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Exploitation of Cymbopogon Citratus to Synthesise Silver Nanoparticles for the Assessment of Antioxidant, Free Radical Scavenging and Reducing Power Activity: A Green Approach

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Abstract

The advent of nanotechnology revolutionized various sectors including medicine. Tradtionally, nanoparticles are synthesised by chemical and physical methods commonly utilized in laboratories and industries which are accountable for toxin production as by products and high energy consumption. Green synthesis of nanoparticles has gained more interest due to its eco-friendly processing and is considered as an essential measure to minimize the ill effects of conventional synthesis methods. Scientists, nowadays, are more focused to biosynthesise silver nanoparticles which acquired attention because of its antimicrobial and antioxidant activities. The present review describes the plant mediated synthesis of silver nanoparticles by leaf extract of Cymbopogon citratus which contain numerous bioactive components that avail H+ ions readily to reduce silver nitrate for synthesising consistent spherical shaped silver nanoparticles in the range of 5-35 nm in a single step. Silver nanoparticles exhibit considerable antioxidant, free radical scavenging and reducing power properties that may serve as an antidote for oxidative stress associated diseases, however advanced exploration is obligatory in this aspect.

Keywords: Characterization; Cymbopogon citratus; Green synthesis; Nanotechnology; Silver nanoparticle

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Introduction

Nanotechnology is a far reaching field of science having control over manipulation of materials, ranging 1-100nm in size, and their synthesis. The physical, chemical and biological properties of individual atoms or molecules and their corresponding bulk changes at nanoscale which are of core importance [1]. The relatively larger surface area to the volume, stability in chemical processes, enhanced mechanical strength of nanoparticles contribute to aforesaid properties. There is a rapid growth in the unique applications of nanoparticles due to their intensified properties based on their morphology [1,2].

Nanotechnology has its novel applications in sustainable production development in agriculture and is transfiguring (Figure 1) many other fields such as healthcare, biomedical, chemical industries, electronics and IT, drug gene delivery etc. [3]. Nanoparticles can be carbon based, organic and inorganic. Carbon based nanoparticles are classified into grapheme, fullerenes, Carbon NanoTubes (CNT), carbon nanofibres and carbon black. Organic nanoparticles are non-toxic and biodegradable that involves micelles, liposomes, dendrimers, ferritin etc while metal and metaloxide nanoparticles constitute inorganic nanoparticles. Gold(Au), silver(Ag), iron(Fe) , cobalt(Co), zinc(Zn), aluminium(Al), copper(Cu), lead(Pb) and cadmium(Cd) are commonly used metals for nanoparticle synthesis. Among these, silver nanoparticles have attained a special focus of researchers due to its novel properties such as good conductivity, chemical stability, catalytic, antimicrobial, anti-inflammatory and antioxidant activities [1,4]. Silver has been used from ancient times because of its antimicrobial activity across the globe. Romans, Greeks, Persians and Egyptians used silver for food storage purposes whereas in Indian culture, silver utensils are preferred till now for the preparation of "panchamrit" which is composed of curd, Ocimum sanctum and other ingredients [5]. These exceptional properties of silver nanoparticles are being exploited in the fields of agriculture, drug delivery, water treatment, biomedical, food packaging and food safety, photothermal therapy, etc [5,6].

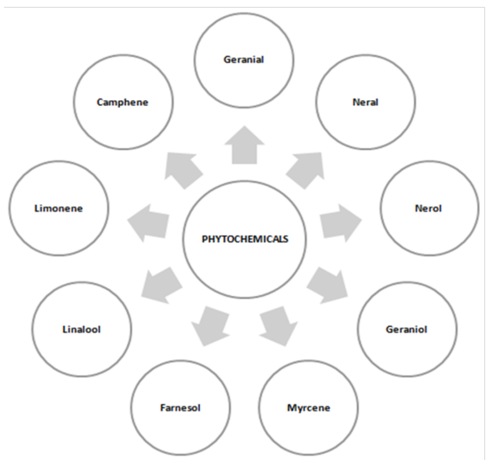

Figure 1: Important Phytochemicals in C.citratus.

