GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *CURCUMA LONGA* PLANT EXTRACT AND THEIR POSSIBLE APPLICATIONS

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Abstract

Due to their wide spectrum antibacterial activity against distinct microorganisms and different mechanisms of action that may reduce the growth of resistant bacteria, silver nanoparticles may be a promising solution for the control and treatment of bacterial infections in livestock. Nanoparticle synthesis from plant extracts is tentatively offering a path for large-scale production of commercially appealing nanoparticles, such as silver nanoparticles (AgNP). In this study we report our preliminary findings regarding the phytofabrication of silver nanoparticles (AgNPs) by Curcuma longa, emphasizing on their antioxidative characteristics as expressed by the hydrogen peroxide scavenging activity and the possible applications of silver green synthesized nanoparticles in the agri-food sector. The green synthesis of AgNPs was performed by mixing curcuma extract with $AgNO_3$ solution (1 mM) in a 1:9 proportion and adjusting the pH to 10, using NaOH 0.1N solution. The solution was kept under dark conditions at room temperature. The color of the curcuma extract varied from bright yellow to pale yellow when the $AgNO_3$ was added and finally changed to dark red-brown after 24 hours, indicating complete reduction of silver ions to AgNPs. The ability of green synthesized AgNPs to scavenge hydrogen peroxide was evaluated using UV-VIS spectroscopy, by incubating the curcuma-AgNP with hydrogen peroxide. The biosynthesis of silver nanoparticles using Curcuma longa extract was confirmed by UV-Vis spectroscopy. The absorption spectra of AgNPs covered a wide range between 330-500 nm with a prominent peak at 428 nm, indicating the formation of AgNPs because this value is consistent with the range of the surface plasmon resonance (SPR) for AgNPs. Additionally, the spectroscopic analysis showed that the curcuma AgNP extract (500 µg/ml) has moderate hydrogen peroxide scavenging activity. Green synthesis of silver nanoparticles using Curcuma longa is a promising nanotechnological strategy that enables the simple, cost-effective and ecological production of silver nanoparticles that may be used afterwards for a multitude of applications, including the development of alternative antimicrobial agents by exploiting the antibacterial activity of Curcuma longa extracts and silver ions against pathogens, in the agri-food sector.

Key words: green synthesis; plant extract; silver nanoparticles; antioxidant activity; animal husbandry

INTRODUCTION

Nanotechnology is the area of studies devoted to the designing, making, application and implementation on a large scale of these nanometric structures. Moreover, the use of nanoparticles, particles with at least one aspect in the range 1–100 nm and elevated surface to volume ratios), is now widespread in areas such as medicine, industry, agriculture and pharmaceuticals [1], [2], [3], [4].

This promising and emerging technology has an enormous potential of revolutionizing the agri-food sector. Due to their physio-chemical characteristics such as size, distribution and morphology, as well as catalytic activity, optical properties, electronic, magnetic characteristics and of course antibacterial characteristics, the potential of noble metal nanoparticles, particularly silver

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nanoparticles (AgNPs), has been widely exploited [5], [6]. The wide spectrum antibacterial activity against distinct microorganisms and different mechanisms of action that may reduce the growth of resistant bacteria are making silver nanoparticles a promising solution for the control and treatment of bacterial infections in livestock [7], [8].

Curcumin, a plant-based product extracted from "turmeric", has been extensively used in medicine due to its non-toxicity and various therapeutic properties such as anti-oxidant, anti-inflammatory, analgesic, anti-inflammatory, anti-bacterial. Due to its high total polyphenol content, which may favorise the reduction of silver ions and synthesis of Ag NP, several protocols have been tested for the green synthesis (phytofabrication) of AgNPs from curcumin [9], [10].

In this study we report our preliminary findings regarding the phyto-fabrication of silver nanoparticles (AgNPs) by *Curcuma longa*, emphasizing on their anti-oxidative characteristics as expressed by the hydrogen peroxide scavenging activity and the possible applications of silver green synthesized nanoparticles in the agri-food sector.

MATERIAL AND METHOD

Chemical and plant extract

Pure organic turmeric powders were purchased from a local grocery store. $AgNO_3$ (99.98%) was used as a silver precursor, and was acquired from Merck (Darmstadt, Germany).

Preparations of the aqueous plant extract

The aqueous extract of turmeric powder was prepared by mixing 6.8 g of organic turmeric or curcumin powder with 100 mL distilled water. Subsequently, the mixture was boiled at 80°C for 15 minutes. After cooling at room temperature, the mixture was filtered using Whitman filter paper no.1 and centrifuged at 6000 G-force for 15 min, at 25ºC.

