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TETRADENIA RIPARIA-MEDIATED SYNTHESIS OF NANO-GOLD PARTICLES

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Plant-mediated synthesis of metallic nanoparticles is a growing area of interest in the fields of green synthesis and nanotechnology. The present work studied the synthesis of nano-gold particles using *Tetradenia riparia* flower, leaf and stem extracts derived using methanol and water. The synthesized nanoparticles were characterized using UV-visible spectroscopy, scanning electron microscopy (SEM), energy dispersive x-ray (EDX) analysis and Fourier transform infrared (FTIR) spectroscopy. UV peaks for nano-gold synthesized from methanol and aqueous extracts were observed at 535 nm and 540 nm, respectively. SEM analysis revealed monodispersed spherical nano-gold particles in the diameter range of 10-35 nm. The presence of elemental gold in the nanoparticles was confirmed by EDX. FTIR analysis indicated the presence of terpenoids and pyrones which were responsible for reducing, capping and stabilizing of the nano-gold particles.

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1. Introduction

In recent years there has been much interest in the use of plants for the synthesis of metal nanoparticles (<100nm) in simple, environmentally friendly and cost effective ways as opposed to conventional physicochemical methods. Plants are also easily available and safe to handle during this process. Secondary metabolites derived from plant extracts are responsible for the reduction of metal salts into their respective nanoparticles [1]. Several types of metal nanoparticles have been successfully synthesized using plant extracts for example gold, silver, nickel, cobalt, zinc, platinum, palladium and copper [2]. Infra-red spectroscopy has revealed that terpenoids, pyrones, flavones, aldehydes, amides and carboxylic acids are mainly responsible for metal nanoparticle synthesis. These nanoparticles have several applications. In medicine, the use of nanoparticle-drug complexes is advantageous due to extended half-lives, longer circulatory periods and deep penetration of desired organs and tissues with elevated concentrations of relevant drugs [1]. Nanoparticles have demonstrated cytotoxic action against carcinomas [3-7] and broad-spectrum anti-microbial activity in human and animal disease [8-11] and wastewater effluent [12]. They have also demonstrated their effectiveness in crop protection and agriculture [13, 14].

Numerous reports illustrate the synthesis of gold nanoparticles using various plant species [15-19] but this has not been reported in *Tetradenia riparia*, an important medicinal species of South Africa. *T. riparia* (misty plume, ginger bush, *iboza*) is a large deciduous shrub belonging to the family Lamiaceae. It is found along river banks, forest margins, dry wooded valleys and hillsides in KwaZulu-Natal, Northern Province and Mpumalanga in South Africa to Swaziland, Namibia, Angola, Uganda and tropical east Africa into Ethiopia [20]. In addition to the plant's many aromatic qualities, it is mainly the leaves that are used in the preparation of traditional remedies for the management of tuberculosis [21], respiratory infections [22], stomach ailments [23], diarrhoea, influenza, malaria and headaches [24]. The pharmacological effects of the leaf extracts are purported to be the result of various secondary metabolites including essential oils

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particularly abundant in terpenoids [25, 26] and pyrones [27]. The present study was undertaken to investigate the biosynthesis of nano-gold using various extracts of *T. riparia*.

2. Experimental

2.1 Preparation of plant extracts

Fresh leaves, stems and flowers of *Tetradenia riparia* were collected from the University of KwaZulu Natal gardens (S29º48'59.8", E30º56'37.4") in July 2013. All plant parts were dried in a laboratory oven at 40°C for 48 h. Thereafter, the dried leaves, stems and flowers were separately crushed in an electric blender (Ottimo, China). Twenty g of each plant part were separately extracted in each of cold water and methanol on a mechanical shaker for 24 h. Following extraction, each extract was filtered through Whatman no.1 filter paper.