Remarkable properties of silver nanoparticles led to the interest of scientific community for its synthesis by top to bottom or bottom to up approaches. Bottom to up approach or constructive method involves chemical and biological methods in which atoms self-assemble into clusters to form a nanoparticle whereas bulk materials are broken down into fine particles by lithographic techniques in top to bottom or destructive approach. Large quantities of silver nanoparticles can be synthesised by chemical method but it utilizes non-ecofriendly chemicals [1,2]. Therefore, to counter this limitation, green synthesis of nanoparticles is being adopted which is based on the principles like minimizing the waste produce, reducing derivatives and utilizing non-toxic solvents during nanoparticles synthesis to uplift environment friendliness by following sustainable procedures [7]. Green synthesis involves various biological systems such as bacteria, fungi, plant extracts and biomolecules like amino acids and vitamins for synthesizing not only silver nanoparticles but others like gold and graphene too [4]. Because of easy processing in nanoparticles production on large scale and the presence of natural phytochemicals capable of reducing metal salts into metal nanoparticles, green synthesis of metallic nanoparticles by plant extracts is preferred over bacteria and fungi mediated synthesis [1,8].

Plant extracts from various plants such as Azadirachta indica, Aloe barbadensis, Avena sativa, Citrus limon, Osimum sanctum Coriandrum sativum, Brassica juncea and Cymbopogon citratus have been utilized for the synthesis of silver and gold nanoparticles [7]. Cymbopogon citratus commonly known as lemon grass is a perennial tall grass, belonging to Gramineae family, is native to India and Sri Lanka with an economic life span to 5 years [9]. Various investigations suggest that C. citratus extract composition varies on the basis of their geographical origin [10]. Despite of the difference, studies have revealed that there are several reducing agents, reproducibly found, responsible for the reduction of silver ions into silver nanoparticles in plant extract of C. citratus including carbohydrates, terpenol, phenols, flavanoids, phenols etc. [11].

Based on the antimicrobial and antioxidant properties of silver, and higher antibacterial potential along with the presence of bioactive components in Cymbopogon citratus, studies were conducted for the C. citratus mediated synthesis of silver nanoparticles. In light of recently published findings, the present review summarizes C. citratus mediated silver nanoparticles synthesis and its antioxidant properties, free radical and reducing power potential.

Phytochemical Screening of Cymbopogon Citratus

Substantial study has been done for selecting plants with novel properties that could be of welfare to mankind. C. citratus was reported to be potent on the basis of its antibacterial potential out of six selected plants viz, Azadirachta indica, Plumria obtuse, Capsicum annum, Phylanthus emblica, Sapindus mukorossi and Cymbopogon citratus. Previous researches suggest that there are antimicrobial bioactive components that are present in plants such as terpenoid and flavonoid and high amount of such phenolic compounds was reported in the findings [11]. Soliman et al, (2017) in their study for evaluating the production and quality of lemongrass reported the total phenolic content as 7.55mg GAE/g with 1.96mg CE/g flavonoid along with the essential oil bearing 0.66% of its amount in leaf extract. Essential oil was characterized by the abundance of monoterpenes (96.37%), sequiterpenes (1.25%) and diterpenes(0.21%). Researches also confirmed the presence of geranial, neral, myrcene, citronellene, limonene oxide, geranoil and linalool [12]. Of all the essential oil components, citral, the combination of neral and geranial isomers [13], was identified as the major essential oil component with the reported percentage of 79.69% which reflected the high quality of lemongrass [12]. In addition to antimicrobial properties of C. citratus, it adds on the antioxidant and free radical scavenging effects. In the comparative phytochemical study in Pterospartum tridentatum, Gomphrena globosa and Cymbopogon citratus, Roriz L. et al., (2014) found flavonoids (7798.96 µg/g dw) as the major group in C. citratus because of which C. citratus showed highest inhibition to β-carotene bleaching and lipid peroxidation than P. tridentatum and G. globosa [14].

Reducing power of any compound gives the measurement of its antioxidant potential. Lawrence et al., (2015) studied antioxidant activity of C. citratus essential oil and found a reducing power value of essential oil being $p<0.01$ comparable to the standard value and reported the inhibitory action of essential oil against Nitric Oxide (NO) [15]. DPPH being a stable free radical at room temperature has ability to accept hydroxyl radical or an electron to form a stable diamagnetic molecule and so DPPH free radical reduction method has been the model for conducting study on antioxidant activity of phytochemicals. The antioxidant potential of the plant is demonstrated by parameter IC50 (half maximal inhibitory concentration) which determines the amount of inhibitory compound of a plant extract required to inhibit the DPPH radical processing by 50%. Boeira et al., (2018) showed that the antioxidant activity of phytochemicals by DPPH method was higher than their activity by the ORAC method in their investigation based on bioactive compounds of lemongrass and their antioxidant activity in fresh chicken sausage [16].

