

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

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### Abstract

Gold is the oldest dental restorative material, used for dental repairs for more than 4000 years and remains an important metal included in the dental sector. In a world where nanoparticle importance has been well established and preparation of nanoparticles has become much easier, it is important to assess if these nanoparticles can be extracted from plants as well. Along with its extraction, analysis of each property of the nanoparticle is essential. Pepper and eucalyptus remain two of the most important ingredients used in ayurveda and can be easily found in every household. The aim of this study was to extract gold nanoparticles using Eucalyptus and Piper longum and evaluate the antibacterial activity of the derived gold nanoparticles. Preparation of plant extract was done following which, extraction of gold nanoparticles was performed. Antiinflammatory properties of the gold nanoparticles were tested by albumin denaturation method and compared against the anti-inflammatory gold standard, Diclofenac sodium. The protein denaturation levels were measured, and the data was compiled. From this study, it can be concluded that gold nanoparticles derived from pepper and eucalyptus can be used as a potential source of anti-inflammatories.

**Keywords** - Eucalyptus, antiinflammatory, Nanoparticles, novel technique, Gold, Piper longum.

### Introduction

Chrysotherapy, the use of gold in medicine, has been practiced since ancient times. Ancient cultures such as Egypt, India and China used gold to treat diseases such as smallpox, skin ulcers, syphilis and measles [1 - 3]. Gold is currently used in medical devices, including pacemakers and gold-plated stents [4, 5] for heart disease; gold implants for the middle ear [6] and gold alloys in dental restorations [7, 8]. In recent decades, several organo-gold complexes have emerged with promising antitumor, antibacterial, antimalarial, and anti-HIV activities [9-11].

Although dental alloys are used and most of the dental alloy metals used are non-precious alloys, passivation occurs over time. With the additional use of gold in dental alloys, it has been found that gold remains most important in providing corrosion resistance to the alloy [12].

Gold is the oldest restorative material in dentistry, having been used for over 4,000 years to restore teeth. These early dental applications were based on aesthetics, not chewing. The use of gold in dentistry remains significant today, with annual worldwide consumption generally estimated at around 70 tons [13]. However, with the widening range of alternative materials available for dental restorations, it was deemed

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appropriate to review the gold-based technologies available today, thus emphasizing the superior properties that competing materials must exhibit if the gold must be the gold of its present use. The desire to move to new gold-based dental techniques are also being promoted.

Gold is used in restorative dentistry and orthodontics both as a pure metal and in alloys with precious and base metals. The use of this pure gold is limited to the direct filling of small occlusal cavities and there are no standards regarding the application and properties of direct filling gold. However, the pure gold used in this application is very soft (HV 25), has a very low yield strength of 0.2% (30 MPa) and a high elongation (45%). As a result, it can be cold worked very easily, an essential condition for filling cavities. Gold dental fillings are suitable for small cavities due to their low mechanical resistance to chewing forces. In recent years, pure gold has been used through the electroforming process. Electroformed inlays and onlays are suitable for bonding into cavities after plating. Dental restorations such as porcelain copings for crowns and bridges can be electroformed from pure gold. Unfortunately, there is no standard for this process, and it is becoming a common technique in modern dentistry.

In previous literature, it was observed that herbally derived nanoparticles have had anti-inflammatory, antioxidant and antimicrobial properties which could be utilized in further treatment modalities [14- 19]. The scope for nanoparticles is very high and can be regarded as a future modality of treatment.

Beyond this, if longevity, functionality, esthetics and biocompatibility, as well as ease of fabrication are considered important requirements, the best materials for dental restorations remain approved high-gold alloys. It is no coincidence that gold has always been defined as the standard material in which all competing materials are tested and developed. Interestingly, when practicing dentists were asked what kind of restorative material they

preferred, the answer was always gold, with a few exceptions. However, attention is increasingly turning to a wide range of alternative restorative materials. These new materials include titanium and cobalt/nickel-based alloys and all-ceramic crowns. The latter has excellent aesthetic properties but lacks the long-term clinical approval of gold.

Some of the challenges facing conventional therapies are low bioavailability and inherent toxicity. This seriously compromises the therapeutic effect of many other beneficial drugs. Nanosystems that modify the pharmacological and therapeutic properties of molecules are designed to overcome some of these limitations. Research efforts in this area have resulted in innovative nanodevices and nanostructures for applications including diagnostics, biosensing, therapeutics, and drug delivery and targeting [20 - 36].

