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GREEN SYNTHESIS OF NANO-SILVER PARTICLES FROM LEAF AND STEM EXTRACTS OF *IBOZA* (*TETRADENIA RIPARIA*)

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Abstract

Background: Secondary metabolites derived from plant extracts can be used in the reduction of metal salts into their respective nanoparticles using simple, environmentally friendly and cost effective green synthesis techniques. Metal nanoparticles have important applications in medicine and agriculture. The leaves of *Tetradenia riparia* (*Iboza*), an important medicinal species in South Africa, are reported to contain various terpenoids and pyrones which can be used in the reduction of silver nitrate ($AgNO_3$) to nano-silver particles.
Materials and Methods: Fresh leaves and stems of *Iboza* were oven-dried, crushed, extracted in water and methanol and filtered followed by incubation with $AgNO_3$. Synthesized nano-silver particles were characterised using ultraviolet visible (UV-Vis) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray (EDX) spectroscopy and Fourier transform infrared (FTIR) spectroscopy.
Results: The bio-reduction of metal ion to base metal was rapidly conducted in a single step and at room temperature and pressure. UV-Vis spectra showed the characteristic surface plasmon resonance band of the synthesized nano-silver particles at 410 nm for all extracts. SEM analysis revealed predominantly aggregated spherically-shaped nano-silver particles with a size range of 20-50 nm and an average diameter of 26 nm. The presence of elemental silver in the nanoparticles was confirmed by EDX at 3 keV. As revealed by FTIR analysis, the reducing agents included terpenoids and pyrones which were responsible for reducing and capping the nano-silver particles.
Conclusion: Both methanol and aqueous-derived extracts of *Iboza* leaves and stems can be used to synthesize nano-silver particles. FTIR evidence suggests that the reduction of the silver ions and the synthesis of the nanoparticles may have been actioned by various terpenoid and pyrone compounds found in the plant parts.

Key words: *Tetradenia riparia*, plant extracts, UV spectroscopy, SEM, EDX, FTIR.

Introduction

Tetradenia riparia (misty plume, ginger bush, *Iboza*) is an important medicinal species of South Africa belonging to the family *Lamiaceae*. It is a large deciduous shrub found along river banks, forest margins, dry wooded valleys and hillsides in KwaZulu-Natal, Northern Province and Mpumalanga in South Africa. Its distribution also extends to Swaziland, Namibia, Angola, Uganda and tropical east Africa into Ethiopia (Codd 1985). It is mainly the leaves of *Iboza* that are used in the preparation of traditional remedies for the management of tuberculosis (Asiimwe et al., 2013), respiratory infections (York et al., 2012), stomach ailments (Okem et al., 2012), diarrhoea, influenza, malaria and headaches (Hutchings et al., 1996). The leaves are reported to contain various phyto-compounds such as terpenoids (Campbell et al., 1997; Van Puyvelde and de Kimpe, 1998) and pyrones (Van Puyvelde et al., 1987). It is these secondary metabolites that are purported to contribute to the pharmacological effects of the leaf extracts.

In recent years, there has been much attention devoted to the use of secondary metabolites derived from plant extracts in the reduction of metal salts into their respective nanoparticles (Mittal et al., 2013). Gold, silver, nickel, cobalt, zinc, platinum, palladium and copper nanoparticles have been successfully synthesized using plant extracts (Kharissova et al., 2013) in simple, environmentally friendly and cost effective ways as opposed to conventional physicochemical methods. Applications of metal nanoparticles include improved drug delivery (Mittal et al., 2013); broad-spectrum anti-microbial activity in human and animal disease (Ali et al., 2011; Lara et al., 2011; Saxena et al., 2011; Singh et al., 2010; Singhal et al., 2011) and wastewater effluent (Duran et al., 2007); cytotoxic action against carcinomas (Fortina et al., 2007; Jacob et al., 2011; Safaepour, 2009; Subramanian, 2012; Sukirtha et al., 2011); and crop protection (Khot et al., 2012; Perez-de-Luque and Rubiales, 2009).

Numerous reports illustrate the synthesis of silver nanoparticles using various plant species (Bar et al., 2009; Dubey et al., 2009; Kumar et al., 2010; Mallikarjuna et al., 2011; Mittal et al., 2012; Nabikhan et al., 2010; Vanaja and Annadurai, 2012) but in the case of *Iboza*, only the synthesis of gold nanoparticles has been reported (Shaik et al., 2014). Therefore, the present study was undertaken to investigate the synthesis of nano-silver particles using leaf and stem extracts of *Iboza*.

