL of obtained AgNPs solutions and placed in a thermostat at 25 °C for imbibition. After 12 h of imbibition, seeds were placed in the agar layer of the phenotyping plate, according to Levenets et al. 2021. Phenotyping of seedlings architecture was carried out using morphological traits: lengths of primary, seminal roots, and coleoptiles. Morphological traits were measured using the ImageJ software (Hohn and Bektas 2020).

Statistical analysis

Each experiment was performed at least in triplicate. The results were expressed as mean ± standard deviation (SD). The analysis of variance (ANOVA) followed by Tukey's multiple range test was performed using STATISTICA (StatSoft, USA). A value of $P < 0.05$ was considered significant.

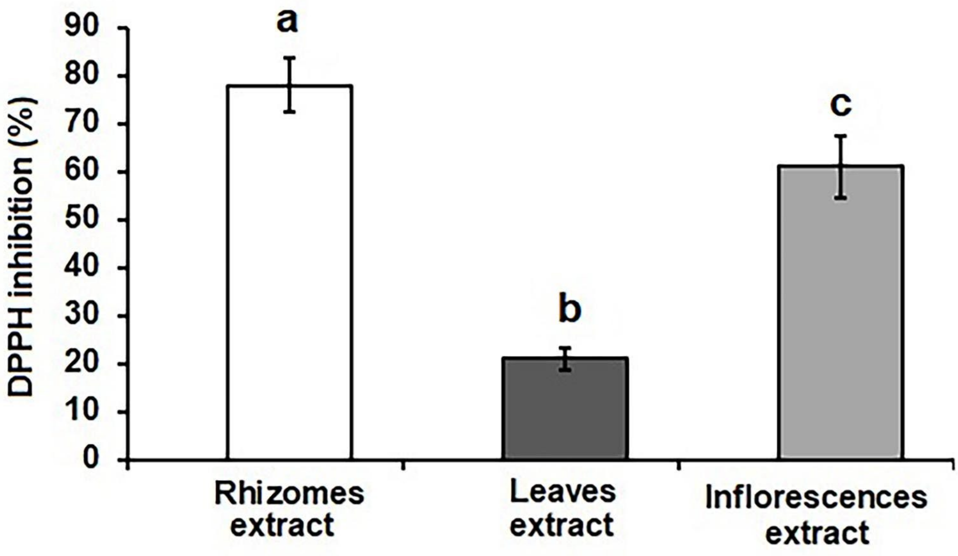


Fig. 2 The antioxidant activities of *L. squamaria* aqueous extracts from rhizomes, leaves and inflorescences tissue evaluated by DPPH method. Means followed by the same letters were not significantly different at $P < 0.05$ according to the Tukey's multiple range test

Results

The total antiradical potential of *L. squamaria* rhizomes, leaves and inflorescences filtered aqueous extracts were investigated. The relatively stable radical DPPH is broadly used to test the capacity of natural compounds to act as free radical scavengers or hydrogen donors and thus to assess antiradical activity (Santos et al. 2012). The activity of antioxidant molecules in all experimental *L. squamaria* aqueous extracts was detected by DPPH reduction after 30 min (Fig. 2). The extract from rhizomes tissue showed the highest level of DPPH inhibition. – 79%. Quantitative analysis revealed that the aqueous extracts from leaves and inflorescences tissues reduced DPPH with significantly less intense (21% and 63%, respectively) than the rhizomes extracts.

The formation of AgNPs in aqueous extracts from different organs: rhizomes (RE-AgNPs), leaves (LE-AgNPs) and inflorescences (IE-AgNPs) in compositions with silver nitrate was monitored in dynamics by detecting characteristic of surface plasmon resonance (SPR) of AgNPs in the range of 200–700 nm (Fig. 3a, b, c). The inset in Fig. 3 shows a resulting colour of the reaction compositions – change from pale to deep dark brown in 90 min at room temperature, which is regarded as a preliminary indication of RE-AgNPs, LE-AgNPs, and IE-AgNPs formation.

