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Original research article

Fruit waste (peel) as bio-reductant to synthesize silver nanoparticles with antimicrobial, antioxidant and cytotoxic activities

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A B S T R A C T

Since last decade, biogenic synthesis of metal or metal-oxide nanoparticles is emerging as an alternative method, which is environment friendly, simple and safe to use. In this article, fruit waste (peel) extract (FWE) of three citrus fruits *viz. Citrus limon*, *Citrus sinensis*, and *Citrus limetta* were used as bio-reductant for green and sustainable synthesis of silver nanoparticles (AgNPs). As-synthesised AgNPs were characterized by using UV–vis spectroscopy, Dynamic light scattering, and High Resolution Transmission Electron Microscopy. TEM studies revealed 9–46 nm size range of synthesized AgNPs. The antimicrobial and antioxidant activities were also studied by using Agar well diffusion method and DPPH Assay, respectively. Nanoparticles showed good antimicrobial activity against both Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria. Further, bioactivity assays revealed selective cytotoxicity (anticancer) of the nanoparticles against human lung cancer cell line A549. The nanoparticles are able to induce cancer cell specific apoptosis at G0/G1 phase of cell cycle. The results showed potential mechanism of action of nanoparticles *via* augmentation of antioxidant system in cancer cells. Over all, this study show multifaceted potential bioactivities of nanoparticles generated from fruit waste.

Introduction

Nowadays green chemistry is an emphasized area of research and require some additional efforts for the implementation of sustainable methods in order to achieve the desirable products as well as to minimize and further eliminate the waste materials produced (Anastas and Warner, 1998; Raveendran et al., 2003). Nanotechnology, an emerging field of research not only concise to materials instead include biological, medical and engineering also, hence introduced nanobiotechnology, a novel multidisciplinary approach which serves as domineering technique in developing and congregation of the nanoparticles *via* green methods for biomedical applications (Iravani et al., 2014). Nanoparticles have an ample of applications in various fields such as energy science, catalysis, light emitters, photo-electrochemical, single electron transistors, mechanics, optics, electronics, optoelectronics, non-linear optical devices, space industries, chemical industries, biomedical sciences, drug delivery, tissue engineering, food, health care and cosmetics (Gupta and Gupta, 2005; Iravani et al., 2014; Scaramuzza et al., 2016; Wang and Herron, 1991). Among various nanoparticles such as Ag, Au, Ce, Pd, Pt and Zn, (Ahmed et al., 2016b, 2017a) AgNPs are well known for their constructive and beneficial inhibitory biomedical properties such as antibacterial (Sharma et al., 2009), antifungal (Panáček et al., 2009), antiviral (Trefry and Wooley, 2012), larvicidal activity, anti-inflammatory (David et al., 2014), and anti-angiogenesis (Baharara et al., 2014; Veerasamy et al., 2011) against different microbes, bacteria and fungi. Amid them, AgNPs also exhibits good catalytic activity with favourable thrombolytic as well as anticoagulant applications (Azeez et al., 2017b).

Conventionally, variable methods have been developed till date such as physical methods including thermal decomposition (Esumi

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et al., 1990), evaporation-condensation or laser ablation (Scaramuzza et al., 2016) and chemical methods involving reduction of solution to form metal clusters (Goia and Matijevic, 1998), photochemical reactions in reverse micelles (Taleb et al., 1997), sonochemical (Zhu et al., 2000), electrochemical (Rodríguez-Sánchez et al., 2000), radiation assisted (Henglein, 1999, 2001) and recently *via* microwave assisted techniques (Chen et al., 2013). As these methods utilized precarious and environmentally lethal chemicals, required high energy, or low material conversions as well as tedious purification, hence arose different issues while going through these processes mainly regarding stabilization and aggregation of nanoparticles (Iravani et al., 2014). On the other hand, biogenic synthesis is an eco-friendly and sustainable one-pot synthesis route to synthesize the AgNPs. This method provides advancement over physical as well as chemical method as there is no need to use toxic chemicals, high energy, high temperature and pressure. Generally, plant leaf extract (Ahmed et al., 2016c), fruit peel extract (Bankar et al., 2010), microbes (Fariq et al., 2017; Hulkoti and Taranath, 2014), fungi (Anand et al., 2015) and enzymes (Liu et al., 2016) have been used as a source to synthesize nanoparticles. Furthermore, the agricultural wastes (Kumar et al., 2012), plant-derived pigments (Adelere and Lateef, 2016) and metabolites of arthropods (Lateef et al., 2016e) have also been used as a source of green synthesis of metallic nanoparticles.

This is due to the easy availability of plants, cost effectiveness, rapidity and proficient production. Recent literature revealed the enthusiasm of green route to synthesize AgNPs, for example, *Azadirachta indica* leaf extract (Ahmed et al., 2017b), Banana peel extract (Bankar et al., 2010), Mangosteen leaf (Veerasamy et al., 2011), *Citrus sinensis* peel (Kahrilas et al., 2014), *Mangifera indica* leaf (Philip, 2011), *Terminalia arjuna* leaf (Ahmed and Ikram, 2015), *Murraya koenigii* leaf (Philip et al., 2011), *Punicia granatum* peel (Ahmad et al., 2012), *Cinnamomum zeylanicum* leaf (Gauthami et al., 2015), and so on as given in Table 1.