2.2 Biosynthesis of nano-gold

Five ml of each plant extract was added to 45ml of 1mM chloroauric acid ($HAuCl_4$) (Sigma, South Africa). The solutions were left to incubate at room temperature until a final colour change of ruby red was observed indicating the formation of gold nanoparticles.

2.3 UV spectroscopic analysis

The absorbance of each reaction mixture was measured using a spectrophotometer (Beckman DU 500 UV-VIS) in a wavelength range from 300 to 600 nm. Instrument parameters were single scan mode, medium scan speed, sampling interval of 5 nm and a slit width of 5 L.

2.4 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 gold nanoparticles 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.

2.5 Energy Dispersive X-ray (EDX)

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

2.6 Fourier transform infrared (FTIR) spectral analysis

Each sample was centrifuged at 10000 rpm (Beckman Coulter Avanti J-E Centrifuge) for 30 min. 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}.

3. Results and discussion

3.1 UV spectroscopic analysis

Table 1 Absorbance and time to final colour change for nano-gold synthesis in various T. riparia extracts.

Solvent	Extract	Original colour of extract	Time to final colour change (min)	Absorbance (nm)
Methanol	Flower	dark green	20	535
	Stem			
	Leaf			
Water	Flower	light brown	180	540
	Stem			
	Leaf			

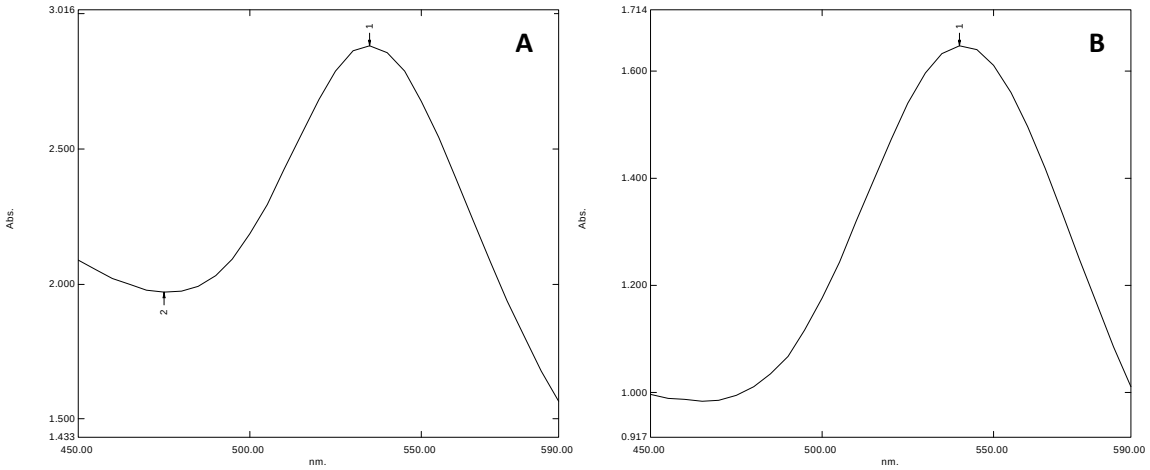

Fig. 1. UV-VIS absorption spectra of nano-gold synthesized from aqueous flower extract (A) and methanol stem extract (B) of T. riparia.

Addition of methanol and aqueous extracts of *T. riparia* flowers, leaves and stems to 1 mM chloroauric acid resulted in the appearance of a ruby-red colour in each solution after 20 mins for methanol extracts and 3 hours for aqueous extracts (Table 1). The colour change from dark green and light brown to ruby red indicated the formation of nano-gold. 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. Therefore, colour change in the methanol extracts occurred more quickly than in the aqueous extracts. Surface plasmon resonance (SPR) is the collective oscillation of electrons in the conduction band on a nanoparticle surface [19]. 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 [28]. The characteristic SPR band of the gold nanoparticles centred at 535 nm for methanol extracts and 540nm for aqueous extracts (Figure 1) (all spectra not shown). Nanoparticle size can be judged by the position and the width of the plasmon resonance peak [29]. In all spectra, the plasmon resonance peaks were narrow which can be related to the very small sized nano-gold particles synthesized (10-35 nm – see SEM later). Although the source of the plant extract is known to influence the characteristics of nanoparticles [30], notably, both the methanol and aqueous extracts for all plant parts had similar peak widths demonstrating that the size of the nano-gold particles synthesized between them did not differ.