The morphology and size range of phytosynthesized AgNPs from rhizomes and inflorescences extracts were evaluated by scanning electron microscopy. Analysis of SEM images (Fig. 4) showed that both types of phytosynthesized AgNPs were well-dispersed with a spherical shape for RE-AgNPs and a polygonal shape for IE-AgNPs. In this way, the optical characteristics of AgNPs are mostly influenced by the size distribution of nanoparticles, which correlates with UV-Vis data. The morphological analysis of the obtained SEM micrographs showed that the average size of phytosynthesized AgNPs varied from 15 to 40 nm for RE-AgNPs and from 10 to 40 nm for IE-AgNPs.

The anticandidal effect was determined by agar diffusion assay with rhizome extract, inflorescence extract, RE-AgNPs, and IE- AgNPs (Fig. 5). The RE-AgNPs displayed the highest anticandidal action, as evidenced by the diameter of the inhibition zone was 20 mm. Meanwhile, the IE-AgNPs showed moderate anticandidal potential with a 15 mm inhibition zone. At the same time, growth inhibition was not recorded in variants with initial extracts.

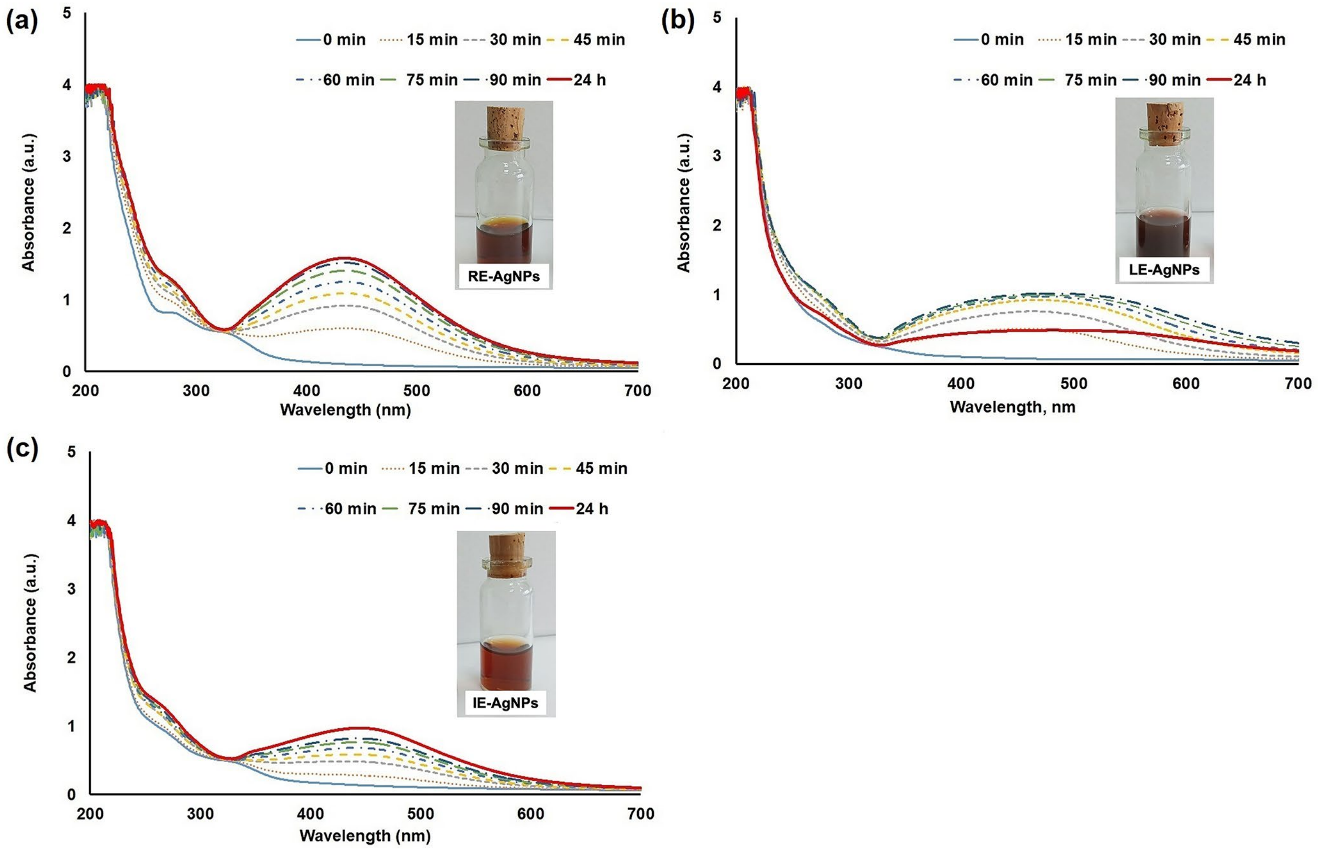


Fig. 3 UV-Vis absorption spectra of experimental solutions recorded as a function of time: AgNPs solutions from extracts of rhizomes (a), leaves (b) and inflorescences (c). The inset shows the color of the resulting solutions of RE-AgNPs, LE-AgNPs, and IE-AgNPs – visible manifestation of the SPR effect

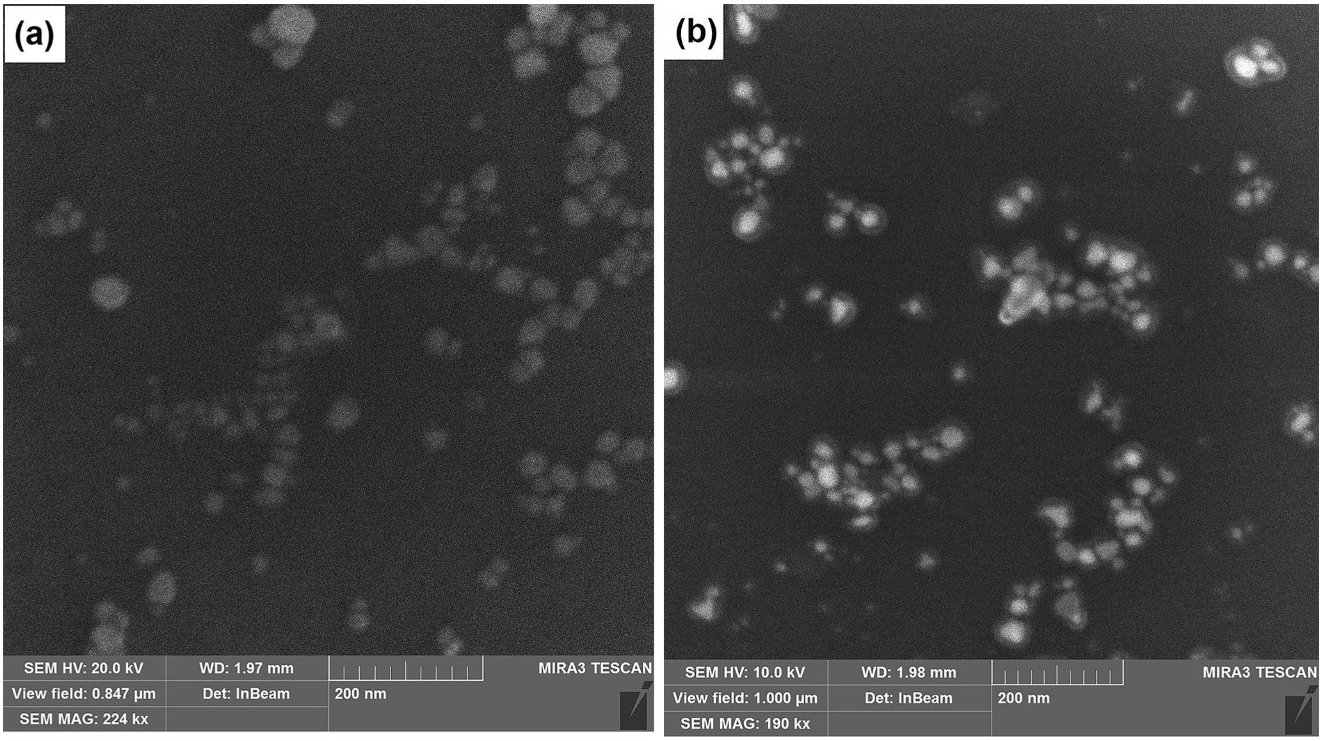


Fig. 4 Scanning electron microscopy micrograph images of AgNPs phytosynthesized using aqueous extracts of *L. squamaria* rhizomes (a) and inflorescences (b)