Flavonoids have a strong antioxidant and free radical scavenging activities which was supported by Pereira R.P. et al, (2009) in their investigation on antioxidant effects of Melisa officinallis, Matricaria recutita and Cymbopogon citratus where Melisa officinalis presented best antioxidant effect whereas flavonoids and phenolic compounds from C. citratus showed considerable activity against TBARS production induced by lipid peroxidation promoting agents [17].

Green Synthesis of Silver Nanoparticles

There are several routes to synthesise silver nanoparticles via bottom-up and top-down approaches which involves physical, chemical and biological processes [18].

Chemical reduction method

This is the simplest method which utilise reducing agent for the reduction of a metal salt into metal nanoparticles in presence of a solvent. Metal precursor, reducing agent and stabilizing agent are the key components for silver nanoparticle synthesis [19]. Khan Z. et al., (2011) prepared silver nanoparticles by reducing silver nitrate with aniline which acts as an adsorbing agent as well in an aqueous solution of CTAB [19]. Distinct organic and inorganic reducing agents such as sodium citrate, sodium borohydride, hydroquinone, Tollen's reagent, N, N-dimethyl formamide (DMF), polyethylene glycol etc are the commonly used. Effective stabilizing agents involving polyvinyl alcohol, polyvinylpyrrolidone, polyethylene glycol, polymethylmethacrylate and polymethacrylic acid avoid agglomeration of nanoparticle synthesis and protect from their sedimentation and loss of surface properties [8,20]. Large quantities of nanoparticles can be synthesised by this method but chemicals utilized are toxic and non-eco-friendly that could be threat to life [21].

Physical method

This method has faster processing and do not involve non-eco-friendly chemicals unlike chemical method. Out of various processes involving physical vapour condensation, mechanical milling, laser ablation, arc discharge, sputtering and evaporation condensation, laser ablation and evaporation-condensation are the most important physical processes of nanoparticle synthesis. Evaporation-condensation is carried out using tube furnace at atmospheric pressure but requires large area for its processing, high amount of energy and more time to achieve thermal stability. The small ceramic heater could be used for silver nanoparticle synthesis to evaporate source material because of the sheer temperature gradient to that of tube furnace [20]. While laser ablation involves the irradiation of the metal submerged into solvent by a laser beam to synthesise nanoparticles from the concerned metal [2]. Solati E. et al., (2013) in their experiment irradiated silver metal plate with a laser pulse of wavelength 1064 nm in acetone to synthesise silver nanoparticles [22]. Comparably, Tsuji T. et al., (2008) prepared silver nanoparticles from a silver plate by performing laser ablation in Polyvinylpyrrolidone (PVP) and showed that addition of PVP leads to increase in efficiency and stability of nanoparticles [23]. Pulsed photoacoustic study of silver nanoparticles produced by laser ablation showed reduced yield of Ag-NPs during the first hundreds of laser and this production rate remain almost constant which necessities the use of high energy lasers which are quite expensive [24].

Biological method

In view of various disadvantages of chemical and physical methods [21,24] of nanoparticle synthesis involving toxic by products and chemicals, high cost and power utilization, researchers shifted their interest towards green synthesis of nanoparticles which is a single step process that requires low energy, low cost for its initiation and is eco-friendly as well [7]. Green synthesis utilizes several biological systems including microorganisms, plant extracts and small biomolecules (vitamins and amino acids) [8].

