

Evaluation of antimicrobial activity of synthesised silver nanoparticles using *Thymus kotschyanus* aqueous extract

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Abstract: Development of a green chemistry process for the synthesis of silver nanoparticles (AgNPs) has become a focus of interest. Characteristics of AgNPs were determined using techniques, such as ultraviolet–visible spectroscopy (UV–vis), Fourier transform infrared (FTIR) analysis, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy and X-ray diffraction (XRD). The synthesised AgNPs using *Thymus kotschyanus* had the most growth inhibition against gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilise*, while the growth inhibition of AgNPs at 1000–500 µg/ml occurred against *Klebsiella pneumonia* and at 1000–250 µg/ml of AgNPs was observed against *E. coli*. The UV–vis absorption spectra confirmed the formation of the AgNPs with the characteristic peak at 415 nm and SEM micrograph acknowledged spherical particles in a nanosize range. FTIR measured the possible biomolecules that are responsible for stabilisation of AgNPs. XRD analysis exhibited the crystalline nature of AgNPs and showed face-centred cubic structure. The synthesised AgNPs revealed significant antibacterial activity against gram-positive bacteria.

1 Introduction

Nanoparticles (NPs) are the unique form of materials, which are included 1–100 nm dimensions or less. The therapeutic applications of silver NPs (AgNPs) are currently being increased due to their potential of the silver ions in the nanoscale range [1]. In medicine, silver incorporated compounds such as silver sulphadiazine have been widely used as an antimicrobial agent for the microbial elimination of surface tissues or external wounds. Another application of silver is as an antiseptic coating in medical devices such as wound dressing wounds, urinary catheters, and endotracheal breathing tubes. Actually, the antimicrobial potential of inorganic AgNPs is dramatically stronger than those of the corresponding bulk structures [2, 3].

Generally, metallic NPs are synthesised from inorganic metals by several chemical methods including gas condensation, attrition, precipitation, implantation, pyrolysis and hydrothermal treatment [1, 4]. These methods employ many chemical reagents or consume excessive energy to progress the NP synthesis reactions by a multi-step process. Chemical precursors and toxic solvents in the reaction pots are ultimately considered as hazardous wastes, threatening the living environment and human health [5]. Currently, an alternative method has developed based on the usage of biological reagents, which have more advantages over the chemical synthesis. Despite the chemical synthesis protocols, biologically-based methods provide eco-friendly and easily-handleable condition using low-cost materials [6–8]. These methods are known as green synthesis in which a living system such as bacteria, fungi, algae and plant extracts are often responsible for the reduction of metals for NPs formation [9]. Besides, synthesis of various types of metal NPs using plant extracts is found to be more efficient, easily controllable due to the existence of various metabolites like emulsifying, capping and stabilising agents [10].

In traditional medicine, *Thymus* genus is employed for treating spasms, inflammation, and bloat in the ruminant digestive system [11]. Amongst current known 300–400 species, *Thymus kotschyanus* (*T. kotschyanus*) (common thyme) is widely used in Iranian traditional medicine [12]. Thyme is an Iranian flora, naturally distributed in the mountainous semi-grazing areas and grows as the perennial subshrubs [13]. In the current years, the therapeutic potential of thyme extract was approved by many

studies such as the treatment of retinal neovascularisation, immunodeficiency syndrome, infection and cancer [14–16].

AgNPs have a wide usage in the biological and chemical process, due to increasing absorption capacity to the various ligands such as toxins, enzymes, virus, and chemicals [17]. Considering that AgNPs have exhibited a great antimicrobial activity against the most resistant pathogens, this research focused on the green synthesis of these NPs using *T. kotschyanus* as reactive agents in the aqueous phase. Furthermore, biosynthesis of AgNPs by *T. kotschyanus* extract is a first study to develop a cost-benefit method for making AgNPs. Therefore, our study highlights the green synthesis of metal NPs by thyme extract with their antimicrobial bioactivity as a different formulation compared with the traditional application.

2 Experimental section

2.1 Preparation of plant extract

A wild-grown species of *T. kotschyanus* were collected from mountainous areas around Khorramabad city, Lorestan province, Iran. The plant leaves were washed with distilled water, air-dried and ground into fine pieces. To prepare the extract, 10 g of leaf powder was transferred in 100 ml of sterile distilled water and boiled for 45 min in 70°C. The mixture was then filtered by filtrate paper and the extract was kept for NPs synthesis.