3.2 Scanning Electron Microscopy (SEM)

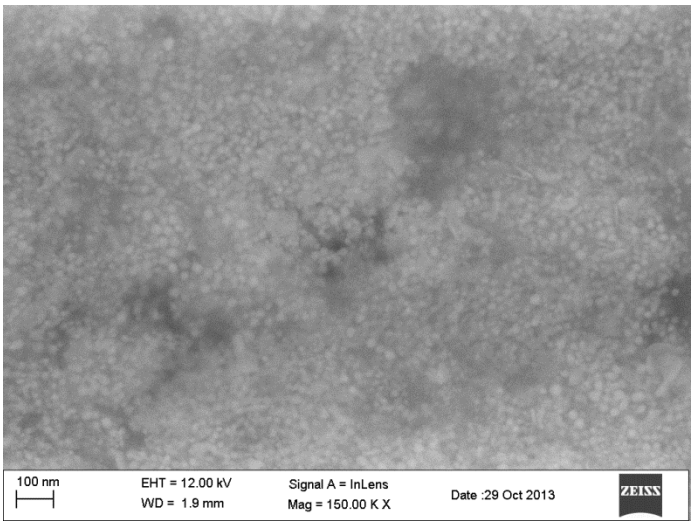

Fig. 2. Representative SEM image of nano-gold particles synthesized from methanol leaf extract of T. riparia

The shape and size of the nano-gold particles from *T. riparia* leaf extracts are depicted in Figure 2. The shape of metal nanoparticles can significantly influence their optical and electronic properties [31]. In general, the particles were uniformly spherical. The size ranged from 10-35 nm for all plant parts in both methanol and aqueous extracts (data not shown) and the average diameter was found to be 26 nm. Similarly shaped nano-gold particles were synthesized using *Terminalia catappa* leaf extract [15] and *Lycopersicon esculentum* callus extract [16]. The nano-gold particles are predominantly monodispersed and stable. The inherent functional group capping, caused by the organic material derived from the plant extraction process, offers stability and prevents agglomeration [32].

3.3 Energy Dispersive X-ray (EDX) analysis

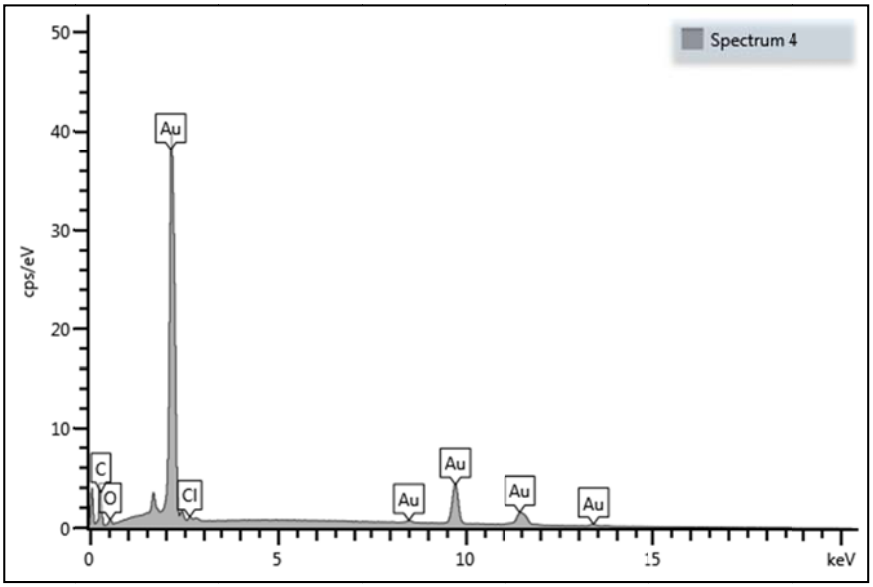

Fig. 3. EDX spectrum of synthesized nano-gold particles using methanol leaf extract of T. riparia

