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Green Synthesis of Gold Nanoparticles Using Eucalyptus and Piper Longum and Its Subsequent Antioxidant Activity Evaluation

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Abstract

Gold continues to be one of the oldest dental restorative materials which has been used for dental repairs for more than 4000 years and remains an important metal in the dental sector. In a world where the importance of nanoparticles has been well established and its preparation has become much easier, it is important to analyse if these nanoparticles can be extracted from a plant based source as well. Along with its extraction, assessment of each property of the nanoparticle is essential. A few ingredients used in Ayurveda which can also be found being used in almost every household is pepper and eucalyptus and over the years, its importance has remained constant, if not showing an increase. The aim of this study was to extract gold nanoparticles using Eucalyptus and Piper longum and evaluate the antioxidant activity of the derived gold nanoparticles. Preparation of plant extract was done following which, extraction of gold nanoparticles was performed. Antioxidant properties of the gold nanoparticles were tested by a DPPH assay method and compared against the antioxidant gold standard, butylated hydroxytoluene (BHT). The percentage of absorbance was calculated and data was analyzed. The results demonstrated the presence of elemental gold. Both, plant extract derived AuNPs exhibited significant free-radical scavenging activity indicating that they possess antioxidant effects.

Keywords - Eucalyptus, antioxidant, Nanoparticles, Novel technique, Piper longum.

Introduction

Chrysotherapy, or the use of gold in treatment, has been around for a very long time. Gold was utilized by ancient cultures in Egypt, India, and China to heal ailments like smallpox, skin ulcers, syphilis, and measles (1, 2, 3). Currently, gold is used in medical devices such as pacemakers, gold-plated stents, and middle ear implants to treat cardiac illness. Gold alloys are also used in dental restoration (7, 8). Several organogold compounds with promising anticancer, antibacterial, antimalarial, and anti-HIV properties have surfaced in recent years (9, 10 ,11).

Even though dental alloys are currently in use and the bulk of them are made of non-precious alloying metals, passivation can develop with time.

It has been discovered that gold continues to play a crucial role in supplying the alloy's corrosion resistance despite its auxiliary use in dental alloys (12). Given that it has been used for tooth repairs for more than 4,000 years, gold is the most historic restorative material in dentistry. These early dental applications prioritized appearance over masticatory function. Today, gold is still widely used in dentistry, with yearly usage generally estimated to be around 70 tonnes worldwide (13).

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To highlight the exceptional performance that competing materials must exhibit if they are to displace gold from current uses, it is thought appropriate to review the current gold-based technology available today. This is because there are an increasing number of alternative materials available for dental repairs. Additionally highlighted are fresh gold-based dental innovations. In both orthodontics and restorative dentistry, gold is utilized either as a pure metal or in alloys with other noble and base metals. This application of pure gold is restricted to direct fillings of small occlusal cavities, and neither its use nor the characteristics of direct filling gold are standardized. However, the pure gold employed in this application has a very low 0.2% proof stress (30 MPa), a high elongation (45%), and is quite soft (HV 25). Because of this, it can be cold worked extremely readily, which is a need for filling a hollow. Dental gold fillings are appropriate for minor cavities since they do not have a strong mechanical resistance to masticatory pressures. In recent years, the electroforming procedure has been employed with pure gold. After a porcelain veneer has been applied, electroformed inlays and onlays can be glued into cavities. Pure gold can be electroformed into tooth restorations like porcelain-veneered copings for crowns and bridgework. Unfortunately, despite the fact that this procedure is quickly becoming a commonplace one in contemporary dentistry, there is no standard for it.

Despite all of this, the ideal material for dental restorations is still an approved high gold alloy if lifespan, functionality, aesthetics, and biocompatibility are regarded as significant requirements. It is no accident that gold is consistently designated as the benchmark material to be evaluated against in all testing and development of rival materials. It's interesting to note that, with a few notable exceptions, when professional dentists are asked what kind of restoration material they prefer for themselves, the response is always gold. However, the wide variety of alternative restorative materials is drawing more and more attention. These innovative materials include dental crowns made entirely of ceramic, titanium, and cobalt/nickel base alloys. Although the latter have good aesthetic qualities, gold has long-term clinical approval. In previous literature, it was observed that herbally derived nanoparticles have had the anti-inflammatory, antioxidant and antimicrobial properties which could be utilized in further treatment modalities [14- 28]. The scope for nanoparticles is very high and can be regarded as a future modality of treatment.