In the investigation by Fayaz M. et al., (2010) on silver nanoparticles synthesis by using fungus Trichoderma viridae and their synergistic effect with antibiotics, they showed that the antibiotics have better antimicrobial effects in the presence of silver nanoparticles [25]. Kumar G. et al., (2011) synthesised silver nanoparticles using bacteria Pseudomonas aeruginosa BS-161R. Silver cations were reduced on treating silver nitrate with Pseudomonas aeruginosa culture at room temperature producing nanoparticles of size 13nm which exhibited strong antimicrobial activity against gram-positive and gram-negative bacteria [26]. The researches confirmed the strong antimicrobial activities of silver at nanoscale. Similarly researchers have synthesised silver nanoparticles exploiting various other bacterial species such as E.coli, Lactobacillus casei, Bacillus cereus, Arthrobacter gangotriences, Bacillus amyloliquefaciens, Bacillus indicus, Bacillus cecembensis, Enterobacter clocae, Pseudomonas proteolytica etc [7]. However, low yield of nanoparticles and culturing microbes under natural conditions are the major drawbacks of bacterial synthesis of silver nanoparticle along with limited shapes of synthesised AgNPs [8,27].

Keeping in view of the aesthetic sense, plant extract mediated nanoparticle synthesis has drawn attention of researchers due to its eco-friendly, non-pathogenic, cost efficient and simple processing, and majorly due to the presence of naturally occurring phytochemicals used as reducing agents. Researchers have exploited various plants for synthesizing silver nanoparticles including Azadirachta indica, Medico sativa, Aloe barbadensis, Citrus lemon, Brassica juncea etc. Dwivedi R. (2013) synthesised silver nanoparticles using two medicinal plants viz Jasminum grandifollium and Cymbopogon citrullus. They utilized natural reducing and capping agents found in leaves which rapidly reduced silver cations to silver nanoparticles with color changing to dark brown from pale yellow during reaction [28]. Recently Keshari et al., (2020) synthesised silver nanoparticles using leaf extract of Cymbopogon citratus. The silver nanoparticles synthesis was confirmed by visible colour change resulting to reddish brown. Their phytochemical analysis of C. citratus confirmed the presence of phenols, tannins, carbohydrates, proteins, saponins, flavonoids, glycosides, and terpenoids [29]. A simple protocol was followed to synthesise silver nanoparticles from the aqueous solution of silver nitrate using C. citratus leaf extract (Figure 2).

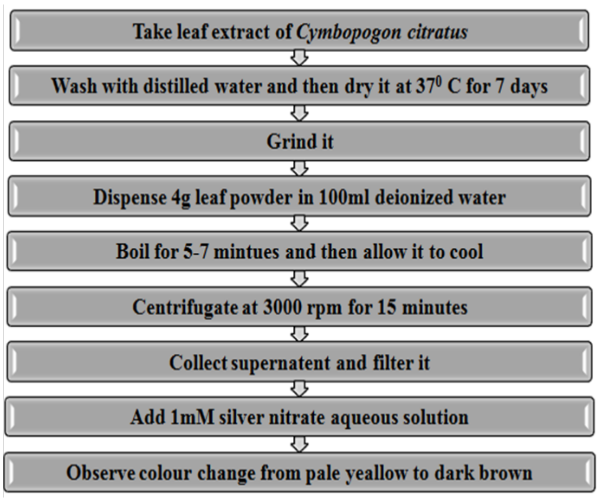

Figure 2: Protocol for synthesis of silver nanoparticles using Cymbopogon citratus.

Characterization of Silver Nanoparticles

Different shape and size of nanoparticles are responsible for their different physical and chemical properties and therefore it becomes important to analyse the synthesised nanoparticles using various analytical tools including UV-Vis spectroscopy, SEM, TEM, FTIR, XRD, DLS etc.

UV-Visible spectroscopy and x-ray diffraction spectroscopy (XRD)

UV-vis spectroscopy is useful for primary characterization of silver nanoparticles which checks the stability of nanoparticles and can also be used for monitoring size of silver nanoparticles [4]. The silver nanoparticle produced by Cymbopogon citrullus showed absorption peaks at 198-206nm on UV-Vis spectroscopy in the range of 190-1100nm [28]. Whereas XRD spectroscopy calculates the crystalline size of a nanomaterial and confirmed the crystalline C. Citratus mediated silver nanoparticle formation by obtaining peaks at 2θ valus of 38.060, 44.230 and 67.430 [26].