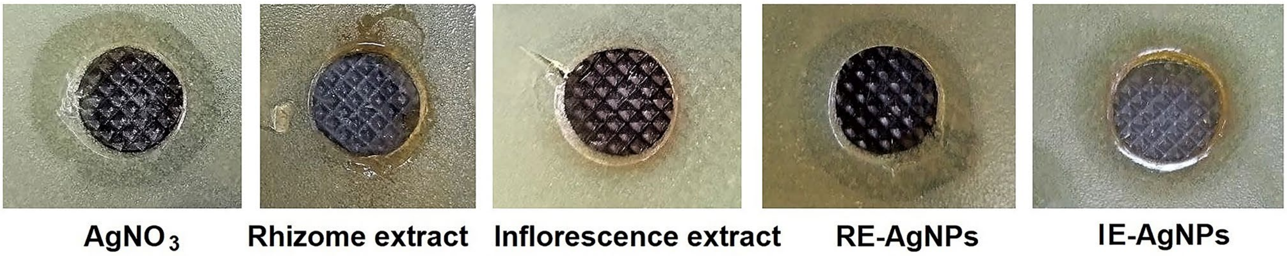


Fig. 5 Antifungal activity of plant extracts and phytosynthesized AgNPs against *C. albicans*

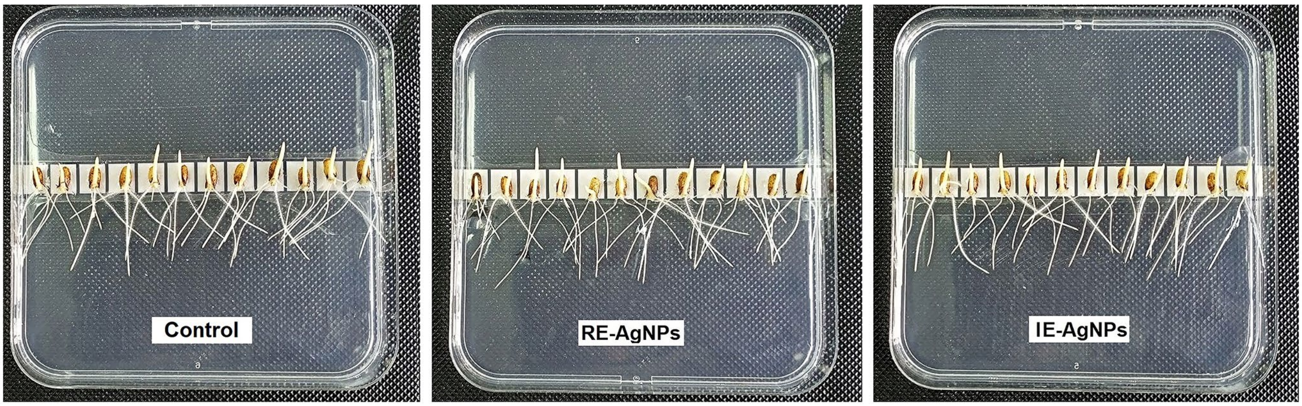


Fig. 6 Agar-phenotyping plates with wheat seedlings on 2nd day of growth

The influence of phytosynthesized AgNPs on the growth of wheat seedlings was examined by evaluating their effect on the lengths of primary, seminal roots, and coleoptiles at the agar-phenotyping plate after seed treatment with rhizomes (RE-AgNPs), and inflorescences (IE-AgNPs) experimental solutions (Fig. 6). The results of the present study indicate that RE-AgNPs and IE-AgNPs promote the seed germination and subsequent seedling growth of emmer wheat compared with control variant (Fig. 7). The most pronounced effect was exhibited by RE-AgNPs experimental solution. Under RE-AgNPs treatment, the length of primary roots was increased by 27%, length of seminal roots – by 23% and length of coleoptiles – by 33%.