Material and Methods

Preparation of Plant Extracts

Fresh leaves and stems of *Iboza* were collected from the University of KwaZulu Natal gardens in July 2013. A voucher specimen was deposited in the Ward Herbarium at University of KwaZulu-Natal (Accession number 17872, Genus number 7339000). The collected plant parts were dried in a laboratory oven at 40°C for 48 h. Thereafter, the dried leaves and stems were separately crushed in an electric blender (Ottimo,

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China). Twenty g of each plant part were separately extracted in 200 ml each of cold water and methanol on a mechanical shaker for 24 h. Following extraction, each extract was vacuum-filtered through Whatman no.1 filter paper.

Synthesis of Nano-Silver Particles

Five ml of each plant extract was added to 45ml of 1mM silver nitrate ($AgNO_3$) (Sigma, South Africa). The solutions were left to incubate at room temperature until a final colour change of dark brown was observed, indicating the formation of nano-silver particles.

Characterization of Nano-Silver Particles

Characterization of nano-silver was performed as outlined below.

UV-Vis Spectroscopic Analysis

After 24 h of incubation with silver nitrate, the absorbance of each reaction mixture was measured using a spectrophotometer (Beckman DU 500 UV-VIS) in a wavelength range of 350-600 nm and 380-490 nm for nanoparticles synthesized from aqueous and methanol extracts, respectively. Instrument parameters were single scan mode, medium scan speed, sampling interval of 5 nm and a slit width of 5 L.

Scanning Electron Microscopy (SEM)

A small quantity of each sample was placed onto carbon-coated copper stubs to create a thin film. The solvents were allowed to completely evaporate. The size and shape of the nano-silver particles were determined using a scanning electron microscope (ZEISS FEGSEM Ultra Plus) with EHT of 12kV and WD of 1.9 mm. Images were recorded for visual inferences.

Energy Dispersive X-Ray (EDX)

The presence of elemental silver was determined using EDX analysis (Oxford Instruments AZTEC Analysis Software) on the SEM.

Fourier Transform Infrared (FTIR) Spectral Analysis

Each sample was centrifuged at 10000 rpm (Beckman Coulter Avanti J-E Centrifuge) for 30 min at 4ºC. The supernatant was discarded and the resulting pellet was dried in a laboratory oven at 60°C until completely dry. Spectroscopic measurements of the pellets were determined using a PerkinElmer FTIR Spectrum One spectrophotometer in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

Results and Discussion

UV-Vis Spectroscopic Analysis

Addition of methanol and aqueous extracts of *Iboza* leaves and stems to 1 mM silver nitrate resulted in the appearance of a dark brown colour in each solution, indicating the formation of nano-silver, after 6hr for methanol extracts and 18hr for aqueous extracts (Table 1). The methanol extract colour changes occurred more quickly than the aqueous extracts probably because methanol is a less polar solvent than water so the secondary metabolites in the methanol extracts would have had a significantly higher solubility than in water.

Table 1: Absorbance and time to final colour change for nano-silver synthesis in different *Iboza* extracts.

Solvent	Extract	Original colour of extract	Time to final colour change (hr)	Absorbance (nm)
methanol	stem and leaf	green	6	410
water	stem and leaf	light brown	18	410

The spectra for nano-silver synthesized using aqueous extracts are much more defined than those synthesized using methanol extracts (Figure 1). Nevertheless, the characteristic surface plasmon resonance (SPR) band of the synthesized nano-silver particles centred at 410 nm for all extracts. SPR is the collective oscillation of electrons in the conduction band on a nanoparticle surface (Suman et al., 2014). These electrons conform to a specific vibration mode dependent on particle size and shape and therefore exhibit characteristic optical absorption spectra in the UV–VIS region (Fayaz et al., 2010). The position and the width of the plasmon resonance peak can be used to estimate nanoparticle size (Raveendran et al., 2006). In this study, the plasmon resonance peaks were narrow which can be related to the very small nano-silver particles synthesized (20-50 nm – see SEM later). Although, the source of the plant extract is known to influence the characteristics of nanoparticles (Kumar and Yadav, 2009), notably, both the leaf and stem extracts had similar peak widths demonstrating that the size of nano-silver particles synthesized did not differ between them. This was also verified by SEM.

