South African Journal of Botany 119 (2018) 172–180

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Biosynthesis and characterisation of antimicrobial silver nanoparticles from a selection of fever-reducing medicinal plants of South Africa

M.E. Lediga [a], T.S. Malatjie [a], D.K. Olivier [b], D.T. Ndinteh [c], S.F. van Vuuren [a,*]

[a] Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa
[b] Research and Development, Seda Essential Oil Business Incubator (SEOBI), 19 Mountain street, Derdepoort 0186, South Africa
[c] Department of Applied Chemistry, Faculty of Sciences, University of Johannesburg, PO Box 17011, Doornfontein, Johannesburg 2028, South Africa

ARTICLE INFO

Article history:
Received 22 February 2018
Received in revised form 1 August 2018
Accepted 16 August 2018
Available online xxxx

Edited by B-E Van Wyk

Keywords:
Brine shrimp lethality assay
Fever
Green synthesis
Minimum inhibitory concentration
Toxicity

ABSTRACT

Most developing countries, including South Africa, depend strongly on traditional medicine for a therapeutic outcome and have therefore employed numerous medicinal plants to treat fevers. Therefore, it is imperative that fever-reducing medicinal plants are investigated to establish their efficacy and to determine their potential as sources of new antimicrobials. The incorporation of nanotechnology in antimicrobial research with reference to medicinal plants is a growing domain. The interest in silver nanoparticles (AgNPs) encompasses the tested hypothesis that the chemical combination of silver with medicinal plant extracts results in nanoparticles with enhanced antimicrobial properties in comparison with plant extracts alone. This study investigated the antimicrobial properties from 10 medicinal plants of commercial significance used traditionally for the treatment of fever in South Africa and their potential for enhanced antimicrobial efficacy when incorporated within AgNPs. Plant extracts and AgNPs were tested against fever-related pathogens, i.e., two Gram-positive pathogens; *Listeria monocytogenes* (ATCC 19111) and *Enterococcus faecalis* (ATCC 29212) as well as two Gram-negative pathogens; *Klebsiella pneumoniae* (ATCC 13883) and *Acinetobacter baumannii* (ATCC 19606) using the broth microdilution method. Chemical characterisation of AgNPs included Ultraviolet–visible (UV–Vis) spectroscopy, dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), and Transmission electron microscopy (TEM). The toxicity profiles of the AgNPs were evaluated using the brine-shrimp lethality assay (BSLA). Silver nanoparticles of both *Eucomis autumnalis* and *Sclerocarya birrea* display a dramatic increase in antimicrobial activity against the four test pathogens compared to the respective aqueous plant extracts. The greatest difference in antimicrobial activity was observed against *E. faecalis* where an increase in antimicrobial activity of at least 50-fold when *E. autumnalis* aqueous sample were compared with the AgNP-counterparts. Cytotoxicity of both AgNP samples from the BSLA emerged at less than 50% mortality, the results obtained in this study justify the use of selected fever-reducing plant extracts with the biosynthesis of AgNPs as promising antibacterial agents with low toxicity.

1. Introduction

Medicinal plants remain a trusted source of healthcare for the treatment of fevers and related ailments (Nascimento et al., 2000). The commendable antimicrobial properties of medicinal plants have made them a focus in the discovery of future antibiotics. Given that fever is typically the earliest symptom of an infection and usually the driving factor for a patient to seek healthcare, fever-reducing plants are among the most frequently used medicinal plants, with a high majority of these plants already recognised as having commercial value. However, despite their outstanding potential and availability, only a few medicinal plants have been commercialised (Van Wyk, 2008). This highlights the need for further research into the development of medicinal plants, especially those of commercial significance (Van Wyk, 2008).