Poor bioavailability and inherent toxicity are two problems with traditional medicines. Many otherwise helpful medications' therapeutic efficacy has been severely hampered by them. To get around some of these restrictions, nanoscopic systems that modify the pharmacological and therapeutic properties of molecules are being developed. Innovative nanodevices and nanostructures have been created as a result of research in this field for use in applications like medication delivery and targeting, biosensing, and diagnostics (29-37)

Numerous of these nanoparticles can enter tiny capillaries and are absorbed by the cells because of their small size (38). Many are also known to be biodegradable, immune system-unsusceptible, and biocompatible. Quantum dots (Q-dots) and gold nanoparticles (AuNPs), two prominent examples, may also have distinctive optical and electrical properties (39), making it possible to monitor their intracellular movement and location (39; 40).

Although the increased permeability and retention (EPR) phenomenon is used by nanoparticle-based treatments to transport drugs into malignancies. Not all cancers can benefit from this impact, especially when relatively big nanoparticles are delivered (41).

In addition, because heat diffusion from hot particles increases the injured tissue area with prolonged exposure times, selective photothermolysis is not achieved for tiny

tumours or single metastatic cells (42). Therefore, new techniques for selective nanoparticle distribution must be created in order to accomplish successful therapy (43).

The production of gold nanoparticles in a variety of sizes and shapes has been described using a number of different techniques in the literature. The most common synthetic approach uses citrate as a reducing agent to chemically reduce gold salts like hydrogen tetrachloroaurate (45). Monodisperse gold spherical nanoparticles with a diameter between 10 and 20 nm are created using this technique. However, this process produces bigger gold particles with diameters between 40 and 120 nm with low yields, frequently leading to polydisperse particles (46). In contrast, monodisperse AuNPs with diameters between 30 and 100 nm have been synthesised using a seeding method. The process is based on the use of AuNPs' surface as a catalyst for the reduction of Au3+ by hydroxylamine. Murphy and colleagues used this seed-mediated growth strategy after that to manage the shape and size of the nanoparticles (47). Gold salt growth solution, rod-shaped micellar template (cetyltrimethylammonium bromide; CTAB), reducing agent (ascorbic acid), and a small amount of silver ions for shape induction were combined with borohydride-reduced gold nanoparticle seeds (3-4 nm diameter) to produce spherical or rod-like gold nanoparticles (48). Additionally, they have enhanced this technique to obtain monodispersed, multiple-shaped AuNPs in yields that are higher than previously reported.

Other techniques for creating AuNPs include physical reduction (49) (producing large-scale hollow Au nanostructures), photochemical reduction (48) (producing cubic AuNPs), biological reduction (50) (using peptide amphiphile molecular hydrogels to create different shapes of AuNPs), and solvent evaporation methods (51) (2D Au super lattices).

The aim of this study was to extract gold nanoparticles using Eucalyptus and Piper longum and evaluate the antioxidant activity of the derived gold nanoparticles. Null hypothesis of the study would be that gold nanoparticles do not possess any antioxidant activity.

Materials And Methodology

Preparation of Plant Extract

One gram of Eucalyptus (Eucalyptus teriticornis) along with one gram of Piper longum was taken in a beaker containing 100 mL distilled water and mixed. Both the powders are commercially available products of the leaves of the plants. The solution was boiled using the machine, Labquest HME 500, at 60 degrees for 15 min, following which the solution was passed through a filter paper, thus yielding us the plant extract of desire.

Preparation of Gold Nanoparticles using the Plant Extract

Gold chloride solution 5mL was taken and added to 80mL of the plant extract and the solution was allowed to undergo continuous shaking in circular motions. After 3 days of periodically checking the level of the nanoparticles, the solution was centrifuged for 10 minutes. The agglomerate of gold nanoparticles at the bottom of the test tubes were isolated from the supernatant liquid.

Antioxidant Activity

DPPH assay was used to test the antioxidant activity of biogenic synthesized gold chloride nanoparticles. Diverse concentrations (2-10 µg/ml) of pepper and eucalyptus leaf extract interceded gold chloride nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control. The percentage of inhibition was determined from the following equation.

$$\% \, inhibition = \frac{Absorbance \, of \, control - Absorbance \, of \, test \, sample \, \times 100}{Absorbance \, of \, Control}$$