Due to their small size [25-28,37], some of these nanoparticles can enter smaller capillaries and be taken up by cells. Many are also known to be biocompatible, undetectable by the immune system, and biodegradable. In addition, many may possess unique optical and electrical properties [38], prime examples include quantum dots (Q-dots) and gold nanoparticles (AuNPs), allowing their intracellular transport and localization to be tracked [39,40].

While nanoparticle therapies use enhanced permeability and retention (EPR) to deliver drugs to tumors. Not all tumors are sensitive to this effect, especially when relatively large nanoparticles are delivered [39]. Additionally, selective photothermal lysis cannot be achieved for small tumors or single metastatic cells because thermal diffusion from hot particles increases the area of damaged tissue and prolongs exposure time [40]. Therefore, other methods of selective delivery of nanoparticles need to be developed for effective therapy [39].

Various methods of synthesizing gold nanoparticles of different shapes and sizes are described in the literature. The most popular synthesis is the chemical reduction of gold salts

such as tetrachloric acid using the reducing agent citrate [42]. This method produces spherical monodisperse gold nanoparticles with diameters between 10 and 20 nm. However, the yield of larger (40–120 nm) gold particles produced by this method is low, often resulting in polydisperse particles [43]. reported synthesis of monodisperse AuNPs with diameters between 30 and 100 nm using a seeding method. The method is based on the reduction of Au<sup>3+</sup> by hydroxylamine using the surface of AuNPs as a catalyst. Next, Murphy and colleagues used this seed-mediated growth method to control the shape and size of the nanoparticles [44].

Seeds of reduced borohydride gold nanoparticles (3–4 nm in diameter) were mixed with gold salt growth solution, micellar rod matrix (cetyltrimethylammonium bromide; CTAB), reducing agent (ascorbic acid) and a small amount of silver ions Mixture to shape induction produces spherical or rod-shaped gold nanoparticles [42]. They also improved this method to produce monodisperse and multiform AuNPs with higher yields than previously reported [45,46]. Other methods for the synthesis of AuNPs include physical reduction [47] (large-scale hollow Au nanostructures), photochemical reduction [48] (cubic AuNPs), bioreduction [48] (for the production of amphiphilic peptide hydrogels of different shapes) AuNPs and solvent evaporation techniques [50] (2D Au superlattice). The aim of this study was to extract gold nanoparticles using Eucalyptus and Piper longum and evaluate the anti-inflammatory activity of the derived gold nanoparticles. The null hypothesis of the study would be that gold nanoparticles do not possess any anti-inflammatory 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.

### Anti-inflammatory Activity

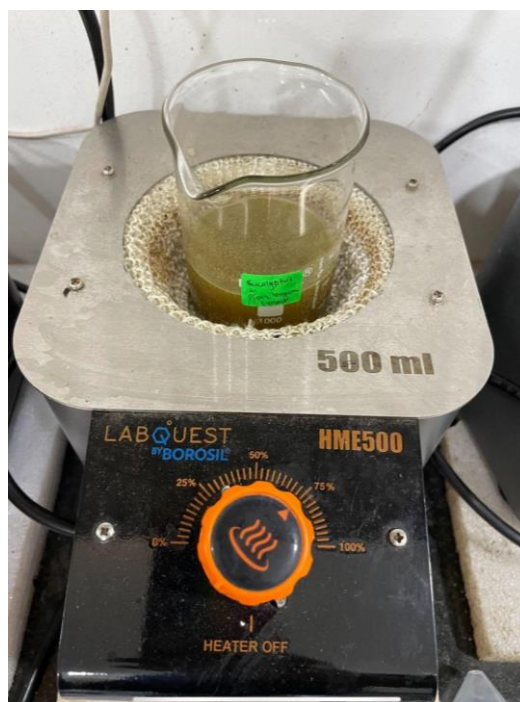
#### Albumin Denaturation Assay

The anti-inflammatory activity for gold nanoparticles from plant extract was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of nanoparticles of various fixation (10µL,20µL,30µL,40µL,50µL) was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled, and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control. Percentage of protein denaturation was determined utilizing following equation,