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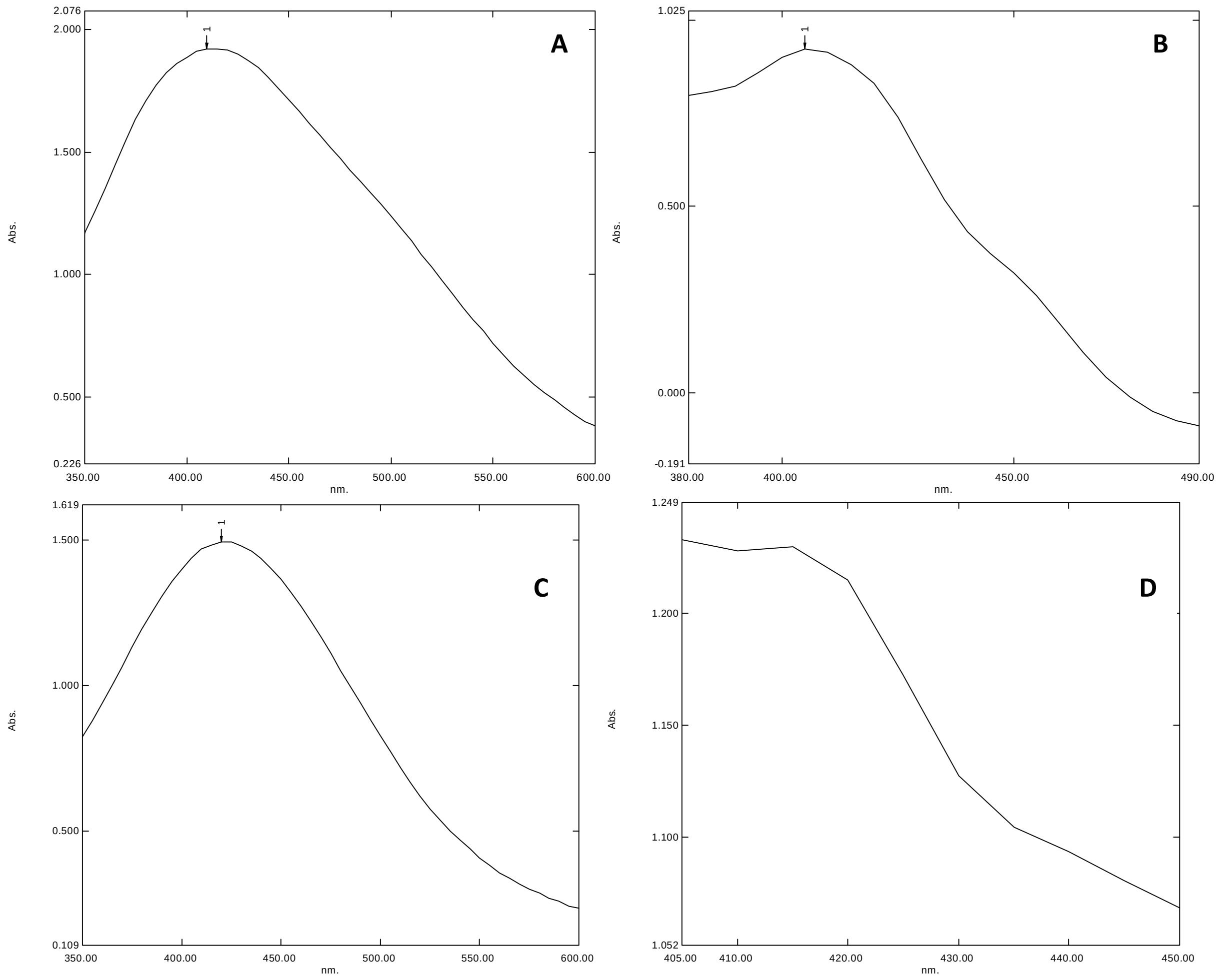

Figure 1: UV-VIS absorption spectra of nano-silver synthesized from aqueous (A) and methanol (B) leaf extracts, and aqueous (C) and methanol (D) stem extracts of *Iboza.*

Scanning Electron Microscopy (SEM)