Phytofabrication of silver nanoparticles

The phytofabrication of AgNPs through green synthesis was performed according to the methodology described by [10], with some modifications. Briefly, 1 ml of curcuma aqueous extract was mixed with 9 ml of AgNO3 aqueous solution (1 mM) (1:9 proportion), under moderate stirring, at room temperature. The pH of the solution was adjusted to 10, using NaOH 0.1N solution and the mixture was kept under dark conditions at room temperature until the complete synthesis of AgNPs. The color of the curcuma extract varied from bright yellow to pale yellow when the AgNO3 was added and finally changed to dark red-brown after 24 hours, indicating complete reduction of silver ions to AgNPs.

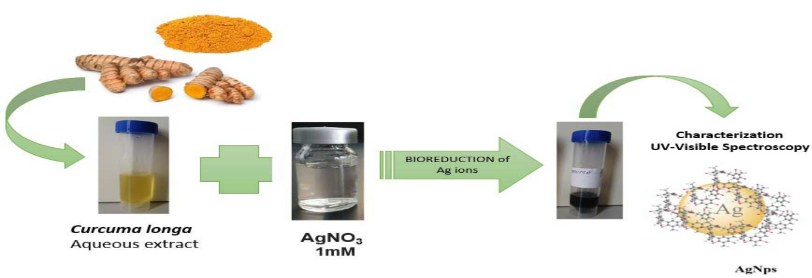

Fig. 1 Green synthesis of silver nanoparticles (AgNps) using *Curcuma longa* extract

Hydrogen peroxide scavenging activity

The ability of green synthesized AgNPs to scavenge hydrogen peroxide was determined according to the method described by [11]. Briefly, 300 µl of synthesized AgNPs (500 µg/ml) was added to a hydrogen peroxide solution prepared in 0.1M phosphate buffer saline (pH 7.4, 40 mM and 0.6 ml). After 10 minutes, the absorbance of the control (hydrogen peroxide solution) was measured at 230nm against blank solution containing phosphate buffer without hydrogen peroxide. *Curcuma longa* aqueous extract was used for comparison. The percentage scavenging of hydrogen peroxide of AgNPs was calculated using the following formula:

$$\% \text{ scavenged } (H_2O_2) = [(Ao - A1) / Ao] \times 100$$

Ao- the absorbance of the control, A1- Absorbance of the test sample

RESULTS AND DISCUSSIONS

Spectroscopic analysis

The UV-VIS spectroscopy may provide useful information regarding size, shape, and stability in aqueous suspensions of the green synthesized silver nanoparticles. Changes in color, from pale yellow to dark red-brown are corresponding to the excitation of the surface vibration of plasmon in the metal nanoparticles generated during green synthesis, indicating the formation of Curcumin-AgNPs. The absorption spectra of AgNPs covered a wide range from 330 to 500 nm with a prominent peak at 428 nm, indicating the formation of AgNPs because this value is consistent with the range of the surface plasmon resonance (SPR) for AgNPs.

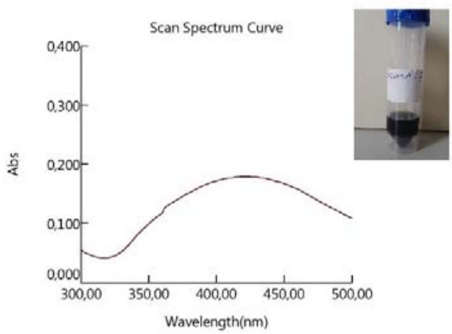

Fig. 2 Characterization of AgNPs by UV-Visible spectroscopy

Antioxidative activity

In our case, the spectroscopic analysis confirmed that green synthesized AgNPs by *Curcuma longa* presented 47.45% hydrogen peroxide scavenging activity, while the *Curcuma longa* extract presented 44.3% hydrogen peroxide scavenging activity. This results proved that the curcuma AgNP extract (500 µg/ml) has moderate hydrogen peroxide scavenging activity.

For the synthesis of nanoparticles, chemical, physical and biological techniques have been employed, however, the chemical and physical techniques can be rather expensive because they require high cost equipment and specific conditions (high temperature and high pressure), moreover generating toxic by-products [12], [13], [14].

