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Catalytically and biologically active silver nanoparticles synthesized using essential oil

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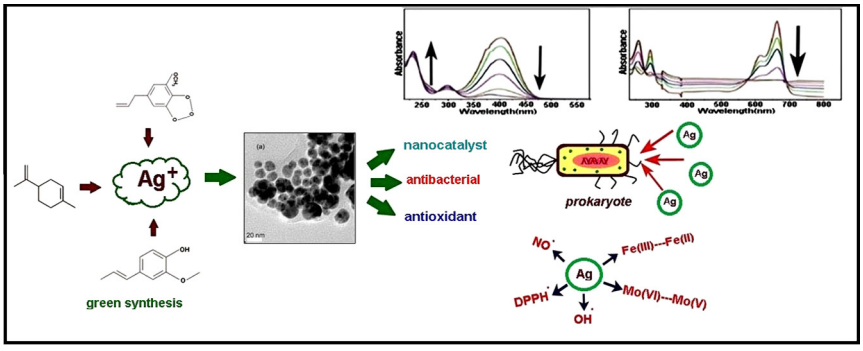
HIGHLIGHTS

- Essential oil is used for the synthesis of silver nanoparticles.
- Rapid, cost-effective, environmentally benign method is suggested.
- Efficiency of the nanocatalyst is portrayed in the degradation of a cationic dye and an organic pollutant.
- The synthesized biogenic silver nanoparticles act as potent free radical scavengers and antibacterial agent.

GRAPHICAL ABSTRACT



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ABSTRACT

There are numerous reports on phytosynthesis of silver nanoparticles and various phytochemicals are involved in the reduction and stabilization. Pure explicit phytosynthetic protocol for catalytically and biologically active silver nanoparticles is of importance as it is an environmentally benign green method. This paper reports the use of essential oil of *Myristica fragrans* enriched in terpenes and phenyl propenes in the reduction and stabilization. FTIR spectra of the essential oil and the synthesized biogenic silver nanoparticles are in accordance with the GC–MS spectral analysis reports. Nanosilver is initially characterized by an intense SPR band around 420 nm, followed by XRD and TEM analysis revealing the formation of 12–26 nm sized, highly pure, crystalline silver nanoparticles. Excellent catalytic and bioactive potential of the silver nanoparticles is due to the surface modification. The chemocatalytic potential of nanosilver is exhibited by the rapid reduction of the organic pollutant, para nitro phenol and by the degradation of the thiazine dye, methylene blue. Significant antibacterial activity of the silver colloid against Gram positive, *Staphylococcus aureus* (inhibition zone – 12 mm) and Gram negative, *Escherichia coli* (inhibition zone – 14 mm) is demonstrated by Agar-well diffusion method. Strong antioxidant activity of the biogenic silver nanoparticles is depicted through NO scavenging, hydrogen peroxide scavenging, reducing power, DPPH and total antioxidant activity assays.

Introduction

Molecular manipulation at the nanoscale has undergone progressive changes ever since its dawn before 4th century AD. Though the methods of nanoparticle (NP) synthesis extends over

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a wide range, green synthesis methods has gained wider acclaim owing to the increasing awareness on the undesirable changes of the environment posed by several synthesis strategies. Phytosynthesis of nanoparticles uses phytoconstituents of the plant parts as reducing and capping agents. This ecofriendly method does not demand the use of high temperature, pressure or energy [1]. Development of a facile, pure, specific phytosynthetic route leads to the use of volatile aromatic oil. Kumar et al. [2] have synthesized metal NPs embedded paints based on vegetable oil. The auto oxidation of drying oils have been used for the reduction of metal salts to synthesize metal NPs. Castor oil, palm oil and coconut oil have been used for the synthesis of metal NPs [3–6]. In the first report on essential oil mediated synthesis of metal NPs, Sheny et al. [7] have used *Anacardium occidentale* as a reducing and capping agent for the synthesis of hexagonal gold NPs.

The synthesis of silver (Ag) on a nanoscale is of importance, due to its thermal conductivity, chemical stability, catalytic and antibacterial activity [8,9]. The surface effects and quantum effects of nanoAg affects their chemical reactivity [10] and gives them unique mechanical, optical and electric properties [11]. Recent reports [12–17] suggest biological method for synthesis of antifungal, antibacterial, antioxidant, robust, biocompatible Ag NPs. Size, shape, surface area to volume ratio and the nature of the surface modifier act as limiting factors of the synthesized Ag NPs imparting adequate therapeutic potency.

Exploitation of the antibacterial potential of nanoAg can be traced back to ancient times. Oligodynamic action coupled with a broad spectrum of targeted bacteria prompts the use of Ag based nanostructured materials as efficient antibacterial agents [18]. The rapid multiplication and proliferation of drug resistant bacteria is curbed by Ag NPs fortifying its use in burn treatments, water filters and as an antimicrobial finish on fabrics [15]. Antioxidant nanoAg repairs reactive oxygen species (ROS) damage to cell components and cell function disruption enhancing the immune system. The conjoint application of nanoAg as an antibacterial and an antioxidant agent ensures its use as a biomedical nanoproduct. NanoAg extends its aptness as a catalyst in pollution treatment, due to its small size, larger surface area to volume ratio and greater accessibility of the surface atoms. Ag NPs have attracted the interests of scientists in its use as a competent catalyst in the reduction of major pollutants of the dye industry that pose serious health risks.