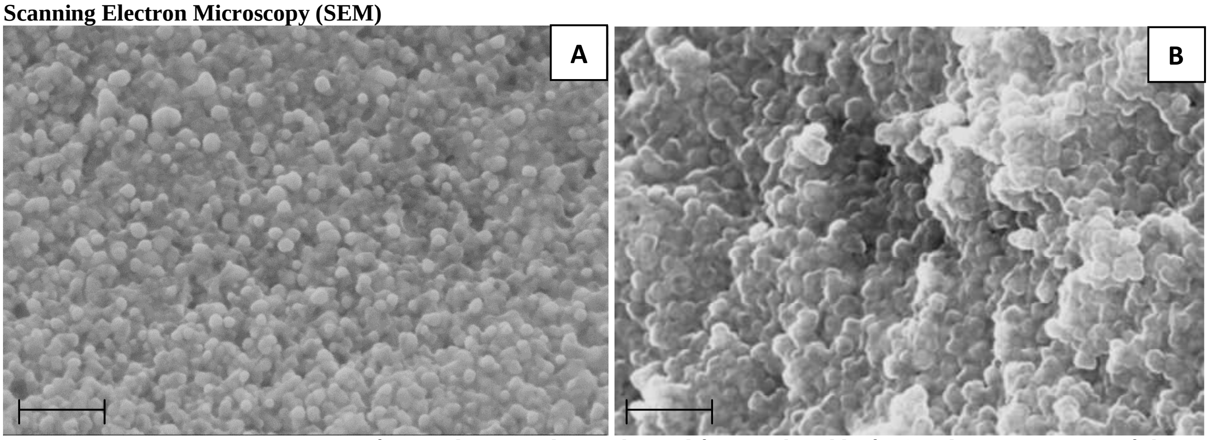

Figure 2: Representative SEM image of nano-silver particles synthesized from methanol leaf (A) and stem (B) extract of *Iboza*. Bar = 100 nm.

The shape and size of the nano-silver particles from *Iboza* leaf extracts are depicted in Figure 2. In general, the particles were spherical. The shape of metal nanoparticles can significantly influence their optical and electronic properties (Kim et al., 2007). The size ranged from 20-50 nm for nano-silver synthesized from both leaf and stem methanol and aqueous extracts. The average diameter was found to be 26 nm. The nano-silver particles were predominantly aggregated and the nanoparticles produced by the leaf extracts were more distinct than those obtained from stem extracts. It is possible that the concentration of the secondary metabolites, which have the effect of nanoparticle stabilization and non-agglomeration (Morones et al., 2005), was higher in the leaf extracts compared to the stem extracts. The lighter colour of the aqueous and methanol stem extracts compared to that of the leaf extracts observed during the extraction process may also be an indication of the lower concentration of eluted secondary metabolites in leaves. Spherically shaped nano-silver particles were also synthesized using *Coleus aromaticus* (Vanaja and Annadurai, 2013), *Ocimum sanctum* (Mallikarjuna et al., 2011) and *Syzygium cumini* (Kumar et al., 2010).