Discussion

There is limited information about the chemical composition of *L. squamaria* aerial and underground parts (Malaník et al. 2019). Initial phytochemical studies have shown iridoid glycosides and phenolic acids attend in the whole plant (Swiatek and Dombrowicz 1976) and, subsequently, iridoid glycosides, β-sitosterol, and fatty acids have been determined in the seeds (Grabias et al. 1993). Further, the presences of phenylethanoid glycosides and benzoic acid has been identified in the underground parts of the plant (Daňková et al. 2016). Recently, there is fragmentary information that the *L. squamaria* contains iridoids, phenylethanoid glycosides, organic acids, monosaccharides, fatty acids. The more thorough research of the bioactive metabolites was not conducted, so additional studies of quantitative and especially qualitative profile are needed (Bokov et al., 2020). The recorded differences in DPPH antiradical activity can affect the experimental optimization of operating parameters needed for the phytosynthesis of silver nanoparticles from different organs of *L. squamaria*.

The change in colour of experimental solutions was likely due to collective oscillation of conduction electrons of AgNPs produced in RE-AgNPs, LE-AgNPs, and IE-AgNPs reaction composition, which darkens steadily with their age (Ansari et al. 2021). The gradual formation of sharp peaks at 488 and 434 nm in UV-Vis spectra can be assigned to SPR, developed by *L. squamaria* reducing and stabilizing agents, which mediated reduction of Ag ions in RE-AgNPs and IE-AgNPs reaction compositions. At the same time, a gentle peak was recorded for the LE-AgNPs reaction mixture, which completely disappeared after 24 h. For the future applied research were used AgNPs produced with rhizomes and inflorescences extracts.

The resembling size diapason between 10 and 40 nm for spherical phytosynthesized nanoparticles, obtained using extracts from *Catharanthus roseus* leaves as bioreductant molecules source, was previously reported by Keshari (Keshari et al. 2021). Several authors indicated that the spherical forms of AgNPs demonstrated the highest bactericidal effectiveness, which provides the maximum reactivity and stability to obtain the potent antibacterial efficiency (Raza et al. 2016). At once, the shape of AgNPs could impact their bioavailability and cytotoxicity. For example, the spherical AgNPs used in biomedical applications are more bioassimilated than the elongated or polygonal shape particles lying parallel to the cell membrane. (Adabi et al.,2017; Marinescu et al. 2020).

Previously the anticandidal activity of the phytosynthesized by alcoholic extract of tulsi (*Ocimum tenuiflorum* L.) leaves AgNPs was evaluated for opportunistic human fungal pathogens *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*. *Candida albicans* is the predominant

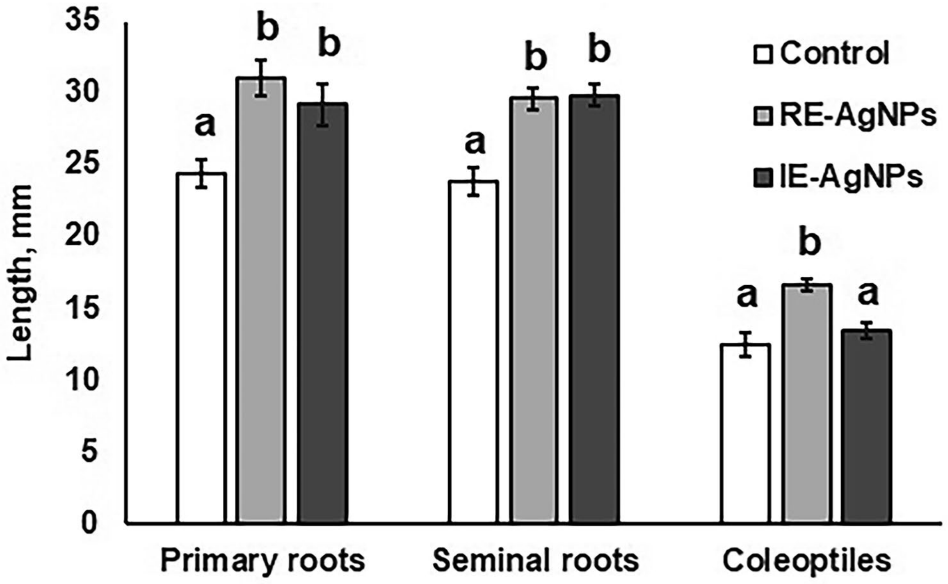


Fig. 7 Effect of phytosynthesized AgNPs on growth traits of wheat seedlings. Means followed by the same letters were not significantly different at $P < 0.05$ according to the Tukey's multiple range test

cause of virtually all types of candidiasis (Khatoon et al. 2015). The agar diffusion test is used to qualitatively assess the efficacy of diffusible biocides. The score for this test is based on the level of growth both under and around the specimen. The zone of inhibition around the test solution is measured and any growth present underneath the sample is assessed (Hauck et al. 2010).