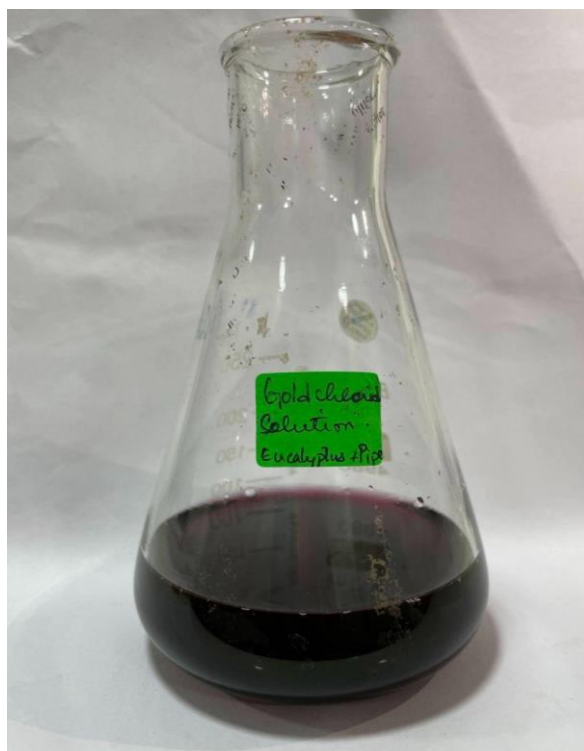
$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of Control}} \times 100$$



**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

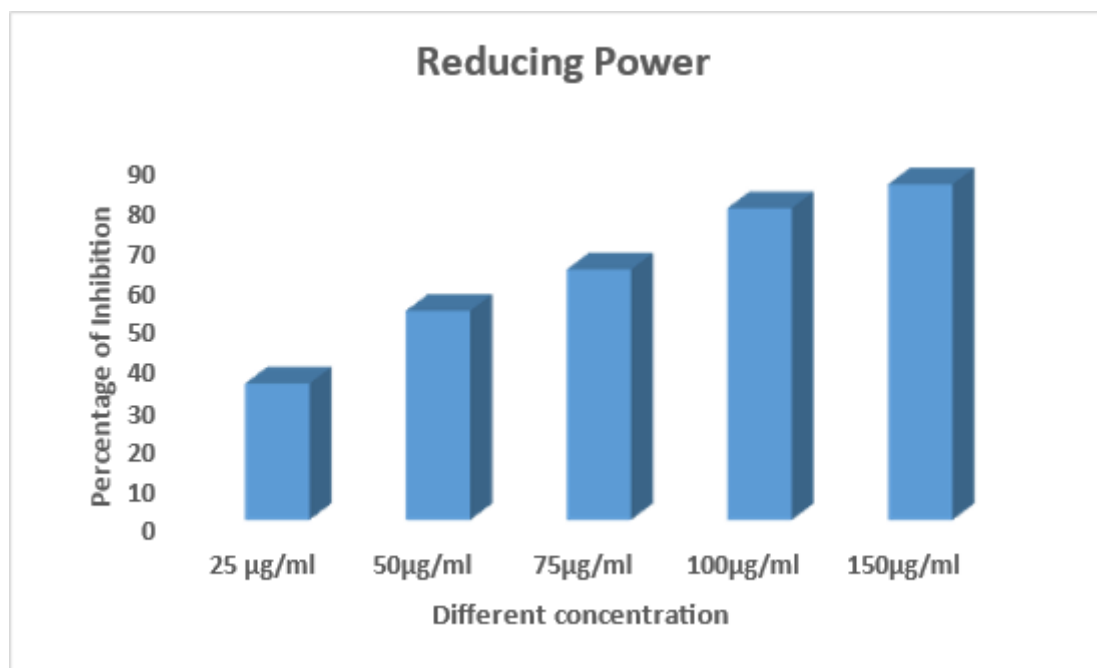
### Visual Observation

The reaction mixture of 1 mM aqueous solution of gold chloride with plant extract exhibits a pink color indicating reduction of gold ions to gold nanoparticles (Fig 1). Initially, the mixture turned brownish pink. After that, the color of the solution vigorously changed into pink while increasing the incubation time from 30 min to 48 h. The color of the solution is stable without change of intensity indicating that the reduction process was complete. Color change occurred in the reaction mixture due to the

excitation of surface plasmon resonance in the nanoparticles. This important observation indicates the reduction of the gold ions and the biosynthesis of gold nanoparticles. Previously, Singaravelu et al. [51] reported that the gold nanoparticle synthesis process was started at 1 h and the entire process was finished at 15 hours. Kannan et al [52] started the synthesis of gold nanoparticles at 50 min and completed the process within 48 hours of incubation time. However, in the current study, the synthesis of the gold nanoparticles process was rapidly started at 50 min for Piper Longum and Eucalyptus respectively and completed at 36 hours of incubation time.