As the demand for nanoparticles has grown exponentially, research in the field of nanotechnology has also been reoriented in the direction of developing new methods for the synthesis of metal nanoparticles, through techniques that are environmentally friendly. Green synthesis, which is defined as the use of environmentally compatible materials such as bacteria, fungi and plants in the synthesis of nanoparticles, is a low-cost, safe and "green" approach that has been developed to synthesize stable metal nanoparticles with controlled size and shape [15].

According to some reports [16], [17], plant-based synthesis, that uses peel, leaf, root, inflorescence and fruit, is more efficient for the synthesis of stabilized nanoparticles as compared to microbe-based synthesis. Synthesis of nanoparticles from biologically derived extracts provides several benefits, such as fast synthesis, high yields and, most importantly, the absence of expensive downstream processing needed to generate the nanoparticles. As a result, nanoparticle synthesis from plant extracts is tentatively offering a path for large-scale production of commercially appealing nanoparticles.

Nanoparticles have been used for some time in the medical field as diagnostic and therapeutic tools, and more recently, their possible applications have begun to be explored in the field of veterinary medicine, animal husbandry and of course the food industry [18]. In the husbandry sector, nanoparticles have the potential to improve nutrition [19] and even replace the use of antibiotics [8]. AgNPs have been shown to have a potential wide spectrum of antibacterial activity against distinct microorganisms, their mechanism of action being effective even on antibiotic-resistant bacteria [20], [21] (Fig. 3). Silver nanoparticles presented high interest for the veterinary field as well, their biomedical applications including early clinical diagnosis, treatment, targeted anticancer drug delivery, medical device coating, wound dressings, smart bandages [22], [23], [24].

Furthermore, the increasing demand for enhanced fresh food shelf life and the need for improved foodborne illnesses protection has led to the emergence of active food packaging used in the food industry. Due to their specific proprieties (i.g antimicrobial, anti-fungal, anti-yeast), silver nanoparticles can be combined with both edible and non-degradable polymers to create "smart" food packaging [25], [26], [27].

Nanotechnology has the potential to revolutionize the agriculture sector as well, its applications being beneficial for plant growth and dissease control (Fig. 3). Metalic nanoparticles have been used for the development of environmentally friendly biocides that may be used in organic farming against phytopathogens. Silver nanoparticles are one of the most commonly used nanomaterials in the agricultural field, having multiple applications such as diagnosis, target drug delivery, pest control, virus disease, delivery system of pesticides (nanopesticides) [28], [29].

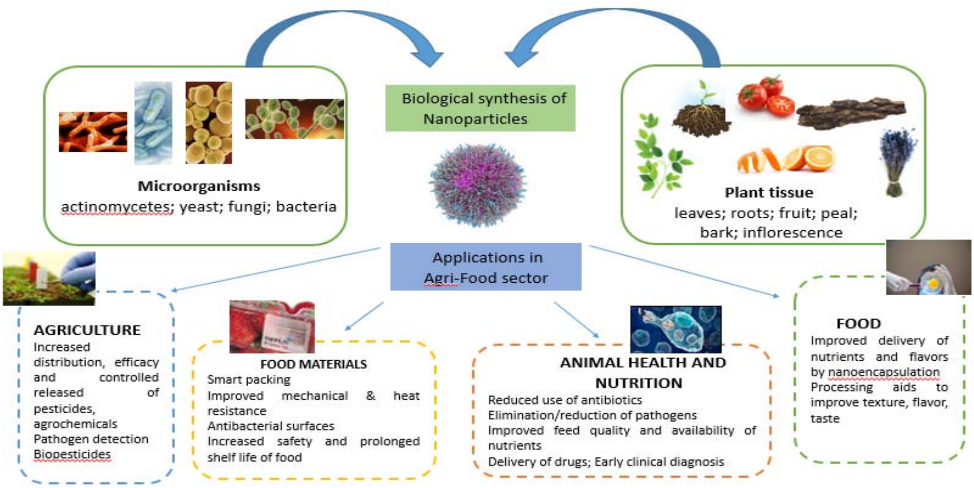

Fig. 3 Graphical presentation of biological synthesis of nanoparticles and their applications in agri-food sector

CONCLUSIONS

Green synthesis of silver nanoparticles using *Curcuma longa* is a promising nanotechnological strategy that enables the simple, cost-effective and ecological production of silver nanoparticles that may be used afterwards for a multitude of applications, including the development of alternative antimicrobial agents by exploiting the antibacterial activity of *Curcuma longa* extracts and silver ions against pathogens, in the agri-food sector.

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