The EDX spectrum recorded from the nano-gold particles showed a strong signal of gold at 2.2 keV and weaker signals at 8.5, 9.7, 11.4 and 13.4 keV (Figure 3) confirming the presence of elemental gold. A similar spectral profile for gold has been reported [19]. Weaker signals of carbon, oxygen and chlorine are also evident. The presence of carbon is likely to be from the support grid coating. Signals of carbon and oxygen can also be the result of x-ray emission from organic compounds capping the nanoparticles. Chlorine is likely a trace residue from $HAuCl_4$ used in the reduction process.

3.4 Fourier transform infrared (FTIR) spectral analysis

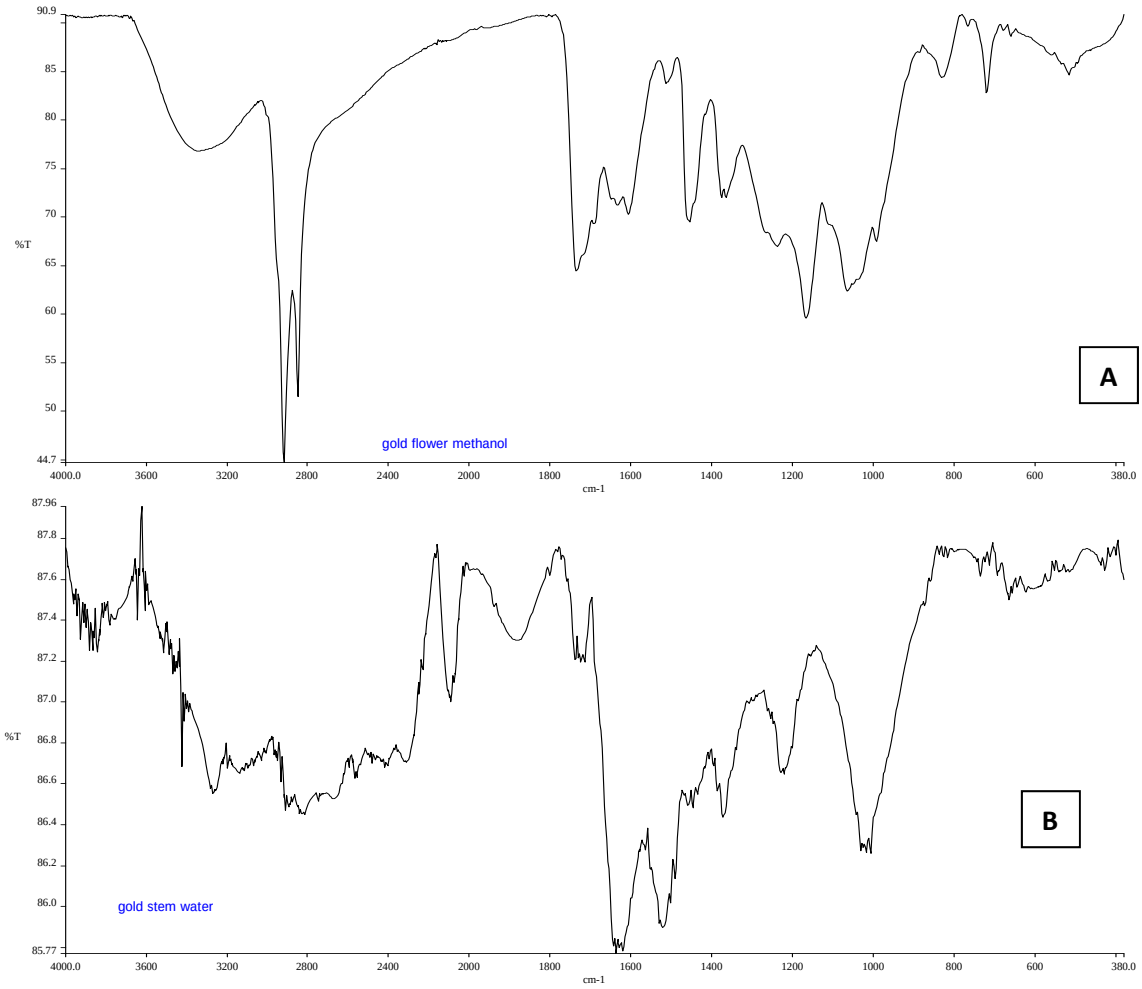

Fig. 4. FTIR spectra of nano-gold synthesized from methanol flower extract (A) and aqueous stem extract (B) of T. riparia.

FTIR spectroscopy was performed to determine the bio-groups that bound to the nano-gold particle surface. The known active ingredients in *T. riparia* are terpenoids viz. terpineol, fenchol, perillyl alcohol, caryophyllene and ibozol [25, 33] and pyrones viz. fenchone, tetradenolide and umuravumbolide [25, 26, 34]. In Figure 4, the nano-gold particles synthesized using methanol flower extract (A) and aqueous stem extract (B) respectively, shows various medium to strong absorption peaks. The spectra of methanol and water look somewhat different to each other possibly as a result of the differing polarities and solubilities of these solvents. However, closer inspection reveals peaks at similar wavenumbers. Spectrum A shows prominent peaks at 3350, 2920, 2860, 1740, 1600, 1450, 1360, 1160 and 1060 and spectrum B shows prominent peaks at 3250, 2810, 2090, 1870, 1710, 1640, 1510, 1370, 1210 and 1110. The peaks at 2920-2810 correspond to aliphatic -CH stretching, the peaks at 1450-1360 suggest –CH bending whilst the peaks at 1210-1060 correspond to -CO stretching. The peaks at 3350-3270 correspond to alcohol -OH group indicating the possible involvement of the known terpenoids. The peaks at 1740-1710 suggest -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 2090-1870 are possibly the result of -C=C and -CH bonds present in the ring structure of the terpenoids. It is possible that the terpenoids and pyrones played a role in the reduction of aqueous gold ions to gold nanoparticles. The synthesized nano-gold particles were surrounded by secondary metabolites belonging to the terpenoid and pyrone groups preventing agglomeration and influencing stability of the solution.

All methanol and aqueous-derived nanoparticles displayed similar FTIR spectra (data not shown). Nanoparticle characteristics can be influenced by different sources of extracts since they contain different concentrations and combinations of organic reducing agents [35]. However, the present investigation has demonstrated that both the methanol and aqueous extracts for all plant parts had similar FTIR spectra. This indicates that the profile of secondary metabolites in *T. riparia* flowers, leaves and stems is similar and therefore has the same effect on the synthesis of stable nano-gold particles.

4. Conclusion

This study has provided a simple and rapid method for the biosynthesis of nano-gold particles offering a valuable contribution to the areas of green synthesis and nanotechnology, with possible environmental, biotechnological and biomedical applications. The secondary metabolites present in *T. riparia* perform the dual function of formation and stabilization of nano-gold particles using both methanol and aqueous-derived extracts of flowers, leaves and stems. The reduction of the gold ions and the stabilization of the nanoparticles are believed to occur through the action of various terpenoid and pyrone compounds found in all the plant parts tested. The size of the nanoparticles varied from 10-35 nm with an average diameter of 26 nm. This study supports the efficiency of such synthetic procedures using environmentally benign natural resources as a cost-effective alternative to chemical synthesis.

Acknowledgments

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