2.2 Biosynthesis of AgNPs

For the synthesis of AgNPs, 40 ml of *T. kotschyanus* extract was mixed with 40 ml of AgNO₃ solution and stirred continuously for 30 min. The formation of NPs resulting from AgNO₃ reduction was monitored by observation of the colour change in the mixture after the overnight incubation at the room temperature. Lastly, separation of prepared NPs was performed by centrifugation of the reaction mixture at 8000 rpm for 10 min and the NPs were then washed and dried at 60°C.



Fig. 1 Colour changes in the reaction solution
 (a) Aqueous extract of thyme leaf, (b) Aqueous solution of 1 mM AgNO₃, (c) Reaction mixture (plant extract + AgNO₃ solution) after AgNPs formation

2.3 Characterisation of NPs

The NPs powder, AgNO₃ and crude extract of *T. kotschyanus* were subjected for analysing possible structural evidence involved in the reducing AgNO₃ using spectroscopy instruments such as ultraviolet-visible (UV-vis), Fourier transform infrared (FTIR), and energy-dispersive X-ray (EDX) as well as scanning electron microscopy (SEM). The UV-vis spectrums were drawn using a UV-vis spectrophotometer instrument (Jenway 6100, Dunmow, Essex, UK) in the range of 300–700 nm. FTIR spectroscopy was carried out for predicting responsible groups for NPs synthesis and stabilisation. The FTIR spectra were recorded for the *T. kotschyanus* extract and AgNPs in the range of 4000–400 cm⁻¹. An X-ray diffractometer (Philips X'PERT PROMPD) with Cu K α radiation (1.5409 Å) was used to study the size and crystalline structure of the particles. The dried AgNPs were also analysed by SEM and EDX spectroscopy (Mira3).

2.4 Evaluation of antimicrobial activity

The antibacterial activity of the synthesised AgNPs was studied by disc diffusion method. Some standard bacterial strains including *Staphylococcus aureus* (PTCC 1112), *Bacillus cereus* (PTCC 1556), *Escherichia coli* (PTCC 1330) and *Klebsiella pneumonia* (PTCC 1053) were applied. The discs were impregnated with about 35 μ l of the synthesised AgNPs in various concentrations (100–1000 μ g/ml). A loopful of bacterial cells was spread on Mueller-Hinton agar plates, AgNPs-impregnated discs were then placed over the surface of plates and incubated at 37°C for 18–24 h [18]. The zone of inhibition around the discs was measured and compared with amikacin standard disc as a positive control.

3 Results and discussion

3.1 Biosynthesis of AgNPs

The biosynthesis of AgNPs was detected through a visual colour change in the solution. At first, the AgNO₃ and thyme extract solutions were clear and relatively light red, which followed by AgNPs formation, the solution colour turned to dark brown (Fig. 1). Some chemical compounds in the *T. kotschyanus* extract such as polyphenols, alkaloids, flavonoids, saponins, and steroids are responsible for reducing various metal ions to the corresponding NPs.

3.2 UV-vis spectroscopy

Fig. 2 presents the UV-vis spectral profile of AgNO₃, plant extract solution and respective reaction mixture. The difference of spectra curves could be attributed to excitation of surface plasmon vibrations originated from the presence of AgNPs in the reaction mixture [18]. The UV-vis spectrum of colloidal solutions of AgNPs synthesised from *T. kotschyanus* extract had an absorbance

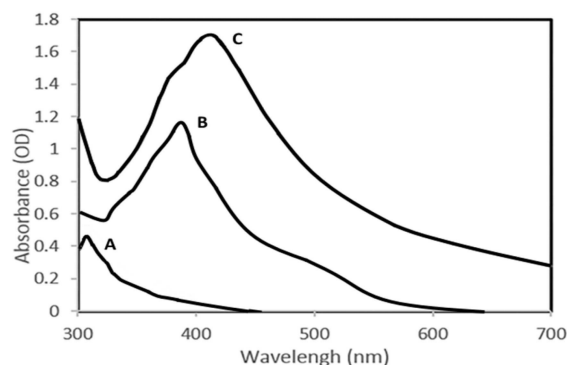


Fig. 2 UV-vis spectra of AgNPs
 (a) AgNO₃ solution, (b) *T. kotschyanus* extract, (c) AgNPs solution

peak at 415 nm. The frequency and width of the surface plasmon absorption depend on the size and the shape of the metal NPs as well as on the dielectric constant of the metal itself and the surrounding medium [19, 20]. Besides, the shift of the absorption wavelength observed in the spectra could be correlated with the colour change of the colloidal AgNPs solution.