Nanotechnology is suggested to be the basis of many of the main technological advancements of the 21st century. The field delivers innovative solutions to research questions in a large number of sciences including biomedicine, drug–gene delivery and electronics, to name a few (Sadeghi and Gholamhoseinpoor, 2015). Metallic nanoparticles (MNPs) are of interest because of the improved properties that result from their size and morphology (e.g., larger surface area to volume ratio) which in turn can be successfully incorporated into antimicrobial applications (Sulaiman et al., 2013; Adavallan and Krishnakumar, 2014). Among all noble MNPs, silver nanoparticles (AgNPs) have gained interest due to their unique biological properties such as antibacterial, antifungal, antiviral and anti-inflammatory activities among others (Kuppusamy et al., 2015). In this instance, AgNPs have been shown to

* Corresponding author.
E-mail address: Sandy.vanvuuren@wits.ac.za (S.F. van Vuuren).

https://doi.org/10.1016/j.sajb.2018.08.022
0254-6299/

Table 1
Medicinal plant selection with reference to traditional use to treat fever.

Plant samples	Family	Plant Part	Voucher specimen No.	Reference
Adenia gummifera Burtt Davy.	Passifloraceae	Leaves	SVV 247	Kraft et al., 2003
Artemisia afra Jacq.	Asteraceae	Leaves	SVV 173	Van Wyk et al., 2009
Dodonaea viscosa Jacq.	Sapindaceae	Leaves	HS 223	Van Wyk et al., 2009
Eucomis autumnalis (Mill.) Chitt.	Hyacinthaceae	Roots	MVSKK 002	Kose et al., 2015
Gunnera perpensa L.	Gunneraceae	Roots	MVSKK 005	Kose et al., 2015
Lippia javanica Spreng.	Verbenaceae	Leaves	SVV174	Van Wyk et al., 2009
Pelargonium sidoides DC.	Geraniaceae	Root	212,105	Van Wyk, 2008
Sclerocarya birrea Hochst.	Anacardiaceae	Bark	NZ22	Felhaber, 1997
Sutherlandia frutescens (L.) R.Br. ex W.T.Aiton	Fabaceae	Leaves	312,010	Aboyade et al., 2014
Tulbaghia violacea Harv.	Alliaceae	Roots	SVV 248	Van Wyk et al., 2009

exhibit noteworthy antimicrobial activity against a range of Gram-positive and Gram-negative bacteria (Ahmed et al., 2016; Kingslin and Ravikumar, 2016). This is of particular interest to antimicrobial researchers as modifications could curb the onslaught of the ever-increasing resistance problem posed by infectious diseases.

Generally, AgNPs are prepared by a variety of chemical and physical methods. Most of these methods are quite expensive, result in high rates of energy consumption and involve the use of toxic and perilous chemicals that are potentially hazardous to the environment creating biological risks (Roy and Das, 2015). Therefore, the need for the development of economic, eco-friendly and simple approaches for the synthesis of AgNPs has turned researchers towards green chemistry, and in this instance green nanotechnology (Rajan et al., 2015). Using plant extracts to synthesise AgNPs is more advantageous than using microbes or culture specimens because it eliminates the elaborate process of maintaining cell cultures (Roy and Das, 2015). Plant extracts act as both reducing agents and capping agents for the reduction of silver and stabilisation of NPs. This phenomenon is attributed to the presence of phytochemicals within the plant extracts such as proteins, flavonoids, alkaloids, sugars and phenolic acids. Many researchers around the globe have reported on the biosynthesis of AgNPs using plant extracts (Mukunthan et al., 2011; Bharani et al., 2012; Banerjee et al., 2014; Kotakadi et al., 2014; Kumar et al., 2014; Kalaiarasi et al., 2015; Khan et al., 2016; Chiguvare et al., 2016; Zhang et al., 2016) where this type of green synthesis of MNPs is done through a simple, quick one-pot synthesis procedure. The combination of the plant extracts with silver nitrate in the one-pot green synthesis of AgNPs is believed to result in increased antimicrobial effects, enhancing the antimicrobial properties of the individual plant extract (Durán et al., 2016).