**Table 1.** Albumin Denaturation Assay - Percentage of Inhibition of Plant Derived AuNPs (Standard - Diclofenac Sodium)

Test	Concentration (ug/ml)	Plant Derived AuNPs (%)	Standard (%)
BSA ASSAY	10	49	48
	20	57	60
	30	72	69
	40	75	78
	50	87	81



**Figure 4.** Percentage of Inhibition at Different Concentrations of Gold Nanoparticles

### Anti-inflammatory Activity

Bovine serum albumin (BSA) accounts for approximately 60% of all animal serum proteins. It is commonly used in cell culture, especially when protein supplementation is needed, and other serum components are not needed. BSA denatures upon heating and begins to express antigens associated with type III hypersensitivity reactions associated with diseases such as rheumatoid arthritis, glomerulonephritis, serum sickness, and systemic lupus erythematosus. The inflammatory response involves a complex sequence of enzyme activation, mediator release, fluid extravasation, cell migration, tissue breakdown and repair, aimed at host defense, and is normally activated in the most disease states. Chronic inflammatory diseases such as rheumatoid arthritis remain among the most important health problems for the world's population. Currently, although synthetic drugs dominate the market, a toxic factor cannot be excluded for these drugs. Its long-term use can lead to serious adverse effects during chronic administration. There is currently a great deal of interest in finding nanoparticles with anti-inflammatory activity, which could lead to the discovery of novel therapeutic agents that not

only suppress inflammation but also ameliorate the course of various disease states in which the inflammatory response is present. The use of animals in experimental pharmacology research poses certain problems, such as ethical concerns and the lack of justification for the use of animals when other suitable methods are available or can be explored. Therefore, in this study, a protein denaturation bioassay was chosen to evaluate the anti-inflammatory properties of eucalyptus and pepper derived AuNPs in vitro. Denaturation of tissue proteins is one of the causes of inflammation and arthritis. The formation of autoantigens in some diseases may be due to protein denaturation. Drugs that prevent protein denaturation would aid in the development of anti-inflammatory drugs.

An increase in the absorbance of the test sample relative to the control indicates that the protein is stable, ie. Plant extract derived AuNPs and the reference reagent, diclofenac sodium, inhibit heat-induced protein denaturation. The present results demonstrate that pepper and eucalyptus extracts mediated inhibition of protein denaturation by AuNPs in a concentration-dependent manner.

The maximum effect of AuNPs (150 µg/mL) was found to be greater than that of standard

diclofenac sodium. From the current study, it can be concluded that AuNPs mediated by plant extracts have good anti-inflammatory effects on protein denaturation.

Protein denaturation is closely linked to the occurrence of an inflammatory response and leads to various inflammatory diseases such as arthritis. Tissue damage may be due to denaturation of the protein components of the cells or of the intercellular matrix. Thus, the substance's ability to inhibit protein denaturation suggests a clear potential for anti-inflammatory activity. Plant extracts can inhibit protein denaturation. The ability of different plants such as *rachis* inflorescence, endosperm, leaves and pericarp of *B. racemosa* to inhibit albumin protein denaturation, the inhibition ranged from  $43.33 \pm 0.002\%$  to  $70.58 \pm 0.004\%$ , indicating anti-inflammatory properties.

## Conclusion

Gold nanoparticles have been extensively studied in nanostructures for enhanced biomedical applications. Nanosystems and nanomaterials based on gold nanoparticles are

sweat-resistant modifications for drug delivery. Many studies have shown that pepper and eucalyptus derived AuNP have broad therapeutic effects such as anti-inflammatory and antibacterial activities due to inhibition of AA, COX, LOX, cytokine, and NF- $\kappa$ B metabolism.

From this study, it can be concluded that gold nanoparticles derived from pepper and eucalyptus can be used as a potential source of anti-inflammatories.

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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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