Myristica fragrans (*M. fragrans*) is an evergreen tree, successfully cultivated in South India. There are reports [19,20] on the hepatoprotective and anti cancer activity of Myristicin and anti-inflammatory property of *t* – Caryophyllene; the essential oil constituents. The leaf oil exhibit significant antimicrobial and larvicide activity and cytotoxic activity against MCF-7 breast cancer cell line and A 357 epidermal skin cancer line [19].

In the present study, Ag NPs are synthesized using the essential oil extracted from leaves of *M. fragrans*. The catalytic potential of the synthesized Ag NPs is tested by studying the degradation of para nitro phenol (4NP), an anthropogenic pollutant and methylene blue (MB), a thiazine dye. The antibacterial activity of the biogenic Ag NPs against the pathogenic *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) is studied using Agar well diffusion method. The in vitro antioxidant activity of Ag NPs is investigated through a series of assays.

Materials and methods

Fresh green leaves of *M. fragrans* were collected from Thiruvananthapuram. Silver nitrate ($AgNO_3$), 4NP and sodium borohydride ($NaBH_4$) were procured from Sigma–Aldrich; MB and acetone from Merck were used. Demineralised water has been used throughout the experiment.

300 g fresh leaves of *M. fragrans* are hydro distilled in a Clevenger apparatus, yielding 3 mL essential oil (yield 1%). The aromatic oil, diluted using acetone (1:170) is used for the synthesis of Ag NPs. 1 mL of diluted oil is added, with vigorous stirring to 30 mL of 2.14×10^{-4} M $AgNO_3$ at 100 °C and pH 7 to get the colloid a_1. The experiment is repeated with 2, 3, 4, 5 mL diluted oil to get colloids a_2, a_3, a_4 and a_5, respectively. Colour change from yellow to golden yellow and to red, indicated the enhanced formation of Ag NPs, with quantity of diluted oil. 2 mL diluted oil is added with vigorous stirring to 30 mL boiling solution of $AgNO_3$ (2.14×10^{-4} M) at varying pH conditions of 7, 8, 9 and 10 to obtain colloids a_2, a_6, a_7 and a_8, respectively.

Catalytic activity of Ag NPs

The efficiency of Ag NPs as heterogeneous catalyst in the reduction of 4NP and MB using $NaBH_4$ is investigated. The stock solutions, 7.1×10^{-3} M 4NP and 0.25 M $NaBH_4$ are prepared. After stirring 1 mL each of 4NP and $NaBH_4$ in 23 mL aqueous medium, 1 mL of Ag nanocatalyst is added and vigorously stirred. UV–vis spectra are recorded after regular intervals of time to study the degradation of 4NP. The procedure is repeated with 9.9×10^{-2} M $NaBH_4$ and 10^{-3} M MB. The progress of the rapid degradation of the cationic dye is studied through UV–vis spectra taken at regular intervals.

Antibacterial and antioxidant potential of Ag NPs

Agar-well diffusion method is used for the antibacterial studies of green synthesized Ag NPs. Mueller–Hinton plates are seeded with *S. aureus* and *E. coli*. Wells of 10 mm are bored and samples of 25, 50,100-μL concentration are added. After incubating at 37 °C for 24 h, the antibacterial activity is assayed by measuring the diameter of the zone of inhibition formed around the well. Experiments are done in triplicate and mean values are presented. Gentamycin is used as the positive control [21,22].

Free radical scavenging activity, reducing power, total antioxidant activity of the green synthesized Ag NPs is exemplified through nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power activity, 2,2-diphenyl -1-picryl hydrazyl (DPPH)) assay and total antioxidant activity (description of the procedure of antioxidant assays is given in Supplemental information). Absorbance of the Ag NP bound coloured complexes of radicals or ions are determined (A_{test}) in addition to the absorbance of a similar mixture without the test solution ($A_{control}$). Percentage inhibition (%inhibition) is ascertained using the equation,

$$\%\text{inhibition} = [(A_{control} - A_{test})/A_{control}]100 \quad (1)$$

Instrumentation

UV–vis spectra are recorded using Perkin–Elmer Lambda-35 spectrophotometer with a scanning speed of 480 nm/min and a slit width of 1 nm. TEM samples are prepared by dropping the Ag colloid on carbon coated copper grids. The images are taken using TecnaiG^2 30 TEM after the evaporation of the solvents. XRD pattern is obtained using XPERT-Pro diffractometer operating at 30 mA current and 40 kV voltage for the scanning angle 2θ from 20° to 100°. The diffraction pattern is obtained by irradiating with Cu Kα radiation with λ of 1.5406 Å. FTIR spectra are recorded using IR Prestige-21 Shimadzu spectrophotometer. GC–MS analysis was carried out on a HP Chem Gas Chromatogram fitted with a DB5 silica column coupled with a model 5973 mass detector. Essential oil components were determined on comparison of the mass spectra with Wiley 275.L database. (GC–MS spectra given in Fig. S1 of Supplemental information).