3.3 SEM and EDX

The AgNPs synthesised by aqueous *T. kotschyanus* extract was morphologically characterised using SEM. Analysis of the SEM micrograph showed that the most of AgNPs were spherical along with few aggregates in some points (Fig. 3). As shown in Fig. 3, spherical NPs are ranged from nanoscale particle size to large aggregates. There are unknown causes that could affect monodispersity and the stability of NPs in a complex reaction mixture like plant extracts. It is found that the shape of metal NPs can be affected by pH, temperature, electrostatic force and other physiochemical factors. Thus, according to the findings of Markus *et al.* [21] aggregation occurred due to the interaction of concentrated AgNPs with plant-derived organic materials like proteins and polysaccharides. Therefore, in the preparation of the AgNPs sample for SEM, NP aggregation and nucleation could occur for AgNPs. EDS analysis of NPs showed that the strongest peak appeared at 3 keV is associated with aggregated AgNPs nanocrystals. On the other hand, EDS revealed Ag element formed no Ag oxide in the crystallisation process.

3.4 FTIR spectroscopy study

The FTIR analysis displayed functional groups of biomolecules that have a possibly critical role in the formation and stabilisation of NP crystals. As shown in Fig. 4a, which presents the FTIR spectrum of crude extract, a broad signal was observed about 3377 cm⁻¹ can be attributed to O–H or C–N stretching vibration modes in primary amines of aromatic compounds [22]. The others peaks, 2941.54, 1452.09 and 669.32 cm⁻¹ observed can be associated with C–H bond in alcohols and phenols, carbonyl present in the plant extract.

Fig. 4b presents the FTIR spectrum of the AgNPs that exhibits chemical shifts in a number of adsorption peaks compared with the plant extract. The results suggest that plant-derived bioactive compounds could provide a satisfying condition for the synthesising of the AgNPs, capping of their crystals and maintaining without the using chemical catalysts or other stabilisers. Correspondence of the role of biological compounds, proteins, and exopolysaccharides, polyphenols and alkaloid is well known that can bind to AgNPs through numerous functional groups and therefore caused the stabilisation of AgNPs [23].

3.5 XRD analysis

The crystalline properties of AgNPs were studied by the XRD analysis (Fig. 5). The XRD pattern of AgNPs synthesised showed four distinct diffraction peaks at 2θ value ranging from 10 to 80 of 38.08°, 44.27°, 64.42° and 77.36°, corresponding to the crystalline

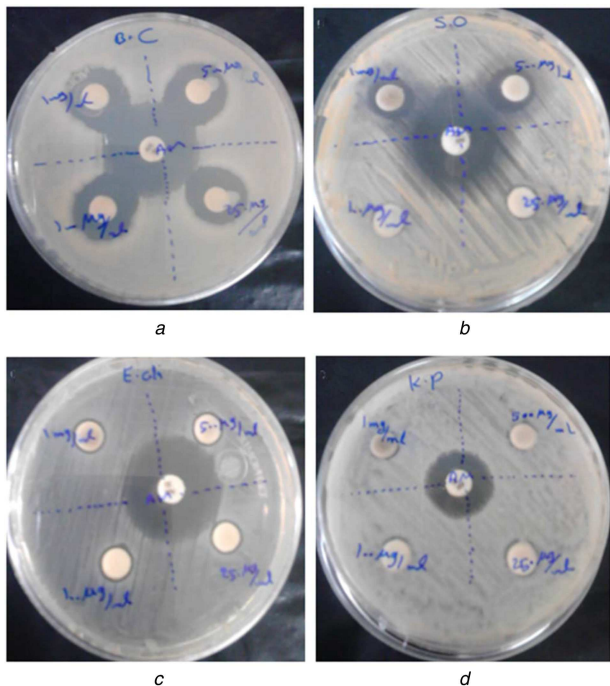


Fig. 6 Antibacterial effect of the AgNPs against bacteria (a) *B. cereus* S., (b) *S. aureus*, (c) *E. coli*, (d) *K. pneumonia*

Table 1 Bacterial growth inhibition biosynthesised AgNPs based on disc diffusion method

AgNPs, µg/ml	Zone of inhibition, mm			
	<i>S. aureus</i>	<i>B. cereus</i>	<i>K. pneumonia</i>	<i>E. coli</i>
1000	10.33 ± 1.2	20.33 ± 3.25	5 ± 1.63	7.33 ± 1.38
500	9.67 ± 1.65	20 ± 2.58	2.67 ± 0.41	7 ± 1.06
250	8.33 ± 1.47	19 ± 2.14	—	2.33 ± 0.06
100	8 ± 1.07	18.67 ± 2.28	—	—

condition. Hence, *T. kotschyanus* is well known as one of the most important herbs with different uses in traditional medicine, our success for biosynthesis AgNPs by this plant extract can be valuable from the view of both safety and economic development.

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