Figure 1. Measurement of Plant Extract using Eucalyptus and Piper Longum

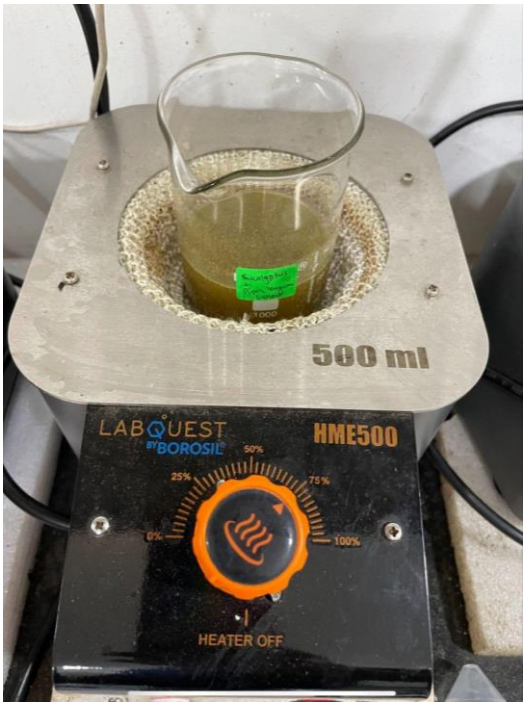

Figure 2. Preparation of Plant Extract using Eucalyptus and Piper Longum

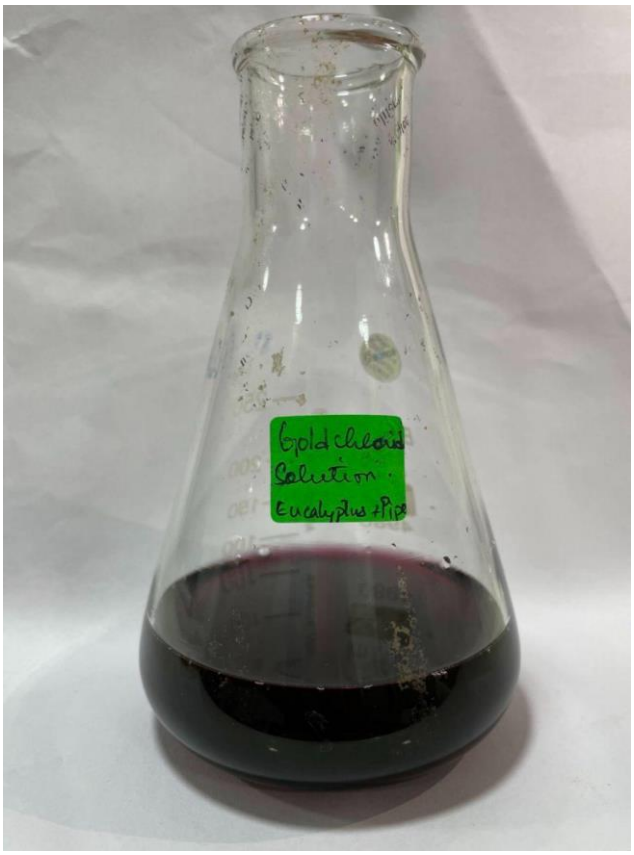

Figure 3. Preparation of Gold Nanoparticles using Eucalyptus and Piper Longum

Result & Discussion

Visual Observation

The reaction mixture of 1 mM aqueous gold chloride solution and plant extract was pink, indicating the reduction of gold ions into gold nanoparticles (Figure 1) The mixture first turned brownish pink. Then, the color of the solution changes strongly to pink, while the incubation time increases from 30 min to 48 h. The solution is color stable with no change in intensity, indicating the completion of the reduction process. A color change occurred in the reaction mixture due to the excitation of surface plasmon resonances in the nanoparticles. This important observation suggests the reduction of gold ions and the biosynthesis of gold nanoparticles. Previously, Singaravelu et al reported that the gold nanoparticle synthesis process started in 1 h and the whole process was completed in 15 h. Kannan et al started gold nanoparticle synthesis at 50 minutes and completed the process within 48 hours of incubation. However, in the present study, the gold nanoparticle synthesis process started rapidly at 50 min for Piper Longum and Eucalyptus, respectively, and ended with an incubation time of 36 h.

Table 1. Antioxidant Properties of Plant Derived AuNPs by DPPH Free Radical Assay

Test	Concentration (ug/ml)	Plant Derived AuNPs (%)	Standard (%)
DPPH ASSAY	10	51.8	56.3
	20	59.9	61.4
	30	62	69
	40	70.7	73
	50	87	81

The antioxidant activity of formulated AuNPs and the seaweed extract was estimated by evaluating the percentage inhibition of DPPH radicals. The DPPH radical scavenging activity of AuNPs was found to be directly proportional to the concentration of the plant extract and its synthesized AuNPs. The results exhibited significant inhibition of DPPH free radicals in a dose-dependent manner at 10, 20, 30, 40 and 50ug/ml. AuNPs showed marked radical scavenging activity when compared to gold standard. The results revealed that the plant extract derived AuNPs were found to be potent which was comparable with that of standard ascorbic acid by a significant amount.

Conclusion

In this present study, we have reported an eco-friendly method for green synthesis gold nanoparticle synthesized from plant extract. The results demonstrated the presence of elemental gold. Plant extract derived AuNPs, exhibited significant free-radical scavenging activity indicating that they possess antioxidant effects.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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