EDX Analysis

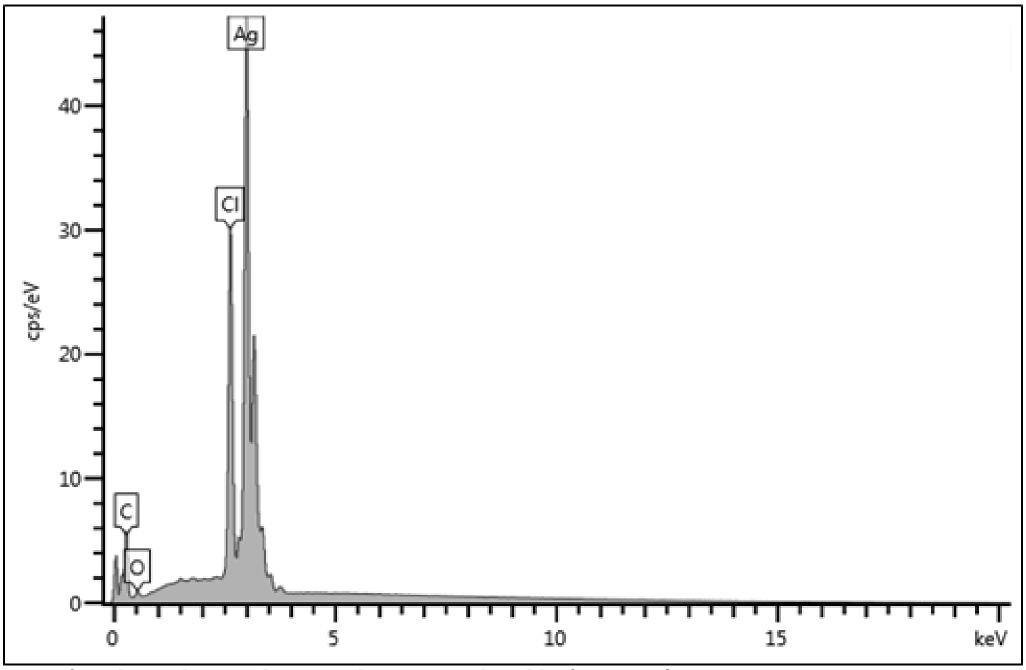

Figure 3: EDX spectrum of synthesized nano-silver particles using methanol leaf extract of *T. riparia*

The EDX spectrum recorded from the synthesized nano-silver particles showed a strong signal of silver at 3 keV (Figure 3) confirming the presence of elemental silver. Metallic nano-silver particles generally show typical optical absorption peak at approximately 3 keV due to surface plasmon resonance (Magudapatty et al., 2001). A similar spectral profile for silver was reported for nanoparticles synthesized from *Jatropha curcas* (Bar et al., 2009) and *Coleus aromaticus* (Vanaja and Annadurai, 2013). Weaker signals of carbon, oxygen and chlorine are also evident. These could be the result of x-ray emission from organic compounds capping the nanoparticles. The presence of carbon is also likely to be from coating of the support grid prior to viewing.

FTIR Spectral Analysis

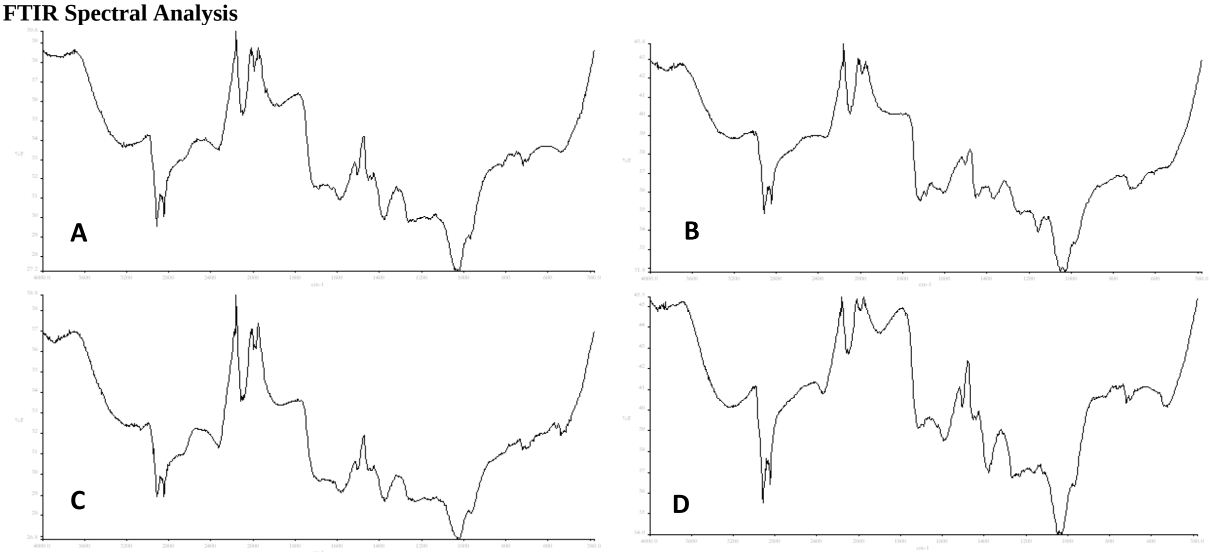

Figure 4: FTIR spectra of synthesized nano-silver particles from aqueous and methanol extracts respectively, of *Iboza* leaves (A and B) and stems (C and D)