Previously research with AgNPs phytosynthesised from *Lotus lalambensis* aqueous leaf extract demonstrated inhibition of *C. albicans* morphogenesis and suppression of the virulence factors – adhesion and the formation of *C. albicans* biofilm. Onward, AgNPs inhibited the activities of antioxidant enzymes of *C. albicans*. Microscopic investigation revealed degradation in the cell wall ultrastructure in AgNPs-treated *C. albicans* (Abdallah and Ali 2021). Jalal et al. (2019) previously reported that the phytosynthesized by the using seed extract of *Syzygium cumini* AgNPs strongly inhibited the propagation, germ tube and biofilm formation and most importantly secretion of hydrolytic enzymes such as phospholipases, proteinases, lipases and hemolysin by *C. albicans*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, and *C. krusei*. Reduced biofilm formation as a possible mechanism of AgNPs anticandidal force is also determined by Lara (Lara et al. 2020).

Considering that the exact mechanism of bactericidal and antifungal effects of AgNPs is still not well described, after detecting anticandidal activity, was performed a germination test to evaluate the toxicity of AgNPs to plant cells. The structure and physio-chemical properties of the AgNPs (shape, size, and surface coating) and experimental design (concentration, exposure time, method of exposure, and experimental plant species) are the priority features that determine the inhibitory or stimulatory effect of AgNPs on plant germination, early development and subsequent growth (Singh et al. 2020).

A few researchers have reported different affirmative responses of crops to AgNPs priming. AgNPs had no effect on germination but increased the number of seminal roots and stimulated the yield of wheat cv. NARC-2009 (Razzaq et al. 2016). AgNP priming can increase α-amylase activity, resulting in higher soluble sugars to support seedlings' early growth. In addition, priming with AgNPs stimulated the activation of aquaporin genes in germinating seeds in *Oryza sativa* L. cv. KDML 105 (Mahakham et al. 2017). In addition, various investigations have previously reported that seed priming with AgNPs stimulated seed germination, causing several biochemical changes in the seed, such as dormancy, hydrolysis, or growth-inhibitory metabolites, imbibition and enzyme activation (Singh et al. 2020).

Conclusions

The present work highlights that it is possible to obtain green AgNPs using an aqueous extract of different organs of parasitic plant *Lathraea squamaria*. Underground rhizomes and aboveground inflorescences also act as a best source for the formation of AgNPs. It was characterized the phytosynthesized AgNPs by UV-Vis spectroscopy and scanning electron microscopy (SEM). The phytosynthesized AgNPs with aqueous extract of *L. squamaria* rhizomes and inflorescences were crystalline with a spherical and polygonal shape with diameters from 15 to 40 nm. The phytosynthesized AgNPs are stable and shows a significant antimicrobial effect against *C. albicans*. Furthermore, the phytosynthesized AgNPs have shown significant potential in enhancing the seed germination and early seedling development of emmer wheat (*Triticum dicoccum* Schrank.). The green synthesized AgNPs show excellent bactericidal activity against the gram negative bacteria and moderate activity against the gram positive bacteria. Our findings indicate that green synthesized AgNPs using the *L. squamaria* source will allow unique opportunities in the direction of biomedical applications and sustainable crop production.

Author contribution O.S. and V.K. conceived the project, designed the study, and edited the manuscript, Yu.Yu. and P.Z. performed the experiments with *C. albicans*, T.L. analysed the data of agar-phenotyping, M.K. wrote the first draft of the manuscript, V.D. and M.S. performed and analysed SEM data,.

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Declarations

Conflict of interest There exists no conflict of interest among the authors.

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