As per green synthetic strategy, renewable as well as non-toxic materials should be used and we here too utilized a completely environmentally benign method in order to synthesize the AgNPs from the fruit waste *i.e.* peel, generated from Citrus fruits namely, *Citrus limon* (lemon), *Citrus limetta* (mosambi) and *Citrus sinensis* (orange). India ranks sixth in the production of citrus fruits and so as produce their waste materials *i.e.* peel. Citrus fruits are widely cultivated in tropical and subtropical regions in dried and arid condition. *C. limetta* is also known as sweet lime and in India it is popular as Mosambi, having a large number of benefits regarding health issues, like scurvy, peptic ulcer, diabetes, skin, hair, etc. Similarly, *C. sinensis* and *C. limon* have medicinal properties and hence found vast applications in various fields regarding health problems such as cancer, cormunative, stomach imbalance, diuretic and colic, bacterial infection and weak immunity besides cosmetics, skin and hair (Hussain et al., 2015; Nisha et al., 2014). Moreover, these citrus fruit waste have potent antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Shigella flexineri*, *Candida parapsilosis* and *Candida albicans* (Chabuck and Chabuck, 2014; Dubey et al., 2011; Panáček et al., 2009). Furthermore, antioxidant property of AgNPs from green methods have also been observed by many researchers and scientists mainly on leaf and flower extract (Aksoy et al., 2013; Phull et al., 2016). Cytotoxicity or cell viability of AgNPs have been observed by some researchers (Foldbjerg et al., 2011) but in this article, antioxidant and cytotoxicity was observed on blood cell as well as lung cancer cell line A549, of biologically synthesized AgNPs *via* a green and sustainable route from the waste materials produced, mainly for environmental concerns.

Materials and methods

Fresh lemons (*Citrus limon*), S1, oranges (*Citrus sinensis*), S2 and mosambi (*Citrus limetta*), S3 were procured from a local market in New Delhi, India. Silver nitrate was purchased from Merck Chemicals, Mumbai, India and was used as purchased. Mackonkey broth and Mackonkey Agar was purchased from HiMedia, Mumbai. Fresh blood around 5 ml have been drawn from healthy individual as per the protocol number CUPB/cc/14/IEC/4483, Government of India. For blood cells, RPMI-1640 media having 5% fetal calf serum (FCS), and RBC lysis buffer (1×) were obtained from Invitrogen. CellROX® Deep Red from Invitrogen. All the cell lines were acquired from National Cell Repository, NCCS, Pune, India. For lung cancer cell line, A549, DMEM media augmented with 10% fetal bovine serum (FBS) and 1× antibiotic solution with 1% Pentsrip ($50\,U/ml^{-1}$ penicillin G, $50\,Ig/ml^{-1}$ streptomycin sulfate and $1.25\,lg/ml^{-1}$ amphotericin B) were obtained from Invitrogen. DMSO solution, Dihydroethidium (DHE) and Butylhydroxytoluene (BHT) all were purchased from Sigma-Aldrich and used without further purification. All the glassware was washed thoroughly with chromic acid followed by distilled water and then transferred to oven for being dried before the experiment.

Table 1
Comparison of different source and their biosynthesized nanoparticles.

Material required	Morphology	Size	Applications	References
Leaf extract of *Adiantum raddianum*	Spherical, Truncated triangles, and Decahedron	10.9 nm	Toxicity against larvae of malaria and filariasis vector	Govindarajan et al. (2017)
Lactobacillus bacteria	Spherical	30–100 nm	Antioxidant activity	Dakhil (2017)
Activated sludge micromycetes, *Fusarium nivale*, *Fusarium oxysporum, Penicillium glabrum*	–	more than 100 nm 30–50 nm 20–30 nm	Metal ion toxicity	Tyupa et al. (2016)
Bacillus safensis LAU 13	–	~8.3 nm	–	Lateef et al. (2015)
Leaf and seed extracts of *Synsepalum dulcificum*	Spherical	4–26 nm	Antimicrobial, catalytic, anticoagulant and thrombolytic applications	Lateef et al. (2016a)
Pod extract of *Cola nitida*	Spherical	12–80 nm	Antioxidant activity and phytochemical contents of *Amaranthus caudatus*	Azeez et al. (2017a)
Cocoa pod husk extract	Spherical	4–32 nm	Antimicrobial, antioxidant and larvicidal activities	Lateef et al. (2016d)
Nest extract of paper wasp (*Polistes* sp)	Hexagonal, rhomboid, 3-D triangular, rod and spherical	12.5–95.55 nm	Antimicrobial, catalytic, anticoagulating and thrombolytic applications	Lateef et al. (2016b)
Cell-free extract of *Bacillus safensis* LAU 13	Spherical	5–95 nm	Anti-candida, anti-coagulant and thrombolytic activities	Lateef et al. (2016f)