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FTIR spectroscopy was performed to determine the bio-groups that bound to the nano-silver particle surfaces. In Figure 4, the nano-silver particles synthesized using aqueous (A) and methanol (B) leaf extract and aqueous (C) and methanol (D) stem extract respectively, shows various medium to strong absorption peaks. These spectra are similar to each other and reveal peaks at similar wavenumbers. The peaks at 2900-2810 correspond to aliphatic -CH stretching, the peaks at 1450-1380 suggest –CH bending whilst the peak at 1050-1010 correspond to -CO stretching. The peak at 3250 corresponds to alcohol -OH group indicating the possible involvement of the known terpenoids. The peak at 1700 suggests -C=O carbonyl stretching caused by the presence of this functional group in the known pyrones. The alkene stretching for the peaks at 1640-1510 and 2090-1890 are possibly the result of -C=C and -CH bonds present in the ring structure of the terpenoids. It is likely that the terpenoids and pyrones played a role in the reduction of aqueous silver ions to silver nanoparticles. The known active compounds in *Iboza* are pyrones viz. fenchone, tetradenolide and umuravumbolide (Campbell et al., 1997; Sabitha et al., 2012; Van Puyvelde and de Kimpe, 1998) and terpenoids viz. terpineol, fenchol, perillyl alcohol, caryophyllene and ibozol (Campbell et al., 1997; Zelnik et al., 1978). Mukunthan and Balaji (2012) reported that nanoparticle characteristics can be influenced by extracts arising from different sources as they comprise different concentrations and combinations of organic reducing agents. However, in the present investigation, all methanol and aqueous-derived nanoparticles displayed similar FTIR spectra. This indicates that the profile of secondary metabolites in *Iboza* leaves and stems is similar resulting in similar effects on the synthesis of nano-silver particles.

Proposed Mechanism of Nano-Silver Synthesis

A possible mechanism by which nano-silver was synthesized using *Iboza* extracts is given in Figure 5. Ag^+ ions in solution are reduced by the secondary metabolites (terpenoids and pyrones) in the plant extracts to create Ag^0. The Ag^0 species cluster together and assemble as nano-silver particles being stabilized with the aid of capping agents as identified using FTIR spectroscopy.

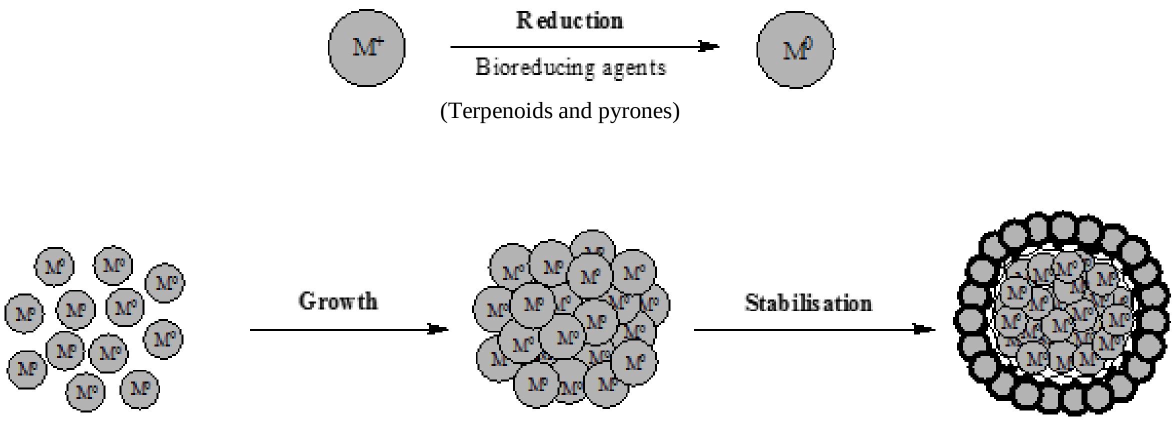

Figure 5: Proposed mechanism for the synthesis of nano-silver particles using *Iboza* extracts (M = Ag). Adapted from Mittal et al. (2013).

Conclusion

Both methanol and aqueous-derived extracts of *Iboza* leaves and stems enabled the synthesis of nano-silver particles. The reduction of the silver ions and the synthesis of the nanoparticles are believed to occur by the action of various terpenoid and pyrone compounds found in the plant parts tested. The size of the nanoparticles varied from 20-50 nm with an average diameter of 26 nm. By providing a simple and rapid method for the biosynthesis of nano-silver particles, this study supports the effectiveness of such synthetic techniques using environmentally benign natural resources as a cost-effective alternative to chemical synthesis.

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