The safety of AgNPs on human health remains unclear, with many studies giving conflicting results. Dos Santos et al. (2014) has, however, shown that the toxicity of AgNPs is highly dependent on several morphological and physicochemical characteristics of the nanoparticles. These include the size of the nanoparticles as well as the coating of AgNPs, where polysaccharide coatings, for example, lower the risk of cytotoxicity.

The aim of this study was to synthesise AgNPs from aqueous extracts of a selection of commercially relevant fever-reducing plants of South African origin as a means to enhance their efficacy. Characterisation was accomplished using different techniques as to assess their size and morphological properties. Along with this, antimicrobial studies were performed using the minimum inhibitory concentration (MIC) assay to evaluate the synthesised AgNPs for their antimicrobial efficacies. Lastly, the level of cytotoxicity of the synthesised AgNPs was assessed through the brine shrimp lethality assay (BSLA).

2. Materials and methods

2.1. Collection and preparation of aqueous plant extracts

Plant materials with voucher numbers (leaves, roots or barks) were selected on the basis of traditional use for fever and ethnobotanical importance (Table 1) and were obtained from the Walter Sisulu Botanical Garden (Johannesburg) with the assistance of Mr. Andrew Hankey, a specialist horticulturalist, who also assisted with plant identification and authentication. *Eucomis autumnalis* and *Gunnera perpensa* were sourced from Thaba-putsoa and Sehlaba-thebe National Parks in Lesotho respectively, with the aid of relevant collection permits. The collected plant parts were washed thoroughly using sterile water to remove adhered soil and debris. The plant material was completely dried at room temperature and thereafter ground into fine powder and stored in an air-tight container at room temperature for further use. For extraction, 5 g of dried plant material was mixed with 100 mL sterile deionised water and heated for 10 min to mimic traditional use. The mixture was allowed to cool at room temperature, filtered and stored at 4 °C until required for AgNPs synthesis and antimicrobial determination. Aseptic conditions were maintained in all instances to prevent microbial contamination.

2.2. Preparation of silver nanoparticles

The synthesis of AgNPs were carried out by modification of a method previously reported by Amini et al. (2017). The prepared aqueous extracts (Table 1) (10 mL; concentration: 50 mg/mL) was added to 90 mL of 1 mM silver nitrate ($AgNO_3$) (Sigma–Aldrich) to give a final stock concentration of 5 mg/mL. The solution was then heated to 80 °C for 10 min while stirring. A control which consisted of $AgNO_3$ without the plant extract was also incorporated. The appearance of a brownish-black colour was observed within a few minutes of adding the extracts, indicating the confirmed formation of AgNPs (Kuppusamy et al., 2015).

2.3. Characterisation of silver nanoparticles

2.3.1. UV–VIS spectroscopy

The synthesis of AgNPs was monitored through UV–Vis spectroscopy at a wavelength range of 400–600 nm operated at a resolution of 1 nm using Perkin-Elmer Lambda-25 spectrophotometer at a scan speed of 930 nm/min. Distilled water was used as a blank and $AgNO_3$ as the control. The samples were diluted 1:10 with distilled water to avoid errors due to high absorbance of the solution. The spectrophotometer was equipped with "UV Winlab" software to record and analyse the data. The obtained spectral data was plotted using Origin Pro software version 8.1.

2.3.2. Attenuated total reflectance-Fourier-transform infrared spectroscopy (ATR-FTIR)

The FTIR measurements were carried out to identify the functional groups on the biomolecules responsible for the reduction and capping of AgNPs (Nayak et al., 2016). The spectra were recorded using a FTIR spectrophotometer (Perkin-Elmer instruments Ltd., USA), at a resolution of 4 cm^{-1} with the sample in solution form. The spectra were scanned in a wavelength range of 4500–500 cm^{-1} by taking 25 scans per sample.