Scanning electron microscopy (SEM) and transmission electron microscope (TEM)

SEM employs focusing of fine electron beam to the sample with electrostatic or electromagnetic lenses to obtain high resolution images of the surface of sample whereas an electron beam is transmitted through the sample that generates scattered and unscattered electrons which are focused by electromagnetic lenses in order to generate electron diffraction and images of varying darkness depending upon the density of unscattered electrons [30]. In previous studies, SEM images confirmed the presence of uniform spherical silver nanoparticles and showed the presence of large silver nanoparticle that might be resulted due to aggregation of nanoparticles while TEM marked the presence of spherical shaped polycrystalline silver nanoparticles synthesised by using C. citratus with varying sizes ranging from 5 to 35nm [29].

6.3. Dynamic light scattering (DLC)

It analyses size distribution profile of nanoparticles in which the sample is subjected to laser beam following detection of fluctuations of scattered light, with some scattering angle, resulting from Brownian motion of particles. A study analysed the synthesised silver nanoparticles using Coptis chinensis by DLC and observed the mean diameter of AgNPs in optimum condtions which was 135.8 nm. [31].

Fourier transform infra-red (FTIR)

It identifies the potential functional groups of phytocompounds which are responsible for the reduction and capping of silver nanoparticles. FTIR analysis of C. citratus silver nanoparticles (CCAgNPs) showed absorption band at 3349cm-1 for carboxylic acid, 2952cm-1 for alkane, 1616cm-1for primary amines, 1418cm-1 for aromatic compounds [29].

Energy dispersive x-ray spectroscopy (EDX)

This technique quantitatively and qualitatively analyses the elements that may have their involvement in nanoparticle synthesis. In a study of synthesising silver nanoparticles from plant leaf extract of C.citratus, the elemental profile showed a higher count at 3keV [32].

Antioxidant, Free Radical Scavenging and Reducing Power Properties of Silver Nanoparticles

Previous studies have shown that silver nanoparticles exhibit antioxidant activity by antioxidant determining methods involving spectrophotometric methods, electrochemical methods, chromatographic methods, biosensors method and nanotechnological methods. Halliwell and Gutteridge have defined antioxidants as the substances that have ability to prevent, delay or remove oxidative damage to a target molecule. An ideal antioxidant is active even at low concentrations and has ability to react with free radicals. In vivo antioxidant response is usually generated by the synthesis of various enzymes such as superoxide dismutases, glutathione peroxidases, catalases etc, or by supplying flavonoids, polyphenols, niacin, vit-C, D, E and A, elements etc [33]. To determine antioxidant properties of silver nanoparticles, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method is used usually. Many researchers determined the antioxidant activity of silver nanoparticles synthesised from plant extracts. Lately, Keshari et al., (2020) evaluated antioxidant activity of silver nanoparticle, C. citratus and vitamin C by DPPH method and it was found that C. citratus extract has maximum antioxidant activity with 53% among the three and vit C and silver nanoparticle with 51% and 48% antioxidant activity respectively. Free Radical scavenging activity of the three was also determined by using hydrogen peroxide, hydroxyl and superoxide free radicals where silver nanoparticles showed considerable free radical scavenging activity. The reducing power assay revealed that silver nanoparticles have lesser reducing power than C. citratus extract [29]. Similarly, Ajayi E et al., (2017) reported a considerable free radical scavenging activity of silver nanoparticles of C .citratus with their IC50 value of 30.60 µg/ml whereas rutin and vit C found to have an IC50 of 15.63µg/ml [32]. The lesser antioxidant activity of silver nanoparticle than plant extract is due to the presence of phytochemicals [33].

Conclusion

Significant progresses have been made in synthesis and processing of nanoparticles in last few decades. Green approach of nanoparticle synthesis using plant extracts bestow an economic and energy efficient method which generate non-toxic products and hence safer to the beings. Among the wide range of metal nanoparticles, silver nanoparticles are most common because of its high electrical and thermal conductivity and promising antioxidant, antimicrobial, free radical scavenging and catalytic properties which make them suitable for biomedical applications and hence developed interest of the scientific community towards its biosynthesis. The extracellular synthesis of silver nanoparticles using leaves of Cymbopogon citratus seems to be quite easy, sustainable and a rapid process that results in uniform silver nanoparticle production. The antioxidant, free radical scavenging and reducing power activities of these conveniently synthesised silver nanoparticles make them a potent candidate for various applications including pharmaceuticals and biomedical but thorough investigations regarding this are still required.

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